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## Postharvest physiological and microbial impact of hydroxy quinoline citrate as 'Cherry Brandy' rose vase solution biocide.

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### ABSTRACT

One of the major applied biocides in the cut flower market and industry is hydroxy quinoline citrate (HQC). This compound is the most recommended biocide for cut rose flowers. Beside its ethylene inhibiting behavior, its major function is to control microbial proliferation and consequently improve cut flower water relation. Beside microbial proliferation control, biocides could affect cut flower's quality and physiology in various aspects. In order to study HQC impact on various microbial and physiological aspects, 'Cherry Brandy' roses were treated with HQC (200, 300 and 400 mg l<sup>-1</sup>) and sterilized distilled water (control). As a result, effects of HQC 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 during this study. Results indicated that HQC significantly reduces vase life of 'Cherry Brandy' rose flowers compared to control. This is while all concentrations of this compound completely prevented microbial proliferation in vase solutions. On the other hand its low concentration application improves fresh weight gain and solution uptake during the first week of vase life. Although this compound did not have a visual and serious side effect, but it declined leaf membrane permeability and chlorophyll fluorescence of the treated flowers. Controversially chlorophyll content of HQC treated flowers increased during vase life.

**Keywords:** *Bacillus*, chlorophyll content, chlorophyll fluorescence, membrane permeability, microbial proliferation, water relation.

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### 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 extended 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], and sodium hypochlorite [4, 16, 18].

One of the major biocide applied in the cut flower business is hydroxy quinoline which is used as two forms of hydroxy quinoline sulphate [3] and hydroxy quinoline citrate (HQC) [4, 16, 18, 19, 20]. Beside being a biocide, HQC is known to inhibit ethylene production in cut flowers [21] and cut surfaces of flower stems [22]. This is while; being a germicide is its major function [6, 18].

Although previous studies have investigated the biocide role of HQC, but they 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 this article we have focused on some of the mentioned physiological properties when HQC is applied as a biocide in vase solution 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 got 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: hydroxy quinoline citrate (HQC) (200, 300 and 400 mg l<sup>-1</sup>), or sterilized distilled water (control).

### *Experimental condition:*

Cut rose flowers were kept in a laboratory with a maximum and minimum temperature of 25 ± 2 °C and 21 ± 2 °C, respectively, relative humidity (RH) of 55 ± 5 %, and light intensity of 4 μmol mm<sup>-2</sup> s<sup>-1</sup> 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].

### *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<sup>-1</sup> day<sup>-1</sup>) = (S<sub>t</sub> - S<sub>t-1</sub>); where, S<sub>t</sub> is weight of vase solution (g) at t = day 1, 2, 3, etc., and S<sub>t-1</sub> is weight of vase solution (g) on the previous day [11, 12, 23].

*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 [23]. Relative fresh weight was calculated as:  $RFW (\%) = (W_t/W_{t0}) \times 100$ ; where,  $W_t$  is weight of stem (g) at  $t = \text{day } 0, 1, 2, \text{ etc.}$ , and  $W_{t0}$  is weight of the same stem (g) at  $t = \text{day } 0$  [11, 12].

*Microbial Count:*

Microbial count was determined by taking 1ml vase solution samples during the first 6 days of the experiment, at 2 days intervals with 3 replications. One ml 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 35°C 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<sup>-1</sup> (CFU ml<sup>-1</sup>) [23].

*Microbial Identification:*

After plate counting, obtained colonies were studied and separated by their apparent morphological differences which resulted in 2 bacterial isolates. Afterwards bacterial isolates were studied by their morphological and biochemical characteristics in order to define their genus.

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, acetoin (VP), nitrate reduction, arginine dihydrolase and H<sub>2</sub>S production from cysteine [24, 25].

*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 [26]. 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 ( $F_v/F_m$ =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) [27]. Leaves were maintained in darkness for 20 min by a special clip before measurement of  $F_v/F_m$ . Minimal fluorescence ( $F_0$ ) was measured under a weak pulse of modulating light over a 0.8 s period, and maximal fluorescence ( $F_m$ ) was obtained after a saturating pulse of 0.7 s at 8000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ .  $F_v$  is the difference between  $F_0$  and  $F_m$  [27, 28].

