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Comprehensive Microbial Study on Biocide Application as Vase Solution Preservatives for Cut 'Cherry Brandy' Rose Flower

Mohammad Mahdi Jowkar[1,2], Nader Hassanzadeh[3], Mohsen Kafi[4*] and Ahmad Khalighi[1]

1. Department of Horticultural Sciences, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran.

2. Department of Horticulture, College of Agriculture, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran.

3. Department of Plant Pathology, College of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran.

4. Department of Horticulture and Landscape Design, College of Agricultural Science and Engineering, University of Tehran, Karaj, Iran.

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Abstract

Disturbance in water relations is the major causes of vase life reduction and senescence in cut flowers. This problem is mainly due to microorganism proliferation in the vase solution which leads to vascular occlusion and reduction in solution uptake by cut flowers. Therefore a comprehensive study was conducted to evaluate the biocidal effect of nano silver particles (NSP) and compare it with some previously applied biocides. Roses (cv. 'Cherry Brandy') were treated in a completely randomized design with: colloid of NSP, citric acid, aluminum sulfate, hydroxyquinoline citrate (HQC), calcium hypochlorite, sodium hypochlorite (NaOCl), tap water, or sterilized distilled water as vase water or solution. Longest vase life was observed in flowers treated with nano silver particles, aluminum sulphate and citric acid, respectively. Nano silver particles, HQC and calcium hypochlorite were the most effective treatments in controlling microbial population followed by aluminum sulfate as the second effective treatment. Nano silver particles, HQC and calcium hypochlorite completely inhibited the microbial growth during the first six days of experiment. Moreover, aluminum sulfate retarded microbial growth, proliferation and growth rate more efficiently than others. Each treatment allowed proliferation of a specific microbe. In general, two yeasts, six fungi, and 26 bacterial colonies were isolated from different vase solutions. Among the isolated fungi, one isolate was *Trichoderma harzianum* and the five other were different strains of *Fusarium solani*. Identified bacterial isolates were *Bacillus* sp., *Coccus* spp., *Streptomyces* sp., *Pectobacterium* sp., *Burkholderia* sp., and *Pseudomonas* sp. *Bacillus* was the most wide spread microorganism in most treatments. Identified *Bacillus* sp. isolates were *B. polymexa*, *B. subtilis*, *B. megaterium* and *B. circulans*. Since nano silver significantly improved vase life and effectively controlled microbial proliferation in vase solution, our results suggest that nano silver application could be considered as a biocidal preservative solution for rose cut flowers.

Keywords: Aluminum sulfate, *Bacillus subtilis*, Calcium hypochlorite, Hydroxyquinoline citrate, Nano silver, Sodium hypochlorite, *Trichoderma harzianum*.

Abbreviations: HQC, Hydroxyquinoline Citrate; **NS,** Nano Silver; **NSP,** Nano Silver Particles; **NaOCl,** Sodium Hypochlorite.

* Corresponding Author, Email: mkafi@ut.ac.ir

Introduction

Rose is one of the most important cut flower in the ornamental market. One of the problems that this cut flower encounters is vase life reduction after harvest. Many factors affect cut flowers' postharvest life which are mainly: dehydration (Knee, 2000; Lu et al., 2010), assimilates and substrates losses (Ichimura et al., 2005), ethylene induced senescence (Liao et al., 2000), and cell programmed death (Eason et al., 2002). Between the mentioned, dehydration and loss of water balance have a major role. Water relation disruption and consequently dehydration is caused by many factors such as heat stress after harvest, blockage of vascular bundles, loss of turgidity as a result of transpiration after flower cutting and a decline in water uptake ability.

It has been reported that vase solution bacteria cause vase life decline by various means such as i) obstruction of cut flower stem basal end (Ohta and Harada, 2000; Bleeksma and van Doorn, 2003; He et al., 2006; Robinson et al. 2007; Liu et al., 2009a, Ratnayake et al., 2012; Suwannateep et al., 2013; Iftikhar et al., 2016), ii) release of pectinases and toxic compounds, and iii) ethylene production (Williamson et al., 2002). Besides vase life reduction, loss of turgidity and disruption of water relation cause predominantly physiological disorders such as lack of flower opening (Bleeksma and van Doorn, 2003), bent neck (Bleeksma and van Doorn, 2003; Muriithi and Ouma, 2011; Iftikhar et al., 2016), premature senescence (Macnish et al., 2008), leaf wilting accompanied by improper opening and wilting of flowers (Torre and Fjeld, 2001; Bleeksma and van Doorn, 2003). Considering the fact that vase solution microorganisms interrupt water relation of rose cut flowers and eventually results in various post-harvest defects in cut flowers, reduction of microbial proliferation has a great importance in postharvest studies of cut flowers particularly in roses.

