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Pre - Harvest and Post - Harvest applications of Salicylic Acid and Methylchlorflurenol to Modify Petal Senescence in Calendula Officinalis L.

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Abstract: ***Flowering buds of Calendula officinalis plants were provided pre- harvest treatments with 40 and 20µM concentrations of salicylic acid (SA) and methylchlorflurenol (a morphactin, MOR) in separate experiments to find out their effects on flower diameter and certain physiological changes like sugars (reducing and non- reducing), starch and protein contents as well as activities of α- amylase and peroxidase (POD). One of these experiments was further extended to find out how scapes of these treated and untreated flowers behaved when put in vase solutions of SA, MOR, SUC, SA+MOR+SUC and double distilled water (DDW, control). Pre- harvest MOR treatment was responsible for maximum flower size followed by SA in comparison to control. Flower diameter was also slightly higher in those cases having post- harvest PGR treatments; the maximum value was observed in plants having SA+ MOR+ SUC treatment. These PGR's were effective in lowering the degradation of starch and protein and they minimized the accumulation of reducing and non- reducing sugars in comparison to untreated flower petals. PGR's were also able to maintain higher POD activity while α- amylase activity was slightly lower. These degradations and other symptoms of petal senescence were slowed down individually by SA and MOR and also in combination as SA+ MOR+ SUC.***

Keywords: Petal senescence, flower diameter, starch and sugars, total soluble protein, α-amylase activity, peroxidase activity.

I. INTRODUCTION

The phase occurring between maturity and death of entire flower or flower part is known as flower senescence. It is responsible for the metabolic degradation and removal of petals after it has attracted pollinators for sexual development, signals the initiation of ovule development and seed production [1]. Organized breakdown of polysaccharides, proteins, lipids and nucleic acids can be witnessed during petal senescence like that of leaves [2]. This process will result the formation of sugars, amino acids and amides that can be transported to other plant parts [3- 4].

A cut flower may be defined as flower or an inflorescence containing more than one floral unit in the opened or unopened state which is harvested and marketed for ornamental purpose. Cut flowers lose their freshness and turgidity quickly than uncut flowers. When detached from plants, cut flowers and scapes get very limited supply of water and nutrients (inorganic and organic). Sucrose, an important metabolite and organic nutrient, is the source of carbon and energy and is an essential requirement to maintain quality of cut flowers [5]. The osmotic concentration of petal cell sap can be increased by providing sucrose in holding solution [6].

The longevity of cut flowers can be increased and the onset of senescence can be delayed by the application of various plant growth regulators (PGR's) like cytokinins, auxins, salicylic acid, morphactins, gibberellins, polyamines, etc [7].

Salicylic acid (SA) belongs to an unique group of compounds commonly known as plant phenolics. They possess an aromatic ring bearing a hydroxyl group or its functional derivative [8]. Among various effects, SA is also known to regulate flowering [9] and may be involved in flower longevity as the conversion of 1- aminocyclopropane -1- carboxylic acid (1- ACC) to ethylene is blocked by it in pear cell suspension culture [10]. Only a few studies have been carried out with SA for delaying petal senescence [11- 12]. Morphactins are synthetic PGR [13] and can also regulate petal senescence by lowering protein degradation and membrane damage [11, 14].

It was realized to undertake a novel study involving SA and methylchlorflurenol (MCF, a morphactin, MOR) to make a comparison between the two regarding their effectiveness to regulate petal senescence in vase solutions of those flowers which already had pre-harvest treatments with SA and MOR. Selected vase solutions were not only individual SA and MOR but SA, MOR and sucrose were also included in the combined form to assess the effect. It was also thought to find out effects of these PGR's on the flower diameter and some physiological changes when pre- harvest applications were made at flower bud stage. *Calendula officinalis* plants on which some earlier studies carried out in our laboratory was selected to carry out this investigation.

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II. MATERIAL AND METHODS

Saplings of *Calendula officinalis* L. were raised in the experimental beds from soaked seeds. Uniform saplings were transferred to 3 experimental beds, each measuring 1×3 meters during 1st week of November in the wire net enclosure of departmental garden.