*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:*

HQC application has an adverse effect on vase life of Cherry Brandy roses and significantly reduced vase life compared to control (sterilized distilled water) (Table 1). Vase life reduction was greatly affected as HQC concentration increased, causing the least vase life of 8.22 days for 400 mg l<sup>-1</sup> HQC treated flowers. This was while, van Doorn [16] saw a beneficial effect by adding HQC to rose vase solution and he reported that vase life was restored by adding HQC to vase solution of rose flowers when *Narcissus* flowers were kept in the same vase. Although in our study HQC decreased vase life, Jones and Hill [19], and Marousky [18] observed beneficial effect

of this biocide on vase life of ‘Gabrielle’ and ‘Better Times’ roses, respectively by applying the same concentration as us.

**Table 1: Effect of hydroxy quinoline citrate on vase life of cut ‘Cherry Brandy’ rose**

Treatment	Vase life (day)
Hydroxy Quinoline Citrate 200 mg <sup>l</sup> <sup>-1</sup>	10.00 b <sup>†</sup>
Hydroxy Quinoline Citrate 300 mg <sup>l</sup> <sup>-1</sup>	9.00 cd
Hydroxy Quinoline Citrate 400 mg <sup>l</sup> <sup>-1</sup>	8.22 d
Sterilized Distilled Water (Control)	11.67 a

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

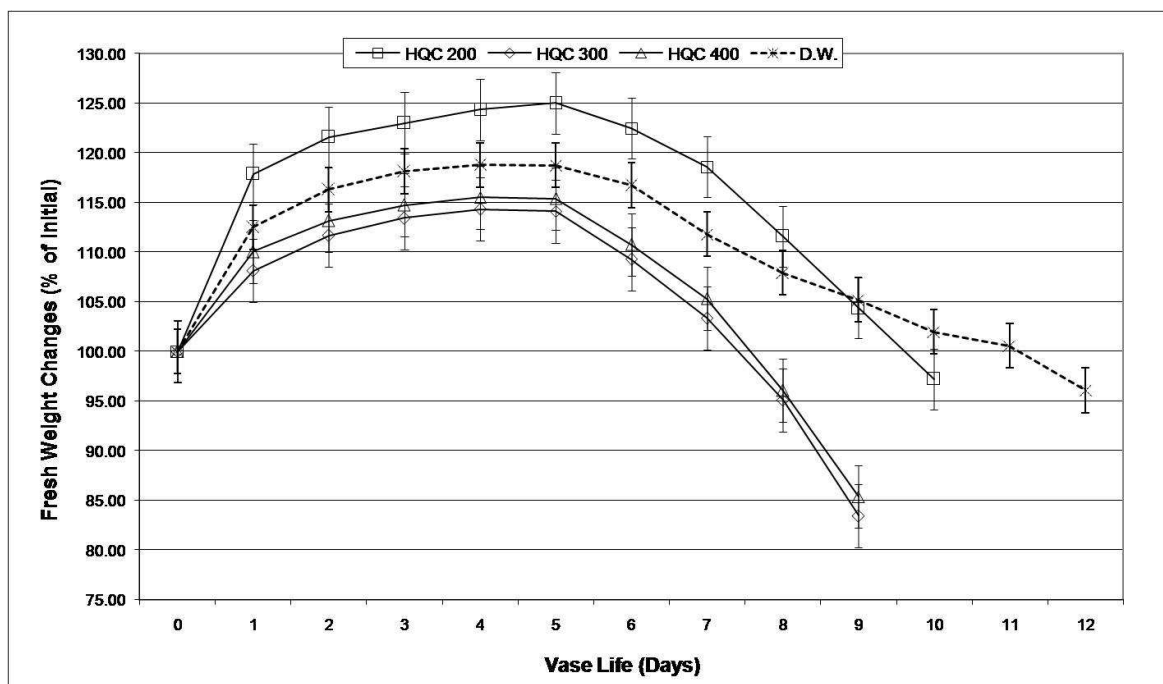
*Side effects:*

Generally, effective concentrations of biocides can be toxic to many flowers [6, 23, 29]. Van Doorn *et al.* [29] concluded that at none toxic concentrations none of the applied compounds had constant and high anti-bacterial effect.

