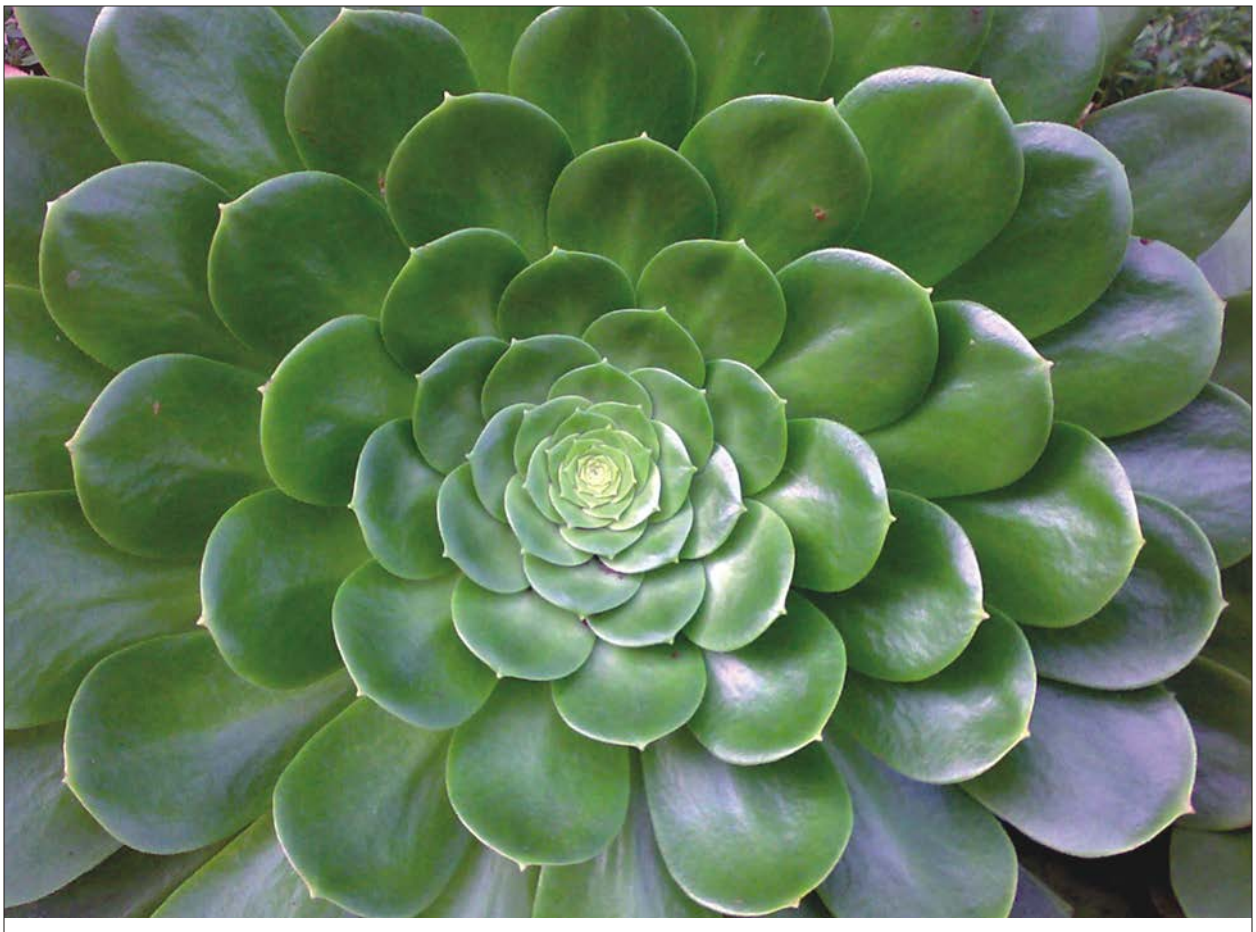

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Effect of Cycloheximide on Senescence and Postharvest Performance in *Hemerocallis fulva* cv. Royal Crown