A. Experiment no. 1

Flowering buds numbering 35- 40 were tagged in each experimental bed, which were treated either with salicylic acid (SA, 40µM) or methylchlorflurenol (a morphactin, MOR, 40 µM) using a glass automizer. Flowering buds maintained as control were sprayed with double distilled water (DDW). Cross diameter of tagged flowers was measured after 10 days. Developed flowers 15 days after pre- harvest treatments were cut under water inside a bucket and brought to the laboratory. After removing leaves from flower twigs, scapes were recut under water to have an uniform length of 14 cm. Scapes were transferred to 100ml Borosil- make conical flasks, each having 30ml holding solution. Five conical flasks were used for taking each holding solution and three scapes were placed in each flask. Selected holding solutions were: distilled water (DDW, control), salicylic acid (40µM), methylchlorflurenol (MOR, 40µM), sucrose (SUC, 0.1M), [SA (40µM) + MOR (40µM) + SUC + (0.1M)]. Flower diameter and moisture content were noted at 0, 3 and 6- day while cumulative uptake of vase solution was recorded after 6- day. Petal samples were collected to find out the amount of reducing and non- reducing sugars; starch and protein as well as peroxidase and α- amylase activity.

B. Experiment no. 2

About 30 days after first experiment, flower buds of *C. officinalis* were tagged again (35- 40 flowering buds in each experimental bed) and pre- harvest treatments were given again with 20 µM concentration of both SA and MOR. Separate flower buds were sprayed with DDW to maintain as control sets. Flower diameter was noted again after 10 days of treatments. Samples were also collected to find out the amount of reducing and non- reducing sugars, starch and total soluble protein. Besides these, α- amylase activity and POD activity were also recorded.

C. Estimation of starch and sugars

The method recommended by Hart and Fisher [15] was followed for the estimation of starch and sugars .One hundred milligram sample was extracted in 10 ml DDW and centrifuged at 5000 rpm (2124 RCF) for 10 min. in a Remi centrifuge. The residue left after the separation of aqueous extract was later used for the quantification of starch. Aqueous extract was used for the determination of reducing and non- reducing sugars using anthrone- H_2SO_4 reagent. They were quantified in terms of glucose. Detail procedure has been described elsewhere [12].

D. α- Amylase activity

The specific activity of α- amylase was determined following the method of Bernfeld [16]. Amount of petals used for the assay was 100 mg and starch was used as a substrate. The protein content of the enzyme extract was quantified using Coomassie brilliant blue dye G- 250 by the method of Bradford [17]. Detail procedure can be seen in a paper of Khokhar et al. [12].

E. Determination Of Guaiacol Peroxidase (Pod) Activity

Total peroxidase activity was measured by the method of Maehly [18] and it was expressed in terms of per mg protein per 10 min. Protein was estimated from the same extract following the procedure of Bradford [17] at 595 nm using uv- vis spectrophotometer (Systronics, Double beam Spectrophotometer 2203, India).

F. Estimation Of Protein Content

Total soluble protein was estimated by the method of Bradford [17] as mentioned earlier. Bovine serum albumin (BSA) was used for making standard curve which was used to find out the amount of protein.

III. RESULTS

Results related to pre- harvest treatments to flower buds of *Calendula officinalis* with 20 and 40µM concentrations of salicylic acid (SA) and methylchlorflurenol (a morphactin, MOR) have been presented in Table 1-7.

A. Flower Diameter

Table 1 shows values of flower diameter 10 days after treatments. Flower diameter found to be considerably higher in plants having either SA or MOR treatments in comparison to control. Further, plants having MOR application showed maximum size of flower among various stages compared to control and SA. Table 2 shows flower diameter of *C. officinalis* plants subjected to applications

of SA (20µM), MOR (20µM) and double distilled water (DDW) to tagged flower buds at different experimental beds. Here also, diameter was recorded 10 days after treatments. Observations based upon four different stages of flowers indicated maximum values with morphactin followed by salicylic acid and control.

B. Reducing And Non- Reducing Sugars

Variations in the amount of reducing and non- reducing sugars reveal slightly higher amount in petals of untreated plants in comparison to treated ones (Table 3). Flowers having either 40µM or 20µM pre- harvest application of SA exhibited significantly lower values of reducing sugars in petals than those having MOR and double distilled water (DDW) treatments. The amount of non-reducing sugars was also slightly lower in petals having both 40 and 20µM SA treatments but the difference was non- significant in case of 40µM concentration. Petals of all treated and control plants, however, showed much higher quantities of non- reducing sugars than reducing sugars.

C. Starch And A- Amylase Activity

Flower petals of those *C. officinalis* plants having treatments of 40µM concentration of two plant growth regulators (PGR's) at flowering bud stage showed maximum quantity of starch and α- amylase activity in case of morphactin followed by salicylic acid (Table 4- 5). Petals developed from untreated flower buds (control) were unique in having least values of starch and α- amylase activity. Recorded amounts were significantly different.