As a side effect of HQC application, Marousky [18] saw that ‘Better Times’ roses treated with HQC turned blue. In *Narcissus tazetta* flowers, all HQC treatments caused proximal end of cut flowers to shrink and turn brown, consequently causing a loss in quality by day-3 [23]. In contrast, Knee [6] considered HQC to be one of the safest biocides for *Alstromeria*, carnations and roses. In our study, the only visible side effect of HQC on ‘Cherry Brandy’ rose flowers, was vase life reduction.

*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 4 days, relative fresh weight had a slight increase until day-5, which reached the maximum point and then weight reduction continued until the flowers vase life ended. This decrease was sharp in HQC treatments while in control flowers the trend continued at a slow rate.



**Figure 1: Relative fresh weight trend of cut ‘Cherry Brandy’ rose flowers treated with HQC. 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.**

Between the treatments 200 mg<sup>l</sup><sup>-1</sup> HQC had the most weight gain throughout the experiment until 2 days before vase life termination of its flowers. After 200 mg<sup>l</sup><sup>-1</sup> HQC treated flowers, control flowers had the most weight gain throughout the experiment. This was while the other 2 concentrations of HQC had an adverse effect on fresh weight gain of their flowers resulting in a significant lower relative fresh weight gain and a faster fresh weight loss. Therefore at the end of vase life, relative fresh weight of the other 2 concentrations of HQC reached around 85% of

the initial fresh weight. This was while fresh weight of 200 mg<sup>l</sup><sup>-1</sup> HQC and control flowers was almost the same amount of their initial fresh weight at the end of vase life.

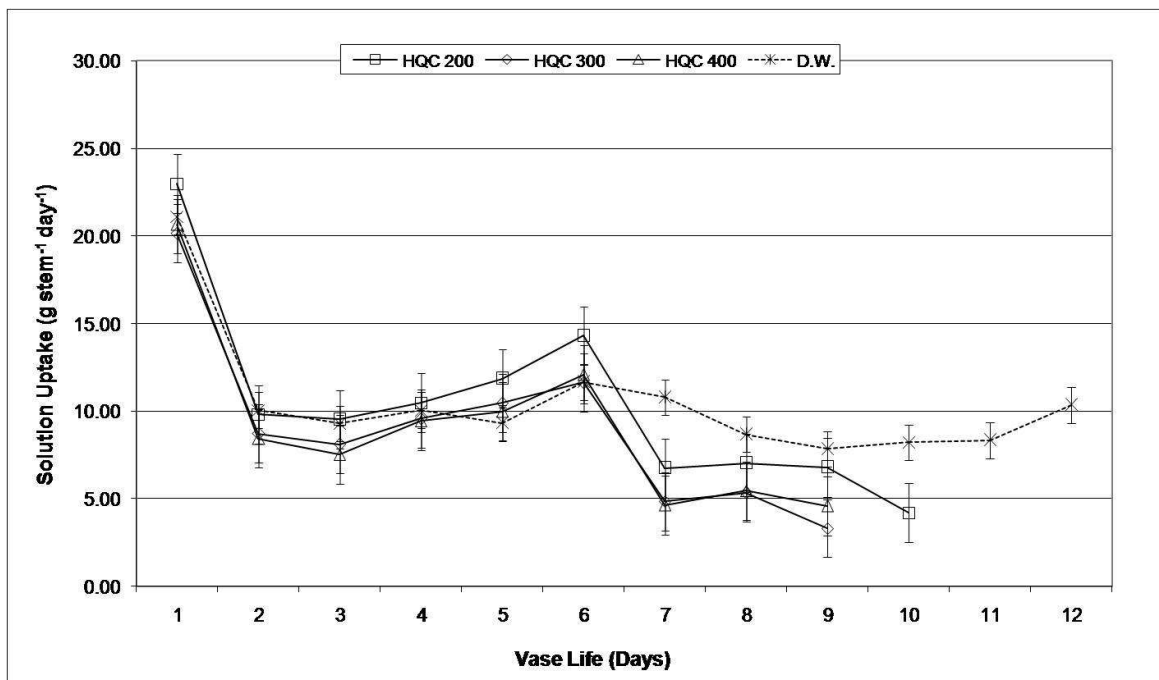
Van Meeteren *et al.* [30] observed a decrease in fresh weight of deionized treated cut flowers during the first 1–3 days of vase life. This decrease in fresh weight was not seen either in sterilized distilled water nor in HQC treated flowers of our experiment. Instead they all reached their maximum fresh weights on day-5. In fact in our experiment, sterilized distilled water had a better fresh weight change than high concentrations of HQC accompanied by a higher relative fresh weight throughout the experiment (Fig. 1) and upon vase life termination, sterilized distilled water had a significant higher relative fresh weight.

