

Effect of salicylic and ascorbic acids on post-harvest vase life of Chrysanthemum cut flowers

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Abstract: Flower vase life is one important aspect in determining the flower quality in the cut flower chrysanthemum. The use of ascorbic or salicylic acid as preservative solutions was expected to have an impact on the prolonged duration of the cut flower freshness. The research was designed in two parallel experiments. The first dealt with salicylic acid, while the second focused on ascorbic acid. Both experiments were arranged in a factorial completely blocked randomised design. Two chrysanthemum varieties, i.e., 'Reagent Sunny' and 'Yellow Fiji' were the first factor and concentrations of the salicylic or ascorbic acids, i.e., 0, 100, 200 and 300 ppm became the second factor. The results showed the termination of the flower freshness was recognised by the wilted leaves and petals, and petal colour changes. In all the treatments, the colour change of the wilted petals of each variety fell in the same colour, indicating the direction of the colour changes was not affected by the ascorbic/salicylic acids. Ascorbic acid at 200 ppm induced longer flower longevity than the control with delayed chlorophyll degradation in both tested cultivars. In the salicylic acid applications, a 100-ppm solution induced longer flower freshness only in the 'Reagent Sunny'. In higher concentrations, salicylic acid solutions induced shorter flower freshness with faster chlorophyll degradation and basal tissue damage in the 'Yellow Fiji'.

Keywords: flower freshness; preservatives; wilting; variety; chlorophyll content

Chrysanthemums (*Dendranthema grandiflora* Tzvelev) are one of the famous ornamentals cultivated all over the world. As a cut flower, the capability of the harvested stem to freshly withstand under room temperature for a long period is an important factor in determining the value of the crop (Bayat, Aminifard 2017). Many reports regarding the vase life and quality of cut flowers have dealt with the application of various preservatives to the vase water resulting in the postponement of the cut flower senescence (Clark et al. 2010; Dole et al. 2013). The additional preservatives usually contain a germicide, growth regulators, ethylene/ABA synthesis inhibitors, carbohydrates and some minerals compounds that were essential to prolong the vase life of cut flowers (Banjaw 2017).

The vase life termination, for many cut flowers, is attributed to the wilting of the leaves and petals (Azizi et al. 2015). Water blockage in the stem end is the main factor of the imbalance between the water loss and water uptake by cut flowers (Ahmad et al. 2014; In et al. 2016) but also by growth conditions. For instance, the vase life of cut roses that are grown hydroponically during the winter period often ends at an early stage of maturation due to petal wilting or neck bending. In addition, the vase life of cut roses from different growers varies markedly despite identical postharvest conditions. To elucidate the underlying mechanisms, the relationship between preharvest environmental factors, post-harvest morphological and physiological factors, and the vase life of cut roses were determined. Cut

roses (*Rosa hybrida* L. cv. 'Fuego'). Thus, the water balance between the transpiration and water uptake is an essential factor in determining the longevity of cut flowers (Dung et al. 2016). Water blockage can be caused by several factors, such as air-trapped inside the pit cell membranes resulting in the prevention of water flow (an air emboli) (van Ieperen et al. 2002), physical wounds that cause tissue decay (Wang et al. 2014) and vascular occlusions by microbial activities (Kazemi et al. 2012; Nemati et al. 2013). Among them, microbial activities are the most predominant factor of tissue decay resulting in stem end blockage (Jowkar et al. 2012, Hashemabadi 2014). Several authors have also reported that the microbial activity might produce ethylene and toxic compounds that accelerate senescence (Basiri et al. 2011; Bhaskar et al. 2017). The blockage of the water uptake then results in wilting (Sudaria et al. 2017).

Several compounds, i.e., ascorbic acids (vitamin C), have been observed to have effects on increasing the water uptake in cut flowers and prolonging the vase life of cut flowers, like lisianthus (Azizi et al. 2015), snapdragons (Abdulrahman et al. 2012), gerberas (Mehdikhah et al. 2016), and gladioli (Ravanbakhsh et al. 2016). Ascorbic acid has been reported to play an important role in the photosynthesis and with other plant growth and development processes, especially in the electron transport system (Gallie 2013). Vitamin C has been related to several biological compounds, including enzyme cofactor, antioxidant and being an electron transporter in the plasma membrane and chloroplast (Szarka et al. 2013). Several studies also indicated that high endogenous ascorbic acids also increased the antioxidant system and protected the plant against oxidative damage (Smirnoff 2000; Conklin 2001).

