



Research Article

Extending The Vase Life of Peruvian Lily (*Alstroemeria* Spp.) With 1-Methylcyclopropene and Ascorbic Acid

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Abstract

Alstroemeria spp. is one of the highly demanded cut flowers in the local and global cut flower market. Short vase life of flowers and leaves, petal wilting, petal drop, and transparency of petals are major postharvest problems. The objective of the present study was to extend the vase life of *Alstroemeria* spp. with 1-methylcyclopropene and ascorbic acid. Freshly cut flowering stems of *Alstroemeria* spp. were treated with 1-methylcyclopropene (0.25 ppm) and ascorbic acid (57 mM) alone and in combination of the two, for six hours. Distilled water was used as the control. Postharvest concentrations of anthocyanin, chlorophyll and glucose in flowers were best maintained when treated with a combination of 1-methylcyclopropene and ascorbic acid, compared to all other treatments. Percentage fresh weight loss was same among treatments. The best treatment to extend vase life of *Alstroemeria* spp. is the combination of 1-methylcyclopropene and ascorbic acid, which extended the vase life by additional seven days compared to the control.

Keywords: *Alstroemeria*; ascorbic acid; 1-methylcyclopropene; vase life

Introduction

Alstroemeria spp., commonly known as Peruvian Lily is a highly demanded cut flower, available in numerous and vibrant colours (Choon, 2012). *Alstroemeria* flowers are highly sensitive to ethylene. The ethylene induced postharvest degradation symptoms of *Alstroemeria* spp. include; petal wilting, transparency of petals, petal drop, premature yellowing of leaves and short life of leaves (Choon, 2012). These symptoms reduce the economic value of *Alstroemeria* spp. cut flowers.

Ethylene (H₂C=CH₂), is a colourless, flammable gas having a sweet taste and odour. Ethylene is plant hormone influencing growth, development, ripening, and senescence (aging) of the whole plant and plant organs (Francis, 2014). The ethylene induced postharvest degradation of cut flowers can be mitigated by applying chemicals, which inhibit ethylene biosynthesis and/or ethylene receptors.

1-methylcyclopropene (1-MCP), an inhibitor of ethylene receptors, is structurally similar to ethylene that has a ten-fold more affinity to ethylene receptors than ethylene

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(Blankenship, 2003). In many other cut flower species such as; rose, carnation and chrysanthemum the vase life and the postharvest quality has been maintained through treatments of 1-MCP and ascorbate (Abri *et al.*, 2013; Blankenship and Dole, 2002).

In senescing cut flowers supernumerary free radicals and reactive species of molecular oxygen cause oxidative stress in cells that lead ultimately to cell death (Thompson *et al.*, 2006). These reactive oxygen species (ROS) are; superoxide radical, hydrogen peroxide, hydroxyl radical, alkoxyl radical and singlet oxygen (Khan and Panda, 2002; Panda, 2002). These reactive oxygen species have the capacity to degrade almost all cell components including membrane lipids, proteins and DNA (Hendry, 1993, Casano *et al.*, 1994). Antioxidants such as, ascorbic acid, phenolic and polyphenolic compounds are known to neutralize and scavenge these free radicals protecting plants from oxidative stress induced senescence.

Ascorbic acid (vitamins C) is a product of D-glucose metabolism in higher plants (El-Kobisy *et al.*, 2005). Ascorbic acid is a powerful antioxidant due to its ability to donate electrons to neutralize ROS protecting plants from oxidative damage (Cherut, 2009; Conklin, 2001). Ascorbic acid also serves as a co-factor for many enzymes contributing to the detoxification of ROS (Barth *et al.*, 2006). The antioxidant effects of ascorbic acid were evident in number of cut flower species in which it improved growth, delayed flower opening, extended vase life and stimulated the accumulation of carbohydrate (Bedour *et al.*, 2011). Ascorbic acid also extended the vase life of rose and red ginger flowers (Abri *et al.*, 2013; Ieamtim *et al.*, 2008).

In many other cut flower species such as the vase life and the postharvest quality has been maintained through treatments of 1-MCP and ascorbic acid alone (Abri *et al.*, 2013; Blankenship and Dole 2002). However, the use of 1-MCP and ascorbic acid in combination has not been widely studied for the inflorescences of *Alstroemeria* spp. Therefore, the current study will evaluate the effects of different concentrations and exposure durations of 1-MCP and ascorbic acid, alone and in combination on postharvest longevity and quality of *Alstroemeria* spp. inflorescences.

Materials and Methods

Inflorescences

Inflorescences of red *Alstroemeria* spp. were harvested from fields of commercial growers in Meepilimana (N 6° 54', E 80° 47'), Sri Lanka. Inflorescences were harvested when at least three florets were completely red. At the laboratory, stems were trimmed to 20 cm as measured from the top of the inflorescence. The lower 10 cm of the stems were defoliated. Again 2 cm of the stem were cut off under water to avoid air embolism. Stems of inflorescences were placed in beakers with 200 ml dH₂O, which were then placed in chambers (40 x 30 x 30 cm), made from sealed

300 gauge clear polythene. Inflorescences were subjected to treatments inside the polythene chambers.

Experiment 1: Effect of variable concentrations of 1-MCP and different exposure durations on postharvest longevity and quality of *Alstroemeria* spp.

Treatments

To generate 1-MCP, Ethyl Bloc™ powder was placed in 2.5 mL micro-centrifuge tubes to which dH₂O was dispensed with a micropipette to obtain 1-MCP (aq) concentrations; 0.25, 0.5 and 0.75 ppm. Control had only dH₂O without the addition of Ethyl Bloc™ powder in micro-centrifuge tubes. The micro-centrifuge tubes were placed inside the polythene chambers and the chambers were sealed air tight. One group of chambers remained sealed for 6 hrs while the second group for 24 hrs. After treatments, qualitative changes were observed and phytochemical analyses were done for the inflorescences at one-day interval for 4 days.