*Solution Uptake*

There was a high solution uptake in all treatments on day-1 (Fig. 2) after which there was a decrease. The highest solution uptake was seen in 200 mg<sup>l</sup><sup>-1</sup> HQC treated flowers throughout the experiment until day-6.

During the first 6 days of the experiment, in HQC treatments only 200 mg<sup>l</sup><sup>-1</sup> had a higher solution uptake compared to control (Fig. 2). With HQC concentration increment the ability of flowers to uptake solution decreased. This was while there was not a significant difference between solution uptakes of the other two HQC concentrations. Unlike our findings, Marousky [18] observed that in ‘Better Times’ roses application of 200 mg<sup>l</sup><sup>-1</sup> HQC increases water absorption and stomal closure. This was while in our study, application of HQC had an adverse effect on solution uptake. Controversially van Doorn [16] found a beneficial effect by HQC application on ‘Sonia’ roses. He managed to overcome the effect of daffodil mucilage on water up take reduction of ‘Sonia’ roses by adding HQC to vase solution.

Solution uptake trend by rose cut flowers in previous studies tended to increase initially and then decrease [7, 12]. Throughout our experiment there were two critical points of maximum solution uptake which all treatments followed (Fig. 2). Those days were day-1 and day-6. The highest solution uptake on both days (day-1:which is exactly after rehydration of flowers, and day-6) belonged to 200 mg<sup>l</sup><sup>-1</sup> HQC. This was while from day-6 onwards solution uptake dropped dramatically in all HQC treatments bellow control level and remained at a very low amount. This finding implies that HQC could have beneficial effect on solution uptake of ‘Cherry Brandy’ rose if it is applied at low concentrations for short period.



**Figure 2: Vase solution uptake trend of cut ‘Cherry Brandy’ rose flowers treated with HQC. There is a high solution uptake in all treatments on day-1. After that, there is another point of maximum solution uptake which all treatments follow.**



*Microbial count:*

In cut flowers, HQC is known to inhibit ethylene production of flowers [21] and cut surfaces of flower stems [22]. However, being a germicide is its major function [6, 18]. As seen in table 2, HQC application as ‘Cherry Brandy’ rose vase solution preservative completely prevented microbial proliferation and therefore HQC vase solutions did not contain any microbes even after 6 days of experiment. The same was observed in Jowkar’s [23] previous findings. In his experiment HQC was one of the most effective compounds for controlling microbial growth and proliferation of narcissus vase solution. Van Doorn [16] has also showed that HQC prevented microbial proliferation in rose vase solution. On the other hand van Doorn and Perik [4] showed that HQC limits microbial proliferation in stems of ‘Sonia’, ‘Ilona’, ‘Polka’ and ‘Frisco’ roses bellow detection limit. They concluded that HQC and low pH prevent vascular blockage by reducing the number of bacteria in the stem. Bleeksma and van Doorn [10] have also found that HQC suppressed bacterial growth and prevented the increase in ultrasonic acoustic emissions frequency within the treated stems.

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 [23].