Aside from ascorbic acid, salicylic acid (SA) has also been studied for extending the cut flower vase life, like gladioli (Ravanbakhsh et al. 2016), alstroemerias, gerberas, lilliums, roses, polianthes (Bayat, Aminifard 2017), carnations (Kazemi et al. 2012) and coral vines (Hariri et al. 2019). The substance has been included in the regulation of plant growth and development especially in response to environmental stresses (Fragniere et al. 2011). Furthermore, its role in the ion uptake and transport (Shen et al. 2016) photosynthetic rate, stomatal conductance and transpiration have also been confirmed (Rivas-San Vicente et al. 2011). SA is also involved in promoting disease resistance by modulating the plant response to local and endemic disease

resistance to various pathogenic attacks and a wide range of oxidative stresses (Wani et al. 2017). Used as a preservation solution on cut flowers, SA reduced the rate of respiration and ethylene evolution, postponed the occurrence of the respiration and ethylene peaks, and inhibited the chlorophyll degradation in leaves, thereby inducing a prolonged vase life and the longevity of individual flowers (Roodbaraky et al. 2012). The use of ascorbic acid to prolong the flower freshness in the cut flower chrysanthemum has been reported by Budiarso (2019), however, the environmental conditions during the study were not clearly stated and the use of SA under one set of specific environmental conditions also has not yet been described.

The research was then conducted to evaluate ascorbic acid and salicylic acid on the vase life duration of the chrysanthemum cut flower.

MATERIAL AND METHODS

The research was conducted at the Indonesian Ornamental Crops Research Institute (IOCRI) from December 2017 to May 2018. Two independent experiments were designed. The first dealt with salicylic acid, while the second focused on the use of ascorbic acid. Both experiments were arranged in a two factor-factorial completely randomised design with 10 replications. The first factor was the chrysanthemum varieties, i.e., 'Yellow Fiji' (standard) and 'Reagent Sunny' (spray), while the second factor was the salicylic or ascorbic acid concentration, i.e., 0, 100, 200 and 300 mg/L.

Solutions containing different concentrations (100, 200 and 300 mg/L) of ascorbic acid (L-ascorbic acid, PubChem – 54670067) and salicylic acid (salicylic acid, PubChem – 24899681) were freshly prepared before the treatment application. The flowers were harvested in the morning and directly put into fresh water. The stem was cut with 40 cm of the stalk from the terminal flowers being left. The basal stalks were then immersed into flasks containing 300 mL/flask of the ascorbic or salicylic acid solutions according to the experimental set-up. The flasks were arranged at certain distance to provide space for the flowers so that they were not in physical contact with each other. The experimental set-up was conditioned at an ambient temperature room ($18 \pm 2^\circ\text{C}$ during the night and $23 \pm 2^\circ\text{C}$ during the day time) with proper aeration and protection from direct sunlight. During the whole night, artificial lighting was provided using

22-watt LED lamps (100 lux) that were placed three m above the treated flower stalks.

The parameters observed included the characteristics of the wilted leaves/petals and the colour changes of the floret when wilting, the duration of the leaf and petal freshness and the chlorophyll content of the leaf. The petal colour observation was conducted using the Royal Horticultural Society (RHS) colour chart. The chlorophyll content observation was carried out using a SPAD-502 (Konica-Minolta) chlorophyll meter every two days since the first day of the treatment application until the petals and leaves were defined to be in the wilting stage. The values gained from the chlorophyll meter were then converted into the predicted values of the chlorophyll content using the equation of Davies et al. (2004): $y = 0.001x^3 + 0.0104x^2 - 1.730x + 11.702$ ($r = 0.98$), where y = the predicted chlorophyll content ($\mu\text{g}/\text{cm}^2$) and x = the SPAD reading value. The gathered data were analysed using an analysis of variance (ANOVA) and further analysed by the Least Significant Difference (LSD, $\alpha = 5\%$).

RESULTS AND DISCUSSION

Characteristics of the wilted leaves/petals and floret colour changes

The termination of the flower freshness was visually characterised by the irreversible wilting of the leaves and petals. The fresh leaves and petals had optimum turgidity, rigid tissues along the leaf or petal axis and seated in a certain degree upright from the stem axis (Figure 1A, 1C, 1E). The decrease in the flower freshness started with the wilting of the leaves (Figure 1B) and continued by the petals (Figure 1D, 1F).

