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Impact of floral preservatives on anthocyanin content and vase life of cut *gerbera* cv. Savannah under ambient conditions

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Abstract
A laboratory trial was carried out to investigate the effectiveness of different floral preservatives on extension of vase life of cut *gerbera* cv. Savannah under ambient storage condition. All the cut *gerberas* were precooled at 5^{o}C for 6 hours and followed by pulsing with sucrose 20% + sodium hypochlorite 50 ppm for 12 hours and then kept in preservative floral solutions *i.e.* neem extract, rosemary oil, calcium chloride and their combinations along with sucrose 4%. Using the solution of neem extract 1% + calcium chloride 0.1 % + sucrose 4% significantly maintained water relations and reduced scape bending curvature as compared to all other treatments. Anthocyanin content in ligules and total soluble solids in flower stalk of cut *gerberas* kept in neem extract 1% + calcium chloride 0.1 % + sucrose 4% solution were also highest *i.e.* 6.66 mg Congo Red/g f wt and 10.13 ^{o} Brix respectively. The cut *gerberas* kept in solution of neem extract 1% + calcium chloride 0.1 % + sucrose 4% solution recorded lowest optical density (0.046) which was attributed due to lowest microbial count (3.42 x 10^5 cfu/ml) and resulted in highest vase life of 13.37 days compared to control (4.71 days) with highest microbial count (9.22 x 10^6 cfu/ml).

Keywords: *Gerbera*, floral preservatives, anthocyanin content, vase life

Introduction
Gerbera (*Gerbera jamesonii* Bolus ex. Hook.) belongs to Asteraceae family, the largest family of flowering plants and is one of ten popular cut flowers in the world, according to global trends in floriculture (Soad *et al.,* 2011) [12]. It is in considerable demand in both domestic and export market. The blooms are attractive, suitable for any type of floral arrangements and are available in different shades and hues. Besides floral arrangements, *gerbera* is widely used in bouquets and also in dry flower crafts.

Keeping quality is an important parameter for evaluation of cut flower quality for both domestic and export markets. The postharvest life of cut *gerbera* is very short due to its hollow flower stalk and bigger sized head. The weight of the head and disturbed water relations due to vascular occlusions results in scape bending (Prashanth *et al.,* 2007) [10], a premature senescence apart from normal senescence. The cut *gerberas* are sensitive to microbial contamination at the stem end that shortens their vase life (Balestra *et al*., 2005) [3]. Proper harvesting, postharvest handling and use of suitable floral preservatives improve keeping quality of cut *gerberas* by maintaining the turgidity of scapes. Most floral preservatives contain germicides, ethylene synthesis inhibitors, growth regulators, mineral compounds and carbohydrates that are essential to extend the vase life of cut flowers (Mutui, 2002) [9]. Sucrose is widely used in floral preservatives, which acts as a food source of respiratory substrate and delays the degradation of proteins, improves the water balance of cut flower.

Several attempts have been made to prolong the vase life of cut flowers by using commercial floral preservatives *viz.* Florissant, Chrysal, Bloom life, Petal life, Rose life *etc*. However, these preservatives are costly and out of reach for most of small scale cut flower growers, hence the use of locally available floral preservatives would be handy and cheaper. Essential oils, organic natural substances are safe and environmental friendly due to their strong antimicrobial properties against some pathogens, extends the vase life of cut *gerberas*. These antimicrobial properties of organic compounds are attributed to their high levels of phenolic compounds (Lambert *et al.,* 2001) [7]. But, there was no organized research till now on extension of vase life of cut *gerbera* with postharvest treatments like precooling, pulsing and vase solutions of locally available floral preservatives, essential oils, mineral salts and their combinations so far. In this context, a study to extend the vase life of cut *gerbera* with different postharvest treatments is of greater importance.

