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CALCIUM EFFECTS ON POST-HARVEST ATTRIBUTES AND VASE LIFE OF GLADIOLUS USING DIFFERENT METHODS OF APPLICATION

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Abstract

Gladiolus can play a key role in generating substantial amount of revenue in cut flower trade, however, the rapid loss of quality is a serious threat to its wider adaptation as a commercial crop. Hence both pre and post-harvest management is pre-requisite to extend the vase life of gladiolus. For this purpose, the present study was conducted to determine the influence of calcium concentrations i.e., Distilled water & tap water (Double control), 100, 200, 300 and 400 mM and application methods (holding solution in vase and foliar spray) on post-harvest attributes and vase life of gladiolus spikes in post-harvest Laboratory during the years 2016-2017. The findings of the study revealed that calcium concentrations and application methods had significant effects on all studied traits of gladiolus. Calcium at 200 mM and vase holding solution resulted in extended 1st floret and full spike fading. Likewise, the higher fresh floret weight and senesced floret weight , retention of highest reducing and non-reducing sugars, least electrolyte leakage and extended vase life of gladiolus spikes was also observed at 200 mM calcium concentration and in vase holding solution. The least loss of protein and highest total phenols was also recorded in florets of gladiolus spikes kept in holding solution of 200 mM calcium. It is concluded that application of 200 mM calcium through holding solution was most effective in retaining the quality of gladiolus florets and spikes over extended period and enhanced the vase life of gladiolus spikes.

Key words: Cut flowers, Post-harvest, Electrolyte leakage, Vase life, Calcium.

Introduction

Floriculture is an important and fast growing sector of horticulture, with more than 120 countries involved in floriculture industry (Van Uffelen & De Groot, 2005). The Netherlands is the leading country in worldwide cut flowers trade (Van Uffelen & De Groot, 2005; Van Hemert, 2005) followed by Colombia, Israel, Kenya, Belgium and Zimbabwe (Van Uffelen & De Groot, 2005). It is considered as commercial bulbous plant known as queen of bulbous plants (Lepcha *et al.*, 2007; Bhujbal *et al.*, 2013) and ranked 2nd after rose in Pakistan (Riaz *et al.*, 2007). It is one of the four commercially important and highly demanded cut flowers globally after rose, carnation and chrysanthemum (Bai *et al.*, 2009; Verma *et al.*, 2004). Gladiolus had occupied an area of 7,384.34 ha worldwide with a trade worth of US $ 3,100 million (Liemt, 1999; Lepcha *et al*., 2007). However it can also be used in flower beds as herbaceous perennial flowering plant in landscape gardening as well as is an integral part of flower shows and exhibition (Zubair *et al.*, 2006; Lepcha *et al*., 2007; Cantor & Tolety, 2011). However, the rapid losses associated with gladiolus have serious threats to make it more profitable and commercial crop for end users.

Pakistan has a tremendous potential for its commercial production. The area under gladiolus cultivation has increased from 392.54 ha in 2005 to about 809.37 ha in 2015 (Ramzan *et al*., 2010; Anon., 2015). The increase on commercial basis may help in improving the livelihood of the growers and generating foreign exchange reserves (Saeed *et al.*, 2013). However, the market value of cut flowers is influenced by various factors such as spike length, quality of intact florets/flowers, freshness of flowers as well as post-harvest longevity of cut flowers and spikes (Saeed *et al.*, 2013). Besides quality production of spikes, gladiolus needs optimum post harvest management practices to maintain its market value (Malakouti, 2003). Being highly perishable, cut flowers lose freshness and appearance, which decrease its quality and persistence (Kumar *et al.*, 2017) Thus, it requires proper post-harvest management to retain the quality of gladiolus flower after harvest (Saeed *et al.*, 2013). The loss of post-harvest quality of cut flowers is a challenge for cut flower industry (Faraji *et al.*, 2011). While, the quality attributes of cut flowers cannot be improved further after harvest but the rate of quality losses can be minimized through proper post-harvest management (Saeed *et al.*, 2013). The loss of flower quality and initiation of senescence is characterized by loss in turgidity, cell wall integrity and electrolyte leakage indicating loss in membrane stability (Rubinstein, 2000). Deterioration of cell membrane is one of the major reasons for senescence in cut flowers (Eze *et al*., 1986; Borochov& Woodson, 1989; Itzhaki *et al*., 1990). Various research finding reveals that intra-cellular and or cytosolic changes in concentration of calcium delays the turn-over phenomenon of cellular membrane (Leshem, 1992; Doughari, 2015). Calcium concentration becomes low in cytosol because of intracellular binding or organelles' uptake. In such situation an inhibitory role of extracellular concentrations of calcium is reported (Ferguson, 1984; Leshem, 1992).

Several techniques have been developed to retain the quality of cut flowers with an increase the vase life. The calcium has an important role in regulating plant growth and development (Hepler, 2004) as well as the rate of senescence also depends on the level of calcium in plant tissue and increased calcium concentration has been found to delay the onset of senescence related changes such as protein and chlorophyll degradation, cell wall and membrane stability (Ferguson, 1984; Poovaiah, 1986;

Easterwood, 2002; White & Broadly, 2003). The calcium helps in enzymes activation, enhances antioxidant activities, and delays senescence in gladiolus (Sairam *et al.*, 2011; Singh *et al*., 2013) by declining the level of reactive oxygen species (Agarwal *et al*., 2005).The foliar application of calcium is the simplest technique to delay senescence in flowers (Mortazavi *et al*., 2007). Post-harvest foliar treatment with calcium chloride increases water balance in rose cut flowers and extends its vase life (Hassani & Alimirzaii, 2017). The calcium can also be applied in vase solution for increasing the diameter of xylem wall with an increase in the flow of water thus causing a delay in aging or senescence in cut flowers (Van Ieperen & Van Gelder, 2006). Contamination of vase water pathogens especially bacteria may also add to decline in vase life (Van Doorn *et al.,* 1989) and calcium being present in cell wall can enhance disease resistance (Tobias *et al*., 1993).

Different antimicrobial compounds such as chlorine and silver have been used to reduce bacterial contamination in vase water and enhance the longevity of cut flowers (Beura *et al*., 2001). Post- harvest treatment with calcium chloride retains the fruit quality during storage (Akhtar *et al.*, 2010) delays senescence (Wills &Mahendra, 1989) and retard decay in different fruits (Mahajan & Dhatt, 2004). Likewise, the application of calcium in vase holding solution enhances opening of flower buds in roses and delays senescence (Torre *et al*., 1999). It is observed that calcium addition to the vase solution stabilizes cell membrane (Marschner, 1995) and increases water flow because of its association with pectin in the xylem cell wall (Van Ieperen & Van Gelder, 2006).

Keeping in view the importance of calcium as post-harvest treatment, the present study was initiated to optimize the post-harvest management protocol using calcium for commercially important gladiolus crop. It may help to standardize the optimum calcium concentration as post-harvest treatment to maintain quality attributes and enhance vase life of gladiolus and to assess the effective method of calcium post-harvest application for extending vase life of gladiolus.