In both tested chrysanthemum cultivars, the diminishing flower freshness was also characterised by changes in the colours of the florets. In general, the florets showed a paler colour when wilting compared to the initial point (Table 1). The wilted florets in each tested variety showed the same colour indicating that the ascorbic and salicylic acid application in any given concentration did not direct changes into a certain colour type. These findings reconfirmed



Figure 1. (A) Initial leaf freshness, (B) wilted leaves, (C) initial and (D) wilted flowers of the cv. 'Reagent Sunny', (E) initial and (F) wilted flower petals of the cv. 'Yellow Fiji'

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Table 1. Initial and wilted petal colour of the chrysanthemum cvs Yellow Fiji and Reagent Sunny during the vase life

Variety	Petal colour (RHS colour chart)	
	initial	when wilting
Yellow Fiji	yellow 12B	yellow 6B
Reagent Sunny	red 39D	red Purple 62D

the study of Budiarto (2019) using the same standard type cultivar. Changes in the floret colour after wilting were reported in several cut flowers like orchids (Khan et al. 2015), gerberas (Heidarnezhadian et al. 2017), roses (Zamani et al. 2011) and so on. According to Ichimura (1998), the colour change in a wilted floret is related to the decrease in carbohydrate content in the flower stalks. Carbohydrates are one fundamental component in pigments and other secondary metabolite biosynthesis compound products, including anthocyanin (Asrar 2012). During the vase life, the carbohydrate content decreased to facilitate the respiratory activity of the cells. On the other hand, the supply of assimilates was also limited, since the photosynthetic activity also diminished (Khan et al. 2015). During this situation, the pigment biosynthesis was then also considered lower and resulted in the alteration of the floret colour (Zamani et al. 2011; Heidarnezhadian et al. 2017). Ascorbic acid and salicylic acid play an important role in slowing down the anthocyanin degradation (Heidarnezhadian et al. 2017; Obadamudalige et al. 2019).

Duration of leaves and petal freshness

Ascorbic acid application. The duration of the leaf and petal freshness was defined as the number of days it takes for the leaves and/or petals to wilt during the vase life from the initial state. The analysis of variances conducted on the effects of ascorbic acid on the duration of leaf and petal freshness indicated that no interaction was detected between the ascorbic acid concentrations and the chrysanthemum varieties. The leaf and petal freshness duration between the tested varieties were also not significantly different (Table 2). These conditions inferred that both cultivars showed similar responses to the given treatments, though the genetic constructions were different. Similar post-harvest responses on the flower freshness during the vase life among chrysanthemum cultivars were also reported by Baskaran et al. (2010) and Sharma and Srivastava (2014) with or without the chemical preservation solutions.

These findings also further clarified the study of Budiarto (2019) that the environmental condition (daily ambient temperature), including the artificial lighting for a long day, during the study had little effect on the duration of the leaf and petal freshness. Though not clearly stated, the environmental conditions during the study of Budiarto (2019) and the current study were somewhat similar.

Table 2 also shows that the duration of the leaf and petal freshness was affected by the ascorbic acid application. An average of two-day-longer leaf freshness was detected at a concentration of 200 mg/L ascorbic acid compared to the control. At a higher concentration (300 mg/L), the effects started to decline. A similar phenomenon was observed in the petal freshness in that a longer petal freshness duration was achieved with the 100 mg/L ascorbic acid solution. While in higher concentrations, the increases in the petal freshness were negligible.

Table 2. Duration of the leaf and petal freshness during the vase life of the tested chrysanthemum varieties under the different ascorbic acid solution concentrations

Treatments	Duration of freshness (days)	
	leaf	petal
Variety		
Reagent Sunny	11.27 ^a	13.56 ^a
Yellow Fiji	11.33 ^a	13.71 ^a
Ascorbic acid concentration (ppm)		
0 (control)	10.11 ^a	11.47 ^a
100	10.45 ^{ab}	13.79 ^b
300	12.25 ^b	14.81 ^b
Treatment combinations		
RS at 0 ppm ascorbic acid (control)	10 ^a	11.63 ^a
RS at 100 ppm ascorbic acid	10.43 ^a	13.71 ^{ab}
RS at 200 ppm ascorbic acid	12.56 ^b	14.77 ^b
RS at 300 ppm ascorbic acid	11.71 ^{ab}	15.06 ^b
YF at 0 ppm ascorbic acid (control)	10.12 ^a	11.22 ^a
YF at 100 ppm ascorbic acid	10.51 ^a	14.34 ^b
YF at 200 ppm ascorbic acid	12.73 ^b	14.47 ^b
YF at 300 ppm ascorbic acid	12.44 ^b	14.61 ^b

^{a–b}Values in the same column under each criterion of variety, ascorbic acid concentration and treatment combination followed by different letters differ significantly under the Least Significant Different Test (LSD, $\alpha \leq 5\%$).

