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Evaluation of aluminum sulfate as vase solution biocide on postharvest microbial and physiological properties of 'Cherry Brandy' rose

Mohammad Mahdi Jowkar [a*], Mohsen Kafi [b] Ahmad Khalighi [a] and Nader Hasanzadeh [c]

[a] Department of Horticultural Sciences, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran
[b] Department of Horticultural Science, Faculty of Horticulture and Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[c] Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran

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ABSTRACT

The major cause of vase life reduction in cut flowers is water relation interruption which is mostly due to vase solution microbial proliferation and consequently vascular occlusion resulting in solution uptake reduction. In order to control microbial proliferation, biocides are usually integrated in vase solution preservatives. Beside microbial proliferation control, biocides could affect cut flower's quality and physiology in various aspects. In order to found an easy to use, non toxic and inexpensive compound for large scale application, cut 'Cherry Brandy' roses were treated with aluminum sulfate (100, 200 and 300 mgl^{-1}) and sterilized distilled water (control). Effects of aluminum sulfate application as vase solution biocide and its impact on vase life, water relation, vase solution microbial kind and population beside different physiological parameters such as chlorophyll degradation, chlorophyll fluorescence and membrane permeability were investigated. Results indicated that aluminum sulfate treatment significantly increased vase life and improved postharvest visual quality of this cultivar by retaining leave freshness even at the end of vase life. Controversially solution uptake was reduced at most stages of vase life by aluminum sulfate application while fresh weight was best retained by this compound especially during the second week of vase life. This compound significantly controlled microbial proliferation resulting in zero contamination until day 4. After which a few isolates of Bacillus subtilis, Bacillus polymexa, Pectobacterium sp., Coccus and Fusarium solani were found. Membrane permeability was best maintained by 300 mgl^{-1} aluminum sulfate treatment. Besides that, aluminum sulfate increased leaf chlorophyll content while it resulted in chlorophyll fluorescence reduction during vase life.

Keywords: *Bacillus subtilis*, chlorophyll content, chlorophyll fluorescence, *Fusarium solani*, membrane permeability, water relation.

INTRODUCTION

Cut flowers vase life is affected by several factors such as: cell programmed death [1], ethylene induced senescence [2, 3], dehydration [4, 5, 6, 7], or loss of assimilates and substrates [8, 9]. Among the above mentioned, water relation and balance play a major role in postharvest quality and longevity of cut flowers [7] and water relation interruption during this period is often the reason of short vase life for cut flowers [5].

Water relation interruption is mostly due to microorganism proliferation in vase solution and occlusion in the basal end of the cut flower stem by microbes [5, 10, 11, 12]. Stem blockage could take place by the bacteria [5, 10, 11, 12], or by extra cellular polysaccharides and degradation products of dead cells [10]. Besides vessel blockage, bacteria secrete pectinases and toxic compounds and produce ethylene [13], thereby, accelerate senescence.

It has been shown that beside vase life reduction, disruption of water relation in rose flowers causes some physiological disorders such as bent neck [4, 10, 14], lack of flower opening [10], and wilting of the leaves accompanied by improper opening and wilting of flowers [10, 15]. Therefore, controlling and reducing microbial proliferation is a prerequisite for extending quality and longevity of cut flowers, especially for roses. On the other hand, applied biocides could also severally or moderately affect other physiological properties of cut flowers specially their photosynthetic apparatus function and membrane permeability by their toxic compounds during postharvest development and aging.

In order to prevent microbial proliferation in vase solutions of cut flowers, various compounds and chemicals have been used, namely, silver nitrate [15], silver thiosulphate [3, 16], aluminum sulphate [17], hydroxyquinoline sulphate [3], hydroxyquinoline citrate [4, 16, 18, 19, 20], and sodium hypoclorite [4, 16, 18].

Some of these compounds such as silver nitrate and silver thiosulphate have shown environmental risks and health hazards [21]. While others such as hydroxyquinoline have shown plant phyto-toxemic effects. In the cut flower market there is a great need for preserving solution biocides that control microbial contamination effectively and beside that do not show environmental risks and phyto-toxicity. This need is more crucial for cut rose flowers which hold a very large portion of cut flower market and industry.

Although few studies have investigated a biocide role for aluminum sulfate, but their studies have not been comprehensive and beside their biocidal efficacy, some aspects especially physiological aspects such as chlorophyll degradation, chlorophyll fluorescence and membrane permeability have been unseen. Therefore in order to found an easy to use, non toxic and inexpensive compound for large scale application; we have focused on some of the mentioned physiological properties beside biocidal efficacy of aluminum sulfate as vase solution preservative of cut ‘Cherry Brandy’ roses

MATERIALS AND METHODS

Plant Material:

Rose (*Rosa* × *hybrida*) cv. ‘Cherry Brandy’ (licensed by Rosen Tantau, Germany) were harvested at commercial maturity stage (i.e. outer petals starting to reflex and inner petals have become visible) from rose plants grown in hydroponic perlite in an automatic greenhouse. Flowers were harvested early in the morning and transferred to laboratory within 1 hour after

harvest. Before treatment, all the leaves except the 5 most upper leaves of each flower stem were removed and then stems were recut slantly under water so that all flowers reach a height of 40cm and probable air emboli gets removed.