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ABSTRACT

The present study was conducted to examine the effect of pretreatment with cycloheximide on isolated flowers as well as on cut scapes of *Hemerocallis fulva* followed by their transfer to different holding solutions. The scapes were harvested, brought to laboratory and pulsed for 1 h with different concentrations of cycloheximide (CHI). Following the treatment, they were transferred to Distilled Water (DW) or sucrose+cobalt chloride (DW or SUC+$CoCl_2$). In case of isolated flowers, cycloheximide was sprayed at different concentrations immediately after flower opening, under laboratory conditions. Pulsing the cut spikes with CHI at 0.01 or 0.05 mM CHI before transfer to Suc+$CoCl_2$ enhanced their longevity, promoted the opening of buds in a sustained manner and maintained higher fresh and dry mass of flowers besides maintaining the tissue content of soluble proteins. Pretreatment with CHI did not increase the longevity of isolated flowers instead the increase in vase life was due to the profusion and continuity with which buds bloom into flowers. However, spraying isolated flowers with 0.5 mM CHI increased their longevity upto 3 days as compared to 1 day in unsprayed flowers. In the present study, increased vase life was found associated with decrease in the tissue content of total phenols. Pretreatment of scapes (0.01 and 0.05 mM) as well as spraying isolated flowers with CHI (0.5 mM) before transfer to holding solutions resulted in an enhancement of vase life/flower longevity, maintenance of membrane integrity and improving postharvest performance of cut scapes and isolated flowers of *Hemerocallis fulva*.

Key words: Vase life, soluble proteins, phenols, ephemeral, membrane permeability

INTRODUCTION

The mechanism of petal senescence has been the focus of extensive research. Ephemeral flowers like *Ipomoea* and *Hemerocallis* have been used as effective model systems to study the process of senescence because the events happen quickly (Lay-Yee *et al*., 1992; Sultan *et al*., 2002). *Hemerocallis fulva* (Liliaceae) is an ethylene-insensitive geophyte with a considerable potential in Indian cut flower industry. The commercial life of the flower is typically determined by its perianth as such most of the studies related to flower senescence has focused on the perianth. Flower petals/sepals provide an excellent model system for studies of senescence as they have finite life span, relatively homogenous tissue and that chemical manipulation can be applied without substantial wounding. In addition a number of morphological and physiological changes are evident allowing the process to be readily documented (Wagstaff *et al*., 2002). Multiple processes contribute to produce the visible signs of perianth senescence in this flower system but one of the

most important is that of protein degradation and remobilization (Gulzar *et al.*, 2005; Shahri, 2011). Treatment of flowers with compounds that inhibit protein synthesis, have been found to delay the visible symptoms of petal senescence, revealing that active protein synthesis is required for the execution of cell death in petals (Celikel and van Doorn, 1995; Sultan and Farooq, 1997). The extension of vase life in cut flowers can therefore be achieved by the use of specific protein synthesis inhibitors. Cycloheximide (a protein synthesis inhibitor at the translational level) has been implicated to effectively delay senescence in flowers such as *Consolida, Gladiolus*, *Hemerocallis*, *Ipomoea*, *Iris*, *Narcissus* and *Ranunculus* (Courtney *et al.*, 1994; Jones *et al.*, 1994; Shahri and Tahir, 2010a; Van Doorn *et al.*, 1995). The present study was conducted to examine the effect of pretreatment with cycloheximide in isolated flowers as well as on cut scapes of *Hemerocallis fulva* followed by their transfer to different holding solutions with the aim of extending its vase life.