RS – Regreat sunny; YF – Yellow Fiji

After the stem was cut, the photosynthetic rates diminished and the flower stalks made use of the carbohydrate storage within the organ tissues to maintain the life of the cells (Hossain et al. 2008). During vase life conditions, ascorbic acid was needed more to maintain the cell turgor and promote the longer leaf and petal fresh longevity (Kobayakawa, Imai 2012) as shown in both cultivars (Table 2). According to several reports, ascorbic acid induced a longer flower vase life through several mechanisms. The ascorbic acids play an important role in diminishing the respiration rates and ethylene biosynthesis (Abri et al. 2013). Ascorbic acid especially plays critical roles in the control of reactive oxygen species (ROS) homeostatic pathways as a triggering signal for the ethylene biosynthesis (Obadamudalige et al. 2019). The low respiration rates indicated the capability of the flower stalk to maintain the water content by minimising the water loss due to active biochemical reactions (Abdulrahman et al. 2012). The antimicrobial features of ascorbic acid also maintained the tissue-water conductivity by delaying the stem tissue decay, thus increasing the water supply to upper parts (Bhaskar et al. 2017).

Salicylic acid application. The effects of salicylic acid at the different concentrations on the duration of the leaf and petal freshness during the vase life were depended on the cultivar responses. A longer leaf freshness duration was observed with the cv. 'Reagent Sunny' at 200 mg/L of salicylic acid with negligible differences noted at 300 mg/L (Table 3). In the cv. 'Yellow Fiji', however, the salicylic acid application, in any concentration, shortened the duration of the leaf freshness compared with the control.

Similar phenomena were also observed in the duration of the petal freshness (Table 4). Salicylic acid with a concentration of 100 mg/L increased the petal longevity of the cv. 'Reagent Sunny', yet had a negligible impact on the cv. 'Yellow Fiji'. In higher concentrations, however, the increase in the petal longevity of the cv. 'Reagent Sunny' was insignificant compared to the control. While in the cv. 'Yellow Fiji', a decreased effect was found on the duration of the petal freshness in line with an increase in the salicylic acid concentrations. In higher concentrations than 100 mg/L of salicylic acid, differences between the tested cultivars were observed.

The insignificant differences on leaf and petal longevity in cv. 'Reagent Sunny' and cv. 'Yellow Fiji' when treated with salicylic acid at the concentrations of 200 and 300 ppm indicated that 100 ppm was the high-

est concentration (Table 3 and 4). While the different degrees of leaf and petal longevity between the tested cultivars inferred the specificity of each genotype to salicylic acid solutions especially in higher concentrations. The decrease in the leaf and petal longevity in the cv. 'Yellow Fiji' after being treated with 200 and 300 mg/L salicylic acid indicated that this genotype was more sensitive than the cv. 'Reagent Sunny'. Different responses among the genotypes to salicylic acid during the vase life were also reported in narcissi (Sardoei et al. 2013), roses (Abdolmaleki et al. 2015) and others species.

The shorter leaf and petal freshness of the cv. 'Yellow Fiji' at 200 and 300 mg/L salicylic acid than the control and the cv. 'Reagent Sunny' inferred the substance provided positive effects only in certain concentration ranges. Several reports also indicated that the application of salicylic acids in high concentrations adversely induced cell death, increased the anthocyanin leakage and reduced the vase life (Kazemi et al. 2011). The cell death represented by browning and damaged tissues were observed in the cv. 'Yellow Fiji' basal stem after eight days of vase life (Figure 2A)

Table 3. Interaction effect of the salicylic acid concentrations and the chrysanthemum varieties on the duration of the leaf freshness (days) during the vase life

Varieties	Salicylic acid concentrations (mg/L)			
	0	100	200	300
Reagent Sunny	10.65 ^{aA}	11.33 ^{aAB}	12.67 ^{bB}	13.21 ^{bB}
Yellow Fiji	11.03 ^{aB}	11.45 ^{aB}	9.33 ^{aAB}	8.44 ^{aA}

^{a,b}Values in the same column followed by different lowercase letters differ significantly under the LSD ($\alpha \leq 5\%$).