**Table 2: Effect of hydroxy quinoline citrate on cut ‘Cherry Brandy’ rose vase solution microbial count during day-2, -4, and -6.**

Treatment	Microbial Count <sup>†</sup> (log <sub>10</sub> CFU ml <sup>-1</sup> ) <sup>††</sup>		
	Day-2	Day-4	Day-6
Hydroxy Quinoline Citrate 200 mg l <sup>-1</sup>	0 b <sup>†††</sup>	0 b	0 b
Hydroxy Quinoline Citrate 300 mg l <sup>-1</sup>	0 b	0 b	0 b
Hydroxy Quinoline Citrate 400 mg l <sup>-1</sup>	0 b	0 b	0 b
Sterilized Distilled Water (Control)	4.477 a	6.469 a	9.203 a

<sup>†</sup> Microbe counts, except a zero count, are reported as log<sub>10</sub>x (x = microbe counts).

<sup>††</sup> The number of microorganisms was counted by the standard plate counting method and expressed as Colony Forming Units ml<sup>-1</sup> (CFU ml<sup>-1</sup>).

<sup>†††</sup> 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 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 [33] identified 25 different microorganisms, in this experiment only 2 microbial types were seen. It seems that fewer microbe types were due to lower flower contamination and integrated management applied during flower production.

As HQC completely suppressed microbial growth and proliferation, contamination was only seen in control vase solution. The isolated microorganisms from control vase solution were 2 different kinds of bacterial isolates. This is consistent with other published data [14, 23, 31, 32].

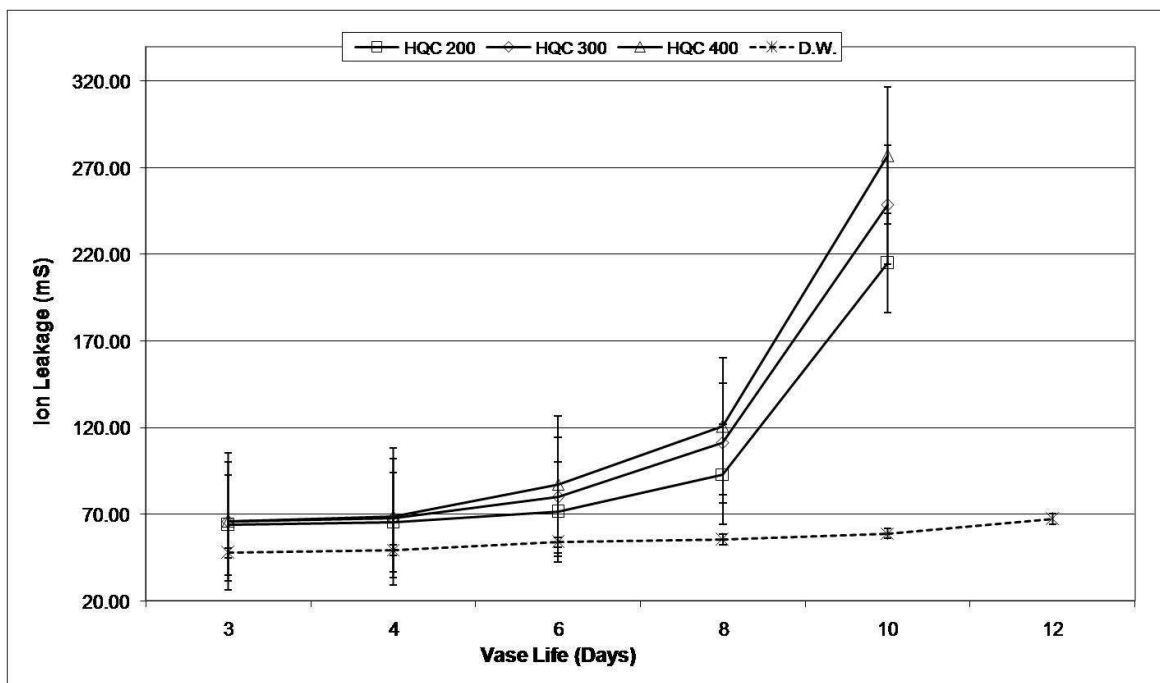
The separated bacterial isolated were 2 different strains of *Bacillus*. This is while in previous studies other different bacterial strains were seen. For example, bacterial strains found in rose stems by van Doorn *et al.* [34] were: Pseudomonads (80 %), Enterobacteria (5-10%); and some other genera such as *Aeromonas*, *Acinetobacter*, *Alcaligenes*, *Citrobacter*, and *Flavobacterium*. In another study they had Pseudomonads and Enterobacteria as the dominant bacterial strains in stems of cut ‘Sonia’ roses [5]. 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’ [33].