MATERIALS AND METHODS

Scapes as well as isolated flowers of *Hemerocallis fulva* cv. Royal crown growing (2010) in the Kashmir University Botanical Garden (KUBG) were used for the study. Scapes were harvested at 1500 h with their first mature bud at one day before anthesis stage (Stage III of flower development). For isolated flowers, mature buds were harvested at 1500 h, one day before anthesis. The scapes as well as buds were immediately transferred to laboratory and processed for the experiment. The scapes were cut under water to obtain a uniform length of 40 cm and pulse treated with different concentrations of cycloheximide (0.01, 0.05, 0.1, 0.25 and 0.5 mM) for 1 h. After pulse treatment the scape ends were washed with distilled water thrice. In each case three scapes were transferred to 250 mL Ehrlenmeyer flasks containing 200 mL of Distilled Water (DW) or sucrose 0.15 M+0.15 mM $CoCl_2$ (SUC+$CoCl_2$). Separate set of five flasks each containing untreated scapes transferred to DW and SUC+$CoCl_2$ represented respective controls. Overall there were 12 treatments including controls. In case of isolated flowers, cycloheximide was supplied as pulse treatment for 1 h and as spray at different concentrations (0.01, 0.05, 0.1, 0.25 and 0.5 mM). They were then held in 15 mL glass vials containing DW. Treatment effects were evaluated by keeping the flowers and scapes in the laboratory at a temperature of 27±2°C under cool white fluorescent light with a mix of diffused natural light (10 W m^{-2}) 12 h a day and RH of 70±10%. The day of harvest was designated as day zero. The average vase life of scapes was counted from the day of harvest and was assessed to be terminated when the last open flower senesced on each scape. The experiment was maintained till the vase life in the last set of scapes was regarded to be terminated. Number of blooms per scape and volume of holding solution absorbed was recorded at regular intervals. Total number of buds on each scape was also counted to express the data on percentage basis. The life of individual flower was regarded to be terminated when the visible signs of senescence (loss of tepal turgidity, change in colour from brick red to yellowish and appearance of water-soaked areas along the tepal margins) appeared. Fresh and dry mass of the flowers was determined on day 3, 6 and 9 of harvest (transfer of scapes to the test solutions). Dry mass was determined by drying the material in an oven for 48 h at 70°C. Proteins were extracted from 1 g perianth tissue drawn separately from different flowers. The tissue was homogenized in 5 mL of 5% sodium sulphite (w/v) adding 0.1 g of polyvinylpyrrolidone and centrifuged. Proteins were precipitated from a suitable volume of cleared supernatant with equal volume of 20% trichloroacetic acid, centrifuged at 1000×g for 15 min and the pellet redissolved in 0.1 N NaOH. Proteins were estimated from a suitable aliquot by the method of (Lowry *et al.*, 1951) using Bovine Serum

Albumin (BSA) as the standard. For the estimation of sugars, α-amino acids and total phenols, 1 g chopped perianth tissue was fixed in triplicate in hot 80% ethanol. The material was macerated and centrifuged thrice. The supernatants were pooled and used for the estimation. Reducing sugars were estimated by the method of (Nelson, 1944) using glucose as the standard. Total soluble sugars were estimated after enzymatic conversion of non-reducing sugars into reducing sugars with 0.2% invertase. α-amino acids were estimated by the method of Rosen (1957) using glycine as the standard. Total phenols were estimated by the method of Swain and Hillis (1959) using gallic acid as standard.

Statistical analysis: The data has been analyzed statistically and LSD computed at $p_{0.05}$ using MINITAB (v 15. 1.2-EQUINOX_Softddl.net) software.

RESULTS

The vase life of an individual flower on the scape after it had opened fully was about 1 day, opening in the morning and wilting in the evening of the same day. This short life of flower is compensated by the profusion and continuity with which buds bloom into flowers. The average vase life of scapes was about 9 days in DW and about 14 days in Suc+$CoCl_2$. Flower senescence was characterized by loss of perianth turgidity, change in colour from brick red to yellowish and appearance of water soaked areas along the perianth margins. The perianth finally collapsed, followed by entire flower abscission. Maximum vase life was recorded in scapes pretreated with 0.01 mM CHI before transfer to DW or Suc+$CoCl_2$ as also in scapes pretreated with 0.05 mM CHI before transfer to Suc+$CoCl_2$. Pretreatment of scapes with CHI at 0.05 and 0.1 mM before transfer to DW resulted in decreased vase life as compared to control (Table 1, Fig. 10a). Pretreatment of scapes with CHI enhanced their postharvest longevity but did not have any effect on longevity of individual flowers. Pulse treatment of isolated flowers with different concentrations of CHI did not have any effect on flower longevity; however spraying flowers with CHI prolonged the flower longevity. Flowers sprayed with 0.5 mM CHI registered an enhanced longevity of about 2 days as compared to unsprayed flowers (Fig. 1). In CHI sprayed flowers water soaked areas did not show up during senescence besides the perianth did not collapse (Fig. 10b). The rate of blooming as well as number of blooms per scape was highest in scapes pretreated with CHI at 0.01 mM concentration. However scapes pretreated with 0.05 mM CHI before transfer to Suc+$CoCl_2$ exhibited a sustained rate of blooming. Higher concentrations of CHI (0.25 and 0.5 mM) prevented the bud opening and promoted premature bud abortion (Table 1). Pretreatment of scapes with CHI resulted in an increase in the volume of holding solution absorbed as compared to controls, however volume of holding solution absorbed registered a decrease in scapes pretreated with 0.25 and 0.5 mM CHI and transferred to Suc+$CoCl_2$. Volume of holding solution registered a decrease with the increase in CHI concentration (Table 2).