Experimental design and treatments:
Following recut, flowers were treated in a completely randomized design of 4 treatments and 9 replications. Treatments applied as vase solutions were: aluminum sulfate [$Al_2(SO_4)_3$] (100, 200 and 300 mgl^{-1}), or sterilized distilled water (control).

Experimental condition:
Cut rose flowers were kept in a laboratory with a maximum and minimum temperature of 25 ±2 ºC and 21 ±2 ºC, respectively; relative humidity (RH) of 55± 5 %, and light intensity of 4 µmol m^{-2} s^{-1} provided by white fluorescent lamps from 07.00 to 20.00 h.

Vase life and side effect evaluation:
During vase life evaluation, cut rose flowers were daily checked and their appearance and condition were recorded to determine the vase life and if the applied chemicals had any side effects. Termination of vase life was recorded when wilting of the outer 5 petals occurred or bent neck was observed [10].

Microbial Count:
Microbial count was determined by taking 1ml vase solution samples at 2 days intervals with 3 replications during the first 6 days of the experiment. 1ml from each sample was diluted in 10 fold serial dilution. 0.1 ml from each concentration of diluted samples was plated on nutrient agar and all were incubated at 35ºC for 48 hours. Microorganisms were counted by standard plate counting method (by counting the number of colonies formed after incubation) to generate the number of colony forming units.ml^{-1} (CFU ml^{-1}) [22].

Microbial Identification:
After plate counting, obtained colonies were studied and separated by their apparent morphological differences. This resulted in 7 bacterial isolates and one fungus. Fungus was cultured on Potato Dextrose Agar and after incubation it was identified according to its morphological characteristics according to Steinkellner [23] and Siddiquee *et al.* [24].The bacterial isolates were purified and then differentiated according to their typical morphological and biochemical characteristics [25, 26].

Bacterial morphological studies were: motility, cell shape, and capsule presence. Bacterial bioassays were: potato soft rot and hypersensitivity test on tobacco. The biochemical tests carried out on isolated bacterial colonies were: gram reaction using KOH, aerobic/anaerobic growth, acid production from glucose, gas production from D-glucose, fluorescent pigments production on KB, oxidase test, catalase test, gelatin hydrolysis, levan, growth at 50°C, growth at 5.7 pH, starch hydrolysis, tween 80 hydrolysis, indol production, methyl red reaction, aceteoin (VP), nitrate reduction, arginine dihydrolase and H_2S production from cysteine [25, 26].

Fresh weight changes:
In order to record fresh weight changes of cut flowers, flower stems were taken out of vase making sure that stem end is not dry and weighted as quickly as possible by a balance on a daily basis. Data were obtained to calculate fresh weight changes (g and %) and relative fresh weight (RFW) changes of the stems [22]. Relative fresh weight was calculated as: RFW (%) =

(W_t/W_{t0})×100; where, W_t is weight of stem (g) at t = day 0, 1, 2, etc., andW_{t0} is weight of the same stem (g) at t=day 0 [11,12].

Solution uptake:
Solution uptake of flowers was measured using a balance by weighting each vase containing its solution without its flowers and correcting the evaporation from the 4 evapo-control vases (vases which did not contain any flowers and were located between the vases that contained flowers at different places) by subtracting the average of 4 evaporation data from solution uptake on a daily basis. Daily vase solution uptake was calculated as: vase solution uptake rate (g stem^{-1} day^{-1})=(S_{t-1}-S_t); where, *St* is weight of vase solution (g) at t = day 1, 2, 3, etc., and S_{t-1} is weight of vase solution (g) on the previous day [11, 12, 22].

Ion Leakage:
Three 2.5 cm diameter discs were taken from leaf of each treatment's flower stalk and placed into 50 ml centrifuge tubes containing 20 ml of 2 bar mannitol solution. Samples were kept at 25°C and dark for 24 h after which electric conductivity was measured and solution's initial electric conductivity was subtracted in order to obtain electrolyte leakage.

Chlorophyll Content:
Total chlorophyll content was measured by non destructive method using chlorophyll meter (SPAD-502, Minolta Co., Japan) which provides a SPAD value [27]. Measurement was conducted with 2 day intervals on 4 different flower stems (replications) in each treatment. For each flower stem, measurement was conducted on the marked spot of distal leaflet of 3 leaves.

Chlorophyll Fluorescence:
The quantum efficiency of open photo system II centers (Fv/Fm=ratio of variable to maximum fluorescence), was measured by a nondestructive method every 2 days with a Opti-Sciences OS-5P pulse amplitude fluorimeter (Opti-Sciences INC., Hudson, NH, USA) [28]. Leaves were maintained in darkness for 20 min by a special clip before measurement of Fv/Fm. Minimal fluorescence (F0) was measured under a weak pulse of modulating light over a 0.8 s period, and maximal fluorescence (Fm) was obtained after a saturating pulse of 0.7 s at 8000 µmol m^{-2} s^{-1}. Fv is the difference between F0 and Fm [28, 29].

Statistics:
Data were analyzed by one way ANOVA using MSTAT-C software and means were compared by the least significant difference (LSD) test at the 0.05 and 0.01 probability level (P=0.05 and 0.01).