Materials and Methods

The "Influence of calcium concentrations and application methods on the post-harvest attributes and vase life of gladiolus spikes cv. White prosperity" was studied in Post-harvest Lab., department of Horticulture, The University of Agriculture, Peshawar during 2016-2017. Fresh cut spikes of gladiolus cv. 'White prosperity' were harvested early in the morning from the field in ornamental nursery, Department of Horticulture. The spikes at the stage with two to three basal buds showing color were cut and sorted out for uniformity of color break. The spikes were re-cut under water with uniform size (90 cm). The spikes were divided into two lots. One lot was allocated to vase holding method and the other to foliar calcium application to the spikes. For this purpose, calcium solutions of 0, 100, 200, 300 and 400 mM strength were prepared, and used as vase holding solution or as foliar spray to the spikes. The spikes were kept in jars containing vase holding solution of different strengths. The other block of spikes kept in distilled water was sprayed with calcium solution of different strengths. The spikes were placed in glass jars, at 25ºC (±2) with relative humidity 70% (±5). Distilled and Tap water were used as double control. The experiment was laid out as completely randomized design (CRD), having two factors i.e., calcium concentrations (Distilled water (control), Tap water (control), 100,200,300 and 400 mM) and application methods (Holding solution and Foliar application). Treatments were repeated three times. Data were recorded on days to 1st floret opening, days to full spike opening , days to 1st floret fading, days to full spike fading, fresh floret weight, senesced floret weight, spike weight loss, loss of protein content, reducing and non reducing sugars, total phenols, electrolyte leakage and calcium content in florets as well as vase life of gladiolus spikes.

Measurements: The spike weight loss was determined by takingfresh weight of spikes at the start of experiment and then spikes were weighed individually after interval and senesced spike weight was recorded. Spike weight loss (%) was determined using the equation 1.

$$\text{Equation 1:} \qquad \text{Spike weight loss} = \frac{\text{Weight of fresh spike} - \text{Weight of semesced spike}}{\text{Weight of fresh spike}} \times 100$$

For loss of protein data, the florets of gladiolus spike from each treatment were taken and dried. The samples were made into fine powder by a grinder. Estimation of nitrogen was done with Microkjeldahl`s method by taking 0.2 grams grounded powder (Sadasivam & Manickam, 1992). The protein content in the florets was obtained by multiplying the nitrogen content with the conversion factor. Protein content of fresh floret was determined at day 1st of the experiment and then after senescence, consequently loss in protein content (%) was worked out for each treatment by using equation 2.

$$\text{Equation 2:} \qquad \text{Protein loss (\%)} = \frac{\text{Proteins in fresh floret} - \text{Proteins in semesced floret}}{\text{Proteins in fresh floret}} \times 100$$

Thereducing sugars were measured by DNS method (Sadasivam & Manickam, 1992). 1 ml of DNS was added to 1 ml sample .The sample was heated for 5 minutes in boiling water bath. One ml 40% Rochelle salt solution was added and then cooled under tap water. Absorbance was recorded against reagent blank at 510 nm. Non reducing sugar content was obtained by subtracting the content of reducing sugar from total soluble sugars.

Total phenol content in different extracts was determined by Slinkard & Singleton (1977) method. Gallic acid was taken as a standard. 1ml of 95% ethanol and 5 ml distilled water was added to 1 ml of methanolic acid. It was

mixed thoroughly with 0.5 ml of Folin-Ciocalteu reagent. Sodium carbonate was added after 5 minutes and the absorbance was measured at 765 nm after 1 hour incubation. A standard curve was obtained by using various concentrations of gallic acid. Electrolyte leakage was determined by the method of Singh *et al*., (2008). For this purpose, 5 flower petal discs having 10 mm diameter from each treatment were put into test tube containing 10 ml distilled water. The tube was incubated at 25°C for 180 minutes and initial ion leakage was measured by conductivity meter. The solution was then boiled in a water bath for 10 minutes to liberate all the electrolytes and then the volume was made 10 ml before final electric conductivity (EC) was measured by using equation 3.

$$\text{Equation 3:} \qquad \text{Electrolyte leakage (\%)} = \frac{C1}{C2} \times 100$$

where, C1 is EC of petals after 180 Minutes room incubation C2 is the final EC of the solution.

The calcium content of Florets was determined in Soil and Environmental Science lab., The University of Agriculture, Peshawar according to the method described by Nan (2007).Calcium concentration in the floret tissue was determined by a colorimetric assay, Calcium L3K® Assay (Diagnostic Chemical Limited (DCL), Oxford, CT).

Statistical analysis: The data were subjected to analysis of variance combined over years as per procedure appropriate for completely randomized design (Jan *et al*., 2009). The least significant difference test was used for significant means comparison.

Results and Discussion

Calcium treatment and phenological development of gladiolus floret and spike

Days to 1st floret opening: According to statistical analysis of the data, different calcium concentrations and calcium application methods resulted in significant variations in days to 1st floret opening (DFO), whereas the interaction between calcium concentrations and application methods was non-significant. Mean data on days to 1st floret opening in response to various concentrations of calcium and application methods are presented in Table 1. The mean data pertaining to various calcium concentrations showed that opening of 1st floret in gladiolus spike was delayed with increasing concentration of calcium up to 200 mM. The maximum time (2.383 days) in 1st floret opening was recorded in spikes of gladiolus treated with 200 mM of calcium, which was statistically similar to the effect of calcium at 300 and 400 mM. The least days (1.333) to 1st floret opening were recorded in spikes sprayed with distilled water. Two years average data regarding application methods revealed that foliar application of calcium on gladiolus spikes resulted in early opening of 1st floret (1.978 days) as compared to (2.086 days) in florets of spikes kept in holding solution of calcium.

Days to full spike opening: Significant variations were observed in days to full spike opening of gladiolus in response to different concentrations of calcium and application methods, however, the interaction between calcium concentrations and calcium application methods was non-significant. Average data regarding days to full spike opening as affected by various calcium concentrations and application methods are in given in Table 1. The two years average data regarding various concentrations of calcium revealed that maximum days (11.153) to full spike opening were recorded when spikes were treated with 200 mM of calcium, which was statistically similar to (11.075 days) with the treatment of spikes with 300 mM calcium. Comparing mean data across two years regarding methods of calcium application, the delayed opening of full spike (9.712 days) occurred in holding solution as compared to (9.553 days) in spikes treated with foliar application method.

The flower opening is vital for the quality and market value of cut flowers. The flower opening is a developmental process, which is followed by fading and senescence (Tripathi & Narendra, 2007). The senescence is started through a programmed cell death (PCD), initiated with flower opening (Van Doorn, 2004; Van Doorn &Woltering, 2008). Unlike leaves, PCD is irreversible in florets (Van Doorn & Weltering, 2008). Different morphological and physiological development takes place during floret opening in a well defined series (Kumar *et al*., 2008). Treatment that slows down the opening of flowers is considered better for quality and longevity of cut flowers (Anjum *et al*., 2001). Calcium delays flowering and senescence of floral organs (Ilias & Rajapakse, 2005). The delay in floret opening of gladiolus spike in current study can be attributed to increased calcium content in florets and the same treatments resulted in maximum vase life (Table 4). The increased vase life could be due to increased water uptake and delayed florets opening (Anjum *et al.,* 2001).