^{A,B}Values in the same row followed by different capitalised letters differ significantly under the LSD ($\alpha \leq 5\%$).

Table 4. Interaction effect of the salicylic acid concentrations and chrysanthemum varieties on the duration of the petal freshness (days) during the vase life.

Varieties	Salicylic acid concentrations (mg/L)			
	0	100	200	300
Reagent Sunny	13.25 ^{aA}	16.12 ^{aB}	15.22 ^{bAB}	14.56 ^{bAB}
Yellow Fiji	13.63 ^{aB}	14.89 ^{aB}	13.13 ^{aAB}	11.53 ^{aA}

^{a,b}Values in the same column followed by different lowercase letters differ significantly under the LSD ($\alpha \leq 5\%$).

^{A,B}Values in the same row followed by capitalised different letters differ significantly under the LSD ($\alpha \leq 5\%$).

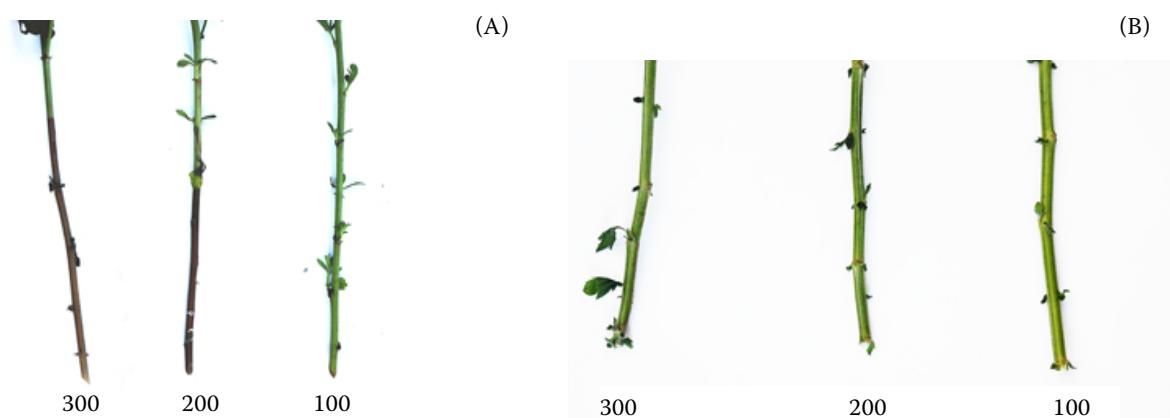


Figure 2. (A) Basal stem of the cv. 'Yellow Fiji' with browning and damaged tissues at the 200 and 300 mg/L salicylic acid solutions (pointing by black arrows) and (B) the normal stem of the cv. 'Reagent Sunny' after eight days of vase life (basal stems are at the top of the images)

at 200 and 300 ppm of the salicylic acid treatments that presumably have a relationship with disturbing the water transport within the stem (Vahdati Mashhadian et al. 2012). While the basal stem of the cv. 'Reagent Sunny' started to deteriorate later after 12 days of vase life.

Chlorophyll content

Ascorbic acid application. The chlorophyll content in the leaves of both tested cultivars in all ascorbic acid concentrations decreased along with the lengthened duration of the vase life (Figure 3). The decrease in the chlorophyll content in the ascorbic acid