D. Guaiacol Peroxidase (Pod) Activity and Total Soluble Protein

POD activity and total soluble protein of the petals having pre- harvest SA and MOR treatments have been presented in Table 6-7. POD activity was highest in samples received MOR application followed by SA and control irrespective of specific concentration of PGR's. Changing the concentration from 40 to 20µM in second experiment, maximum activity was still noticed in petals of MOR treated flowering buds and minimum in control (Table 6). Selected PGR's were also showing their effectiveness in maintaining higher amount of total soluble protein as compared with control petals. However, SA treatments showed higher protein content in comparison to MOR treatments (Table 7).

Post- harvest changes in cut flowers of *C. officinalis* have been presented in Table 8- 14.

E. Flower Diameter and Cumulative Uptake Of Vase Solution

Between 0 to 6th day, diameter of cut flowers exhibited a decreasing trend in both control and treated sets (Table 8). Vase solution comprising 40µM concentration of SA and MOR along with 0.1M sucrose was able to maintain highest values at 3 and 6- day over other solutions. The order of effectiveness among these solutions in maintaining higher values of diameter was (SA + MOR + SUC) > SA > MOR > SUC > control at 3rd day and (SA + MOR + SUC) > MOR > SA > SUC > control at 6- day. Cumulative uptake of vase solutions as presented in Table 9 indicated smaller difference in the volume of absorbed solution. Uptake of MOR was slightly higher than other vase solutions.

F. Reducing and Non- Reducing Sugars

Enhancement in the concentration of reducing and non- reducing sugars was observed during 6 days in petals of flowers maintained as control and treated ones. Therefore, the amount of total sugars was significantly higher at 6- day than at 3- day. However, control flower petals had maximum amount of total sugars while flower petals being placed in 0.1M sucrose solution exhibited least value. Individual PGR (SA and MOR separately) and combined PGR's with sucrose were also effective in lowering the value of total sugars in petals (Table 10).

G. Starch and α- amylase activity

Alteration in the values of starch and α- amylase activity in petals of flowers placed in various holding solutions indicate rapid depletion of the former in the control sets while PGR's and sucrose separately and also in combination could able to minimize this degradation (Table 11- 12). Maximum retention of starch was noticed in petals of cut flowers treated with (SA + MOR + SUC) followed by SUC, MOR, SA and control (Table 11). α- Amylase activity data indicate smaller variations between the treatments as well as control and treatments. However, the activity registered adequate increase between 3 to 6 days (Table 12).

H. Guaiacol peroxidase (POD) activity and total soluble protein

Guaiacol peroxidase activity and protein content in petals of post- harvest treated cut flowers can be seen in Table 13- 14. POD values increased to some extent between 3 and 6- day in all samples. Moreover, control samples showed significantly lower value than the treated ones at a particular stage. Amount of protein, however, decreased significantly between 3 and 6- day stages (Table 14). Protein quantity was the least in controls while it was maximum in (SA + MOR + SUC) treated ones at both the stages. Both PGR's could able to retain some amount of protein in petals.

Table1. Flower diameter, dry weight and moisture content of *Calendula officinalis* L. with pre- harvest treatments of Methylchlorflurenol (a morphactin, MOR, 40µM) and Salicylic acid (SA, 40µM) after 10 days.

Flower stages	Control	SA(40µM)	MOR(40µM)
	Flower diameter (cm)		
Fully opened	6.93	7.50	7.66
70% opened	5.10	6.55	6.65
50% opened	5.77	5.84	6.15
Slightly opened	1.67	1.95	2.11

Dry weight (%), Moisture content (%)

Treatments	Dry weight	Moisture content
Control	14.33	85.67
SA(40µM)	12.66	87.34
MOR(40µM)	11.00	89.00

Table2. Flower diameter, dry weight and moisture content of *Calendula officinalis* L. with pre- harvest treatments of Methylchlorflurenol (a morphactin, MOR, 20µM) and Salicylic acid (SA, 20µM) after 10 days.

Flower stages	Control	SA(20µM)	MOR(20µM)
Flower diameter (cm)			
Fully opened	6.02	7.53	7.64
70% opened	5.66	6.24	6.59
50% opened	4.42	4.90	4.92
Slightly opened	2.68	2.98	3.05

Dry weight (%), Moisture content (%)

Treatments	Dry weight	Moisture content
Control	16.33	83.67
SA(20µM)	14.66	85.34
MOR(20µM)	14.00	86.00

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Table3. *Calendula officinalis* L. showing amount* of reducing, non- reducing and total sugars (in mg/100mg dry weight) in petals having pre- harvest treatments of Salicylic acid (SA, 40 µM and 20µM), and Methylchlorflurenol (a morphactin, MOR,40µMand 20µM).