RESULTS AND DISCUSSION

Vase life:
Results indicate that aluminum sulfate significantly increased vase life of 'Cherry Brandy' rose flowers compared to control (Table 1). The longest vase life was achieved by 100 mgl^{-1} aluminum sulfate application. This was while there was not a significant difference between aluminum sulfate treatments. Although our results indicate the beneficial effect of aluminum sulfate, Knee [6] considered aluminum sulfate as an ineffective biocide for 'Classy' Rose because of vase life reduction by this compound. Van Doorn [16] also found that when Narcissus flowers were kept in the same vase with rose flowers, beside narcissus mucilage, vase life of rose flowers was reduced by aluminum sulfate treatment.

Table 1: Effect of aluminum sulfate on vase life of cut 'Cherry Brandy' rose

Treatment	Vase life (day)
Aluminum Sulfate 100 mgl^{-1}	12.89 a[†]
Aluminum Sulfate 200 mgl^{-1}	12.22 ab
Aluminum Sulfate 300 mgl^{-1}	12.33 ab
Sterilized Distilled Water (Control)	11.67 b

[†]Means followed by the same lower-case letters are not significantly different at the 0.01 probability level using Least Significant Difference (LSD) test.

Side effects:

Generally, effective concentrations of biocides can be toxic to many flowers [6, 22, 30]. Van Doorn *et al.* [30] concluded that at none toxic concentrations none of the applied compounds had constant and high anti-bacterial effect.

Aluminum sulfate did not show any side effects and was completely a safe biocide for cut 'Cherry Brandy' rose flowers. In aluminum sulfate treatment group, leaves of the flower stems were turgid and fresh even 2 days after vase life termination. The vividness of aluminum sulfate treated flowers was so evident that on day-14 lateral auxiliary buds burst and new appeared shoots branched. This is while it has been shown that aluminum sulfate treatment of *Narcissus tazetta* flowers results in bud abortion, yellowing and anthesis failure resulting in a shorter vase life [22].

Although it has been concluded that none of the previously applied biocide compounds had a consistent or high anti-bacterial effects at concentrations that were not toxic to flowers, our experiments findings indicate aluminum sulfate as safe and friendly biocide for vase solution of cut 'Cherry Brandy' rose.

Table 2: Effect of aluminum sulfate on cut 'Cherry Brandy' rose vase solution microbial count during days-2, 4, 6.

Treatment	Microbial Count[†] (log10 CFU ml^{-1})[††]		
	Day 2	Day 4	Day6
Aluminum Sulfate 100 mgl^{-1}	0 b[†††]	0 b	3.322 b
Aluminum Sulfate 200 mgl^{-1}	0 b	0 b	2.539 c
Aluminum Sulfate 300 mgl^{-1}	0 b	0 b	2.128 c
Sterilized Distilled Water (Control)	4.477 a	6.469 a	9.203 a

[†]Microbe counts, except a zero count, are reported as log10x (x = microbe counts).

[††]The number of microorganisms was counted by the standard plate counting method and expressed as Colony Forming Units ml^{-1} (CFU ml^{-1}).

[†††]Means followed by the same lower-case letters are not significantly different at the 0.01 probability level using Least Significant Difference (LSD) test.

Microbial count:

During this experiment, aluminum sulfate was effective to some extent. All concentrations of aluminum sulfate inhibited microbial proliferation by the end of day-4. On day-6, small contamination was observed which showed a decrease with concentration increment, positioning aluminum sulfate 300 mgl^{-1} the least contaminated level of this compound on day-6 (Table 2). Van Doorn and Perik [4] found that aluminum sulfate prevented bacterial proliferation in cut rose stems. On the other hand, van Doorn [16] found that aluminum sulfate had little effect on the number of bacteria when narcissus flowers were placed in rose vases. In Jowkar's experiment [22], aluminum sulfate was among the least effective compounds in controlling microbial proliferation. This was explained by the low solubility of aluminum hydroxides as mentioned by van Doorn [16].

This was while vase solution microbial contamination of sterilized distilled water reached a relatively high count on day-2 (Table 2). As same as our findings, sterilized distilled water did not have any pleasing effect in controlling or reducing microbial population of *Narcissus* vase solution [22]. As the main role of integrated biocide in floral preservatives is to sustain clarity in vase solution and to avoid blockage of xylem elements by microorganisms [6], our results suggest the application of aluminum sulfate and vase solution replacement every 4 days.

Microbial Kind:

In the vase water of cut roses, many different kinds of bacteria, yeasts and fungi have been identified [31, 32]. While in carnation vase solution Zagory and Reid [34] identified 25 different microorganisms, in this experiment only 8 microbial types were seen. It seems that fewer microbe types were due to lower flower contamination and integrated management applied during flower production.

The isolated microorganisms in this experiment were one kind of fungus and 7 different kinds of bacterial isolates. The isolated fungus was a strain of *Fusarium solani*. This was while in *Narcissus tazetta* vase solution, the only fungus found was *Aspergillus* sp. which was due to mulching practice during cultivation [22].