Fresh and dry mass of flowers from scapes pretreated with CHI as well as of isolated flowers sprayed with CHI, registered an increase as compared to respective controls. A higher fresh and dry mass was maintained in samples from scapes pretreated with 0.01 and 0.05 mM CHI (Table 2). Fresh and dry mass of isolated flowers registered a decrease with the progression in time from day 1 to day 3 of transfer; however, the extent of decrease was least in flowers sprayed with 0.5 mM CHI (Fig. 2 and 3). Spraying of isolated flowers with different concentrations of CHI resulted in a decrease in electrical conductivity of ion leachates from perianth discs as compared to control. The

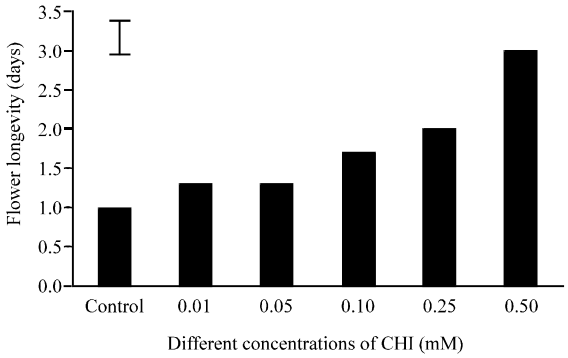

Fig. 1: Effect of different concentrations of cycloheximide spray on longevity in isolated flowers of *Hemerocallis fulva*. Vertical bar represents LSD at $p_{0.05}$

Table 1: Effect of pretreatment with different concentrations of cycloheximide (CHI, 1 h pulse) and subsequent transfer to Distilled Water (DW) and Suc (0.15 M) + $CoCl_2$ (0.15 mM) on vase life, bud abortion and number of blooms per scape at 6, 12 and 18 day (D6, D12 and D18) of transfer in cut scapes of *Hemerocallis fulva*. Each value is a mean of 10 independent replicates

			No. of blooms per scape		
			D6	D12	D18
Treatment	Vase life (days)	Percent bud abortion per scape	(No. of days after transfer)		
Set A (DW)					
Control	9.50	56.45	3.33	4.83	4.83
			(29.80)	(43.50)	(43.50)
0.01 m MCHI	11.83	46.90	3.67	5.83	5.83
			(32.80)	(53.10)	(53.10)
0.05 mM CHI	7.17	74.00	2.17	2.17	2.17
			(26.00)	(26.00)	(26.00)
0.10 mM CHI	5.50	83.00	1.17	1.17	1.17
			(17.00)	(17.00)	(17.00)
0.25 mM CHI	-	100.00	-	-	-
0.50 mM CHI	-	100.00	-	-	-
Set B {Suc (0.15 M) + $CoCl_2$ (0.15 mM)}					
Control	14.33	46.23	3.50	5.00	6.30
			(31.40)	(44.80)	(53.70)
0.01 m MCHI	16.17	35.84	3.67	6.00	7.16
			(32.80)	(32.90)	(64.10)
0.05 mM CHI	17.17	34.05	3.00	5.33	6.66
			(26.80)	(47.70)	(59.70)
0.10 mM CHI	14.00	55.19	2.17	4.17	5.00
			(26.00)	(37.30)	(44.80)
0.25 mM CHI	-	91.01	-	-	-
0.50 mM CHI	-	100.00	-	-	-
LSD at $p_{0.05}$	0.68	3.22	1.19	1.24	1.11

electrical conductivity of ion leachates increased with the progression in time from day 1 to day 3 of transfer, however, the increase was least in samples from flowers sprayed with 0.25 and 0.5 mM CHI (Fig. 4).

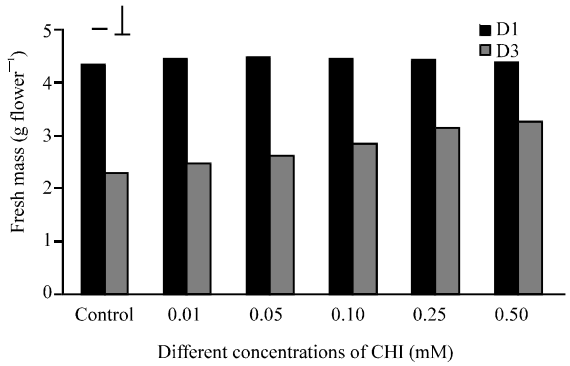

Fig. 2: Effect of different concentrations of cycloheximide spray on fresh mass (expressed as g flower^{-1}) in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