Days to 1st floret fading: Statistical analysis of the data showed that days to 1st floret fading in gladiolus spikes were significantly affected by calcium concentrations and calcium application methods as well as their interaction. Data regarding days to 1st floret fading are given in Table 1. The average for calcium concentrations data across two years, revealed that fading of 1st floret took the maximum days (7.458) when the spikes were treated with 200 mM calcium, followed by (7.391 days) with 300 mM of calcium. The least days to 1st floret fading (5.596 and 5.627) were recorded in spikes treated with distilled and Tap water respectively. Two years mean data for application methods revealed that it took more days (6.693) to 1st floret fading when spikes of gladiolus were held in holding solution containing calcium as compared to (6.508 days) in florets of spikes sprayed with calcium. The interaction between calcium concentrations and application methods (Fig. 1a) showed that 1st floret fading was delayed with increasing calcium concentration up to 200 mM both in holding solution and foliar application method. Although in both methods, the more time was recorded at 200 mM, but the time in 1st floret opening of gladiolus spike was more (4.6%) in calcium containing vase holding solution as compared to foliar application of calcium.

Table 1. Days to 1st floret opening (DFO), days to full spike opening (DFSO), days to 1st floret fading (DFF) and days to full spike fading (DFSF) of gladiolus as affected by various concentrations of calcium and application methods.

Treatments		Phenological development of gladiolus florets and spike			
		DFO	DFSO	DFF	DFSF
Calcium concentrations (C)	Distilled water	1.333d	7.208d	5.596e	11.092e
	Tap water	1.575c	7.192d	5.627e	11.242d
	100 mM	1.992b	10.208c	6.429d	12.910c
	200 mM	2.383a	11.153a	7.458a	14.842a
	300 mM	2.450a	11.075ab	7.391b	14.653b
	400 mM	2.458a	10.958b	7.103c	14.583b
	$LSD_{0.05}$	0.119	0.144	0.049	0.132
Application methods (M)	Holding solution	2.086	9.712	6.693	13.381
	Foliar application	1.978	9.553	6.508	13.060
	Significance	**	**	*	**
Year	Year 1	2.019	9.618	6.605	13.24
	Year 2	2.044	9.647	6.596	13.20
	Significance	NS	NS	NS	NS
Interaction	CxM	NS	NS	** Fig. 1a	** Fig. 1b

NS = Non-significant; *= Significant at p≤0.05; **= Significant at p≤0.01
Means followed by different letter in respective columns are significantly different from each other at p≤0.05

Days to full spike fading: Days to full spike fading of gladiolus spike were significantly influenced by various calcium concentrations and application methods as well as the interaction between calcium concentrations and application methods. The average data pertaining days to full spike fading are given in Table 1. The two years mean data regarding calcium concentrations showed that 200 mM of calcium resulted in the maximum time to full spike fading (14.842 days), followed by (14.653 days) with the application of 300 mM calcium which was statistically at par with the effect of 400 mM of calcium. Early fading of full spike (11.092 days) was observed in spikes treated with distilled water. The mean data for application methods across two years revealed that more days to full spike fading (13.381) were recorded in spikes kept in holding solution of calcium as compared to (13.060 days) in spikes treated with calcium as a foliar spray. The interaction between calcium concentrations and application methods (Fig. 1b) revealed that full spike fading was delayed with increasing concentration of calcium up to 200 mM in both application methods. Further increase in calcium concentration to 300 and 400 mM did not enhanced full spike fading. The maximum delay in full spike fading was recorded with the application of 200 mM calcium with both methods, however delay in full spike fading was (4.3%) higher in holding solution of calcium as compared to foliar application method. The least days to full spike fading (11.08 days and 11.10 days) were recorded with the application of distilled water with both methods of application.

Wilting or fading of petals is regarded as one of the most important symptoms of senescence (Jones *et al*., 1993). Calcium has important regulatory role in physiology of plants (Hepler, 2004) and increases antioxidant enzyme activity as well as regulates senescence in gladiolus flowers (Sairam *et al.*, 2011). Calcium in vase solution delays fading and senescence of cut flowers in gladiolus (Sairam *et al.*, 2011). Calcium reduces the level of reactive oxygen species (ROS) (Agarwal *et al*., 2005) and increases membrane stability, which helps in delaying senescence (Leshem, 1992; Paliyath & Droilldard, 1992; Torre *et al*., 1999; Nan, 2007). These findings are in agreement with Torre *et al*., (1999) who reported that calcium delayed flower fading and regulated senescence in petals of cut flowers in roses.

Physical quality attributes

Fresh floret weight (g): Statistical analysis showed significant variation in fresh floret weight of gladiolus spike in response to calcium concentrations and application methods as well as their interaction. Data regarding fresh floret weight in response to various levels of calcium and application methods are presented in Table 2. The two years mean data regarding various calcium concentrations showed that the maximum fresh floret weight (8.867 g) was recorded in spikes of gladiolus treated with 200 mM of calcium, followed by (8.633 g) with the application of 300 mM calcium, which was statistically similar to the effect of calcium at 400 mM concentration. The least fresh floret weight (7.358 g) of gladiolus spike was found with the treatment of distilled water.The two years average data regarding application methods showed that fresh floret weight (8.364 g) was higher in spikes of gladiolus placed in holding solution of calcium as compared to floret weight (7.925 g) in spikes treated with calcium as foliar spray. The mean data across two years regarding interaction between calcium concentrations and methods of application (Fig. 2a) showed significant variations in fresh floret weight of gladiolus spike. It is clear from the interaction data that fresh floret weight increased with increasing calcium concentration up to 200 mM, whereas further increase in calcium concentration declined the fresh floret weight of gladiolus in both methods. However the fresh floret weight (9.30 g) was higher (9.35%) in spikes treated with 200 mM calcium in holding solution as compared to the spikes that received 200 mM of foliar applied calcium. The least fresh floret weight (7.33 g) was recorded in spikes sprayed with distilled water.

Table 2. Fresh floret weight (FFW), senesced floret weight (SFW) and weight loss (WL) of gladiolus spike as affected by various concentrations of calcium and application methods.