Various compounds and chemicals have been used in order to prevent microbial growth and proliferation in vase solutions of cut flowers. For rose cut flowers some of the most commonly used biocides have been: silver thiosulfate (Liao et al., 2000), silver nitrate (Torre and Fjeld, 2001; Pompodakis et al., 2004), hydroxyquinoline citrate (Solgi et al., 2009; Wu et al., 2016), hydroxyquinoline sulfate (Liao et al., 2000; Lee and Kim, 2012), aluminum sulfate (Singh et al., 2004; Ichimura and Shimizu-Yumoto, 2007; Vasudevan and Kannan, 2014) and sodium hypoclorite (Macnish et al., 2008).

Some of these compounds such as silver thiosulfate and silver nitrate have shown environmental threats and health hazards (Damunupola and Joyce, 2006). Recently new efficient biocides with low toxicity have emerged. Nano silver is one which is more efficient due to higher surface area to volume ratio compared to other forms of silver (Jiang et al., 2004). Formerly nano silver was extensively used as an antibacterial agent in various industrial products such as textile, home appliances, cosmetics and pharmaceutics (Jiang et al., 2004). Recently application of nano silver as cut flower treatment for vase life improvement and ethylene senescence inhibitor has been studied by various researchers on cut flowers such as Rose (Lu et al., 2010; Kader, 2012; Li et al., 2012; Jowkar et al., 2013; Hashemabadi et al., 2014), Lilium (Nemati et al., 2013 and 2014), Carnations (Liu et al., 2009b, Kazemi and Ameri, 2012), Gladiolus (Hasanpoorasl et al., 2014) and Gerbera (Liu et al., 2009a;b; Solgi et al., 2009). In the previous studies, a comprehensive study on biocidal effect of this compound and its application as a vase solution biocidal preservative has not been conducted from microbial aspects. This requirement is more essential on rose cut flowers which hold a very large portion of cut flower market and industry, and its cultivars benefit both from the biocidal and

the ethylene antagonistic result of silver particles.

Regarding the importance of the rose cut flowers in the ornamental business and the influence of vase solution microbes on vase life, and also, in order to find an easy to use, non-toxic and inexpensive compound for large scale application through supply chain (wholesalers), the effects of some conventional biocides and NSP on 'Cherry Brandy' rose vase solution microbial flora and proliferation has been investigated in the present study.

Materials and Methods

Plant material

Rose (*Rosa* × *hybrida*) cultivar namely 'Cherry Brandy' (licensed by Rosen Tantau, Uetersen, Germany) were harvested at commercial maturity stage from rose plants grown in an automatic greenhouse with hydroponic perlite culture system. Commercial maturity stage of flowers was when the outer flower petals starting to reflex and inner petals became visible. Flowers were harvested early in the morning and transferred to experimental laboratory within 1 hour after harvest. All the leaves except for the 5 most upper leaves of each flower were removed and stems were recut slantly under water so that all flowers reach a height of 40 ± 5 cm before treatment in order to remove probable air emboli.

Experimental design and treatments

Cut flowers were treated with different biocides in a completely randomized design of 20 treatments each with 4 replications (4 vases, each containing 3 flowers). Treatments (biocides) applied as vase solutions were: aluminum sulfate (at 100, 200 or 300 mg l^{-1}), citric acid (at 300, 600 or 900 mg l^{-1}), hydroxyquinoline citrate (HQC) (at 200, 300 or 400 mg l^{-1}), sodium hypochlorite (NaOCl) (at 400, 600 or 800 mg l^{-1}), calcium hypochlorite ($Ca(ClO)_2$) (at 400, 600 or 800 mg l^{-1}), colloid of NSP(1, 2.5 and 5 %) (Nanocid L2000, Nano Nasb Pars Co. Ltd., Tehran, Iran), tap water (TW) and sterilized distilled water (SDW) as control. Vase solutions were not changed throughout the experiment and when vase solution reduced, sterilized distilled water was added to reach the volume of 500 ml.

Experimental condition

Cut rose flowers were kept in a postharvest laboratory with a relative humidity (RH) of 55± 5 %, maximum and minimum temperature of 25 ±2 ºC and 21 ±2 ºC, respectively. Light was provided by white fluorescent lamps from 07.00 to 20.00 h with an intensity of 14 µmol mm^{-2} s^{-l}.

Vase life

Flowers were daily checked and their appearance was recorded. Vase life termination was considered when bent neck was observed or five outer petals were wilted.

Microbial population

Microbial population was determined by Plate Count Method as described by Jowkar et al. (2012). In the mentioned, one millilitre of vase solution sample was taken from vase solutions containing cut flowers. Samples were serial diluted up to 10 folds and each dilution were cultured on a broad spectrum nutrient agar medium at 2 days intervals (day-2, day-4 and day-6) and 3 replication. Plated samples were incubated at 35ºC for 48 hours to allow microorganisms growth. Formed colony units after incubation were considered as number of microorganisms present in vase solution and are reported as colony forming units.ml^{-1} (CFU ml^{-1}) (Jowkar, 2006).

Microbial growth

Microbial growth (MG) was calculated as: $MG=Log10\ (M_t-M_{t-1})$; where M_t is the microbe count on the measuring day and M_{t-1} is the microbe count on previous measuring day.