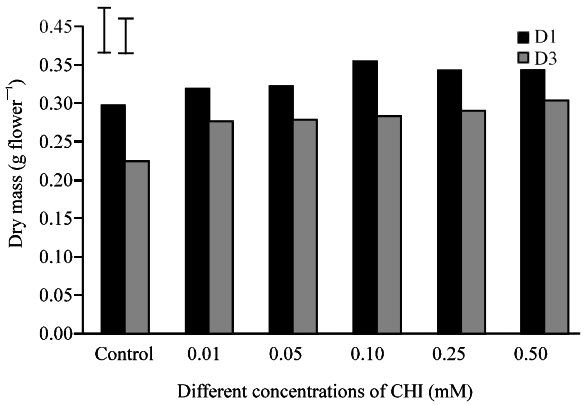

Fig. 3: Effect of different concentrations of cycloheximide spray on dry mass (expressed as g flower^{-1}) in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

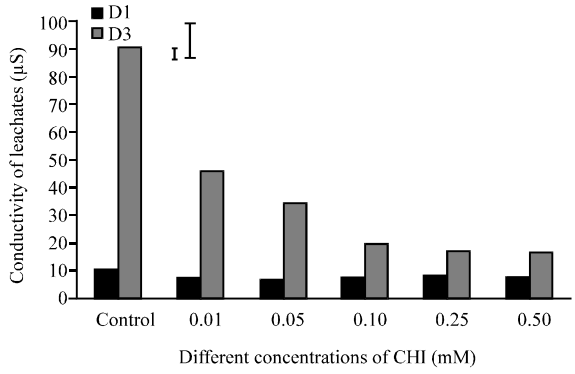

Fig. 4: Effect of different concentrations of cycloheximide spray on electrical conductivity of ion leachates from perianth discs (expressed as µS) in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

Table 2: Effect of pretreatment with different concentrations of cycloheximide (CHI, 1 h pulse) and subsequent transfer to distilled water (DW) and Suc (0.15 M) + $CoCl_2$ (0.15 mM) on volume of holding solution absorbed per scape, fresh and dry mass of flowers at day 3, 6 and 9 (D3, D6 and D9) of transfer in cut scapes of *Hemerocallis fulva*. Each value is a mean of 10 independent replicates

	Volume of holding solution absorbed per scape (mL)			Fresh mass (g flower^{-1})			Dry mass (g flower^{-1})		
Treatment	D3	D6	D9	D3	D6	D9	D3	D6	D9
Set A DW									
Control	5.66	9.66	18.16	2.64	2.51	2.56	0.21	0.20	0.20
0.01 MCHI	9.33	13.50	22.33	3.10	2.73	2.77	0.23	0.22	0.21
0.05 mM CHI	7.83	11.66	19.16	2.97	2.57	2.63	0.23	0.22	0.21
0.10 mM CHI	6.66	10.16	18.00	2.94	2.42	-	0.22	0.21	-
0.25 mM CHI	6.66	9.83	17.33	-	-	-	-	-	-
0.50 mM CHI	6.66	10.00	15.33	-	-	-	-	-	-
Set B Suc (0.15 M) + $CoCl_2$ (0.15 mM)									
Control	7.00	10.00	19.33	2.57	2.32	2.78	0.27	0.29	0.32
0.01 m MCHI	8.50	12.16	23.33	2.67	2.72	3.09	0.28	0.33	0.36
0.05 mM CHI	10.00	15.16	30.33	2.78	3.13	3.67	0.30	0.38	0.44
0.10 mM CHI	8.50	13.00	28.33	3.06	2.71	3.49	0.33	0.35	0.40
0.25 mM CHI	6.66	9.833	18.00	2.38	2.87	3.32	0.27	0.29	0.37
0.50 mM CHI	6.50	9.00	14.66	-	-	-	-	-	-
LSD at $p_{0.05}$	0.34	0.51	0.75	0.77	0.54	0.42	0.09	0.07	0.06