Treatments		Physical quality attributes in gladiolus florets and spike		
		FFW (g)	SFW (g)	WL (%)
Calcium concentrations (C)	Distilled water	7.358d	3.358e	37.308a
	Tap water	7.475d	3.567d	35.069b
	100 Mm	7.992c	5.333c	16.617c
	200 Mm	8.867a	6.838a	8.175d
	300 mM	8.633b	6.789ab	7.750e
	400 Mm	8.542b	6.712b	7.958de
	$LSD_{0.05}$	0.117	0.109	0.314
Application methods (M)	Holding solution	8.364	5.621	17.856
	Foliar application	7.925	5.245	19.769
Year	Significance	*	**	**
	Year 1	8.144	5.446	18.781
	Year 2	8.140	5.419	18.844
Significance		NS	NS	NS
Interaction	CxM	**Fig. 2a	**Fig. 2b	**Fig. 2c

NS = Non-significant; *= Significant at p≤0.05; **= Significant at p≤0.01

Means followed by different letters in respective columns are significantly different from each other at p≤0.05

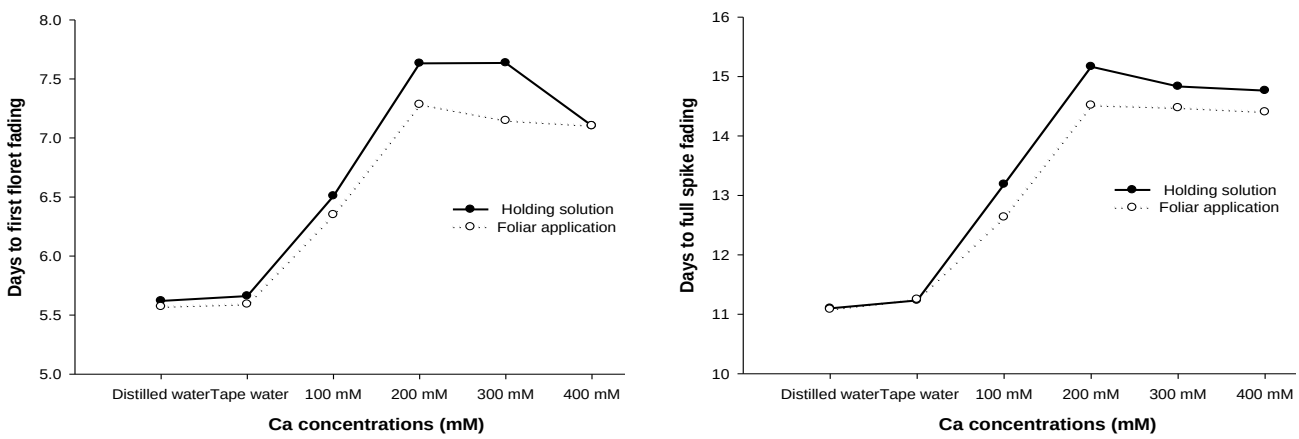

Fig. 1. Influence of calcium concentrations and application methods on (a) days to 1st floret fading, (b) days to full spike fading.

Senesced floret weight (g): Various concentrations of calcium and application methods as well as the interaction between calcium concentrations and application methods significantly affected the senesced floret weight of gladiolus spikes. The mean data of senesced floret weight as influenced by various concentrations of calcium and calcium application methods are presented in Table 2. The two years average data pertaining calcium concentrations showed the highest senesced floret weight (6.838 g) with 200 mM calcium application, which was statistically similar to the weight of senesced floret (6.789 g) with the application of 300 mM calcium. The least senesced floret weight (3.358 g) was recorded in spikes treated with distilled water. The mean data across two years pertaining to calcium application methods revealed that treatment of spikes with holding solution of calcium resulted in higher senesced floret weight (5.621 g) as compared to senesced floret weight (5.245 g) in spikes treated with foliar spray of calcium. The interaction data of calcium concentrations and application methods showed an increasing trend for floret weight of gladiolus during senescence with increase in calcium concentration upto 200 mM in both application methods (Fig. 2b). However senesced floret weight (7.08 g) was 6.92% higher when spikes were treated with 200 mM of calcium through holding solution as compared to the senesced floret weight (6.59 g) in spikes that received foliar applied 200 mM calcium.

Weight loss (%) of spikes: Significant variations in weight loss of gladiolus spikes in response to treatment of calcium concentrations and application methods. Interaction between calcium concentrations and application methods also significantly affected weight loss of gladiolus spikes. Data regarding percent weight loss of gladiolus spikes are presented in Table 2. Mean data regarding different concentrations of calcium revealed that treatment of spikes with 300 mM of calcium resulted in minimum weight loss (7.750%), followed by weight loss (7.958%) with the application of calcium at 400 mM which was statistically similar to the effect of calcium at 200 mM. Weight loss was highest (37.308%) in spikes of gladiolus treated with distilled water (no calcium). Average data across two years for calcium application methods revealed that weight loss (19.769%) was higher in spikes treated with foliar application of calcium as compared to the weight loss (17.856%) in spikes kept in holding solutions of calcium. Average data regarding interaction (Fig. 2c) showed that weight loss was reduced to minimum (6.82%) in spikes placed in holding solution of 300 mM calcium that was close to weight loss (7.20%) recorded in spikes kept in 200 mM holding solution of calcium. Weight loss (37.72 %) was highest in spikes that received no calcium (sprayed with distilled water).

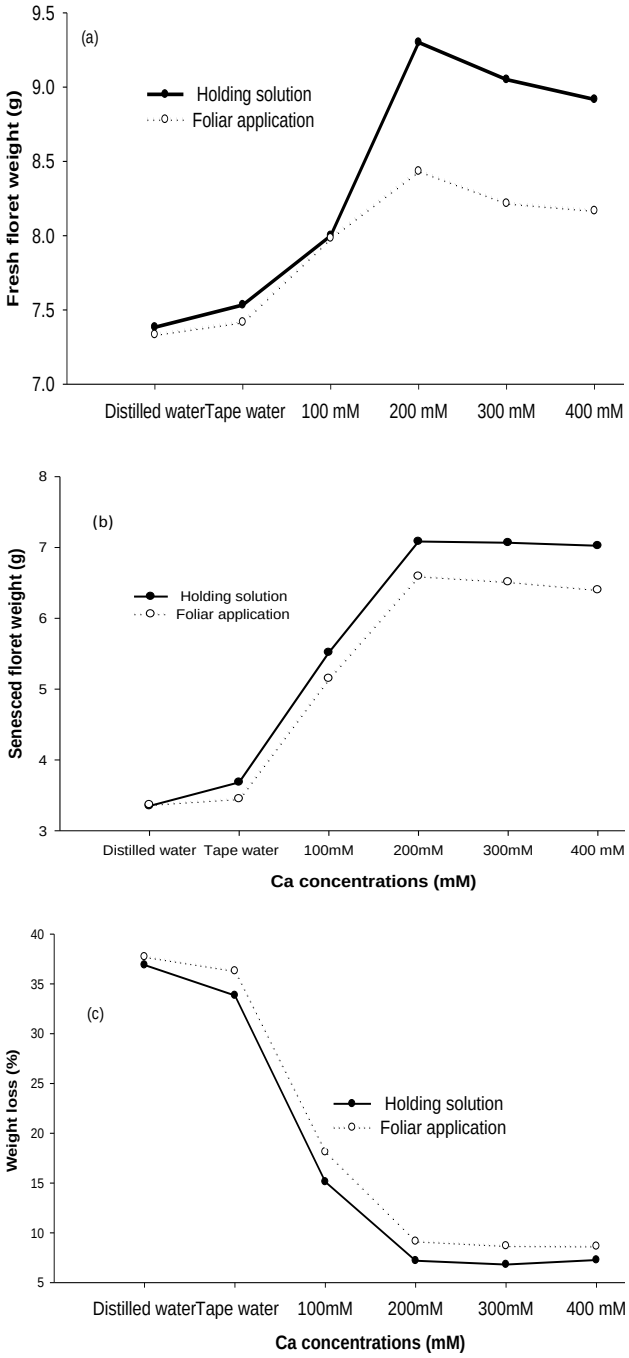

Fig. 2. Influence of calcium concentrations and application methods on (a) fresh floret weight (b) senesced floret weight and (c) weight loss of gladiolus spike.