Treatments	**(A)** 40µM	**(B)** 20µM
Reducing sugar (mg/100mg dry wt)		
Control	7.334±0.247^{A}	3.9133±0.012^{A}
SA	5.691±0.346^{B}	2.8550±0.038^{B}
MOR	6.882±0.218^{A}	3.7233±0.158^{A}
L.S.D(P≤0.05)	0.833	0.756
ANOVA(F2,15)	9.450	5.060
Non reducing sugar (mg/100mg dry wt)		
Control	12.676±0.725^{A}	14.6848±0.958^{A}
SA	11.020±1.251^{A}	8.3222±0.876^{B}
MOR	11.865±0.889^{A}	12.3865±1.189^{A}
L.S.D(P≤0.05)	2.955	3.065
ANOVA(F2,15)	0.714	10.037
Total sugar (mg/100mg dry wt)		
Control	20.011±0.790^{A}	18.5982±0.877^{A}
SA	16.711±1.199^{B}	11.177±1.026^{A}
MOR	18.753±0.980^{AB}	16.1098±1.031^{A}
L.S.D(P≤0.05)	3.026	2.957
ANOVA(F2,15)	2.751	14.818

*Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05).
(A), (B); Data based upon two separate experiments, the second one conducted a month later of the first one.

Table4. *Calendula officinalis* L. showing the amount of starch* (mg/100mg dry weight) in petals having pre - harvest treatments of Salicylic acid (SA, 40 µM and 20µM), and Methylchlorflurenol (a morphactin,MOR, 40µM and 20µM).

Treatments	(A)40µM	(B)20µM
Starch content		
Control	27.760±6.527^{B}	16.824±0.824^{C}
SA	30.338±4.070^{B}	22.805±1.009^{B}
MOR	38.639±3.704^{A}	32.749±1.836^{A}
L.S.D(P≤0.05)	14.859	0.027
ANOVA(F2,15)	1.330	69.157

* Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05).
(A), (B); Data based upon two separate experiments, the second one conducted a month later of the first one.

Table5. Calendula officinalis L. showing α – Amylase activity (unit mg^{-1} protein) in petals having pre - harvest treatments of Salicylic acid (SA, 40 µM and 20µM), and Methylchlorflurenol (a morphactin,MOR, 40µM and 20µM).

Treatments	(40)µM	(20)µM
	α– Amylase activity	
Control	9.059±1.186^{A}	5.464±0.539^{A}
SA	5.298±0.694^{B}	2.641±0.361^{C}
MOR	5.635±0.813^{B}	3.078±0.201^{B}
L.S.D(P≤0.05)	2.780	1.401
ANOVA(F2,15)	5.090	10.687

* Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05).
(A), (B); Data based upon two separate experiments, the second one conducted a month later of the first one.

Table6. *Calendula officinalis* L. showing peroxidase (POD) activity* (Unit/mg protein min^{-10}) in petals having pre- harvest treatments of Salicylic acid (SA, 40 µM and 20µM), and Methylchlorflurenol (a morphactin,MOR, 40µM and 20µM).

Treatments	(A)40µM	(B)20µM
	POD activity	
Control	1.198±0.198^{B}	0.133±0.155^{C}
SA	2.265±0.768^{A}	0.196±0.144^{B}
MOR	2.744±0.496^{A}	0.282±0.031^{A}
L.S.D(P≤0.05)	1.630	0.027
ANOVA(F2,15)	2.142	69.157

* Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05).
(A), (B); Data based upon two separate experiments, the second one conducted a month later of the first one.

Table7. *Calendula officinalis* L. showing the protein content* in (mg/100 mg dry weight) in petals having pre - harvest treatments of Salicylic acid (SA, 40 µM and 20µM), and Methylchlorflurenol (a morphactin,MOR, 40µM and 20µM).

Treatments	(A)40µM	(B)20µM
	Protein content	
Control	3.069±0.337^{B}	1.037±0.385^{C}
SA	5.312±0.695^{A}	3.647±0.976^{A}
MOR	4.882±0.270^{A}	2.059±0.154^{B}
L.S.D(P≤0.05)	1.456	0.218
ANOVA(F2,15)	6.073	329.010

* Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05).
(A),(B); Data based upon two separate experiments, the second one conducted a month later of the first one.

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Table8. *Calendula officinalis* L. showing flower diameter (in cm) and moisture content (%) of petals when scapes were maintained in vase solutions of Methylchlorflurenol (a morphactin, MOR,40µM), Salicylic acid (SA,40µM), Sucrose (SUC,0.1M), and SA(40µM)+ MOR(40µM)+ SUC(0.1M).