Microbe growth rate

Microbe growth rate (MGR) was calculated as: MGR=Log10 [$(M_t-M_{t-1})/ M_{t-1}$]; where M_t is the microbe count on the measuring day and M_{t-1} is the microbe count on previous measuring day.

Microbial identification

After plate counting, obtained colonies were studied and separated by their apparent morphological differences. This resulted in two yeasts, six fungi and 26 bacterial isolates. Fungi were determined by culturing a piece of infected nutrient agar on potato dextrose agar medium (PDA) and incubation at 35ºC for 7 days. The genus of the fungi were determined according to their colour and appearance (Steinkellner, 2004; Siddiquee et al., 2009). Yeast were only recognized during staining and due to their complexity, their genus was not identified. In order to identify the species, bacterial isolates were purified and differentiated according to their typical morphological and biochemical characteristics according to Schaad et al. (2001) and Janse (2005).

Bacterial morphological studies were: cell shape, capsule presence and motility. Bacterial bioassays were: hypersensitivity test on tobacco and potato soft rot bioassay. The biochemical tests carried out on isolated bacterial colonies were: aerobic/anaerobic growth, gram reaction using KOH, acid production from glucose, gas production from D-glucose, fluorescent pigments production on KB, catalase test, oxidase test, gelatin hydrolysis, growth at 5.7 pH, levan, growth at 50°C, starch hydrolysis, tween 80 hydrolysis, indol production, methyl red reaction, nitrate reduction, aceteoin (VP), arginine dihydrolase and H_2S production from cysteine (Schaad et al., 2001; Janse, 2005).

Statistics

Data were analysed by one way ANOVA using MSTAT-C software. Means were compared by the least significant difference test (LSD) at the 0.05 and 0.01 probability level (P=0.05 and 0.01). The correlation between pH and microbial count was computed using SPSS 16.0 software.

Results and discussion

Vase life

Results indicate that all applied biocides except for HQC, sodium hypochlorite and calcium hypochlorite increased vase life of ‘Cherry Brandy’ cut rose flowers when compared to control. The most vase life was observed in NSP treated flowers with a value of 13.78 days (Table 1). Various research (Liu et al., 2009b; Lu et al., 2010; Kader, 2012; Hashemabadi et al., 2014) have recently showed the beneficial effect of NSP application as pulse or vase solution preservative for cut flowers, especially roses. Similar to our findings, Liu et al. (2009b) reported fivefold increase in vase life of ‘Movie Star’ roses by pulse application of NSP compared to deionized water. Lu et al. (2010) also reported a significant vase life improvement by pulse application of NSP on ‘Movie Star’ roses. Kader (2012) has showed an increase for vase life of ‘Tineke’ cut rose flowers. Similarly, Hashemabadi et al. (2014) observed an increase in vase life of ‘Yellow Island’ cut rose flowers by NSP application. Although an increase in vase life was observed in the present study, there was not a significant difference between various applied concentrations of NSP. This was also correspondingly described by Solgi et al. (2009) for gerbera flowers. After NSP, aluminum sulfate and citric acid resulted in the highest vase life compared to the control plants (Table 1). This is while previous reports such as Knee (2000) mentioned aluminum aulfate as an ineffective biocide for ‘Classy’ roses; while Ketsa and Kosonmethakul (2001) reported the beneficial effect of aluminum sulfate on *Dendrobium* orchids. In consistent with our findings, Singh et al.

(2004) reported vase life improvement of seven commercial rose cultivars such as 'Confidence', 'First Red', 'Grand Gala', 'Kiss', 'Pareo', 'Sangria', and 'Starlite' by aluminum sulfate and citric acid application.

Although various reports such as Knee (2000) and Bleeksma and van Doorn (2003) on roses, Wang et al. (2014) on gerbera and Jowkar (2006) on narcissus have been published regarding the beneficial effect of HQC, our findings showed a negative undesirable effect by HQC application on vase life of 'Cherry Brandy' cut rose flowers. Similarly negative effect has been observed on sodium and calcium hypochlorite. This also has been while some research (van Doorn and Cruz, 2000; Singh et al., 2004; Jowkar, 2007; Macnish et al., 2010) have reported the beneficial effect of chlorine on vase life of cut rose flowers.

Microbial population

The biocides that are integrated in floral preservatives sustain solution clarity and avoid xylem elements blockage by microorganisms (Knee, 2000). Among different applied biocides, nano silver, HQC and $Ca(ClO)_2$ were the most effective treatment. They did not allow microbial proliferation until day-6 (Table 2).

Many researchers have found that nano silver application inhibits growth of vase solution microorganisms (Liu et al., 2009a; Solgi et al., 2009; Lu et al., 2010; Li et al., 2012; Kader, 2012; Kazemi and Ameri, 2012; Jowkar et al., 2013; Nemati et al., 2013 and 2014; Hashemabadi et al., 2014; Hasanpoorasl et al., 2014). Among them, Li et al. (2012) reported that nano silver significantly alleviates bacterial related blockage of 'Movie Star' rose xylem vessels. The mentioned reports indicate that when nano silver application was applied as pulse treatment, biocidal benefits were transient (Liu et al., 2009a; Lu et al., 2010; Li et al. 2012; Kader, 2012). In the mentioned case, nano silver pulse treatment inhibited bacteria growth in the vase solution and at cut stem ends during the first days. However numbers of vase solution bacteria increased throughout the vase life (Liu et al., 2009a; Lu et al., 2010). In our study, nano silver inhibited

Table 1. Effect of different biocides on vase life of cut 'Cherry Brandy' rose flowers.