Fresh weight is one of the main qualitative factors that is involved in the freshness, appearance and longevity of cut flowers (Chanasut *et al.*, 2003). Both fresh and senesced floret weight were the maximum with the same treatment in current study. The significantly higher fresh and senesced floret weight with 200 mM calcium treatment in holding solution indicates that calcium treatments decreased the rate of weight loss and delayed senescence (Torre *et al.*, 1999). The relatively superior fresh weight of senesced florets indicate that the vase solution enhanced the relative water content in petals and retained higher florets weight (Mortazavi *et al*., 2007; Cortes *et al.*, 2011). The application of calcium not only enhanced the fresh weight but also decreased the weight loss of the spike. It is suggested that the life of cut flowers can be extended by maintaining weight of florets/flowers (Cortes *et al.*, 2011) by the application of calcium (Mortazavi *et al.*, 2007). The increase in weight loss is also caused by an increase in respiration and or transpiration rate with increased water loss (Ezhilmathi *et al*., 2007). Thus, the decreased weight loss can also be attributed to the influence of calcium on respiration and transpiration, which are significantly lowered by calcium treatments (Hernández-Muñoz *et al*., 2006). The superior performance of calcium application through vase holding solution can be attributed to better uptake of calcium from holding solution in vase that resulted in freshness and less weight loss of flowers and spike (Reddy & Singh, 1996).

Calcium treatment and chemical changes in gladiolus florets

Reducing sugars (mg.g^{-1} floret dry wt.): Reducing sugars in florets of gladiolus spike were significantly affected by calcium concentrations and application methods as well as their interaction. Data pertaining reducing sugars in florets of gladiolus spikes in response to various concentrations of calcium and application methods are shown in Table 3. The average data across two years of various calcium concentrations showed that florets retained the maximum sugar contents (14.033 mg.g^{-1}D.Wt) in spikes treated with 200 mM of calcium, followed by (13.050 mg.g^{-1}D.Wt) with the application of 300 mM of calcium. The least reducing sugars (9.117 mg.g^{-1} D.Wt) were recorded in florets of spikes treated with distilled water. Two years mean data of different application methods revealed that higher reducing sugars (11.573 mg.g^{-1}D.Wt) were observed in florets of gladiolus spike treated with holding solution of calcium as compared to reducing sugars (11.111 mg.g^{-1} D.Wt) found in florets of spike that received calcium through foliar application. The two years average data pertaining interaction (Fig. 3) between calcium concentrations and application methods clearly showed that reducing sugar contents increased in florets with increase in calcium concentration up to 200 mM. Whereas further increase in concentration of calcium resulted in decline of reducing sugars through both application methods i.e., in spikes of holding solution and in spikes that received calcium through foliar spray. However, the maximum reducing sugars (14.15 mg.g^{-1} D.Wt) were recorded in florets of spikes placed in 200 mM holding solution of calcium.

Non-reducing sugars (mg.g^{-1}Dry Wt.): Significant differences were found for non-reducing sugar contents in florets of gladiolus in response to various concentrations of calcium and calcium application methods. Interaction between calcium concentrations and application methods also had significant effect on non-reducing sugars in florets of gladiolus (Fig. 4). Mean data regarding non-reducing sugars in florets of gladiolus as affected by different concentrations of calcium and its application methods are shown in Table 3. Mean data across two years about different concentrations of calcium showed that application of calcium at 200 mM retained highest

amount of non-reducing sugars (22.925 mg.g^{-1}D.Wt), followed by (20.542 mg.g^{-1}D.Wt) with 300 mM calcium. Minimum non-reducing sugars (14.883 mg.g^{-1}D.Wt) were found in the florets of gladiolus spikes treated with distilled water. Two years average data of application methods revealed that non-reducing sugars (18.656 mg.g^{-1}D.Wt) were higher in florets of spikes treated with holding solutions of calcium as compared to (18.214 mg.g^{-1}D.Wt) in florets of gladiolus spike that received foliar applied calcium. It is evident from average data pertaining interaction (Fig. 4) between calcium concentrations and calcium application methods that non-reducing sugars increased with increasing concentration of calcium up to 200 mM, while a decline in non-reducing sugar contents of gladiolus florets was observed with further increase in concentration of calcium to 300 and 400 mM. The maximum non-reducing sugar contents were found with application of 200 mM of calcium in both methods, however non-reducing sugars (23.60 mg.g^{-1}D.Wt) were 5.72% higher in florets of spikes treated with 200 mM of calcium through holding solution as compared to the non-reducing sugar contents (22.25 mg.g-1 D.Wt) in florets of spike sprayed with 200 mM of calcium.

Highly significant reducing and non-reducing sugar contents were retained by florets of spikes placed in holding solution of 200 mM calcium. The non-reducing sugars and reducing sugars are essential constituents of the cells (Halford *et al.,* 2011). Generally, the non-reducing sugars are found as starch or as structural component of the cell wall (Elbein *et al*., 2003). By contrast, the reducing sugars are required for energy metabolism (Dinakar *et al*., 2012). Hence, optimum levels of both the reducing and non-reducing sugars are required for healthy and viable florets (Singh *et al.,* 2008). The senescence of petals is, generally, associated with sugar starvation (Van Doorn, 2004). The similarities found between changes in cell physiology due to sugar starvation and changes in sugars during senescence process also supports the role of sugar starvation as a vital factor responsible for senescence (Van Doorn, 2004). By contrast, treatment with sucrose enhances vase life of flowers by delaying senescence of individual flowers in inflorescence (Eason *et al.,* 1997). Several other sugars such as fructose, trehalose, inositol, and polyethylene glycol (PEG) etc have been found to delay the onset of senescence in petals of different ornamental plant species (Van der Meulen-Muisers *et al*., 1995; Van Doorn & Woltering, 2008). Since, calcium reduces the rate of respiration; it therefore, decreases the depletion of sugars in the tissue (Mahajan and Dhat, 2004). Thus, optimum sugar content in petal improves the post-harvest quality and longevity of cut flowers (Van Doorn, 2004; Eason, 2006). The delayed senescence is also supported by the data of higher total phenols, reduced electrolyte leakage and highest vase life in spikes of gladiolus that received the same treatment.