Treatments	0-Day	3-Day	6-Day
	Flower diameter (cm)		
Initial value	7.04		
Control(DDW)	-	5.43	4.24
SA(40µM)	-	6.61	4.81
MOR(40µM)	-	6.37	4.98
Sucrose(0.1M)	-	6.04	4.62
SA+MOR+SUC	-	6.92	5.42
	Moisture content (%)		
Initial value	90		
Control(DDW)	-	87.30	86.66
SA(40µM)	-	88.33	87.33
MOR(40µM	-	88.66	87.66
Sucrose(0.1M)	-	88.00	87.00
SA+MOR+SUC	-	90.00	89.33

Table9. *Calendula officinalis* L. showing volume of solution absorbed (ml) scape^3 when they were maintained in vase solutions of Methylchlorflurenol (a morphactin, MOR,40µM), Salicylic acid (SA,40µM), Sucrose (SUC,0.1M), and SA(40µM)+ MOR(40µM)+ SUC(0.1M).

Initial volume taken = 30ml

Treatments	Volume of absorbed solution (ml) during 6-day.
Control(DDW)	11.00
SA(40µM)	12.00
MOR(40µM)	12.75
Sucrose(0.1M)	11.5
SA+MOR+SUC	11.5

Table10. Calendula officinalis L. showing amount* of reducing, non- reducing and total sugars (in mg/100mg dry weight) in petals having post - harvest treatments of Salicylic acid (SA, 40 µM), and Methylchlorflurenol (a morphactin, MOR,40µM), Sucrose(SUC,0.1M) and SA(40µM)+MOR(40µM)+SUC(0.1M) after 3 and 6 day stages.

Treatments	0 Day	3 Day	6 Day
		Reducing sugar (mg/100mg dry wt)	
Control	7.334±0.247^{A}	14.238±0.875^{A}(+94.137)	19.102±0.383^{A}(+160.458)
SA(40µM)	5.691±0.346^{B}	12.317±0.871^{AB}(+116.429)	14.616±1.756^{BC}(+156.827)
MOR(40µM)	6.882±0.218^{A}	13.167±0.903^{AB}(+91.325)	16.809±0.000^{AB}(+144.246)
SUC(0.1M)	7.334±0.247^{A}	10.850±0.780^{B}(+47.941)	12.155±0.311^{C}(+65.735)
SA+MOR+SUC	7.334±0.247^{A}	12.582±0.208^{AB}(+71.557)	15.760±0.736^{B}(+114.890)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	0.833	2.106	2.719
ANOVA	9.450 (F2,15)	3.435 (F4,10)	8.910 (F4,10)
		Non reducing sugar (mg/100mg dry wt)	
Control	12.676±0.725^{A}	18.379±0.433^{A}(+44.991)	36.178±8.353^{A}(+185.405)
SA(40µM)	11.020±1.251^{A}	12.087±0.603^{B}(+9.682)	19.654±1.439^{B}(+78.348)
MOR(40µM)	11.865±0.889^{A}	15.472±0.452^{A}(+30.400)	25.238±2.600^{AB}(+112.710)
SUC(0.1M)	12.676±0.725^{A}	6.690±1.131^{C}(-47.223)	15.185±0.460^{B}(+19.793)
SA+MOR+SUC	12.676±0.725^{A}	10.689±0.759^{B}(-15.675)	16.040±1.239^{B}(+26.538)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	2.955	2.279	12.552
ANOVA	0.714 (F2,15)	39.924 (F4,10)	4.694(F4,10)
		Total sugar (mg/100mg dry wt)	
Control	20.011±0.790^{A}	32.618±0.481^{A}(+63.000)	55.280±8.389^{A}(+176.248)
SA(40µM)	16.711±1.199^{B}	24.404±1.295^{B}(+46.036)	34.270±2.804^{BC}(+105.075)
MOR(40µM)	18.753±0.980^{AB}	29.139±0.551^{A}(+55.383)	42.047±2.600^{B}(+124.215)
SUC(0.1M)	20.011±0.790^{A}	17.541±0.353^{C}(-12.343)	27.340±0.140^{C}(+36.625)
SA+MOR+SUC	20.011±0.790^{A}	23.938±0.214^{B}(+19.624)	31.800±0.140^{BC}(+58.913)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	3.026	2.475	12.998
ANOVA(F4,10)	2.751 (F2,15)	34.126 (F4,10)	7.065 (F4,10)

*Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05)

Table11. *Calendula officinalis* L. showing the amount of starch* (mg/100mg dry weight) in petals having pre- harvest treatments of Salicylic acid (SA, 40 µM), and Methylchlorflurenol (a morphactin, MOR,40µM), Sucrose(SUC,0.1M) and SA(40µM)+ MOR(40µM)+SUC(0.1M) after 3 and 6 day stages.