As same as our study, in previous studies, *Bacillus* has been the most common occurring vase solution microorganism [23, 31, 32, 35]. Depending on experiment condition and production system, other dominant types of bacteria have been seen. For example, van Doorn *et al.* [36] 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 [37]. 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 [5] 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 [38]. These facts explain the difference between the microbial contamination in our study and others.

*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 Brandy’ roses. Although in control flowers ion leakage had an increasing trend throughout the experiment, but the increasing amount was very little compared to other treatments. This was while in HQC treatments, ion leakage increment was higher than control and it had a sudden increase from day-6 onwards. During the increment trend, leaf ion leakage increased with HQC concentration increment. This resulted in 2-3 folds increase in leaf ion leakage of HQC treated flowers.



**Figure 3: Leaf ion leakage trend of cut ‘Cherry Brandy’ rose flowers treated with HQC. HQC has harmed membrane permeability and consequently increased ion leakage.**

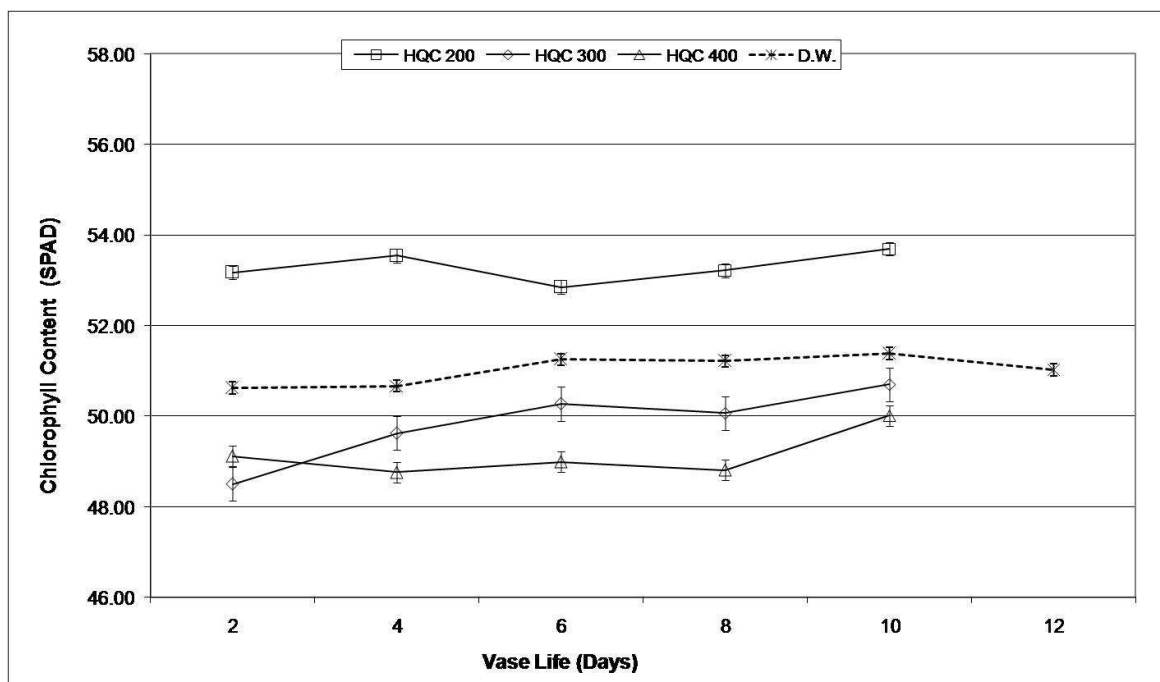
Guiboileau *et al.* [39] have mentioned membrane lipids degradation as leaf senescence progress which results in ion leakage. Maalekuu *et al.* [40] have considered ion leakage as an index of membrane integrity and damage in plants during senescence. Our results confirm this issue and show that leaf ion leakage increases with aging. Like us, Sultan and Farooq [41] have shown that senescence of cut flower is associated with ion leakage increment. Oren-Shamir *et al.* [42] have also found that in cut ‘Mercedes’ rose ion leakage increases during senescence progress. Sood *et al.* [43] found same results for *Rosa bourboniana* and *Rosa 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.* [42] saw that ion leakage trend did not change until day-4 and after that it increased. Sood *et al.* [43] 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.