Treatment	Vase life (Day)
Citric Acid 300 mgl^{-1}	12.44 bcd[†]
Citric Acid 600 mgl^{-1}	11.56 d
Citric Acid 900 mgl^{-1}	11.78 d
Aluminum Sulfate 100 mgl^{-1}	12.89 abc
Aluminum Sulfate 200 mgl^{-1}	12.22 cd
Aluminum Sulfate 300 mgl^{-1}	12.33 bcd
Hydroxy Quinoline Citrate 200 mgl^{-1}	10.00 e
Hydroxy Quinoline Citrate 300 mgl^{-1}	9.00 fg
Hydroxy Quinoline Citrate 400 mgl^{-1}	8.22 g
Calcium Hypochlorite 400 mgl^{-1}	6.44 h
Calcium Hypochlorite 600 mgl^{-1}	6.00 h
Calcium Hypochlorite 800 mgl^{-1}	6.00 h
Sodium Hypochlorite 400 mgl^{-1}	9.55 ef
Sodium Hypochlorite 600 mgl^{-1}	8.44 g
Sodium Hypochlorite 800 mgl^{-1}	8.33 g
Nano Silver 1%	13.78 a
Nano Silver 2.5%	13.22 ab
Nano Silver 5%	13.22 ab
Sterilized Distilled Water (Control)	11.67 d
Tap Water	11.56 d

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

Table 2. Effect of different biocides on cut 'Cherry Brandy' rose vase solution microbial population at days-2, 4 and 6.

Treatment	Microbial Count† (log10 CFU ml^{-1})††		
	Day-2	Day-4	Day-6
Citric Acid 300 mgl^{-1}	1.690 c†††	5.918 b	8.505 b
Citric Acid 600 mgl^{-1}	1.151 d	5.800 c	7.318 c
Citric Acid 900 mgl^{-1}	1.151 d	5.792 c	6.778 d
Aluminum Sulfate 100 mgl^{-1}	0 e	0 f	3.322 h
Aluminum Sulfate 200 mgl^{-1}	0 e	0 f	2.539 i
Aluminum Sulfate 300 mgl^{-1}	0 e	0 f	2.128 j
Hydroxy Quinoline Citrate 200 mgl^{-1}	0 e	0 f	0 k
Hydroxy Quinoline Citrate 300 mgl^{-1}	0 e	0 f	0 k
Hydroxy Quinoline Citrate 400 mgl^{-1}	0 e	0 f	0 k
Calcium Hypochlorite 400 mgl^{-1}	0 e	0 f	0 k
Calcium Hypochlorite 600 mgl^{-1}	0 e	0 f	0 k
Calcium Hypochlorite 800 mgl^{-1}	0 e	0 f	0 k
Sodium Hypochlorite 400 mgl^{-1}	4.175 b	5.360 d	7.321 c
Sodium Hypochlorite 600 mgl^{-1}	0 e	0 f	4.929 f
Sodium Hypochlorite 800 mgl^{-1}	0 e	0 f	4.477 g
Nano Silver 1%	0 e	0 f	0 k
Nano Silver 2.5%	0 e	0 f	0 k
Nano Silver 5%	0 e	0 f	0 k
Sterilized Distilled Water (Control)	4.477 a	6.469 a	9.203 a
Tap Water	1.840 c	4.562 e	6.264 e

†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.

the microbial growth and proliferation throughout the first 6 days of experiment (Table 2). Which could be explained by treatment type (preserving solution) and higher concentration of applied nano silver in our research. Our findings revealed that in order to have a prolonged anti-microbial effect, low-continues application of nano silver with a fine particle size and concentration as vase solution is recommended. This finding is in accordance to Kader's (2012) report. He also found that application of low concentration of nano silver as holding solution, effectively supress the bacterial growth and proliferation compared to the pulse application.

HQC is a widely applied biocide in cut flower industry and has been an effective compound in postharvest research (Knee, 2000). As observed in Jowkar's (2006) findings, HQC was one of the most effective compounds for controlling microbial growth and proliferation. Vase solutions containing HQC did not contain any microbes, even after 6 days of experiment. The same results regarding HQC application was observed in our study (Table 2). Similarly, Bleeksma and van Doorn (2003) found that HQC suppressed bacterial growth within both vase solution and cut flower stem and consequently prevented the increase in ultrasonic acoustic emissions frequency within the treated cut flower stems. Singh et al. (2004) reported that 8-HQC considerably controlled vase solution bacterial growth in seven commercial rose cultivars such as 'Confidence', 'First Red', 'Grand Gala', 'Kiss', 'Pareo', 'Sangria', and 'Starlite'. Wang et al. (2014) reported similar finding on Gerbera. They observed that application of 0.45 mM 8-HQC decreased stem blockage and reduced bacterial growth in cut Gerbera vase solution of cv. 'Hongyan'.