Vase life

Days taken for the shedding of first petal were recorded for each inflorescence.

Percentage Fresh Weight Loss

Fresh weights of the inflorescences were measured daily for the duration of the vase life and percentage fresh weight loss (FWL) was calculated.

Total Anthocyanin Concentration

Total anthocyanin concentration was measured using pH differential method (Giusti and Wrolstad, 2001). Petals were ground, and dissolved in 1% HCl in methanol to obtain slurry. Aliquots were centrifuged at 6000 rpm for 5 min and supernatant obtained. The supernatants were subdivided and resuspended in separate buffer solutions; KCl (pH 1.0) and C₂H₃NaO₂ (pH 4.5). Absorbance at 528 and 700 nm were read using a spectrophotometer (UV-120-00, Shimadzu Co., Japan). The monomeric anthocyanin pigment concentration was calculated (Equations 1 and 2).

Equation 1: Absorbance of the sample = $(A_{528} - A_{700}) \text{pH}_{1.0} - (A_{528} - A_{700}) \text{pH}_{4.5}$

A_{528} = Absorbance of the sample at 528 nm

A_{700} = Absorbance of the sample at 700 nm

Equation 2: Monomeric anthocyanin pigment (mg L⁻¹) = $(A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon)$

A = Absorbance of the sample

MW = Molecular weight (449.2)

DF = Dilution factor (final volume of the sample/initial volume)

ϵ = Molar absorption = 26,900

Total Glucose Concentration

Total soluble sugar concentration was measured using Phenol Sulphuric Acid Method (Roberts and Elias, 2011). Petals were ground, and dissolved in dH₂O to obtain 1%

(w/v) slurry. Aliquots were centrifuged at 6000 rpm for 5 min and supernatant were obtained. Glucose standard series ($0 - 1000 \mu\text{g mL}^{-1}$) were made dissolving appropriate amounts of glucose in dH_2O . To a test tube 400 μL petal supernatant or glucose standard was added, and to which 5% phenol 0.4 mL and 98% sulphuric acid 2 mL were added. The contents in test tubes were vortexed and kept for 10 min to settle. The test tubes were placed in a water bath at 27 °C for 20 min. Absorbance at 490 nm was read using a spectrophotometer (UV-120-00, Shimadzu Co., Japan). Glucose concentrations in samples were calculated using regression equations between glucose concentration and A_{490} for the glucose standard series.

Total Chlorophyll Concentration

Petals (0.5 g) were ground, and dissolved in 80% acetone (aq) to obtain slurry. The slurry was vacuum filtered and filtrate obtained. The extraction and filtration was repeated once again for the material. The filtrate was volumerised to 25 mL with acetone. Absorbance at 645 and 663 nm was read using a spectrophotometer (UV-120-00, Shimadzu Co., Japan). The concentration of chlorophyll was expressed as mg chlorophyll leaf g^{-1} (Equation 3).

$$\text{Equation 3: mg chlorophyll/g tissue} = [20.2(D_{645}) + 8.02(D_{663})] \times V/1000 \times W$$

D = the optical density reading of the chlorophyll extract at the indicated wavelength.

V = final volume

W = fresh weight in grams of the tissue extracted

Experiment 2: Effect of variable concentrations of ascorbic acid and different exposure durations on postharvest longevity and quality of *Alstroemeria* spp.

Treatments

A 200 mL of 57 and 85 mM ascorbic acid were freshly prepared in dH_2O in which the stem ends of inflorescences were placed. Control had dH_2O . The ascorbic acid solution was pulsed for 6 hrs for one group and 24 hrs for the other

group. The vase life, percentage of fresh weight loss and concentrations of anthocyanin, chlorophyll and glucose were measured as described above.

Experiment 3: The effect of 1-MCP and ascorbic acid, alone and in combination on postharvest longevity and quality of *Alstroemeria* spp.

Treatments

Solutions of 1-MCP 0.25 ppm and ascorbic acid 57 mM were prepared and imposed for 6 hrs. The vase life, percentage of fresh weight loss and concentrations of anthocyanin, chlorophyll and glucose were measured as described above.

Statistical Analysis

Analysis of variance (ANOVA), repeated measures ANOVA and Bonferroni post hoc test were performed for the concentrations of chlorophyll, anthocyanin, glucose and arcsine transformed data of vase life and percentage fresh weight loss. Significances were defined at $P < 0.05$. All analyses were performed using SPSS 18.0 statistical software.

Results

Experiment 1: Effect of variable concentrations of 1-MCP and different exposure durations on postharvest quality of *Alstroemeria* spp.

Vase life

Inflorescences of *Alstroemeria* spp. were exposed to three concentrations of 1-MCP (0.25, 0.5 and 0.75 ppm) for six and 24 hours. The longest vase life of eight days was obtained by treating with 0.25 ppm 1-MCP for six hours, which was 2.5-times longer vase life than the respective control ($p < 0.001$; Fig. 1). Vase life was not different whether flowers were exposed to six or 24 hours to 1-MCP ($p > 0.05$; Fig. 1).