Treatments	0 Day	3 Day	6 Day
		Starch content	
Control	27.760±6.527^{B}	16.426±2.371^{C}(-40.829)	10.162±0.781^{D}(-63.393)
SA(40µM)	30.338±4.070^{B}	24.141±1.41^{B}(-20.427)	14.467±0.584^{C}(-52.314)
MOR(40µM)	38.639±3.704^{A}	26.364±0.651^{A}(-31.768)	18.095±1.423^{B}(-53.169)
SUC(0.1M)	27.760±6.527^{B}	24.492±0.128^{B}(-11.772)	20.738±1.558^{AB}(-25.295)
SA+MOR+SUC	27.760±6.527^{B}	26.925±6.730^{A}(-3.008)	23.421±2.360^{A}(-15.630)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	14.859	10.618	2.697
ANOVA	1.330 (F2,15)	14.620 (F4,10)	37.140 (F4,10)

*Mean ± S.E. of mean (n=6).

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Table12. *Calendula officinalis* L. showing α- amylase activity (unit mg^{-1} protein) in petals having post - harvest treatments of Salicylic acid (SA, 40 µM), Methylchlorflurenol (a morphactin, MOR,40µM), Sucrose(SUC,0.1M) and SA(40µM)+ MOR(40µM)+SUC(0.1M) after 3 and 6 day stages.

Treatments	0 Day	3 Day	6 Day
		α- amylase	
Control	9.059 ± 1.186^{A}	5.155 ± 0.401^{A}(-43.095)	5.807 ± 0.306^{A}(-35.898)
SA(40µM)	5.298 ± 0.694^{B}	3.282 ± 0.327^{B}(-38.052)	3.322 ± 0.245^{B}(-37.297))
MOR(40µM)	5.635 ± 0.813^{B}	4.235 ± 0.031^{AB}(-24.845)	5.003 ± 0.570^{A}(-11.216)
SUC(0.1M)	9.059 ± 1.186^{A}	4.779 ± 0.336^{A}(-47.246)	5.501 ± 0.399^{A}(-39.276)
SA+MOR+SUC	9.059 ± 1.186^{A}	4.875 ± 0.723^{A}(-46.186)	5.996 ± 0.359^{A}(-33.812)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	2.780	1.341	0.941
ANOVA	5.090 (F2,15)	29.147 (F4,10)	12.977(F4,10)

*Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05)

Table13. *Calendula officinalis* L. showing peroxidase(POD) activity*(Unit/mg protein min^{-10}) in petals having post - harvest treatments of Salicylic acid (SA, 40 µM), and Methylchlorflurenol (a morphactin, MOR,40µM), Sucrose(SUC,0.1M) and SA(40µM)+ MOR(40µM)+SUC(0.1M) after 3 and 6 day stages

Treatments	0 Day	3 Day	6 Day
		POD activity	
Control	1.198 ± 0.198^{B}	2.005 ± 0.442^{D}(+67.362)	2.783 ± 0.332^{D}(+132.304)
SA(40µM)	2.265 ± 0.768^{B}	3.651 ± 0.340^{C}(+61.192)	4.501 ± 0.279^{C}(+98.720)
MOR(40µM)	2.744 ± 0.496^{A}	5.046 ± 0.294^{B}(+83.892)	5.952 ± 0.167^{B}(+116.910)
SUC(0.1M)	1.198 ± 0.198^{B}	4.011 ± 0.057^{C}(+234.808)	5.152 ± 0.578^{BC}(+330.050)
SA+MOR+SUC	1.198 ± 0.198^{B}	6.545 ± 0.292^{A}(+446.327)	6.760 ± 0.306^{A}(+464.274)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	1.630	0.983	1.131
ANOVA	2.142 (F2,15)	29.147 (F4,10)	17.807 (F4,10)

*Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, (p≤0.05).

Table14. *Calendula officinalis* L. showing protein content* (mg/100 mg dry weight) in petals having post - harvest treatments of Salicylic acid (SA, 40 µM), and Methylchlorflurenol (a morphactin, MOR,40µM), Sucrose(SUC,0.1M) and SA(40µM)+ MOR(40µM)+SUC(0.1M) after 3 and 6 day stages.

Treatments	0 Day	3 Day	6 Day
		Protein content	
Control	3.069 ± 0.337^{B}	0.1430 ± 0.030^{D}(-95.341)	0.1157 ± 0.035^{E}(-96.230)
SA(40µM)	5.312 ± 0.695^{A}	4.2520 ± 0.603^{A}(-19.955)	0.5973 ± 0.036^{AB}(-88.756)
MOR(40µM)	4.882 ± 0.270^{A}	2.7123 ± 0.339^{B}(-44.443)	0.2873 ± 0.017^{B}(-94.115)
SUC(0.1M)	3.069 ± 0.337^{B}	0.7497 ± 0.187^{C}(-75.572)	0.2053 ± 0.002^{B}(-93.311)
SA+MOR+SUC	3.069 ± 0.337^{B}	2.5787 ± 0.644^{B}(-15.976)	0.8993 ± 0.001^{A}(-70.697)
(40+40 µM+0.1M)	-	-	-
L.S.D(P≤0.05)	1.456	1.360	0.076
ANOVA	6.073 (F2,15)	14.620 (F4,10)	177.799 (F4,10)

*Mean ± S.E. of mean (n=6).