Same as our findings, Skutnik *et al.* [44] found that HQC cannot prevent nor retard ion leakage of *Zantedeschia* flowers during senescence. This was while Prashanth and Chandrasekhar [45] saw that hydroxyl quinoline as hydroxyl quinoline sulfate reduces ion leakage of ‘Yanana’ gerbera up to 6% of control. Similar to our finding, the least gerbera ion leakage belonged to 200 mg<sup>l</sup><sup>-1</sup> concentration. Like our findings, Gul *et al.* [46] found that hydroxyquinoline increases ion leakage of *Nerine sarniensis* cv. ‘Red’. It seems that ion leakage increment due to HQC application has been caused by membrane injury and consequently loss of membrane permeability.

*Chlorophyll Content*

Chlorophyll content measurements showed fluctuation during vase life of ‘Cherry Brandy’ roses in all treatments. Generally chlorophyll content of HQC treated flowers increased during vase life (Fig. 4). This was while in control flowers there was not much difference in chlorophyll content change. The only HQC treatment that had higher chlorophyll content than control throughout the experiment was 200 mg<sup>l</sup><sup>-1</sup> concentration. This treatment had also the least chlorophyll content increment.



**Figure 4: Leaf chlorophyll content trend of cut ‘Cherry Brandy’ rose flowers treated with HQC. Leaf chlorophyll levels of HQC treated flowers showed fluctuation during vase life.**

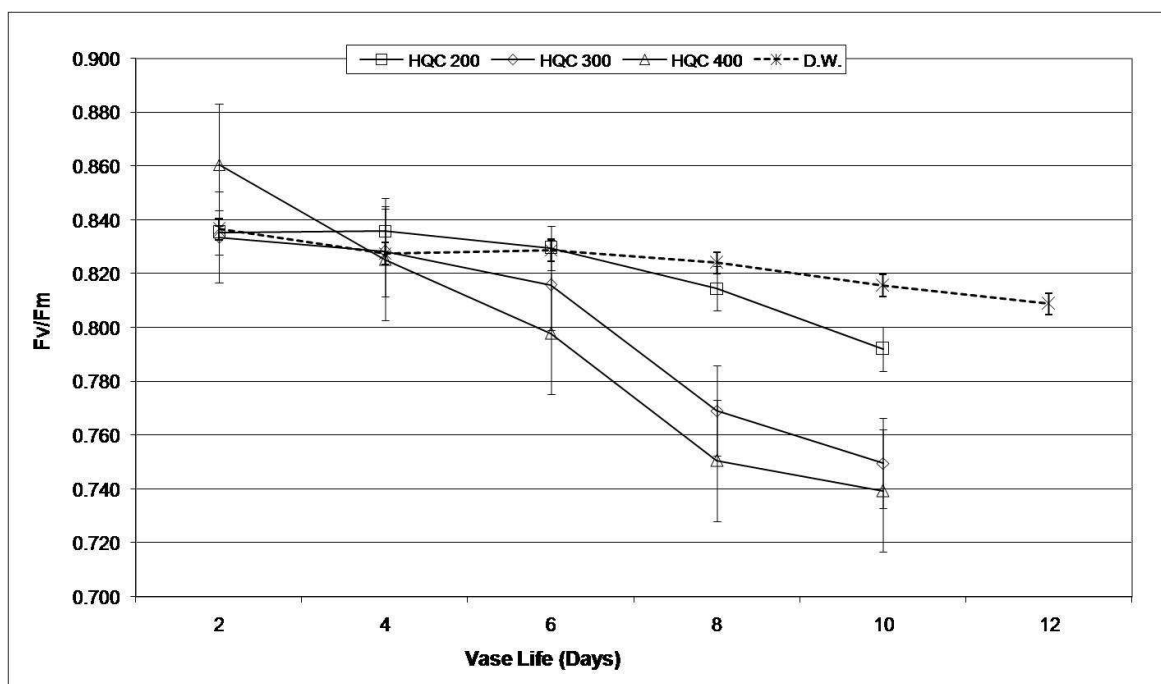
Previously it has been shown that leaf chlorophyll content decreases during senescence [39, 47, 48,]. Senescence delay and chlorophyll preservation has been achieved by various compounds which mostly have growth regulatory behavior such as: GA [48, 49], benzyladenine [50] and tidiazuron [48, 49]. Although in our experiment all HQC treated flowers showed chlorophyll content increment compared to their initial content, previous studies have shown undesirable effect of hydroxy quinoline on leaf chlorophyll content. For example, KiCheol *et al.* [51] found that treating ‘Red Sandra’ roses with hydroxy quinoline reduces photosynthesis rate and leaf chlorophyll content compared with control. Lee and Kim [52] reported the unbeneficial effect of hydroxy quinoline on chlorophyll content of ‘Red Sandra’ roses at 200 mg<sup>l</sup><sup>-1</sup> concentration by chlorophyll content reduction. Similar findings have been seen in other cut flowers. Skutnik *et al.* [44] has also found that HQC cannot retard chlorophyll content loss during senescence.