The soluble protein content initially registered an increase in samples from scapes pretreated with CHI at 0.05 mM concentration as compared to controls. The soluble protein content of samples from scapes pretreated with 0.01 and 0.1 mM CHI before transfer to holding solutions was comparable to that of controls, followed by decrease with increase in CHI concentration. The soluble protein content registered a general increase in samples from scapes pretreated with lower concentrations of CHI (0.01, 0.05 and 0.1 mM) on day 9 of transfer to holding solutions (Table 3). Spraying of flowers with CHI at 0.01 and 0.05 mM concentrations resulted in an increased protein content in tepal tissues while spraying flowers with higher concentrations of CHI (0.1, 0.25 and 0.5 mM) resulted in decreased soluble protein content as compared to control. The soluble protein content registered a general decrease with the progression in time from day 1 to 3 of harvest but the extent of decrease was least in samples from flowers sprayed with 0.5 mM concentration (Fig. 5). The content of α-amino acids registered a decrease in samples from flowers sprayed with CHI as compared to controls, however, the decrease corresponds to increase in the concentration of CHI. As the time progressed from day 1 to day 3 of harvest, the tissue content of amino acids registered an increase irrespective of the treatment (Fig. 6). The tissue content of reducing and total sugars in samples from isolated flowers registered a general decrease as the flowers opened and senesced, irrespective of the treatment. Flowers sprayed with CHI (0.05 mM) registered a sharp decline in sugar status as compared to control (Fig. 7, 8). Spraying flowers with different concentrations of CHI resulted in a corresponding decrease in the tissue content of total phenols as compared to controls. The total phenolic content registered an increase with the progression in time but the increase was least in samples from flowers sprayed with CHI at 0.01 mM concentration (Fig. 9).

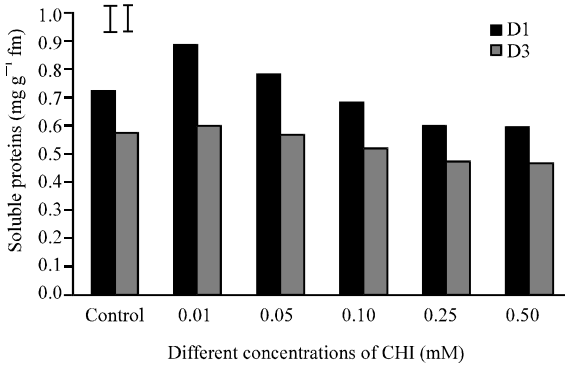

Fig. 5: Effect of different concentrations of cycloheximide spray on the tissue content of soluble proteins (expressed as mg g^{-1} fm) from flag region of perianth tissue in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

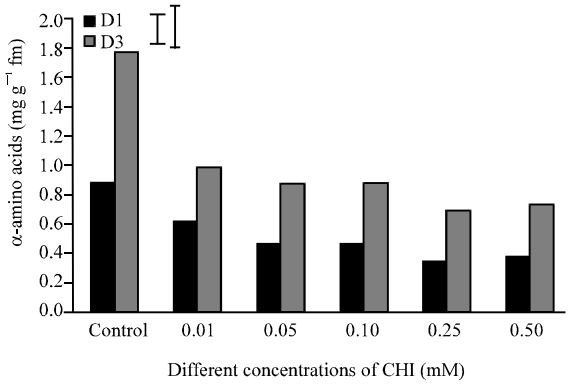

Fig. 6: Effect of different concentrations of cycloheximide spray on the tissue content of α-amino acids (expressed as mg g^{-1} fm) from flag region of perianth tissue in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

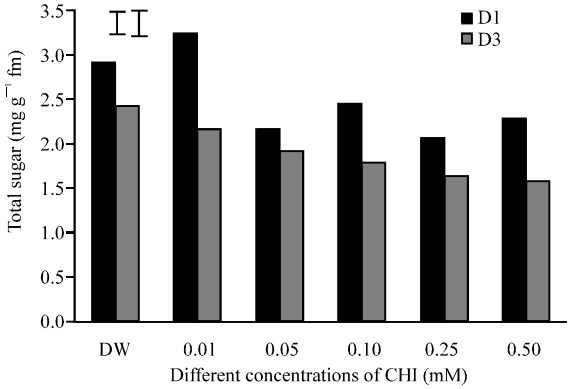

Fig. 7: Effect of different concentrations of cycloheximide spray on the tissue content of total sugars (expressed as mg g^{-1} fm) from flag region of perianth tissue in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

Table 3: Effect of pretreatment with different concentrations of cycloheximide (CHI, 1 h pulse) and subsequent transfer to distilled water (DW) and Suc (0.15 M) + $CoCl_2$ (0.15 mM) on soluble protein content of perianth tissue on day 3 and 9 (D3 and D9) of transfer in cut scapes of *Hemerocallis fulva*. Each value is a mean of 6 independent replicates