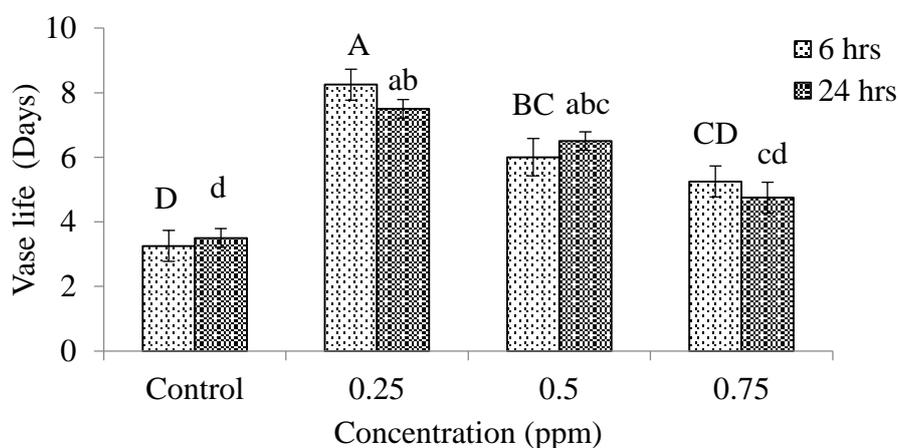


Fig. 1: Effect of 1-methylcyclopropene concentrations; 0, 0.25 and 0.75 ppm for 6 and 24 hrs on the vase life of cut *Alstroemeria* spp. inflorescences. Mean \pm SE ($n=3$) Bars with different letters of same case indicate significant differences between treatments ($p < 0.05$).

Concentration of Anthocyanin

Highest postharvest concentration of anthocyanin in flowers from third day onwards, resulted from applying 0.25 ppm 1-MCP for six hours compared to all other 1-MCP concentrations (Fig. 2A). The concentration of anthocyanin was 168% higher than the control on seventh day ($p < 0.001$; Fig. 2A). Exposure of flowers to 1-MCP for six hours maintained higher levels of anthocyanins compared to 24 hour exposure on seventh day ($p < 0.001$; Fig. 2A).

Concentration of Chlorophyll

Throughout the vase life, exposure of flowers to 0.25 ppm 1-MCP for six hours best maintained the concentration of chlorophyll in leaves; on seventh day 367% higher than that in control ($p < 0.001$; Fig. 2B). At all times for all 1-MCP concentrations, six-hour exposure maintained the higher concentrations of chlorophyll than the 24 hour exposure ($p < 0.001$; Fig. 2B).