*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 HQC treated flowers, chlorophyll fluorescence decreased with concentration increment. In HQC treated flowers with HQC increment, chlorophyll fluorescence declined rapidly as the flowers reached the end of their vase life. The least chlorophyll fluorescence reduction in HQC treated flowers was observed in 200 mg<sup>l</sup><sup>-1</sup> treated flowers. This treatment did not show chlorophyll fluorescence reduction until day-6. This was while in control flowers which had the least chlorophyll fluorescence reduction at vase life termination, chlorophyll fluorescence dropped at day-4. There was also increment in chlorophyll fluorescence of 400 mg<sup>l</sup><sup>-1</sup> treated flowers at day-2 which is due to the damage caused by high concentration of HQC. The toxicity of this treatment caused a dramatically sharp decrease in chlorophyll fluorescence of treated flowers and consequently an increase in chlorophyll content as mentioned before (Fig. 4).

Similar to our findings, Tang *et al.* [47] have reported that with senescence initiation and progress, quantum yield of both photo system I and II decreases. Niewiadomska *et al.* [53] 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.* [54] 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.





**Figure 5: Leaf chlorophyll fluorescence trend of cut ‘Cherry Brandy’ rose flowers treated by HQC application. Generally, chlorophyll fluorescence declines during vase life.**

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 HQC application in cut ‘Cherry Brandy’ rose. Increase in chlorophyll content of HQC 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 HQC absorption to some extent.

### CONCLUSION

Our findings regarding the application of HQC on ‘Cherry Brandy’ roses, questions Reid and Kofranek's [55] recommendations for standardized vase life evaluation due to vase life decline by HQC application on this cultivar. Since they recommended the application of HQC as a control biocide in postharvest studies on cut flowers. But it agrees with incorporation of distilled water in vase life experiments as suggested by them.

As biocides are integrated in floral preservatives to sustain solution clarity and to avoid blockage of xylem elements by microorganisms [6]. From this aspect, HQC was completely effective as it did not allow microbial proliferation until day-6.

Form physiological point of view HQC did not result in vase life improvement. Although solution uptake and fresh weight gain was improved during the first week of experiment by low continues application of HQC. Controversially, its application resulted in physiological deprived function due to membrane permeability and chlorophyll fluorescence decline. Considering different studied aspects, application of lower concentration of this compound could be recommended.

As our report is the first report on physiological changes by HQC application during vase life of cut rose flowers and that no other reports have studies HQC application in such physiological approach, it provides valuable information on different aspects of HQC application as vase solution preservative. This is while further studies are needed to focus on its effect on physiological function of other cultivars in order to fully recommend or oppose its application.

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