Material and Methods

The present study was carried out in laboratory conditions at the Department of Floriculture, College of Horticulture, Mojerla. Cut *gerbera* flowers of cultivar Savannah were obtained from a commercial polyhouse located 15 km away from laboratory. The flowers were harvested when the ray florets at 3/4th opened stage in the morning hours between 7 and 8 am. Immediately after harvest 5-10 cm of basal woody portion was cut under deionized water and were taken to the laboratory. The cut flowers were immediately unpacked, sorted to uniform length (40 cm) and quality of capitulum in order to maintain uniformity within the replications. The flower scapes were precooled at 4±2ºC by placing in precooling chamber and then scapes were kept in the pre-conditioning solution i.e. sucrose 20% + sodium hypochlorite (NaOCl) 50 ppm for pulsing at ambient temperature (22±2ºC).

After completion of pulsing, flower scapes were placed in treatment solution's for the rest of the experimental period to evaluate their vase life under ambient condition. Five flowers were used for each treatment and cut *gerbera* scapes were held in 500 ml glass bottles containing 250 ml of aqueous vase solution. All treatments were replicated thrice for confirmation of results and a same set of treatments were run for biochemical analysis simultaneously. The experimental flowers were held in the laboratory at 22 ± 2°C room temperature, 60 to 70 percent relative humidity and 40 W/84 cool white flourescent tubes using a 12 hours photoperiod. Treatment details are T_1 - Neem extract (*Azadirachta indica)* 1%, T_2- Rosemary oil (*Rosmarinus officinalis)* 5%, T_3- Calcium Chloride ($CaCl_2$) 0.1%, T_4- Neem extract (*Azadirachta indica)* 1% + Rosemary oil (*Rosmarinus officinalis)* 5%, T_5- Neem extract (*Azadirachta indica)* 1% + Calcium Chloride ($CaCl_2$) 0.1%, T_6- Rosemary oil (*Rosmarinus officinalis)* 5% + Calcium Chloride 0.1% ($CaCl_2$) and T_7 with distilled water (control). For treatments T_1 to T_6, 4% sucrose was supplemented and evaluated their vase life. The observations like water uptake (WU g/f), transpirational loss of water (TLW g/f), fresh weight change (FWC %) were recorded (Venkatarayappa *et al*., 1981) [14], scape bending curvature in degrees (Van-Doorn and De-Wittie 1994) [13], anthocyanin content in mg Congo red/gram fresh weight of ligules (Rutland 1968) [11], optical density, microbial count (Bleeksma and Van Doorn 2003) [4] and vase life (Abadi *et al.,* 2013) [1] were determined. Data was statistically analyzed through INDOSTAT software.

Results and Discussion

1. Water uptake (WU): The data presented in Table 1 indicates that water uptake recorded was highest in treatment neem extract 1% + calcium chloride 0.1% (10.17 g/f) followed by neem extract 1% (9.48 g/f), whereas water uptake was significantly low with control (5.65 g/f). The combined effect of neem extract 1% + calcium chloride 0.1% recorded highest WU might be due to their antimicrobial properties which might have inhibited the growth of microbes in vase solution and prevented the blockage of xylem vessels by reducing formation of air cavities. Pulsing of sucrose might have helped in maintaining the water balance and turgidity in cut *gerbera* scapes.

Table 1: Effect of postharvest application of floral preservative combinations on water relations and scape bending curvature during vase life period of cut *gerbera* cv. Savannah at ambient condition

Treatments	Physiological parameters			Physical Parameter
	Water uptake (g/f)	Transpirational loss of water (g/f)	Fresh Weight Change (%)	Scape bending curvature (Degrees)
T_1- Neem extract 1%	9.48^b	9.55^b	93.73^b	4.17^b
T_2- Rosemary oil 5%	8.23^c	8.53^c	87.88^c	5.74^c
T_3- Calcium chloride 0.1%	9.17^b	9.22^b	92.33^b	4.33^b
T_4- Neem extract 1% + Rosemary oil 5%	8.35^c	8.72^c	88.72^c	5.62^c
T_5- Neem extract 1% + Calcium chloride 0.1%	10.17^a	10.05^a	96.65^a	3.17^a
T_6- Rosemary oil 5% + Calcium chloride 0.1%	8.52^c	8.85^c	89.86^c	5.38^c
T_7- Control(Distilled water)	5.65^d	5.87^d	79.22^d	25.01^d
S.Em ±	0.11	0.11	0.72	0.16
**P < 0.01	0.32	0.33	2.06	0.47