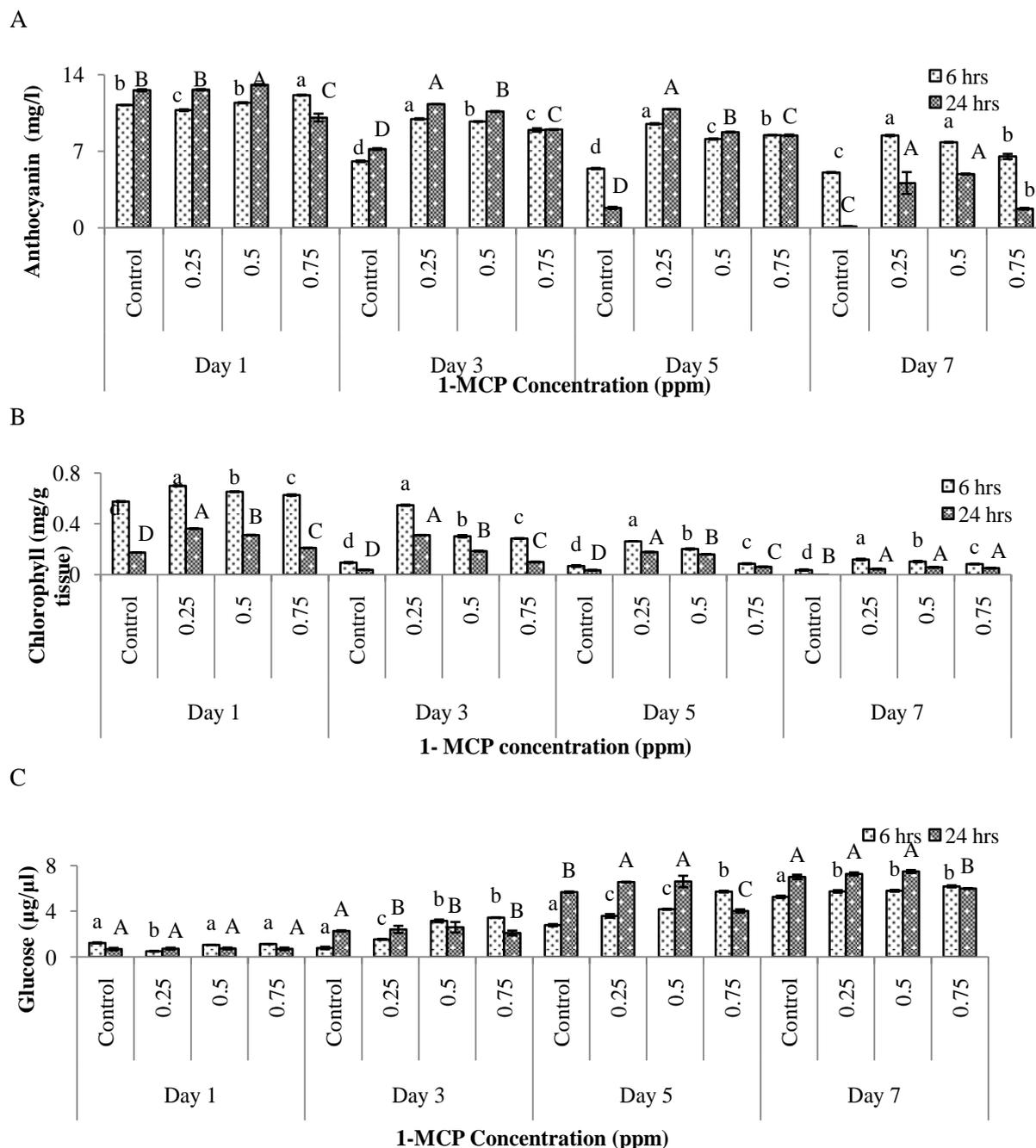


Fig. 2: Effect of 1-methylcyclopropene concentrations; 0, 0.25 and 0.75 ppm applied for 6 and 24 hrs on the concentrations of (A) anthocyanin, (B) chlorophyll and (C) glucose of cut *Alstroemeria* spp. inflorescences measured one, three, five and seven days after treatment. Mean \pm SE ($n=3$). Bars with different letters of same case indicate significant differences between treatments within the given day ($p < 0.05$).

Concentration of Glucose

Higher glucose concentrations were measured in flowers exposed to all concentrations of 1-MCP for six hours compared to the control on third and fifth day ($p < 0.001$; Fig. 2C). However, on the seventh day none of the treatments were significantly different ($p > 0.05$). Levels of glucose were best maintained by treating for 24 hours than for six hours ($p < 0.001$; Fig. 2C).

Percentage Fresh Weight Loss

Lowest fresh weight loss was in inflorescences exposed to 0.25 ppm 1-MCP for six hours compared to the control on third day ($p < 0.05$; Fig. 3). Of the exposure durations, 24 hours caused higher fresh weight loss than exposing flowers to treatments for six hours ($p > 0.05$).

Experiment 2: Effect of variable concentrations of ascorbic acid and different exposure durations on postharvest longevity and quality of *Alstroemeria* spp.

Vase Life

Inflorescences of *Alstroemeria* spp. were exposed to two concentrations of ascorbic acid (0, 57 and 85 mM) for six and 24 hours. The longest vase life of eight days was obtained by treating with 57 mM for six hours, which was 2.6-times longer vase life than the respective control ($p < 0.001$; Fig. 4). Vase life was not different whether flowers were treated for six or 24 hours to ascorbic acid ($p > 0.05$; Fig. 4).

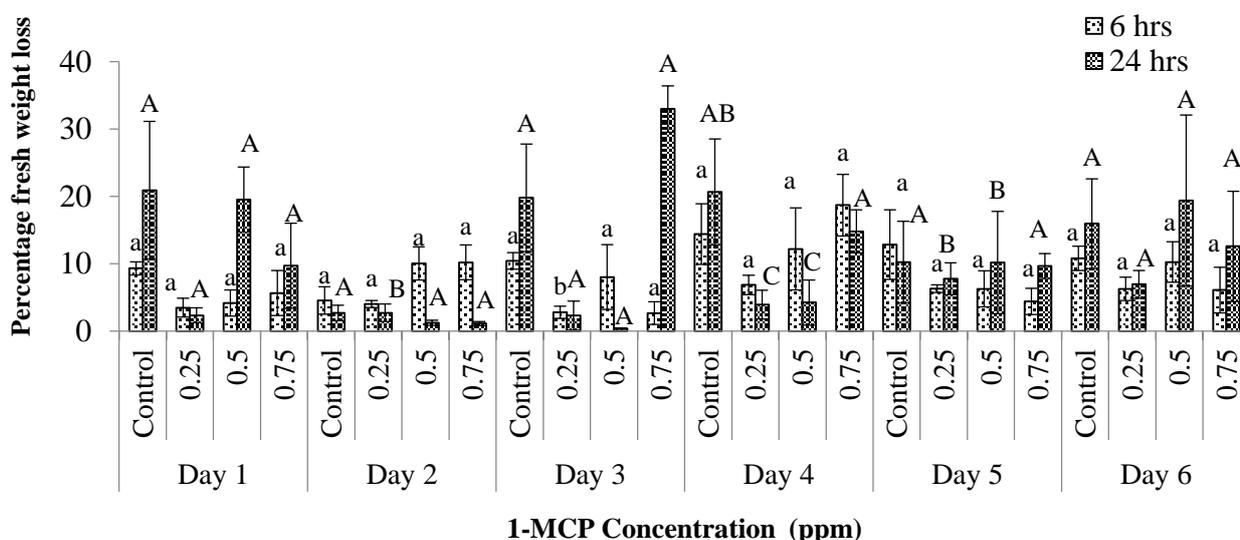


Fig. 3: Effect of 1-methylcyclopropene concentrations; 0, 0.25, 0.5 and 0.75 ppm applied for 6 and 24 hrs on the percentage fresh weight loss was measured for six days continuously after treatment of cut *Alstroemeria* spp. inflorescences. Mean \pm SE ($n=3$). Bars with different letters of same case indicate significant differences between treatments within the given day ($p < 0.05$).

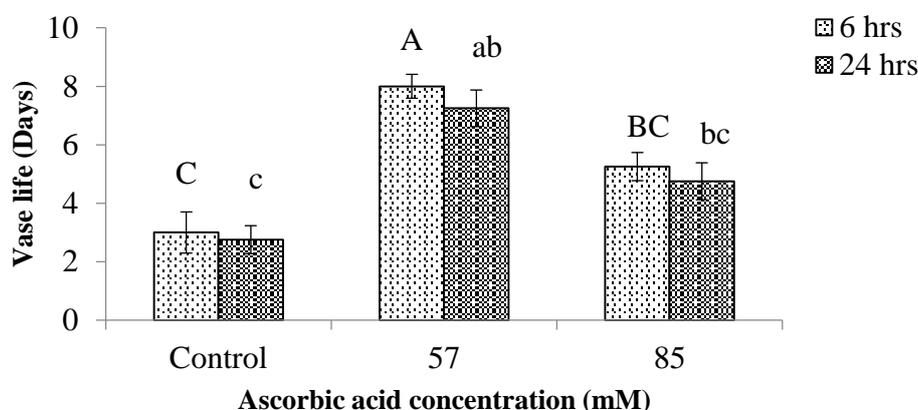


Fig. 4: Effect of ascorbic acid concentrations; 0, 57 and 85mM for six and 24 hrs on the vase life of cut *Alstroemeria* spp. inflorescences. Mean \pm SE ($n=3$) Bars with different letters of same case indicate significant differences between treatments ($p < 0.05$).