Calcium hypochlorite is one of the most common forms of applied chlorine in postharvest chlorination. This compound was completely effective in controlling microbial proliferation throughout our study at all concentrations. Meanwhile for *Narcissus* cut flowers, Jowkar (2006)

found the effectiveness of this compound at high levels (800 mgl^{-1}). Similar to Jowkar's (2006) findings, it was also observed that at the same concentrations, calcium hypochlorite is more effective than sodium hypochlorite.

Following the mentioned treatments, NaOCl and aluminum sulfate were also effective in controlling microbial population and proliferation to some extent. All concentrations of aluminum sulfate inhibited microbial proliferation by the end of day-4. On day-6, small contamination was observed, indicative of a decrease with higher concentrations, therefore 300 mgl^{-1} aluminum sulfate caused the minimum contaminated level on day-6 (Table 2). Similarly, Singh et al. (2004) observed that 300 ppm aluminum sulfate significantly decreased vase solution bacterial number and improved vase life of seven commercial rose cultivars such as 'Confidence', 'First Red', 'Grand Gala', 'Kiss', 'Pareo', 'Sangria', and 'Starlite'. On the other hand, for *Narcissus tazetta*, aluminum sulfate was among the least effective compounds in controlling microbial proliferation (Jowkar, 2006). This could be described by the low solubility of aluminum hydroxides.

Although sodium hypochlorite is a wide spectrum biocide with strong oxidating capability, it is commonly used when the scale of postharvest chlorination is limited. Different studies have shown the positive effect of NaOCl on microbial proliferation prevention (Xie et al., 2008). Bleeksma and van Doorn (2003) observed that NaOCl suppressed bacterial proliferation and consequently decreased the ultrasonic acoustic emissions frequency. Van Doorn and Cruz (2000) reported that NaOCl application as pulse treatment provisionally reduces bacterial counts in cut chrysanthemum flower stems until day-4. Singh et al. (2004) observed that 125 ppm chlorine significantly decreased vase solution bacterial count and improved vase life of seven commercial rose cultivars such as 'Confidence', 'First Red', 'Grand Gala', 'Kiss', 'Pareo', 'Sangria', and 'Starlite'. Beside vase life improvement, NaOCl application as postharvest dip in 200 μl^{-1} for 10s provided the greatest inhibitory effect on *Botrytis cinerea* in 'Akito' and 'Gold Strike' rose flowers (Macnish et al., 2010). In our study NaOCl treatment did not inhibit microbial proliferation efficiently and with a significant difference it was the least effective treatment after sterilized distilled water. Similar to van Doorn and Cruz (2000) reports, we only saw this compound's efficiency until day-4. Compared to Macnish et al. (2010) and Singh et al. (2004), we found efficiency of sodium hypochlorite in higher concentrations. Jowkar (2007) recommended NaOCl as the best treatment for tuberose cv 'Gol Dorosht-e-Mahallat' due to low toxicity and better microbial proliferation control. Macnish et al. (2008) suggested application of aqueous ClO_2 as an alternative antibacterial vase solution agent for many cut flowers such as *Alstroemeria*, *Antirrhinum*, *Dianthus*, *Gerbera* and 'Charlotte' rose.

Most cut flowers vase preserving solutions contain a pH reducing agent. In the present study, we observed a partial microbial proliferation control by citric acid application in vase solution which was not efficient. By reducing the pH of vase solution bellow 2.7 (in 900 mgl^{-1} citric acid) (Table 2), still a high number of the microbes was observed. Our findings are in accordance to Singh et al. (2004); they did not observed desirable microbial control in vase solution of three cut rose cultivars ('Grand Gala', 'Sangria' and 'Kiss') by application of citric acid.

There has been a long dispute regarding the application of sterilized distilled water or tap water as control in postharvest studies of cut flowers. Tap water is the most available vase solution source, while distilled water is not widely accessible. In the present study, tap water was more effective in controlling microbial proliferation compared to the sterilized distilled water (control). Sterilized distilled water had a relatively high microbial

contamination on day-2, compared to tap water which its microbial count on day-4 was almost the same number of sterilized distilled water on day-2. This could be due to the application of sanitary compounds (mostly chlorine derivatives) by Municipal Water Company. Similar to our findings, observed results on cut *Narcissus* study indicate a significant difference in microbial counts between the tap water and sterilized distilled water treatments (Jowkar, 2006). Compared to tap water, sterilized distilled water contained less microbe population, however, neither sterilized distilled water nor tap water had any desirable effect in controlling or reducing microbial population within *Narcissus* vase solution (Jowkar, 2006). Considering the long standing dispute, our results indicate sterilized distilled water as a reliable treatment for postharvest studies of cut flowers.