	Total soluble proteins (mg g^{-1} fm)	
Treatment	D3	D9
Set A (DW)		
Control	0.73	0.79
0.01 mM CHI	0.71	0.82
0.05 mM CHI	0.79	0.93
0.10 mM CHI	0.73	0.80
0.25 mM CHI	0.55	0.73
0.50 mM CHI	0.52	0.71
Set B Suc. (0.15 M) + $CoCl_2$ (0.15 mM)		
Control	0.80	0.95
0.01 mM CHI	0.80	0.88
0.05 mM CHI	0.84	1.00
0.10 mM CH	0.82	0.91
0.25 mM CHI	0.66	0.77
0.50 mM CHI	0.58	0.75
LSD at $p_{0.05}$	0.17	0.14

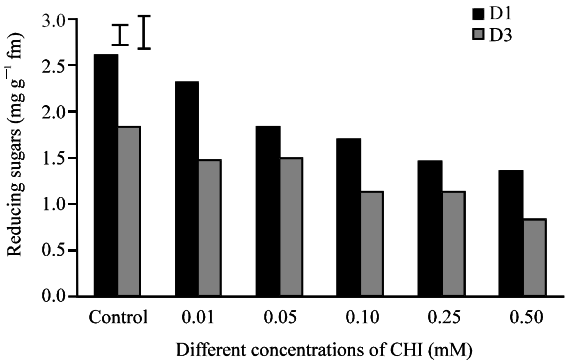

Fig. 8: Effect of different concentrations of cycloheximide spray on the tissue content of reducing sugars (expressed as mg g^{-1} fm) from flag region of perianth tissue in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

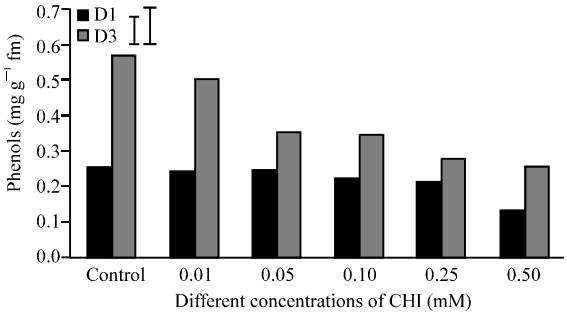

Fig. 9: Effect of different concentrations of cycloheximide spray on the tissue content of total phenols (expressed as mg g^{-1} fm) from flag region of perianth tissue in isolated flowers of *Hemerocallis fulva*. Vertical bars represent LSD at $p_{0.05}$

Fig. 10: (a) Effect of pretreatment with different concentrations of CHI on vase life in cut scapes of *Hemerocallis fulva* at day 12 of transfer to holding solutions. From left to right are flasks containing scapes arranged as DW (control), SUC+$CoCl_2$, 0.01 mM CHI–DW, 0.01 mM CHI–SUC+$CoCl_2$, 0.05 mM CHI–DW, 0.05 mM CHI–SUC+$CoCl_2$, 0.1 mM CHI–DW, 0.1 mM CHI–SUC+$CoCl_2$, 0.25 mM CHI–DW, 0.25 mM CHI–SUC+$CoCl_2$, 0.5 mM CHI–DW and 0.5mM CHI–SUC+$CoCl_2$, (b): Flowers of *Hemerocallis fulva* held in vials containing Distilled Water (DW) after spraying with different concentrations of cycloheximide (CHI, 1 h pulse) at day 3 of transfer. From left to right are arranged vials receiving distilled water (DW, control), CHI (0.01 mM), CHI (0.05 mM), CHI (0.10 mM), CHI (0.25 mM) and CHI (0.5 mM)

DISCUSSION

Pretreatment of scapes at a particular threshold level of CHI (0.01 mM) before transfer to DW or Suc+$Cocl_2$ enhanced vase life by an increment of about 2-3 days. Pretreatment with CHI did not increase the longevity of individual flowers instead the increase in vase life was due to the profusion and continuity with which buds bloom into flowers. However, spraying isolated flowers with 0.5 mM CHI increased their longevity upto 3 days as compared to 1 day in unsprayed flowers. At higher concentrations, CHI (0.25 and 0.5 mM) prevented bud opening in cut scapes. Cycloheximide has been shown to inhibit the flower opening and also delay senescence depending on the stage at which it is included in the experiment (Gulzar *et al*., 2005; Zhuo *et al*., 2005; Shahri and Tahir, 2010a, b). The effect of CHI in delaying the senescence seems to be due to improvement of water balance of cut *Hemerocallis* scapes as pretreatment of scapes with CHI at 0.01 and 0.05 mM concentrations resulted in an increase in volume of holding solution absorbed as compared to controls. However, a decrease in the water uptake was recorded with the increase in CHI concentration. The present results corroborate with those of Van Doorn *et al*. (1995) who also reported decrease in water uptake in *Iris* tepals when pretreated with CHI.