Concentration of Anthocyanin

The postharvest concentrations of anthocyanin in petals were best maintained by treating with ascorbic acid 57 mM for 24 hrs compared to all other treatments after third day ($p < 0.001$; Fig. 5A). Anthocyanin concentration was seven-fold higher in inflorescences treated with 57 mM ascorbic acid than the control on seventh day for both treatment durations ($p < 0.001$; Fig. 5A). After fifth day, in inflorescences treated with ascorbic acid for 24 hours, best maintained the concentration of anthocyanin than the six hour treatment ($p < 0.001$; Fig. 5A).

Concentration of Chlorophyll

Highest concentration of chlorophyll was observed when 57 mM ascorbic acid was pulsed for six hours, compared to all other treatments: Compared to control they were 2000% higher ($p < 0.05$; Fig. 5B).

Concentration of Glucose

Treating inflorescences with 85 mM ascorbic acid for 24 hours best maintained the concentrations of glucose compared to treatment for six hours during latter days of the vase life ($p < 0.05$; Fig. 5C).

Percentage Fresh Weight Loss

On fifth day lowest fresh weight loss was reported for inflorescences treated with 57 mM ascorbic acid for six hours compared to the control ($p < 0.05$; Fig. 6). Percentage fresh weight loss was higher for inflorescences treated with 85 mM ascorbic acid for six hours compared to the control on third day ($p < 0.05$; Fig. 6). On seventh day inflorescences treated with both ascorbic acid concentrations, had higher fresh weight loss than the control for both treatment durations ($p < 0.05$; Fig. 6).