Generally, our experiments findings indicate that most of the microorganisms in the vase solutions were bacteria, which is consistent with other published data [14, 22, 31, 33]. Among the 7 different separated bacterial colonies, 3 were *Bacillus*, 3 were *Coccus* and one colony was *Pectobacterium* sp. This is while in previous studies other different bacterial strains were seen. For example, bacterial strains found in rose stems by van Doorn *et al.* [35] were: Pseudomonads (80 %), Enterobacteria (5-10%); and some other genera such as *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Citrobacter*, and *Flavobacterium* which occurred infrequently. In another study they had Pseudomonads and Enterobacteria as the dominant bacterial strains in stems of cut 'Sonia' roses [36]. Other isolated bacteria from rose vase solution were Fluorescent Pseudomonad and a Nonfluorescent Pseudomonad which reduced flower vase life of cut *Rosa hybrida* cv. 'Cara Mia' [34].

Sterilized distilled water (Control) was only contaminated with *Bacillus* bacteria. This was while aluminum sulfate vase solutions showed more diversity and were contaminated with one isolate of *Bacillus subtilis*, one isolate of *Bacillus polymexa*, one isolate of *Pectobacterium* sp. and three isolates of *Coccus*. It has been shown that *Bacillus subtilis* is one of the most effective microorganisms against *Botrytis cinerea* (the principal causes of pre- and postharvest losses in greenhouses produced roses) [37]. Growth allowance of *B. subtilis* in aluminum sulfate treatment signifies the beneficial effect of aluminum sulfate treatment application during postharvest.

While in our study *Bacillus and Coccus* were the dominant bacteria, in previous studies, *Bacillus* has been the most common occurring vase solution microorganism [22, 31, 32, 38]. Depending on experiment condition and production system, other dominant types of bacteria have been seen. For example, van Doorn *et al.* [39] found *Pseudomonas* species as the dominant microorganism in roses and carnation cut flowers.

Agricultural products microbial flora and population is determined by the products physiological condition and mixture of bacteria, yeasts and fungi covering the product [40]. It has been proved that when cut flowers are placed in vase, bacteria from flower surface transfer to vase solution. For example, van Doorn and de Witte [36] recognized that *Bacillus* and *Staphylococcus xylosus*

transfer from leaves and stems of cut 'Sonia' roses into vase solution. Other sources of microbial contamination are vase water, contaminated vases, containers, or vessels [41]. These facts explain the difference between the microbial contamination in our study and others.

Relative Fresh Weight (% of the initial):

As seen in Fig. 1, there is a general sharp increase in relative fresh weight during the first day of the experiment. During the next days, relative fresh weight of aluminum sulfate treated flowers showed a slight increase until day-5, while control flowers showed a slight increase until day-4. After reaching the maximum point, all treatments showed weight reduction until the flowers vase life ended. The decrease was sharp in control flowers while in aluminum sulfate it reached the initial relative fresh weight point within 7 days. This slight decrease caused 100 and 200 mgl^{-1} aluminum sulfate treatments to reach their initial relative fresh weight point on day-12 (one day before vase life termination), while relative fresh weight of 300 mgl^{-1} treated flowers was always above its initial point. Throughout the experiment, aluminum sulfate 300 mgl^{-1} had the highest fresh weight gain which did not reach its initial point at the end of vase life (Fig. 1). This was while control flowers relative fresh weight during the first 6 days was higher than the other 2 aluminum sulfate treatments. On day-7 relative fresh weight of control flowers reduced bellow all aluminum sulfate treated flowers.