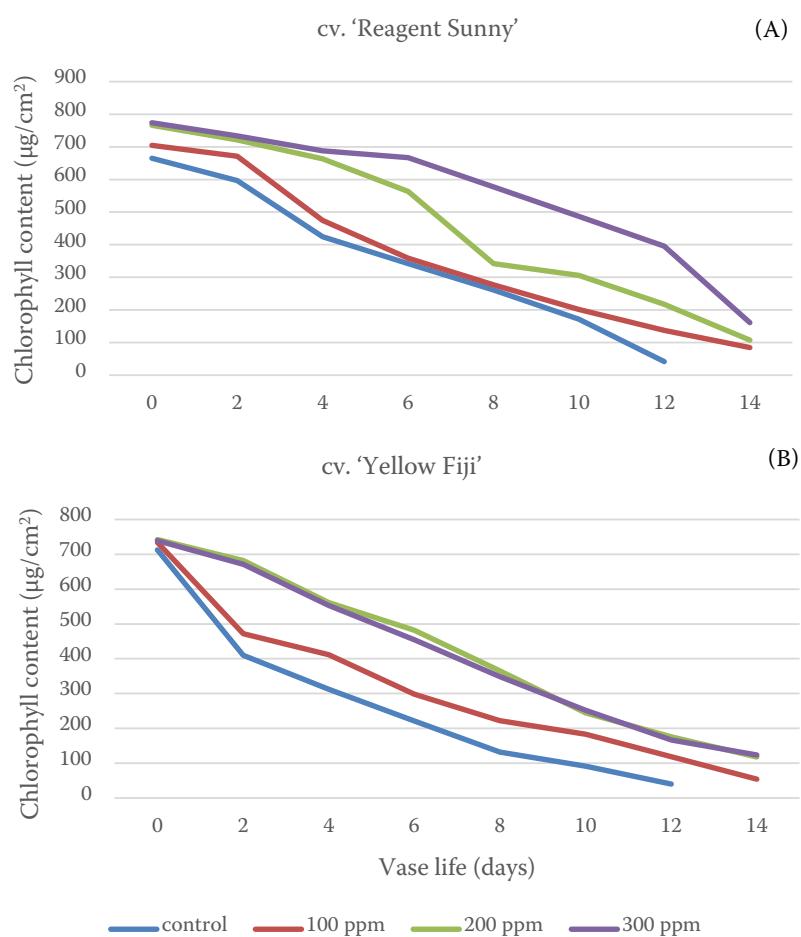


Figure 3. Chlorophyll content of the leaves of (A) the cv. 'Reagent Sunny' and (B) the cv. 'Yellow Fiji' under the different ascorbic acid concentrations during the vase life periods

treatments indicated the chlorophyll degradation (Basiri et al. 2011) is mainly caused by microorganism activities and mineral translocation (Mehraj et al. 2013). The chlorophyll content of the control plants of both tested varieties fell more sharply than the other treatments and the leaves were wrinkly and wilted at 12 days, thus the chlorophyll values were unreadable by SPAD after 14 days of vase life. These conditions inferred that without the ascorbic acid treatment, the chlorophyll degradation was faster compared to the other ascorbic acid treated-flower stalks.

On the ascorbic acid-treated stalks, a decrease in the chlorophyll content was observed as being slower starting after two days on both varieties (Figure 3). The slower chlorophyll degradation was then more obvious on the 200 and 300 mg/L treated stalks. In the cv. 'Reagent Sunny', the flower stalks treated by 300 mg/L of ascorbic acid showed a higher chlorophyll content especially from six to 12 days compared to the 200 mg/L ascorbic acid treatments. While in the cv. 'Yellow Fiji', the effects of these two concentrations on the chlorophyll con-

tent were negligible until 12 days. The slower chlorophyll degradation on the ascorbic acid-treated stalks indicated the preservation effect of the ascorbic acid through a decrease in the stem blockage for water flow and slowing down the respiration rates (Banaee et al. 2013). These conditions then delayed the minerals and organic compound translocations, like chlorophyll (Balouchi et al. 2012), and prolonged the flower longevity during the vase life periods.

Salicylic acid application. Similar to those under the ascorbic acid treatments, the chlorophyll content of both tested cultivars treated with all salicylic acid concentrations also decreased in line with the lengthened duration of the vase life (Figure 4). The different responses of the tested cultivars to the salicylic acid concentrations were observed. In the cv. 'Reagent Sunny', the chlorophyll content of 100, 200 and 300 mg/L salicylic acid-treated stalks decreased with different slopes among the treatments, though the values were higher than control up to 14 days of vase life (Figure 4A). While in the cv. 'Yellow Fiji', the prevention of the chlorophyll degrada-