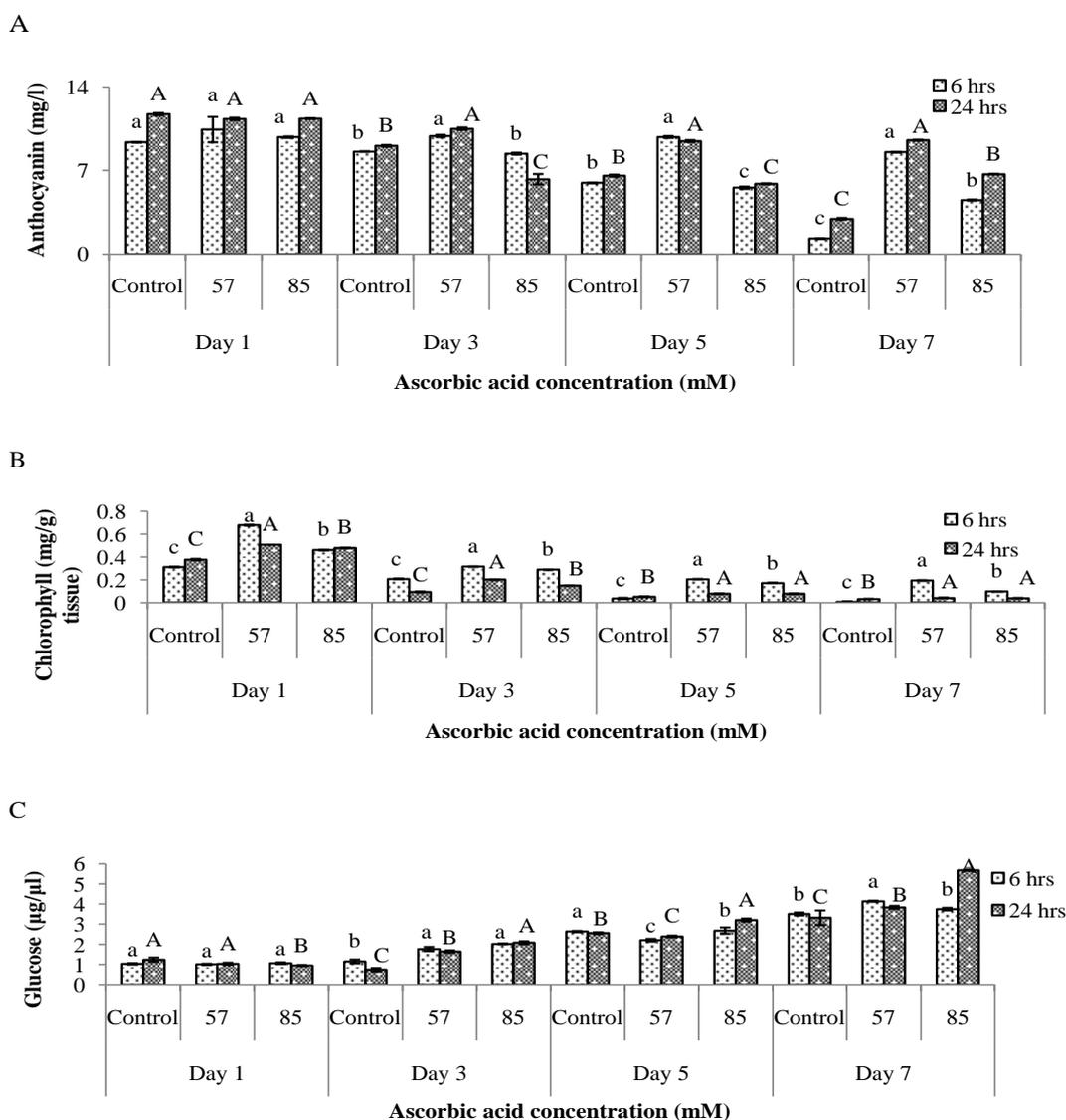


Fig. 5. Effect of ascorbic acid concentrations; 0, 57 and 85 mM pulsed for 6 and 24 hrs on the concentrations of (A) anthocyanin, (B) chlorophyll and (C) glucose of cut *Alstroemeria* spp. inflorescences measured one, three, five and seven days after treatment. Mean \pm SE ($n=3$). Bars with different letters of same case indicate significant differences across treatments within the given day ($p < 0.05$).

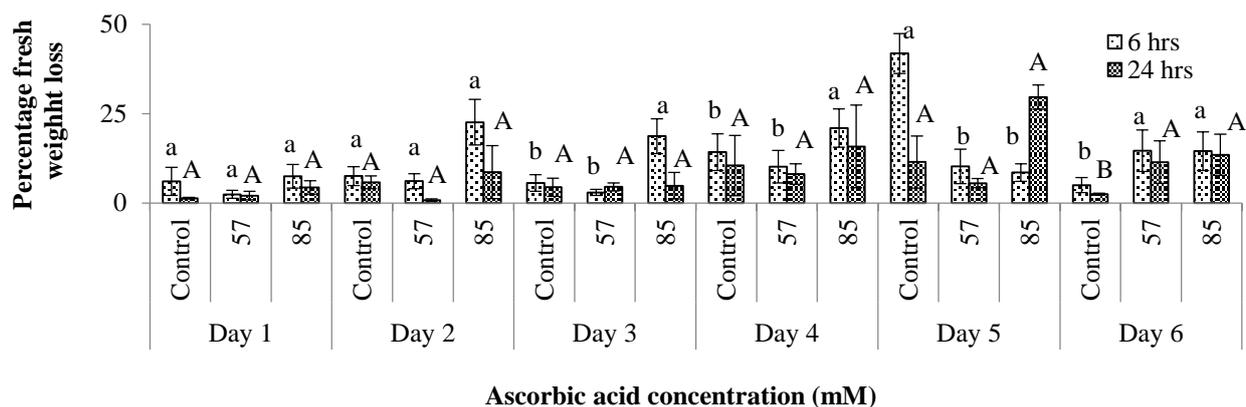


Fig. 6. Effect of ascorbic acid concentrations; 0, 57 and 85 mM applied for 6 and 24 hrs on the percentage fresh weight loss was measured for six days, continuously after treatment of cut *Alstroemeria* spp. Inflorescences. Mean \pm SE ($n=3$). Bars with different letters of same case indicate significant differences across treatments within the given day ($p<0.05$).

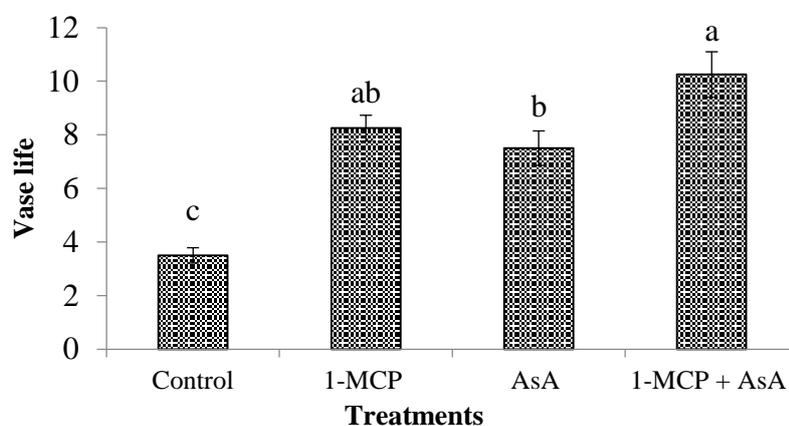


Fig. 7: Effect of treatments; control, 1-MCP (0.25 ppm), ascorbic acid (AsA, 57 mM) and 1-MCP + ascorbic acid for 6 hrs on the vase life of cut *Alstroemeria* spp. inflorescences. Mean \pm SE ($n=3$) Bars with different letters of same case indicate significant differences across treatments ($p<0.05$).

Experiment 3: The effect of 1-MCP and ascorbic acid, alone and in combination on postharvest longevity and quality of *Alstroemeria* spp.

Vase Life

Longest vase life of 10 days, which was two to three-fold longer compared to all other treatments, was obtained when inflorescences were treated with both 0.25 ppm 1-MCP and 57 mM ascorbic acid ($p>0.05$; Fig. 7).

Concentration of Anthocyanin

Inflorescences treated with both 1-MCP and ascorbic acid maintained highest concentrations of anthocyanins compared to all other treatments on third and fifth day ($p<0.05$; Fig. 8). On seventh day, 1-MCP alone and its combined application with ascorbic acid best maintained

the anthocyanin concentration in inflorescences which were 200% and 145% higher than the control ($p<0.05$; Fig. 8A)

Concentration of Chlorophyll

Best concentrations of chlorophyll were maintained when 1-MCP and ascorbic acid were applied together on inflorescences, which was 300 – 400% higher than the control ($p<0.05$; Fig. 8B).