Loss of protein contents (%): Statistically significant variations were observed in protein content of florets in response to various calcium concentrations and application methods, whereas the interaction between calcium concentrations and application methods was non-significant. The data pertaining protein content in response to calcium concentrations and application methods are presented in Table 3. The two years mean data regarding calcium concentrations revealed that the least loss of protein contents (16.682%) was recorded when spikes were treated with 200 mM of calcium, hence highest level of protein contents were retained by the florets treated with 200 mM of calcium, followed by treatment of calcium at 300 mM with (17.052%) loss in protein contents of gladiolus florets. The highest loss of protein content (46.960%) was observed in florets of gladiolus spikes treated with distilled water. The average data across two years regarding application methods revealed that the decline in protein content (28.289%) was more in spikes of gladiolus treated with foliar applied calcium as compared to decline in protein contents (27.566%) in florets of spikes placed in holding solution of calcium.

Table 3. Reducing sugars (RS) and non reducing sugars (NRS), loss of protein content (LPC) and total phenols (TPh) of gladiolus as affected by various concentrations of calcium and application methods.

Treatments		Chemical changes in gladiolus Florets			
		RS(mg.g-1)	NRS(mg.g^{-1})	LPC (%)	TPh (mg.g^{-1})
Calcium concentrations (C)	Distilled water	9.117e	14.883f	46.960a	0.402d
	Tap water	9.175e	15.683e	45.196b	0.419d
	100 Mm	10.892d	18.050d	23.744c	0.555c
	200 mM	14.033a	22.925a	16.682e	0.643a
	300 mM	13.050b	20.542b	17.052e	0.570bc
	400 mM	11.785c	18.525c	17.934d	0.574b
	LSD $_{0.05}$	0.290	0.427	0.762	0.018
Application methods (M)	Holding solution	11.573	18.656	27.566	0.537
	Foliar application	11.111	18.214	28.289	0.518
	Significance	*	**	**	**
Year	Year 1	11.264	18.414	27.999	0.523
	Year 2	11.420	18.456	27.856	0.532
	Significance	NS	NS	NS	NS
Interaction	CxM	**Fig. 3	**Fig. 4	NS	NS

NS = Non-significant; *= Significant at p≤0.05; **= Significant at p≤0.01

Means followed by different letters in respective columns are significantly different from each other at p≤0.05

Table 4. Electrolyte leakage (EL), calcium content in floret (CaF) and vase life (VL) of gladiolus spike as affected by various concentrations of calcium and application methods.

Treatments		EL (%)	CaF (%)	VL(days)
Calcium concentrations (C)	Distilled water	73.279a	0.153d	8.992e
	Tap water	72.400b	0.183d	9.158d
	100 mM	50.043c	1.333c	10.658c
	200 mM	44.843e	1.526a	12.833a
	300 mM	48.372d	1.456b	12.467b
	400 mM	48.813d	1.437b	12.392b
	$LSD_{0.05}$	0.878	0.040	0.150
Application methods (M)	Holding solution	55.233	1.060	11.286
	Foliar application	57.351	0.969	10.881
	Significance	*	**	**
Year	Year 1	56.329	1.017	11.094
	Year 2	56.254	1.012	11.072
	Significance	NS	NS	NS
Interaction	CxM	** Fig. 5	** Fig. 6	** Fig. 7

NS = Non-significant; *= Significant at p≤0.05; **= Significant at p≤0.01

Means followed by different letters in respective columns are significantly different from each other at p≤0.05

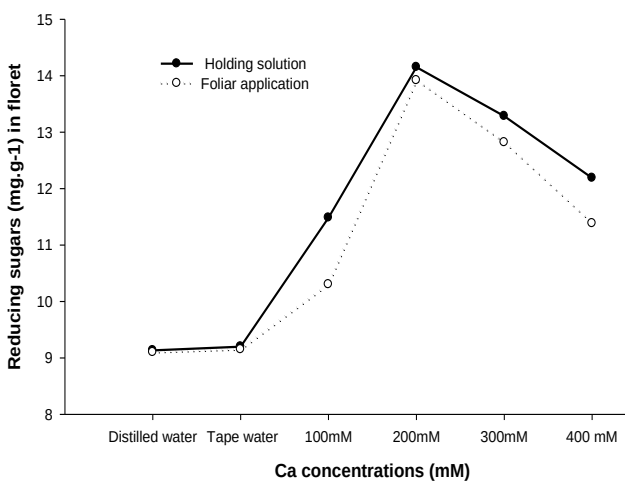

Fig. 3. Influence of calcium concentrations and application methods on reducing sugars (mg. g^{-1} D.Wt) in florets of gladiolus spike.

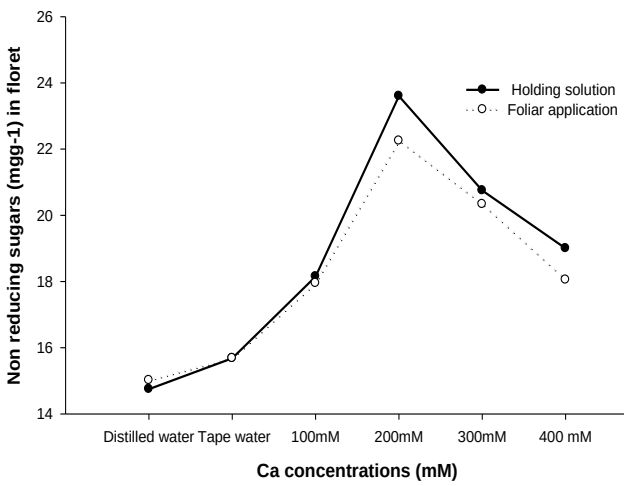

Fig. 4. Influence of calcium concentration and application methods on non-reducing sugars (mg.g^{-1} D.Wt.) in florets of gladiolus spike.

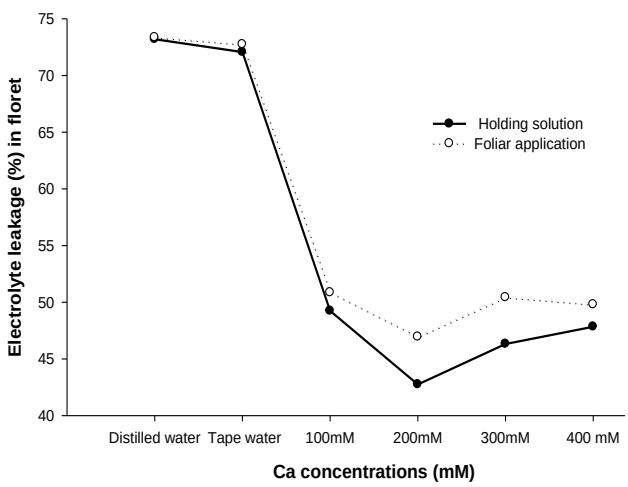

Fig. 5. Influence of calcium concentration and application methods on electrolyte leakage (%) in florets of gladiolus spike.