Means with different letters on the superscript in the same column are significantly different, ($p \leq 0.05$).

IV. DISCUSSION

Results from Table 1 and 2 clearly indicate that pre- harvest applications of PGR's like SA and MOR, which affect plant growth and development in many ways, helped to improve the flower size. The degree of effectiveness among treatments was in the order: MOR > SA > control (DDW). This pattern was also true when scapes were placed in holding solutions as post- harvest treatments (Table 8). Moreover, combined application of SA + MOR + SUC favored further increment in flower diameter. Efficiency of a PGR with sucrose in comparison to their individual effect in minimizing the shrinkage of petals and decrease in flower diameter was reported in our laboratory earlier with *C officinalis* [12]. But, those flowers did not get a pre- harvest treatment as in the present case. Unlike diameter, not much difference was observed in the moisture content of the petals having various applications. However, the value declined a little between 0 to 6 day. Combined application proved to be better in retaining higher moisture content. Pre- harvest and post- harvest treatments with SA and MOR (Table 3 and 10) clearly revealed that both PGR's at both concentrations could check the increment in reducing and non- reducing sugars to some extent. Data were significant mostly after SA application. Individual application of SUC and combined treatment with (SA + MOR + SUC) were very effective in lowering quantities of both kinds of sugars. It was really interesting to notice lesser starch breakdown due to treatments with these PGR's and SUC (Table 4 and 11). Although a lot of differences were noticed in starch content of petals subjected to various treatments and control, α- amylase activity demonstrated smaller variations (Table 5 and 12). Significantly higher level of starch in petals having pre- harvest treatments of MOR and SA may be due to greater synthesis and limited degradation. Flowering process as such requires lot of sugars which can be supplied by the available sugars and also by the depletion of starch. The requirement of sugars increases in case of cut flowers [19], which is associated with senescence. Various reports are available regarding the comparative amount of these sugars [12]. In carnation flowers, concentration of reducing sugars was much higher at maturity but reducing and non-reducing sugars decreased at senescence [20]. While working on Hibiscus rosa sinensis, Trivellini et al.[21] have reported a decline in reducing sugars and increment in sucrose at senescence. However, several studies carried out in our laboratory earlier noticed the accumulation of both kinds of sugars in different cut flowers [11, 14, 4, 22, 23, 12]

As far as POD activity is concerned, samples collected from petals of both pre- harvest and post- harvest treatments indicated comparatively higher activity in the treated ones than in the untreated controls. In the cut flowers of vase solutions also, some increment was noticed between3 to 6- day. Earlier studies on POD indicated marked increase in its activity with the progress of senescence [24]. Higher POD activity is responsible for the breakdown of H_20_2 [25]. In order to control the reactive oxygen species (ROS) level and protect plants from their toxic effects, both enzymatic and non- enzymatic options are available to plants [26]. ROS like singlet oxygen, superoxide radical, hydroxyl radical, hydroperoxyl radical and H_20_2 are produced in plants due to various kinds of abiotic stresses. Most common and well known enzymatic antioxidants are superoxide dismutase, catalase, ascorbate peroxidase, monodehydro ascorbate reductase, dehydroascorbate reductase, guaiacol peroxidase and glutathione reductase. The activity of the enzyme varies depending upon plant species and stress conditions. Severing a flower from the main plant and putting its basal part in the vase solution is an act of giving stress. During 6- day period of the post- harvest experiment, the progress of senescence became faster than the uncut flower, being attached to the plant. The decline in the protein content in the petals of post-harvest maintained scapes during 6- day can be due to a decrease in synthesis and also an increase in degradation as reported earlier in Hemerocallis, Iris and Petunia [27- 29]. In these flowers, a sharp increase in protease activity was observed during petal senescence. The protein breakdown has also been reported in Ipomoea [30], Calendula and Salvia [24] and Chrysanthemum [4]. Polyamines were able to check protein degradation appreciably in Chrysanthemum [4] while a combination of ethanol + sucrose could minimize the protein loss in Calendula and Saliva [24]. Present study has revealed that both SA and MOR applications at pre- harvest and post- harvest stages can retain more soluble protein than control. However, (SA+ MOR + SUC) combination among all applications was the best as it allowed maximum retention of protein.