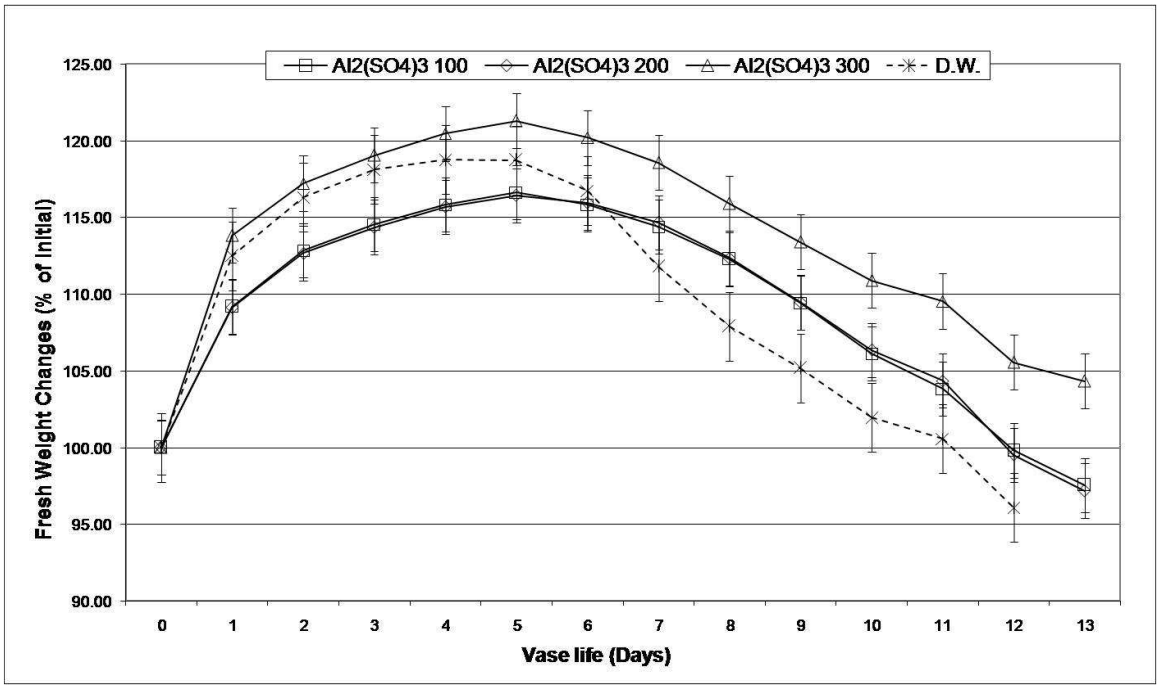

Figure 1: Relative fresh weight trend of cut 'Cherry Brandy' rose flowers treated with aluminum sulfate. There is a general sharp increase in relative fresh weight during the first days of the experiment. After reaching a maximum point there is a reduction until vase life termination.

Although in our experiment aluminum sulfate delayed fresh weight loss (Fig. 1), Knee [6] did not see any delay in fresh weight loss of 'Classy' roses by applying aluminum sulfate as vase solution. On the other hand van Meeteren *et al.* [42] observed a decrease in fresh weight of deionized treated cut flowers during the first 1-3 days of vase life. This decrease in fresh weight was not seen in sterilized distilled water treated flowers of our experiment. Instead the flowers reached their maximum fresh weight on day-4.

According to our observations, aluminum sulfate 300 mgl^{-1} treatment best retained relative fresh weight. Increase in relative fresh weight by aluminum sulfate and fresh weight improvement by this compound was accompanied with bud burst as mentioned before, indicating that 300 mgl^{-1} aluminum sulfate resulted in the best fresh weight retention.

Solution Uptake:

There was a high solution uptake in all treatments on day-1 (Fig. 2) after which there was a great decrease. Although there was not a significant difference between solution uptake in different treatments, generally, solution uptake was higher in control flowers throughout the experiment. In aluminum sulfate treatments, solution uptake decreased with concentration increment during the first week of experiment after which solution uptake increased with concentration increment. When added to vase water under sterile vase water conditions, aluminum sulfate results in partial blockage of water flow in rose stem [16, 33]. This was explained by the low solubility of aluminum hydroxides [16]. In the present experiment the same was seen. Although solution uptake at most days in sterilized distilled water was higher than aluminum sulfate, but there was not a significant difference throughout the experiment. Confirming our findings, Knee saw that aluminum sulfate reduces solution uptake and caused the lowest solution uptake in 'Classy' roses compared to water and HQC [6].

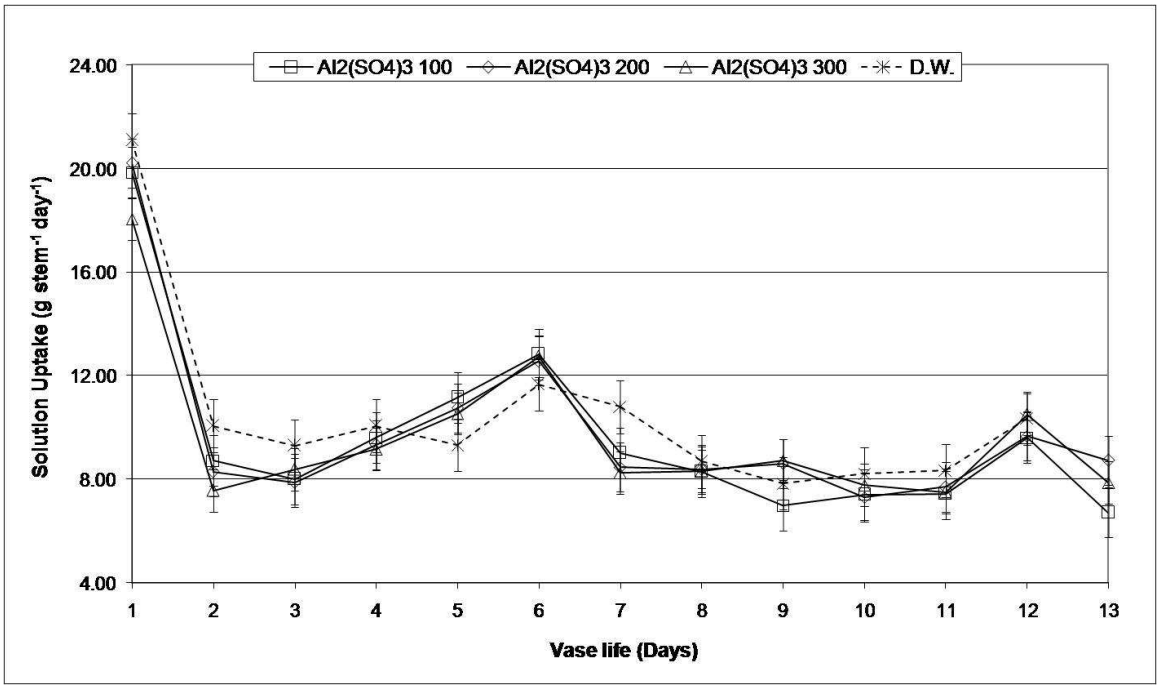

Figure 2: Vase solution uptake trend of cut 'Cherry Brandy' rose flowers treated with aluminum sulfate. There is a high solution uptake in all treatments on day-1. After that, there are 2 critical points of maximum solution uptake which all treatments follow.