Glucose Concentration

Highest glucose concentrations throughout the vase life were observed in inflorescences treated with 1-MCP alone, 93% higher than the control ($p<0.05$; Fig. 8C).

Percentage Fresh Weight Loss

Percentage fresh weight loss were not significantly different among treatment ($p=0.119$; Fig. 9)

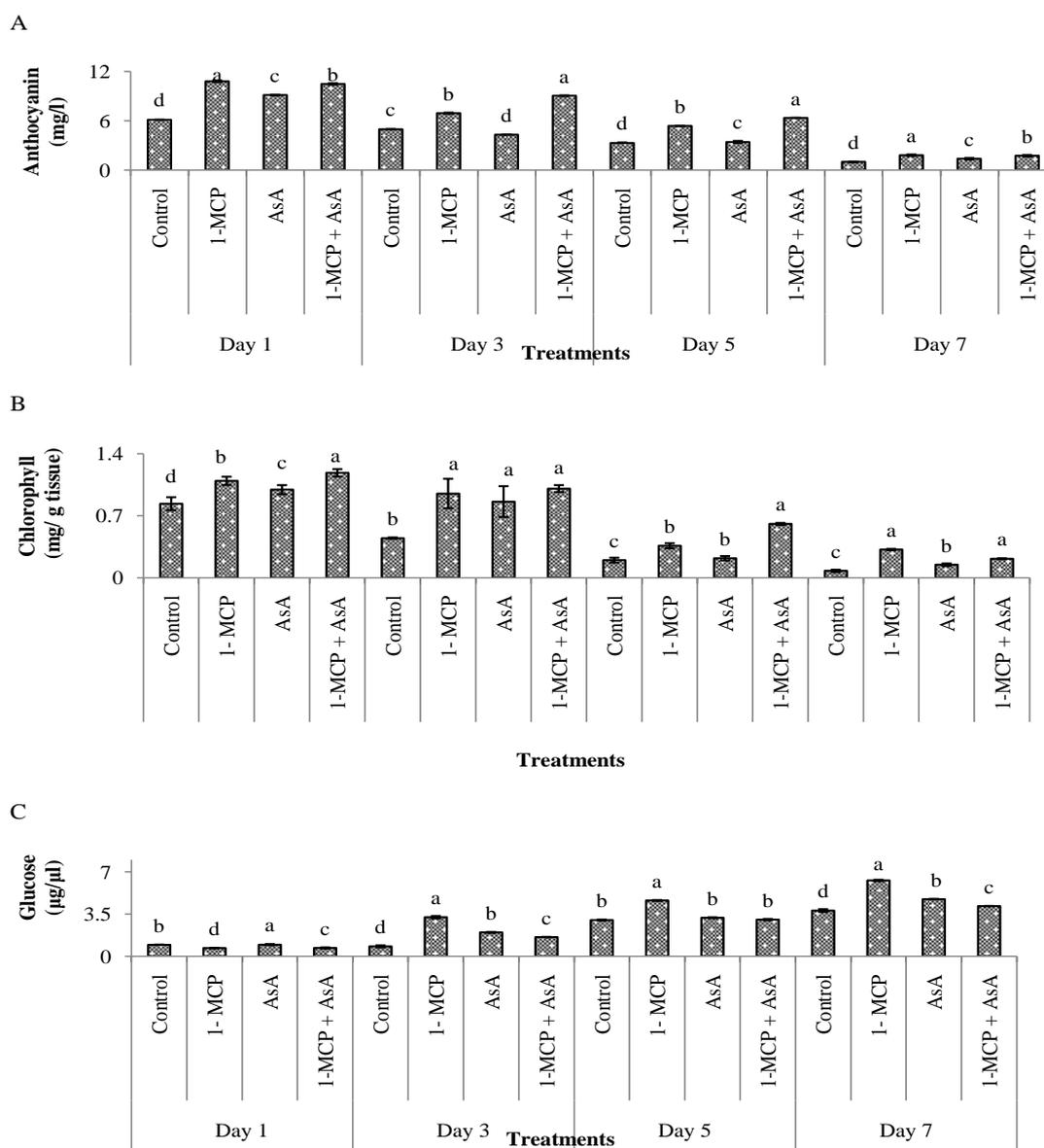


Fig. 8: Effect of treatments; control, 1-MCP (0.25ppm), ascorbic acid (AsA 57mM) and 1-MCP + ascorbic acid for 6 hrs on concentrations of (A) anthocyanin, (B) chlorophyll and (C) glucose cut *Alstroemeria* spp. inflorescences measured one, three, five and seven days after treatment. Mean \pm SE ($n=3$) Bars with different letters of same case indicate significant differences across treatments within the given day ($p<0.05$).