Values are the mean of three replications, Values with same alphabets did not differ significantly

2. Transpirational loss of water (TLW) : The TLW was registered highest in neem extract 1% + calcium chloride 0.1% (10.05 g/f) followed by neem extract 1% (9.55 g/f), and calcium chloride 0.1 % (9.22 g/f) whereas control recorded the lowest TLW (5.87 g/f). This suggests that combined effect of neem extract 1% + calcium chloride 0.1% recorded highest TLW. This might be due to higher water uptake to avoid temporary water stress that led to the increase in membrane viscocity (Faragher *et al.,* 1984) [5] due to their antimicrobial property. The adequate and controlled TLW might have contributed to the retardation of senescence process and hence increased the vase life of cut *gerberas*.

3. Fresh weight change (FWC): The same trend was observed in FWC with highest in neem extract 1% + calcium chloride 0.1% (96.65%) followed by neem extract 1% (93.73%) whereas control registered (79.22%). The better water relations in neem extract at both concentrations is due to its antimicrobial properties, resulting in unplugging of xylem vessels and maintained continuous water relations without any interruption. The combined effect of neem extract 1% + calcium chloride 0.1% recorded maximum fresh weight change might be due to higher rate of water uptake and better maintenance of water equilibrium resulted in higher degree of freshness and pulsing of sucrose also increased mechanical rigidity of scapes by inducing cell wall thickening and lignifications of vascular tissues.

4. Scape bending curvature: It is vivid from the Table 2, that cut *gerberas* held in neem extract 1% + calcium chloride 0.1% recorded lowest scape bending curvature (3.17 degrees) followed by neem extract at 1% (4.17 degrees), calcium chloride 0.1% (4.33 degrees) and highest scape bending curvature was noticed in control (25.01 degrees). It indicates that dual effect of neem extract 1% + calcium chloride 0.1% effectively controlled scape bending curvature might be due to an increase in cell wall resistance (Gregory *et al.*, 1988) [6] and associated with antimicrobial activity or control of metabolism prevented the blockage of xylem vessels by inhibiting formation of air cavities which might have promoted higher water uptake. This increased turgidity and might be possible reason for reduced scape bending curvature in cut *gerberas*.

5. Total soluble solids (TSS) of flower stalk: Table 2 indicates that highest TSS was recorded with neem extract 1% + calcium chloride 0.1% (10.13 ºBrix) followed by neem extract 1% (9.55 ºBrix) and the lowest TSS was noticed with control (7.23 ºBrix). Neem extract 1% + calcium chloride 0.1% recorded highest TSS initially might be due to pulsing of sucrose in vase solution increased the concentrations of glucose, fructose in flower stalk and later on decreased due to action of hydrolytic enzymes as they break down the monosaccharide's as a part of their metabolism with increased rate of respiration.

6. Anthocyanin content of ligules: Table 2 indicates that rosemary oil 5% (5.47 mg Congo Red/g f wt) recorded lowest anthocyanin content whereas neem extract 1% + calcium chloride 0.1% registered highest anthocyanin content (6.66 mg Congo Red/g f wt) followed by neem extract 1% (6.46 mg Congo Red/g f wt). The combined effect of neem extract 1% + calcium chloride 0.1% showed that the anthocyanin content was initially decreased which might be due to dilution of anthocyanin in the expanding cells, however increase in anthocyanin content in later stage might be due to intensification of pigmentation in ligules as water loss is more as the vase life increases (Misoon *et al*,. 2000) [8] in cut carnation.