Microbial growth

Among the treatments that were not able to prevent microbial proliferation, sterilized distilled water had the highest microbial growth during the first 6 days of the experiment (Table 3). After sterilized distilled water, the highest microbial growth during the experiment belonged to the citric acid group. The minimum microbial growth during phase one belonged to the tap water. Throughout the experiment, tap water had a low microbial growth compared to control and the citric acid group. Among the low effective treatments, the least microbial growth during phase two belonged to the aluminum sulfate group, which within the group, 300 mgl^{-1} had the least proliferation. The same was observed for the final microbial growth (Table 3).

Microbial growth rate

Microbial growth rate can show which compound loses it efficiency faster compared to the rest or which compound is more efficient throughout the experiment. The highest microbial growth rate during phase one belonged to citric acid group and the minimum growth was observed in 400 mg l^{-1} NaOCl application (Table 3). During phase II, the maximum growth rate was obtained by distilled water application and 300 mg l^{-1} citric acid, respectively. Although sterilized distilled water had the highest growth rate during phase II, due to its slow growth rate in phase I, it did not have the highest final growth rate. The highest microbial growth rate throughout the experiment was observed in 300 mg l^{-1} citric acid application. After other citric acid concentrations, sterilized distilled water, tap water and 400 mg l^{-1} NaOCl were placed respectively.

pH impact on microbial population

Among the studied treatments, a decrease in citric acid pH had a significant effect on microbial proliferation reduction and control. By a decrease in the pH in different citric acid treatments, microbial count showed significant decrease (Table 4). This was while in the other studied treatments, there was not a considerable change of pH by adding the chemicals and change in concentration. Similar to our findings for citric acid, previous studies have reported positive effect of pH reduction on bacterial proliferation, vase life and xylem occlusion inhibition in cut rose (van Doorn and Cruz, 2000), chrysanthemum (Ohta and Harada, 2000) and gerbera (Schmitt et al., 2013).

Microbial type

Different types of microorganisms such as bacteria, yeasts and fungi have been identified in the vase water and solution of cut flowers. In a study on cut *Narcissus tazetta* vase solution, Jowkar (2006) identified yeast, *Bacillus* spp., *Staphylococcus* spp., *Actinomycetes* and *Aspergillus* spp. as vase solution contaminates. In another study, Jowkar (2007) reported bacteria such as *Streptomyces*, *Bacillus*, *Cocci*, *Aspergillus* and yeasts as the most available microorganisms in vase solution in Tuberose. Carlson et al. (2015) isolated 9

Table 3. Effect of different biocides on cut 'Cherry Brandy' rose vase solution microbial growth and growth rate.

Treatment	Microbial Growth† (log10 CFU ml^{-1})††			Microbial Growth Rate† (log10 CFU ml^{-1})††		
	Growth During Phase I	Growth During Phase II	Final Growth	Phase I Growth Rate	Phase II Growth Rate	Final Growth Rate
Citric Acid 300 mgl^{-1}	5.91 b†††	8.505 b	8.505 b	4.229 a	2.585 a	6.816 a
Citric Acid 600 mgl^{-1}	5.800 b	7.304 c	7.318 c	4.650 a	1.503 b	6.167 b
Citric Acid 900 mgl^{-1}	5.792 b	6.731 d	6.778 d	4.641 a	0.939 c	5.628 b
Aluminum Sulfate 100 mgl^{-1}	-	3.322 h	3.322 h	-	-	-
Aluminum Sulfate 200 mgl^{-1}	-	2.539 i	2.539 i	-	-	-
Aluminum Sulfate 300 mgl^{-1}	-	2.128 j	2.128 j	-	-	-
Hydroxy Quinoline Citrate 200 mgl^{-1}	-	-	-	-	-	-
Hydroxy Quinoline Citrate 300 mgl^{-1}	-	-	-	-	-	-
Hydroxy Quinoline Citrate 400 mgl^{-1}	-	-	-	-	-	-
Calcium Hypochlorite 400 mgl^{-1}	-	-	-	-	-	-
Calcium Hypochlorite 600 mgl^{-1}	-	-	-	-	-	-
Calcium Hypochlorite 800 mgl^{-1}	-	-	-	-	-	-
Sodium Hypochlorite 400 mgl^{-1}	5.330 c	7.317 c	7.321 c	1.155 c	1.957 b	3.147 d
Sodium Hypochlorite 600 mgl^{-1}	-	4.929 f	4.929 f	-	-	-
Sodium Hypochlorite 800 mgl^{-1}	-	4.477 g	4.477 g	-	-	-
Nano Silver 1%	-	-	-	-	-	-
Nano Silver 2.5%	-	-	-	-	-	-
Nano Silver 5%	-	-	-	-	-	-
Sterilized Distilled Water (Control)	6.464 a	9.202 a	9.203 a	1.987 b	2.734 a	4.726 c
Tap Water	4.561 d	6.255 e	6.264 e	2.720 b	1.694 b	4.424 c

†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.

Table 4. Correlation between pH of citric acid and microbial growth in vase solution of 'Cherry Brandy' rose.