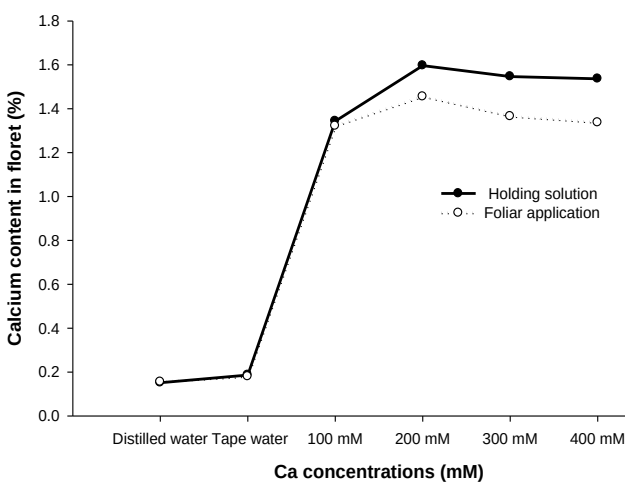

Fig. 6. Influence of calcium concentrations and application methods on calcium content (%) in florets of gladiolus spike.

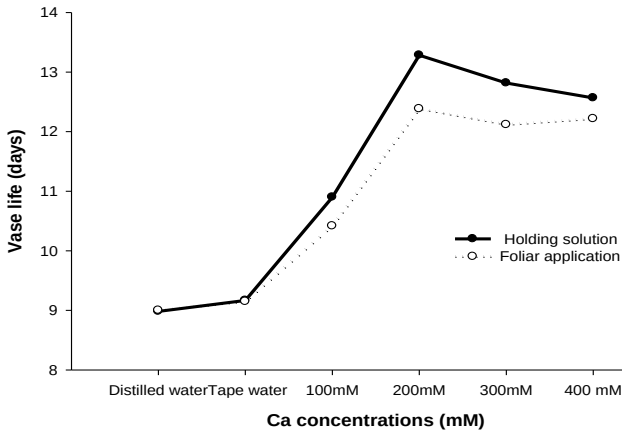

Fig. 7. Influence of calcium concentrations and application methods on vase life (days) of gladiolus spike.

The protein content of the leaf and florets is an important determinant of longevity (Rubinstein, 2000). Since almost all the proteins are present as enzymes in plants especially florets (Fukasawa *et al.*, 2010), the loss of protein content results in significant decline in metabolic activities (Lay-yee *et al.*, 1992; Mittler *et al*., 2004). Thus, the onset of senescence is associated with the degeneration of proteins (Rogers, 2006). It is believed that free radicals degrade the proteins that results in death of tissue (Agarwal *et al*., 2005). The highest protein contents were retained by florets of gladiolus spikes treated with 200 mM of calcium. The calcium is known to enhance strength of cell wall and membrane and helps maintaining chlorophyll and protein contents (Pooviaiah, 1986; White & Broadly, 2003). Calcium decreases and delays the destruction of protein and phospholipid in petals (Torre *et al.*, 1999). It activate enzymes such as membrane bounded Ca^{+2}-ATPase etc. that increases antioxidant activity by reducing free radicals and delays senescence (Agarwal *et al*., 2005; Sairam *et al*., 2011; Singh *et al*., 2013). Thus, the least reduction in protein content in florets with calcium treatment is supported by superior physical quality attributes observed with the same treatment (Table 3).

Total phenols (mg.g^1 dry wt.): The total phenols of gladiolus florets were significantly affected by calcium concentrations and application methods while interaction between calcium concentrations and application methods was not significant. The mean data for total phenols in florets of gladiolus are shown in Table 3. The average data for two years regarding calcium concentrations showed that the highest total phenols (0.643 mg.g^{-1}D.Wt) were recorded in florets of gladiolus spikes treated with 200 mM calcium, followed by (0.574 mg.g^{1}D.Wt) with 400 mM that was statistically similar to the total phenols recorded with 300 mM calcium application. The least total phenols (0.402 mg. g^{-1} D.Wt) were observed in florets of gladiolus spikes treated with distilled water. It is evident from the two years mean data that calcium application through holding solution resulted in the higher phenol contents in florets of gladiolus spikes that was 0.537 mg. g^{-1} D.Wt as compared 0.518 mg.g^{-1}D.Wt in floret of spikes treated with foliar application method.

Both edible and non edible plants contain phenolic compounds and are found to play an important role especially antioxidant activity (Balasundram *et al.*, 2006). The phenolic compounds act as reducing agents and hydrogen donors due to their redox properties and, thus, enhance antioxidant activity (Rice-Evens *et al.*, 1995). The high total phenols in florets of gladiolus with calcium application indicate that calcium decreased the loss of phenolic compounds (Da Silva, 2003). It is reported that higher phenols present in petals of rose is responsible to extend the longevity and maintains its quality (Mwangi *et al*., 2003). The calcium as secondary messenger enhances antioxidant activity and regulates flower senescence in gladiolus by reducing free radicals (Agarwal *et al*., 2005; Sairam *et al.*, 2011; Singh *et al*., 2013). The findings of this study revealed that calcium maintained higher amount of total phenols as compared to control treatment that may contribute to extend the vase life and quality (Sairam *et al.*, 2011). It is also observed that calcium application through holding solution may help in retaining higher total phenols as compared to direct foliar application, probably by greater calcium uptake through the xylem (Starkey & Pedersen, 1997).

Calcium treatment and cell membrane in gladiolus floret

Electrolyte leakage (%): The statistical analysis showed that various concentrations of calcium and application methods as well as their interaction significantly affected the electrolyte leakage. The electrolyte leakage of gladiolus florets in response to calcium concentrations and application methods are given in Table 4. The mean data regarding various concentrations of calcium showed that electrolyte leakage was reduced to the minimum (44.843%) in florets of spikes that received calcium at 200 mM, while the highest electrolyte leakage (73.279%) was recorded in control. The two years mean data pertaining different application methods showed that electrolyte leakage (57.351%) was higher in florets of spikes treated with foliar application of calcium as compared to 55.233% in florets of spikes kept in holding solution containing calcium. It is evident from the interaction data (Fig. 5) between calcium application methods and calcium concentration that with both application methods, electrolyte leakage declined with increase in calcium concentration up to 200 mM, while further increase in calcium concentration to 300 and 400 mM resulted in an increase in electrolyte leakage. The least electrolyte leakage was recorded at 200 mM of calcium with both application methods; however the electrolyte leakage was less (4.19%) in 200 mM holding solution of calcium as compared to the same concentration (200 mM) when applied as foliar spray.