Although solution uptake in previous studies tended to increase initially and then decrease [7, 12], throughout our experiment there were 3 critical points of maximum solution uptake which all treatments followed (Fig. 2). Those days were day-1, 6 and 12. The highest solution uptake on day-1 (which is exactly the day after rehydration of flowers) belonged to control. While on day-6 and 12 the highest solution uptake with a very small un-significant difference belonged to 300 mgl^{-1} aluminum sulfate treated flowers. Having a sharp solution uptake increment on day-12 and consequently continuing solution uptake on the next day, indicates that application of the aluminum sulfate treatment does not disrupt water relation even at the end of vase life.

As solution uptake decreased during the first week with concentration increment and after that it increased with concentration increment, it seems that solution uptake reduction with concentration increment during the first week was due to partial stomata closure by aluminum ions and after that solution uptake increment with concentration increment was due to better microbial control because of higher aluminum hydroxides solubility.

Ion leakage:

Ion leakage trend in all treatments showed an increasing trend during vase life (Fig. 3) indicating membrane permeability reduction with aging in leaves of 'Cherry Bandy' roses. In aluminum sulfate treatments, ion leakage decreased with concentration increment. Although low levels of aluminum sulfate (100 and 200 mgl^{-1}) did not control ion leakage, aluminum sulfate 300 mgl^{-1} suppressed ion leakages compared to control. This resulted 300 mgl^{-1} aluminum sulfate treated flowers to have the most permeable membrane and the least ion leakage. Except for the last days (days 10 and 12) of 100 mgl^{-1} aluminum sulfate treated flowers which showed a great increase, ion leakage difference was not significant between other treatments and control.

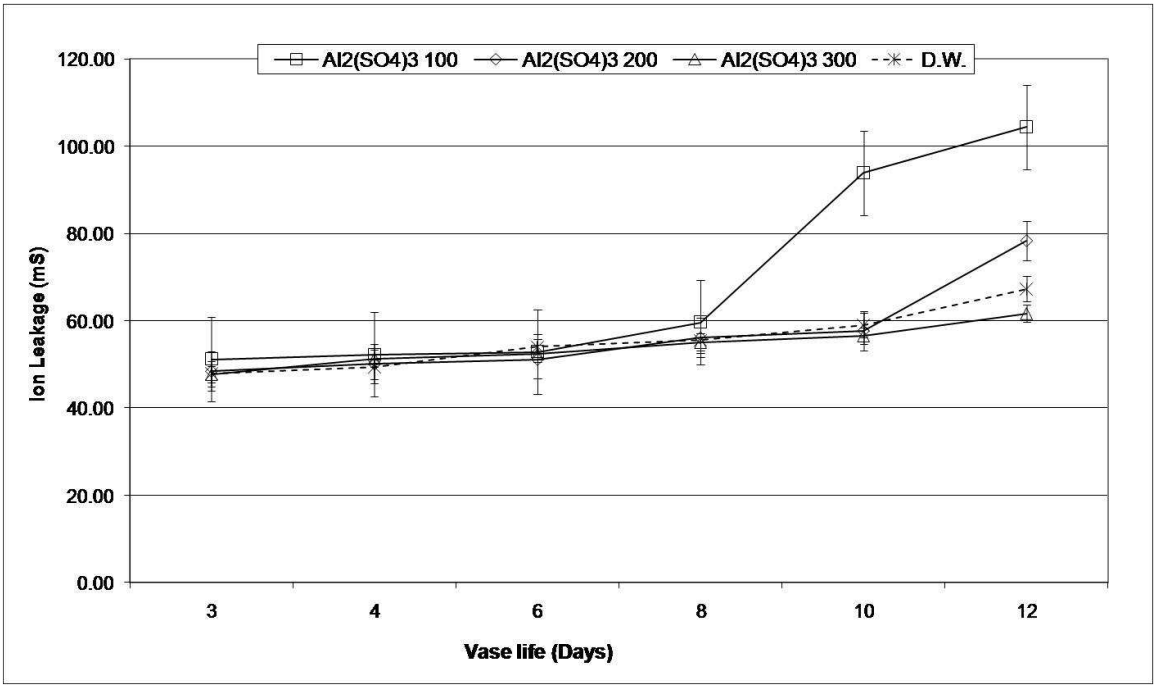

Figure 3: Leaf ion leakage trend of cut 'Cherry Brandy' rose flowers treated with aluminum sulfate. Aluminum sulfate 300 mgl^{-1} best retained membrane permeability.

Guiboileau *et al.* [43] have mentioned membrane lipids degradation as leaf senescence progress which results in ion leakage. Maalekuu *et al.* [44] have considered ion leakage as an index of membrane integrity and damage in plants during senescence. Our results confirm this issue and show that leaf ion leakage increases with aging. Like us, Sultan and Farooq [45] have shown that senescence of cut flower is associated with ion leakage increment. Oren-Shamir *et al.* [46] have also found that in cut 'Mercedes' rose ion leakage increases during senescence progress. Sood *et al.* [47] found same results for *R. bourboniana* and *R. damascene* flowers.