Correlation	Log Growth Phase I	Log Growth Phase II	Log Final Growth
pH	0.861*	0.835	0.842*

* Correlation is significant at the 0.05 level.

bacteria species from cut *Zinnia elegans* vase solution. This is why in the present study more microbial types were seen in vase solution. The isolated microorganisms in the present experiment were 26 bacterial isolates, 5 different kinds of fungi and 2 different kinds of yeasts.

Between the 6 isolated fungi, five different strains of *Fusarium solani* and one isolate of *Trichoderma harzianum* (which has antagonistic effect on other fungi, especially on *Botrytis cinerea*: which is the cause of the most widespread postharvest disease in cut rose flowers) were observed. Previous studies have reported only one kind of fungi (*Aspergillus* sp.) as vase solution contaminant in *Narcissus tazetta* and tuberose (cv. 'Goldorosht-e-Mahallat') (Jowkar, 2006; 2007).

The two kinds of yeasts were found on day-6 in NaOCl vase solutions. It can be assumed that they were originated from the flower stems after NaOCl had lost its efficiency. Similar finding with NaOCl has also been reported on *Narcissus tazetta* and *Tuberosa polyanthus* flowers. In the narcissus study, a broad range of yeasts strains were observed due to mulch application (Jowkar, 2006) while for tuberose few yeast strains were reported as vase solution contaminants (Jowkar, 2007).

Similar to other studies such as Jowkar (2006), Li et al. (2012), Solgi and Ghorbanpour (2015) and Carlson et al. (2015), it was observed that bacteria were the most widespread microorganisms in the vase solution of cut 'Cherry Brandy' rose flowers. Among the 26 different separated bacterial colonies, four of them did not grow when sub-cultured (which usually happens). The other 22 colonies were identified as in table 5

and 6. Among 22 identified isolates, 14 isolates were *Bacillus*, four were *Coccus*, one was *Streptomyces* sp., one was *Burkholderia* sp., one was *Pseudomonas* sp. and one was *Pectobacterium* sp. Different bacterial strains have been reported as cut rose flowers vase solution contaminants. Dominant bacterial strains of cut 'Movie Star' rose flowers were reported as *Aeromonas* sp., *Chryseomonas luteola*, *Comamonas acidovorans* and *Pseudomonas fluorescens* by Li et al. (2012). In another study on cut 'White Naomi' rose flowers, Solgi and Ghorbanpour (2015) reported *Acinetobacter*, *Bacillus cereu*, *Bacillus subtilus*, *Pseudomonads aeruginosa* and *Pseudomonas fluorescens* as the five important preserving solution bacteria. Other reported vase solution bacteria have been *Bacillus pumilus*, *Brevundimonas* sp., *Chryseobacterium* sp., *Chryseobacterium daejeonense*, *Pantoea ananatis*, *Pseudomonas fulva*, *Pseudomonas marginalis*, *Rhizobium radiobacter*, *Serratia ficaria* in cut *Zinnia* vase solution (Carlson et al., 2015); *Bacillus* spp., *Staphylococcus* spp., *Actinomycetes* in cut *Narcissus* vase solution (Jowkar, 2006) and *Streptomyces*, *Bacillus* and *Cocci* in cut tuberose vase solution (Jowkar, 2007).

Table 5. Identification of bacteria found in 'Cherry Brandy' rose vase solution by morphological and biochemical characteristic tests.

Test	Isolated Colony (Code)										
	1-1	**1-2**	**3-1**	**4-1**	**4-2**	**5-1**	**5-2**	**5-3**	**5-4**	**13-1**	**13-2**
Gram Reaction using KOH	+	+	+	+	+	-	+	+	+	-	+
Motility	-	-	-	-	+	+	+w	-	-	-	-
Cell Shape	Circular	Filamentous	Rod	Circular	Rod	Rod	Rod	Circular	Circular	Rod	Rod
Capsule	-	-	-	-	+	-	-	-	+	+	+
Anaerobic growth	-	-	-	-	-	+	-	-	-	+	-
Acid from Glucose	-	+	+	-	-	+	-	-	-	-	-
Gas from D-glucose	-	-	-	-	-	-	-	-	-	-	-
Potato Soft Rot	-	-	+	-	+	+	-	-	+	-	-
Fluorescent pigments on KB	-	-	-	-	-	-	-	-	-	-	-
Oxidase test	+	-	+	+	+	-	-	+	-	-	+
Catalase test	+	-	+	+	+	+	-	+	-	+	+
Gelatine Hydrolysis	-	+	-	-	-	-	+	-	-	+w	+
Levan	-	-	-	-	-	-	-	-	-	-	-
Hypersensitivity test on Tobacco	-	-	-	-	-	-	-	-	-	-	-
Growth at 50°C	NT	-	NT	NT	+	-	-	NT	-	+	+
Growth at 5.7 pH	NT	-	NT	NT	+	NT	-	NT	-	+	-
Starch Hydrolysis	-	-	-	-	+	-	-	-	+	-	+
Tween 80 Hydrolysis	-	+	+	-	+	-	+	-	+	-	-
Indol production	-	-	-	-	-	-	-	-	-	-	-
Methyl Red reaction	-	-	+	-	+	-	+	-	-	+	-
Aceteoin (VP)	+	-	-	+	+	+	+	+	-	-	-
Nitrate reduction	+	-	-	+	+	+	-	+	+	-	+
Arginine dihydrolase	-	+	-	-	+	-	-	-	-	-	+
H_2S production from Cysteine	-	-	-	-	-	-	-	-	-	-	-
Clony Appearance	White	White	Yellow	White	White	Orange	Yellow	White	White	Yellow	White
Identified Result	*Coccus*	*Streptomyces sp.*	*Bacillus polymexa*	*Coccus*	*Bacillus subtilis*	*Pectobacterium sp.*	*Bacillus polymexa*	*Coccus*	*Coccus*	*Bacillus sp.*	*Bacillus megaterium*