V. CONCLUSION

Both experiments with two different concentrations of MOR and SA, showed highest diameter in *C. officinalis* flowers after pre-harvest MOR treatment followed by SA and control. Accumulation of sugars was also noticed in untreated flowers and SA application favoured least accumulation. Flowers having PGR's treatments exhibited higher levels of starch, total soluble proteins and POD activity than control. Higher values of flower diameter were also noticed when SA and MOR were used as vase solutions in which scapes were maintained. Both PGR's as post- harvest treatments helped in decreasing the accumulation of sugars by minimizing the starch breakdown. Combined vase solution of (SA+ MOR+ SUC) was the best in lowering the shrinkage in flower diameter and loss in starch value and protein content.

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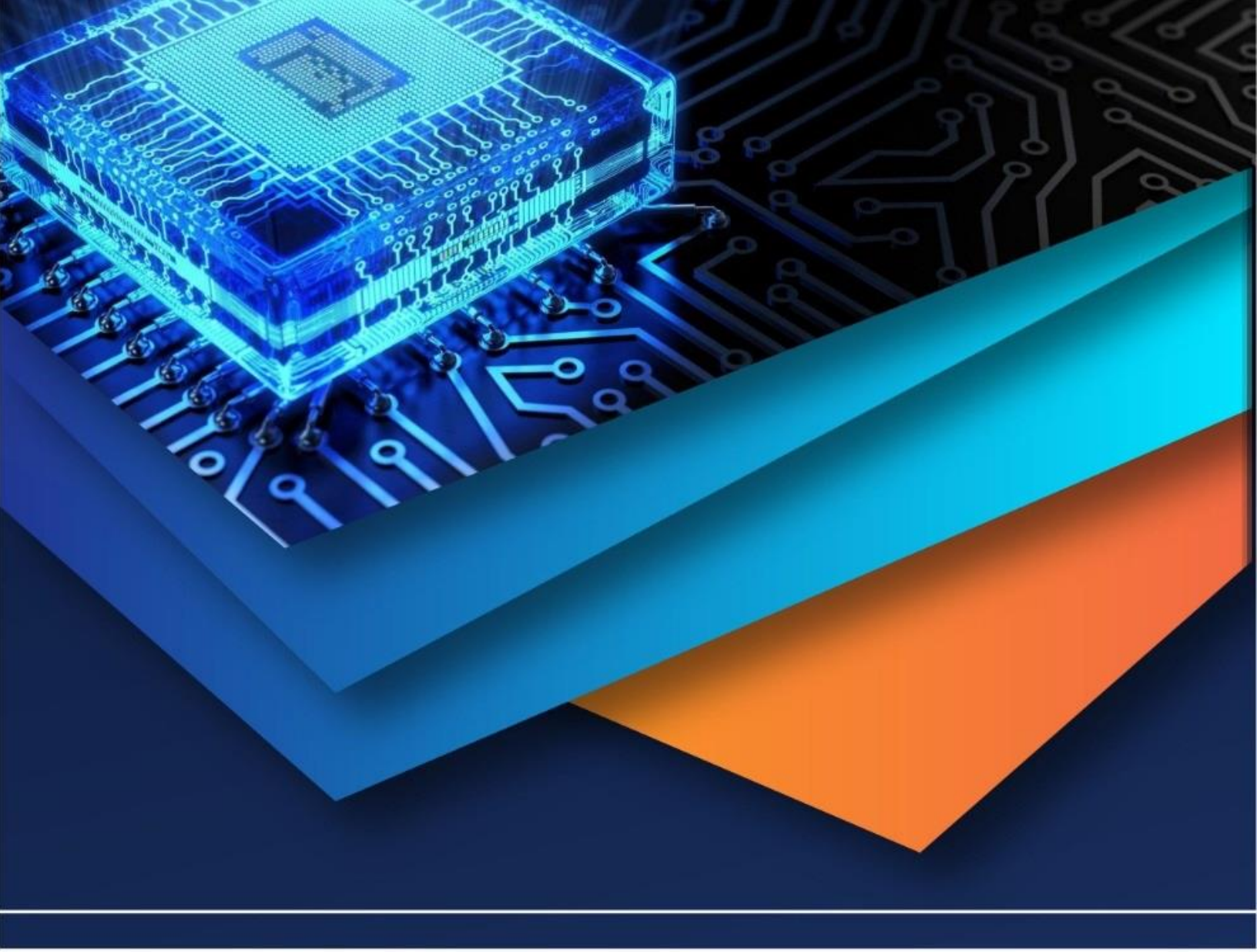

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