Although all reports agree on ion leakage increment during senescence, but different trends have been reported for this issue. In cut 'Mercedes' roses Oren-Shamir *et al.* [46] saw that ion leakage trend did not change until day-4 and after that it increased. Sood *et al.* [47] observed that ion leakage trend in *R. bourboniana* is constant and suddenly increases upon vase life termination

while in *R. damascene* it shows a slight increase during flower development and senescence. Although there is no report on ion leakage of cut roses affected by aluminum sulfate, Khan *et al.*'s finding [48] show that treatment of tulip cut flowers with aluminum sulfate results in cell membrane permeability improvement. Our results indicate that ion leakage trend shows a steady increase during vase life and has been retarded significantly by 300 mgl^{-1} aluminum sulfate application and consequently membrane permeability and vase life has been increased.

Chlorophyll Content:

Although chlorophyll content measurements showed fluctuation during vase life of 'Cherry Brandy' roses, aluminum sulfate increased chlorophyll content (Fig. 4). This increase was only significant in 100 mgl^{-1} aluminum sulfate treated flowers which showed the highest chlorophyll content throughout the experiment. Final chlorophyll content increment reduced with aluminum sulfate concentration increment.

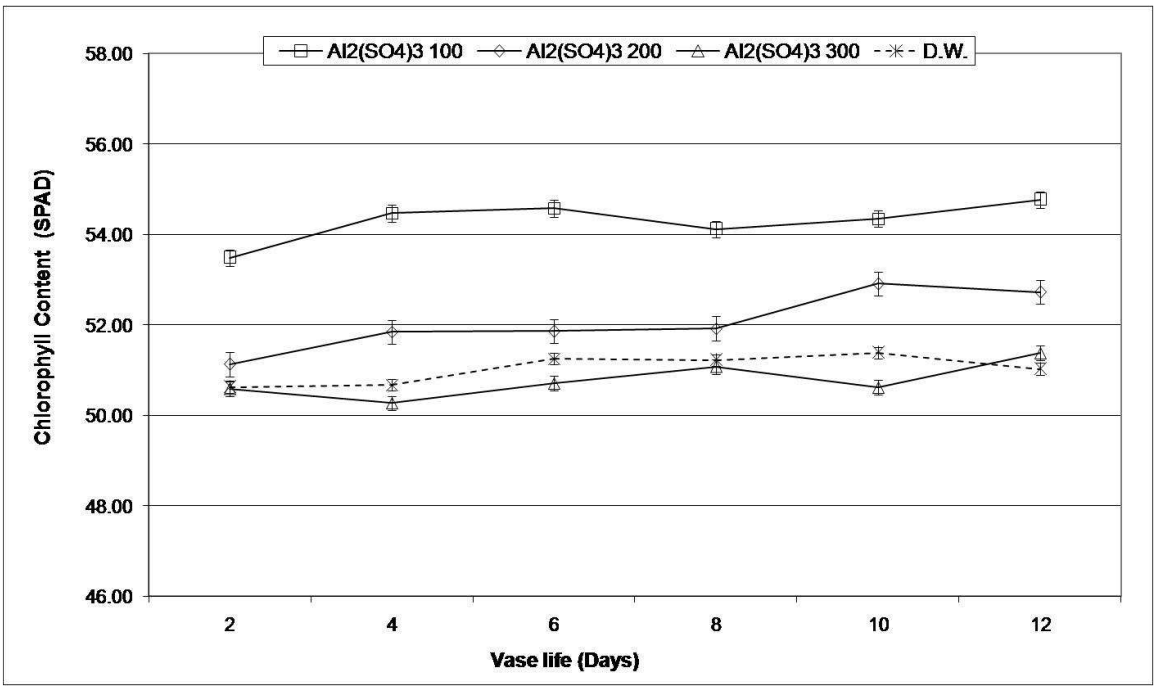

Figure 4: Leaf chlorophyll content trend of cut 'Cherry Brandy' rose flowers treated with aluminum sulfate. While showing fluctuation, aluminum sulfate100 mgl^{-1} significantly increased leaf chlorophyll levels.

Previously it has been shown that leaf chlorophyll content decreases during senescence [43, 49, 50]. Senescence delay and chlorophyll preservation has been achieved by various compounds which mostly have growth regulatory behavior such as: GA [50, 51], benzyladenine [52] and tidiazuron [50, 51]. Beside the mentioned, Khan *et al.*, [48] have shown that treatment of Tulip cut flowers with aluminum sulfate will result in chlorophyll content improvement. As far as our knowledge, the present study is the first report on preservation solution biocidal effect on chlorophyll content of rose flowers. Bolla *et al.* [53] has shown that in 'Euro Red' rose even slight water stress reduces leaf chlorophyll content. We conclude that chlorophyll content retention in 'Cherry Brandy' rose might be to some extend due to water relation improvement (as seen in Fig. 2). Chlorophyll content increment in cut flowers by aluminum sulfate application will have a great impact on commercialization and marketing especially on cut flowers which lose their green appearance of their leaves during vase life and senescence because of chlorophyll degradation.

Chlorophyll Fluorescence:

During vase life, leaf chlorophyll fluorescence of 'Cherry Brandy' rose decreased with aging and consequently reached its lowest level in all treatments at vase life termination. Control flowers had the least chlorophyll fluorescence reduction during vase life (Fig. 5). This was while in aluminum sulfate treated flowers, chlorophyll fluorescence decreased with concentration increment. Within aluminum sulfate treatments, the highest chlorophyll fluorescence at the end of vase life was 0.808 in 100 mgl^{-1} aluminum sulfate treated flowers.