Table 2: Effect of postharvest application of floral preservative combinations on biochemical parameters during vase life period of cut *gerbera* cv. Savannah at ambient condition

Treatments	Biochemical parameters		
	Total soluble soilds (º Brix)	**Anthocyanin content**	**Optical density**
T_1- Neem extract 1%	9.55^b	6.46^a	0.055^c
T_2- Rosemary oil 5%	8.92^c	5.47^c	0.062^b
T_3- Calcium chloride 0.1%	9.37^b	6.14^b	0.056^c
T_4- Neem extract 1% + Rosemary oil 5%	9.02^c	5.77^b	0.061^b
T_5- Neem extract 1% + Calcium chloride 0.1%	10.13^a	6.66^a	0.046^d
T_6- Rosemary oil 5% + Calcium chloride 0.1%	9.18^c	5.93^b	0.059^b
T_7- Control(Distilled water)	7.23^d	5.62^c	0.088^a
S.Em ±	0.12	0.16	0.002
$**P < 0.01$	0.35	0.46	0.005

Values are the mean of three replications, Values with same alphabets did not differ significantly

7. Optical density and Microbial count of vase solution: The lowest optical density (0.046) and microbial count (1.24 x 10^3 cfu/ml) respectively was noticed with neem extract 1% + calcium chloride 0.1% whereas control (distilled water) recorded highest optical density (0.088) and microbial count (4.53 x 10^3 cfu/ml) as shown in Table 2 and 3. The combined effect of neem extract 1% + calcium chloride 0.1% recorded lowest microbial count as they were associated with antimicrobial activity (Nowak and Rundnicki, 1990) that prevented the microbial growth in vase solution which eventually reduced blockage of xylem vessels by inhibiting formation of air cavities and enhanced the vase life of cut flowers.

8. Vase life (days): Data presented in Table 3 indicates that the highest vase life was observed in treatment neem extract 1% + calcium chloride 0.1% (13.37 days) followed by neem extract 1% (11.66 days) whereas control recorded significantly lowest vase life period (4.71 days). Highest longevity of cut *gerberas* in neem extract + calcium chloride is due to the combined effect of neem extract 1% + calcium chloride 0.1% recorded highest vase life, which might have decreased respiration rate (Anjum *et al.*, 2001) [2], increased cell wall resistance (Gregory *et al.*, 1988) [6] and associated with control of microbial activity or control of metabolism (Nowak and Rundnicki, 1990) which promoted higher water uptake and prevented the blockage of xylem vessels by inhibiting formation of air cavities. Pulsing with sucrose antagonizes the effect of ABA which promotes senescence and led to enhanced vase life of cut *gerberas*.

Table 3: Effect of postharvest application of floral preservative combinations on microbial count in vase solution (cfu/ml) during vase life period of cut *gerbera* cv. Savannah at at ambient condition

Treatments	Vase life (Days)	Microbial count	
		0th day	**12th day**
T_1- Neem extract 1%	11.66^b	1.98 x 10^{3b}	4.22 x 10^{5b}
T_2- Rosemary oil 5%	8.56^c	2.67 x 10^{3b}	5.06 x 10^{6c}
T_3- Calcium chloride 0.1%	10.39^b	2.07 x 10^{3b}	4.25 x 10^{5b}
T_4- Neem extract 1% + Rosemary oil 5%	8.66^c	2.47 x 10^{3b}	4.97 x 10^{6c}
T_5- Neem extract 1% + Calcium chloride 0.1%	13.37^a	1.24 x 10^{3a}	3.42 x 10^{5a}
T_6- Rosemary oil 5% + Calcium chloride 0.1%	8.77^c	2.31 x 10^{3b}	4.86 x 10^{6c}
T_7- Control(Distilled water)	4.71^d	4.53 x 10^{3c}	9.22 x 10^{6d}
Mean	9.44	2.46 x 10^3	5.14 x 10^6
S.Em ±	0.45	0.21	0.22
$**P < 0.01$	1.39	0.66	0.67

Values are the mean of three replications, Values with same alphabets did not differ significantly

Conclusion

The treatment neem extract 1% + calcium chloride 0.1% is the most effective floral preservative which can be successfully used to extend vase life of cut *gerberas* under ambient conditions as it has calcium chloride which enhances cell rigidity by thickening of cell wall and antimicrobial activity of both neem extract and calcium chloride extends vase life.

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