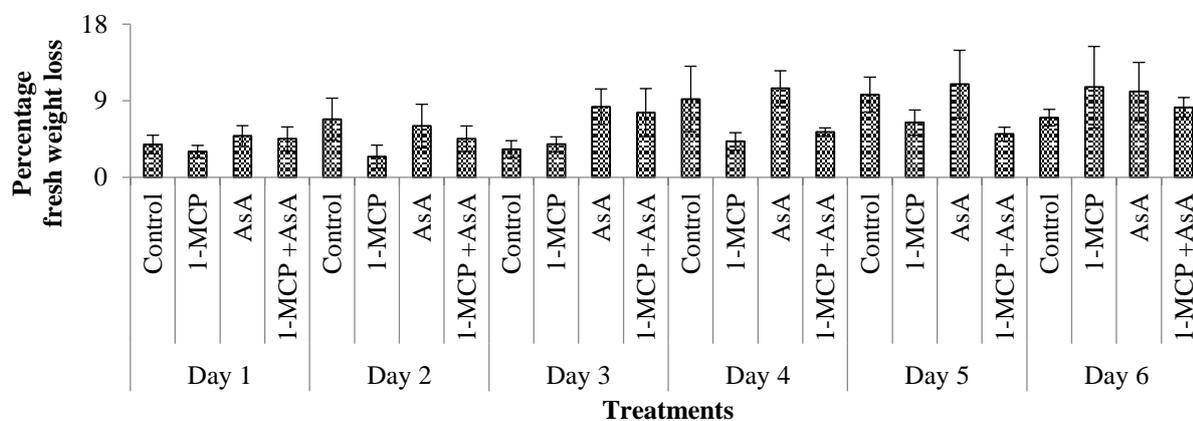


Fig. 9: Effect of treatments; control, 1-MCP (0.25ppm), ascorbic acid (AsA 57mM) and 1-MCP + ascorbic acid for 6 hrs on the percentage fresh weight loss was measured for six days continuously after treatment of cut *Alstroemeria* spp. Inflorescences. Mean \pm SE ($n=3$). Bars with different letters of same case indicate significant differences across treatments within the given day ($p<0.05$).

Discussion

Inflorescences of *Alstroemeria* spp. were exposed to three concentrations of 1-MCP (0.25, 0.5, 0.75 ppm) and two concentrations of ascorbic acid (0, 57 mM, 85 mM) for six and 24 hours through which optimum concentrations, their combined effect and exposure duration were studied on postharvest longevity and quality of *Alstroemeria*.

For postharvest treatment of *Alstroemeria*, combined application of 1-MCP (0.25 ppm) and ascorbic acid (57 mM) for six hrs was the most effective treatment for extending vase life and delaying senescence (Fig. 7-9). Ethylene induced senescence is slowed by 1-MCP since its ability to bind permanently to ethylene receptors present at the time of treatment and by acting as an inhibitor of ethylene biosynthesis (Serek and Sisler, 2001; Serek et al., 1994). Ascorbic acid is an antioxidant that donates electrons to neutralize reactive oxygen species protecting plant cells from oxidative damage, which enhances the flowers ability to withstand numerous postharvest stressors, including water deficit stress and senescence (Jin et al., 2006; Cheruth, 2009; Conklin, 2001). Further, ascorbic acid can control ethylene synthesis, reduce respiration and transpiration rates, pathogen development (Stood et al., 2006). Similarly, vase life significantly extended due to 1-MCP treatment in *Alstroemeria* spp. (Serek et al., 1995) and numerous other cut flower species, including, carnation (Serek et al., 1995), Siam tulip (Chuticudet et al., 2011), lotus (Suanphairoch et al., 2006), white Christmas rose (Chanmanee et al., 2010) and cut foliage species such as *Calathea* (Weerasekara et al, 2013). Vase life of red ginger flowers treated with ascorbic acid too lasted significantly longer than that of untreated flowers (Ieamtim et al., 2008).

The maintenance of green colour in the leaves is an important quality parameter in *Alstroemeria* spp. where combined application of 1-MCP and ascorbic acid best maintained the concentrations of chlorophyll (Fig. 8B). Blocking of ethylene receptors by 1-MCP can inhibit the ethylene induced chlorophyll degradation in leaves of *Alstroemeria* spp. because exogenous ethylene induced rapid degradation of chlorophyll in *Alstroemeria* foliage (Ferrante et al., 2006; Ferrante et al., 2002). The 1-MCP treatments of carnation, pelargonium and *Hibiscus rosa-sinensis* and chrysanthemum prevented leaves from turning yellow while maintaining higher chlorophyll level compared to untreated flowers (Abri et al., 2013; Serek et al., 1998)

Combined application of 1-MCP and ascorbic acid best maintains the postharvest anthocyanin concentration by inhibiting the anthocyanin degradation (Fig. 8A). In *Alstroemeria* spp. colour loss is associated with decrease in anthocyanin concentration (Fig. 8A). Pretreatment with 1-MCP and ascorbic acid preserved colour of flowers (Kongsuwan et al., 2012; Sood et al., 2006). During flower

development and senescence the concentration of total anthocyanins in petals declined in chrysanthemums (Stickland, 1972), *Rosa* × *hybrida* (Ahuja et al., 1963), *Petunia* × *hybrida* (Ferrante et al., 2006), and *Hydrangea macrophylla* (Yoshida et al., 2008). Anthocyanins, too are also powerful antioxidants. Therefore their degradation during senescence makes the flower more vulnerable to oxidative stress (Takahama and Oniki, 1997). This can be the reason, the combination treatment maintain high anthocyanin concentration throughout the period compared to the inflorescences treated with 1-MCP and ascorbic acid alone.

In all 1-MCP and ascorbic acid treated flowers were highly concentrated in glucose (Fig. 8C) which may be due to, 1-MCP and ascorbic acid reduced respiration rates or delayed increases in respiration (Blankenship and Dole, 2002; Sood et al., 2006). This could be the reason, the treatments maintained high glucose concentration.

There was no treatment effect on percentage fresh weight loss may be since both treatments had minimal effects on stomatal function. Similarly, *Curcuma alismatifolia* weight was not affected by application of 1-MCP (Chutichudet et al., 2010)

Conclusion

Best postharvest treatment for cut *Alstroemeria* spp. is the exposure of inflorescences to a combination of 1-MCP 0.25 ppm and ascorbic acid 57 mM for 6 hours which extended vase life up to ten days. The treatment also reduced loss of colour and water from the flowers, while maintaining high anthocyanin and chlorophyll concentrations.

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