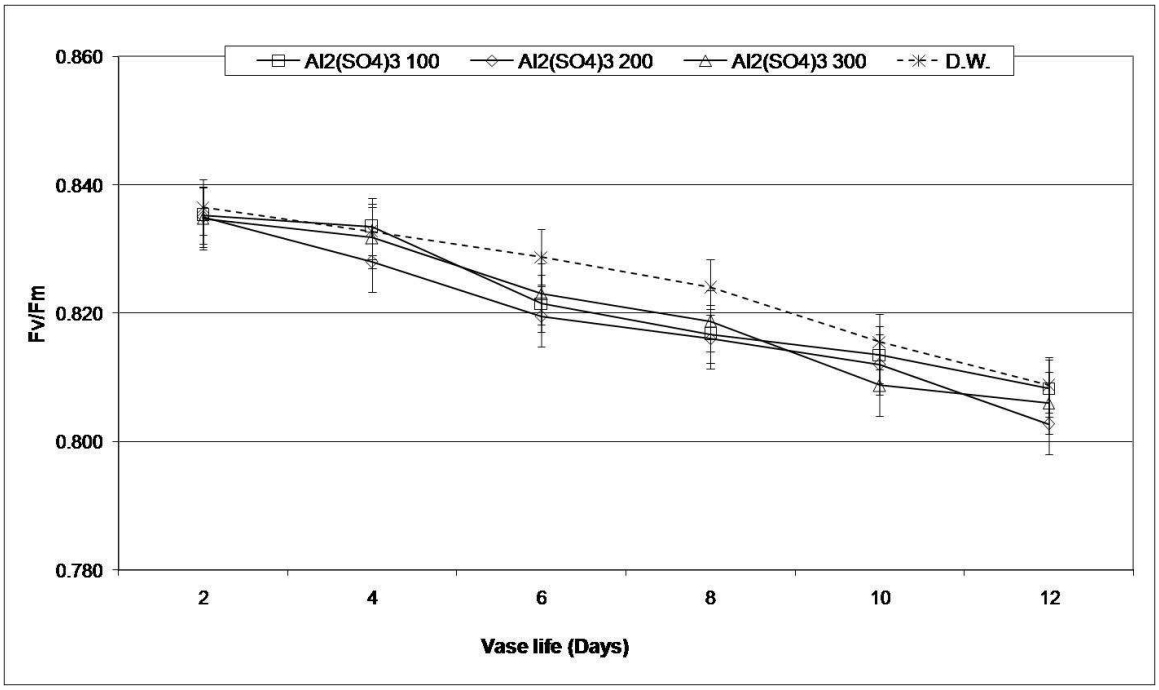

Figure 5: Leaf chlorophyll fluorescence trend of cut 'Cherry Brandy' rose flowers treated with aluminum sulfate. Chlorophyll fluorescence reduces during vase life and with aluminum sulfate concentration increment, chlorophyll fluorescence declines.

Similar to our findings, Tang *et al.* [49] have reported that with senescence initiation and progress, quantum yield of both photo system I and II decreases. Niewiadomska *et al.* [54] have also observed that during senescence quantum yield of photo system II reduces dramatically in tobacco leaves. Our findings on leaves of detached cut rose flower stems are in accordance with the mentioned reports on attached leaves. Controversially Pompodakis *et al.* [55] did not find a correlation between relative chlorophyll fluorescence reduction and vase life reduction of cold stored 'First Red' and 'Akito' rose flowers which seem to be due to low temperature injury of cold stored roses.

Chlorophyll fluorescence reduction indicated that quantum yield of photo system II reduces during vase life and reaches its lowest level at senescence. This fact and our results indicate a successive loss of photosynthetic activity during senescence and aluminum sulfate application in cut 'Cherry Brandy' rose. Considering the beneficial effect of aluminum sulfate treatment, increase in chlorophyll content of aluminum sulfate treated flowers during the experiment could be explained by this fact that flowers have increased their leaf chlorophyll level in order to overcome the loss of photosynthetic activity imposed by aluminum sulfate absorption.

CONCLUSION

Previously it was thought that biocides improve vase life by controlling microbial proliferation and consequently improving solution uptake and water relation. In this study although aluminum sulfate controlled microbial proliferation, but unexpectedly it reduced solution uptake. This was while fresh weight was surprisingly best kept and therefore water relation was sustained and consequently vase life was significantly improved.

Form physiological point of view beside vase life improvement, aluminum sulfate application did not result in any toxicity, controversially it maintain membrane permeability, increase chlorophyll content and freshness of flowers and leaves. Considering different aspects of biocide application (i.e. microbial control, solution uptake, relative fresh weight, flower longevity, and appearance) aluminum sulfate was an efficient treatment.

As our report is the first report on physiological changes during vase life of cut rose flowers and that no other biocides have been studies in such physiological approach, it provides valuable information on different aspects of biocide application and shows the beneficial effects of aluminum sulfate application on cut 'Cherry Brandy' rose flowers. Further study is needed to focus on its effect on water relation aspects especially stomata behavior.

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