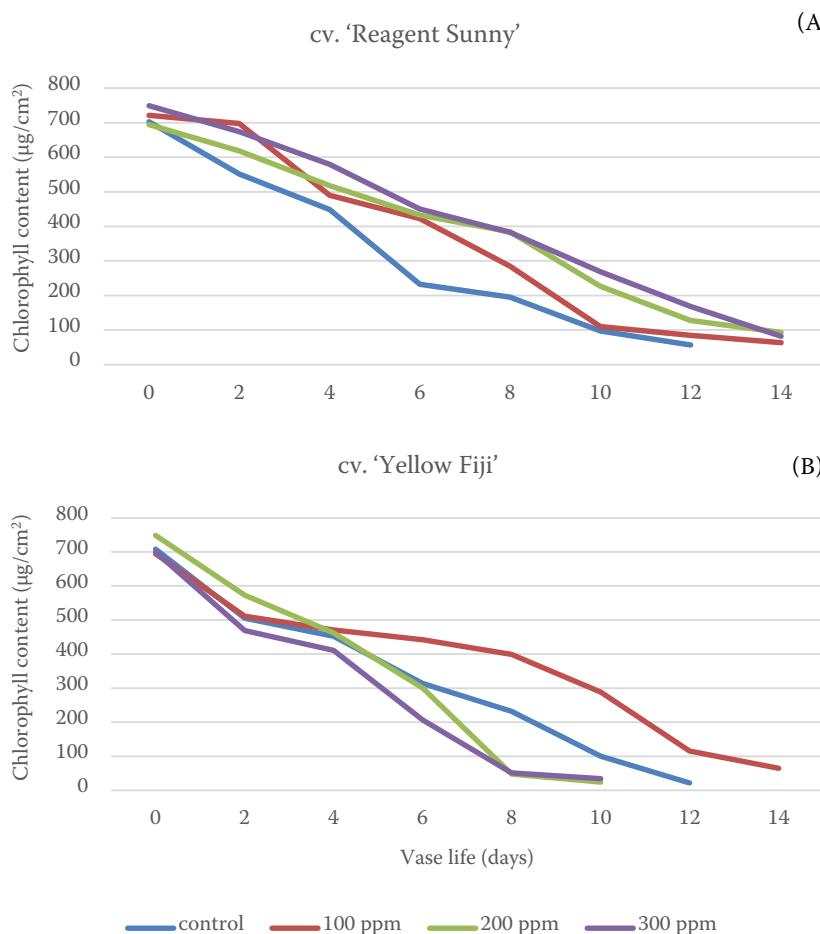


Figure 4. Chlorophyll content of the leaves of (A) the cv. 'Reagent Sunny' and (B) the cv. 'Yellow Fiji' under the different salicylic acid concentrations during the vase life periods

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tion was detected only on the stalks treated with 100 mg/L of salicylic acid. The flower stalks treated with 200 and 300 mg/L of salicylic acid showed chlorophyll degradation faster than the control (Figure 4B) and the values were even unreadable after 10 days of vase life.

The faster decrease in the chlorophyll content in the cv. 'Yellow Fiji' treated with the 200 and 300 mg/L of salicylic acids seemed to have a relationship to the leaf and petal freshness presented in Table 3 and 4 and the damaged tissues of the basal stem presented in Figure 2A. The high salicylic acid concentrations were expected to be the initial cause of the early flower senescence of sensitive cultivars. A high concentration of salicylic acid-induced cell death and contributed to damaging the basal stem tissues (Kazemi et al. 2011). These conditions resulted in the disturbance of the water transport system within the stem. The reduced water uptake induced a water imbalance on the upper part of the tissues and among the organs (Edrisi et al. 2011). By the time the leaves, as the source organs, lost their turgidity, the petals wilted as an indication of the termination of the flower freshness (Marandi et al. 2011).

CONCLUSION

Two tested chrysanthemum cultivars, the cv. 'Reagent Sunny' and cv. 'Yellow Fiji' showed visual changes, including petal colours when wilting during the vase life period. The application of ascorbic and salicylic acid solutions in several concentration levels differently affected the flower longevity of these cultivars. The ascorbic acid induced longer flower freshness in both tested cultivars and, at the concentration of 200 mg/L, the solution gave, on average, two days longer of leaf and petal freshness with a slower chlorophyll degradation compared to those without the ascorbic acid supplementation. On the salicylic acid application, the 200 mg/L solution induced longer flower longevity and a higher chlorophyll content during the vase life period only in the cv. 'Reagent Sunny'. While in the cv. 'Yellow Fiji', the salicylic acid application had a negative impact on the flower freshness. The leaf and petal freshness were less affected at 100 mg/L and the flower longevity with a faster chlorophyll degradation was even shorter in higher salicylic acid concentrations.

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