The present results suggest that pretreatment of scapes with CHI at 0.01 or 0.05 mM concentrations as also spraying flowers with 0.5 mM CHI resulted in an increased fresh and dry mass of flowers. Maintenance of higher fresh and dry mass of flowers could be due to lower respiratory losses as CHI has been found to suppress respiration in certain plant tissues; besides in *Hemerocallis* it has been shown to abolish the peak in respiration at the start of senescence (Ellis and MacDonald, 1970; Bieleski and Reid, 1992). Spraying flowers with CHI resulted in a

decrease in the electrical conductivity of ion leachates of tepal tissues. In various flowers such as *Arum*, *Consolida*, *Ipomoea*, the loss of membrane integrity has been shown to cause an increase in the permeability and leakage during senescence (Van Meeteren, 1979; Halevy and Mayak, 1979; Shahri and Tahir, 2010c). The delay in leakiness of tepal cells due to the application of cycloheximide may be attributed to the fact that it prevented the synthesis of some specific proteins responsible for membrane degradation and the increase in leakiness of ion leachates.

Pretreatment of scapes with CHI (0.01 and 0.05 mM) resulted in an increase in the content of soluble proteins followed by a decrease with increase in CHI concentration. In isolated flowers the extent of decrease in the soluble protein content was least in CHI (0.5 mM) sprayed flowers. An overall decrease in cell protein levels has been found during both ethylene sensitive and insensitive flower senescence. In day lily tepals, a sharp decrease in protein levels preceded the visible symptoms of senescence and cycloheximide delayed the decrease in protein levels and increased the time to visible senescence (Lay-Yee *et al*., 1992). Conversely pretreatment of flowers with CHI resulted in a decrease in the content of α- amino acids. The maintenance of high protein content in the perianth tissue of samples from scapes pretreated with 0.01/0.05 mM CHI may be due to the inhibition of synthesis of specific proteases responsible for protein degradation.

During the current investigation it has been shown that the content of total and reducing sugars was maintained in samples from flowers sprayed with CHI at lower (0.01, 0.05 and 0.1 mM) concentrations. They may be suggested to be accumulated due to reduced metabolic activity as spraying CHI at lower concentrations was ineffective in delaying senescence in isolated flowers. The reduced content of sugar fractions in samples from flowers sprayed at 0.25 and 0.5 mM CHI could be due to utilization of available sugar fractions as the flowers showed an improvement in vase life. Flower maturation and senescence has been shown to be accompanied by a decline in total soluble carbohydrate content in flowers (Paulin and Jamain, 1982; Lukaszewski and Reid, 1989).

Spraying flowers with CHI resulted in a decrease in the tissue content of phenols, particularly at (0.5 mM) CHI concentration. Present results were consistent with our earlier findings on isolated *Hemerocallis fulva* and *Ranunculus asiaticus* flowers in which the increased vase life was found associated with decrease in the phenolic content (Gulzar *et al*., 2005; Shahri and Tahir, 2010b). However, in cut rose petals, the higher content of phenols has been shown to be associated with longer vase life (Mwangi *et al*., 2003).

The present study further revealed that postharvest performance of untreated scapes as also scapes pretreated with 0.01/0.05 mM CHI was better in (SUC+$CoCl_2$) as compared to corresponding spikes transferred to (DW). Sugars have been found to supply respiratory substrates, maintain adequate water balance, decrease sensitivity to ethylene and delay the climacteric ethylene biosynthesis (Ichimura *et al*., 2000; Pun and Ichimura, 2003; Shahri *et al*., 2009, 2010).

CONCLUSION

The present results suggest that the effects of cycloheximide indicate a programme at the cellular level. The fact that cycloheximide delays tepal senescence demonstrates that the synthesis of particular suicide proteins orchestrates the cell death programme, however it is necessary to show that these proteins and their products actually play a causal role. Pretreatment of scapes (0.01 and 0.05 mM) as well as spraying isolated flowers with CHI (0.5 mM) before transfer to holding solutions resulted in an enhancement of vase life/flower longevity, maintenance of membrane integrity and improving postharvest performance of cut scapes and isolated flowers of *Hemerocallis fulva*.

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