Electrolyte leakage is one of the major techniques to determine lesions in cell membrane (Montoya *et al.*, 1994; Gerailoo & Ghasemnezhad, 2011), and an important indicator of senescence in petals and florets (Rubinstein, 2000). The application of calcium resulted in significant decline in electrolyte leakage from the floret. Electrolyte leakage influences selective permeability of a membrane and is an indicator of membrane stability (Montoya *et al.*, 1994; Gerailoo and Ghasemnezhad,

2011). Therefore, post-harvest calcium application has emerged a primary measure to delay senescence as evident from lower electrolyte leakage from the tissue (Torre *et al.*, 1999; Rubinstein, 2000; Nan, 2007). The calcium application is known to improve cell membrane stability and, thus, decreases the electrolyte leakage in cut flowers (Mortazavi *et al.*, 2007). Tuna *et al*., (2007) also reported that treatment of calcium enhanced membrane integrity and prohibited rise in electrolyte leakage. The influence of calcium on membrane integrity is attributed to high antioxidant activities (Singh *et al*., 2013) and, hence, delayed loss of membrane integrity (Sairam *et al*., 2011). It is also established in this study that application of calcium through holding solution is superior to foliar application method, due to greater calcium uptake through the xylem (Gerasopoulos & Chebli, 1999).

Calcium content in floret (%): The calcium content of gladiolus florets varied significantly in response to calcium concentration and application methods as well as the interaction of both the factors. The average data related to the calcium content in florets of gladiolus are shown in Table 4. The average data across two years regarding calcium concentrations showed that highest calcium content (1.526%) was found in florets of gladiolus spikes treated with 200 mM of calcium, followed by (1.456%) with 300 mM calcium that was statistically similar to the treatment of 400 mM calcium. The least calcium content was observed in florets of gladiolus spikes treated with distilled and Tap water. The two years mean data of application methods revealed that calcium contents (1.06%) was higher in florets of gladiolus spikes kept in holding solution containing calcium as compared to 0.969%, recorded in florets of spikes treated with calcium solution as foliar spray. The interaction between calcium concentrations and application methods (Fig. 6) showed that calcium content of gladiolus florets increased with increasing concentration of calcium till 200 mM in holding solution as well as foliar applied calcium, however the calcium content was higher in florets of spikes treated with holding solution of 200 mM calcium as compared to foliar application with the same concentration of calcium.

The calcium is an important constituent of the cell wall (Cosgrove, 2005) and is associated with cell membrane (Luan, 2009). In addition, the calcium also acts as secondary messenger in the cell and hence regulates several physiological activities (Lecourieux *et al*., 2006). The calcium concentration in tissues is positively correlated to quality attributes including post-harvest longevity (Gerasopoulos &Chebli, 1999; Torre *et al.*, 1999) and delays senescence in tuberose, lupines and gladiolus respectively (Anjum *et al*., 2001; Picchioni *et al*., 2002; Reddy and Sarkar, 2016). However, significant variations have been reported regarding the optimum concentration in different tissues (Nan, 2007; Reddy & Sarkar, 2015) as well as method of application (Nan, 2007, Sairam *et al*., 2011). It has been observed that calcium treatment through holding solution resulted in higher calcium content in florets and superior quality attributes (Sairam *et al*., 2011). Since, the calcium is directly taken up by the spike through the xylem (Gislerod, 1997), it is reasonable to observe high calcium content with calcium application in vase solution as compared to foliar applied calcium.

Vase life (days): Significant variations in vase life of gladiolus spikes with calcium concentrations and calcium application methods as well as their interaction. Mean data regarding the vase life of gladiolus spikes in response to calcium concentrations and application methods are presented in Table 4. The average data pertaining various concentrations of calcium showed that application of 200 mM calcium increased the longevity of the spike to maximum (12.833 days) that reduced to (12.467 days) with the application of calcium at 300 mM which was statistically similar to the vase life (12.392 days) recorded with 400 mM of calcium. Minimum vase life (8.992 days) was observed when spikes were treated with distilled water (no calcium). The average data for calcium application methods depicted that calcium treatment through holding solutions resulted in greater extension in vase life (11.286 days) of gladiolus spikes as compared to 10.881 days in spikes treated with calcium as a foliar spray. Data regarding interaction between concentrations of calcium and its application methods (Fig. 7) showed that vase life was increased with increasing concentrations of calcium up to 200 mM, while further increase in calcium did not increase the vase life of gladiolus spike applied through both methods. Though highest vase life was recorded at 200 mM of calcium both with holding solution and foliar applied calcium but longevity of gladiolus spike was more (6.8 %) in spikes present in holding solution of 200 mM calcium as compared to spikes treated with foliar applied 200 mM calcium.

The poor vase life of cut flowers is major concerned in cut flower industry (Reid & Jiang, 2012). The cut flower, being deprived of nutrients and water resources, rapidly senesce (Pun & Ichimura, 2003). Thus, the stalks of cut flowers are, generally, held in nutrients solution to retain flower quality during marketing and display (Asrar, 2012). The application of calcium is also an important technique to increase the vase life of cut flowers (Gerasopoulos & Chebli, 1999). The vase life of gladiolus spikes was significantly influenced by calcium concentration as well as application methods. The calcium enhances the longevity of cut flowers by delaying the degradation of cell membrane (Torre *et al.*, 1999; Rubinstein, 2000) by protecting the cell membrane from damage of free radicals (Agarwal *et al*. 2005; Sairam *et al.*, 2011; Singh *et al*., 2013). In earlier studies, it has reported that calcium treatment extends the vase life of gerbera, roses and gladiolus (Gerasopoulos & Chebli, 1999; Ahmad *et al*, 2011; Capdeville *et al.*, 2005; Sairam *et al.*, 2011). The increase in vase life by calcium is supported by the observation that the highest total phenoles and least electrolyte leakage were also recorded with the same calcium treatment (Tables 3 and 4). However, it is interesting to observe that application of calcium was more affective through holding solution than foliar application. Since, the xylem of the floral stalk is in direct contact with calcium in dipping solution, it is likely to observe greater calcium uptake in this method of calcium application (Gerasopoulos & Chebli, 1999).

Conclusions

It can be concluded form the results that treatment of gladiolus spikes with 200 mM of calcium delayed florets opening and fading, retained fresh and senesced floret weight with maximum reducing, non reducing sugars and total phenols, least electrolyte leakage , least loss in protein content , maximum calcium contents and resulted in an increased vase life of gladiolus spikes. The application of 200 mM calcium was superior as compared to lower or higher concentration of calcium. The application of calcium through holding solution was superior in retaining the quality of gladiolus florets and spike over extended period of time as compared to the application of calcium as foliar spray. Thus holding solutions of calcium was found better method for post harvest calcium application as compared to foliar spray for most of the attributes and vase life of gladiolus spikes. The interaction between calcium concentration and application methods revealed that holding solutions of 200 mM calcium delayed florets fading, retained highest fresh and senesced floret weight, reducing and non reducing sugars with least weight loss, electrolyte leakage and highest calcium content hence extended vase life of gladiolus spikes to the maximum.

Recommendations: The application of 200 mM calcium through holding solution is recommended to retain the quality of gladiolus florets and spike over extended period and to enhance the vase life of spikes.

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