+: Possitive, -: Negative, +w: weakly Possitive, NT: Not Determined.

Table 6. Identification of bacteria found in 'Cherry Brandy' rose vase solution by morphological and biochemical characteristic tests.

Test	Isolated Colony (Code)										
	13-3	**13-4**	**13-5**	**14-1**	**14-2**	**14-3**	**14-4**	**15-1**	**15-2**	**15-3**	**20-1**
Gram Reaction using KOH	+	+	-	-	+	+	-	+	-	+	+
Motility	-	-	-	-	-	-	+	-	+	+	-
Cell Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Capsule	-	+	+	+	+	+	-	+	+	-	+
Anaerobic growth	-	+	+	+	+	+	-	+	-	-	+
Acid from Glucose	-	-	-	-	-	-	+	-	+	-	-
Gas from D-glucose	-	-	-	-	-	-	-	-	-	-	-
Potato Soft Rot	-	+	-	-	+	+	+	+	-	-	+
Fluorescent pigments on KB	-	-	-	-	-	-	-	-	-	-	-
Oxidase test	+	+	-	-	+	+	+	+	+	-	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+
Gelatine Hydrolysis	+	+	+w	+w	+	+	-	+	+	+	+
Levan	-	-	-	-	-	-	-	-	-	-	-
Hypersensitivity test on Tobacco	-	-	-	-	-	-	+w	-	-	-	-
Growth at 50°C	+	+	+	+	+	+	-	+	-	+	+
Growth at 5.7 pH	+	+	+	+	+	+	NT	+	NT	+	+
Starch Hydrolysis	+	-	-	-	-	-	-	-	-	+	-
Tween 80 Hydrolysis	-	-	-	-	-	-	-	-	-	+	-
Indol production	-	-	-	-	-	-	-	-	-	-	-
Methyl Red reaction	-	-	+	+	-	+	-	-	+	+	+
Aceteoin (VP)	-	-	-	-	-	-	-	-	-	-	-
Nitrate reduction	-	-	-	-	-	-	-	-	+	-	-
Arginine dihydrolase	-	-	-	-	-	-	-	-	+	-	-
H_2S production from Cysteine	-	+	-	-	+	-	-	+	-	-	-
Clony Appearance	White	Transparent	White	Orange	Yellow	White	Brown Diffused Pigment	Pink	White	Transparent	White
Identified Result	*Bacillus megaterium*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Bacillus sp.*	*Burkholderia gladioli.*	*Bacillus sp.*	*Pseudomonas sp.*	*Bacillus circulans*	*Bacillus sp.*

+: Possitive, -: Negative, +w: weakly Possitive, NT: Not Determined.

Among the identified *Bacillus* sp. in our study, 2 isolates were *B. polymexa*, 2 isolates were *B. megaterium*, one isolate was *B. subtilis* and one was *B. circulans*. *Bacillus subtilis* was found in 100 mg l^{-1} aluminum sulphate treatment. Previous studies have shown that *B. subtilis* is one of the most effective microorganisms against *Botrytis cinerea* (the main causes of pre- and postharvest damages and losses in

greenhouses produced cut rose flowers). *B. subtilis* growth in aluminum sulfate treatment indicates the useful effect of aluminum sulfate treatments. *Bacillus megaterium* was only found in NaOCl vase solutions.

The most microorganism diversity was seen in NaOCl treated cut rose vase solutions. In the present experiment, *Bacillus* bacteria were the most spread microorganism and was found in different concentrations of NaOCl, aluminum sulfate and tap water treatments. Similar to our findings, *Bacillus* has been the most common microorganism in vase solution in other cut flower studies (Jowkar, 2006; 2007; Carlson et al., 2015; Solgi and Ghorbanpour, 2015).

The most common bacteria in citric acid vase solution treatments were *Streptomyces* sp. and *Coccus*, after which a colony of *B. polymexa* was observed. This was while in narcissus citric acid vase solution, *Actinomycetes* were one of the most broad spread microorganisms, which were then substituted by *Bacillus* sp. (Jowkar, 2006). In his study, *Pectobacterium* sp. and *Bacillus subtilis* were only observed in aluminum sulfate 200 and 100 mgl^{-1} concentrations, respectively. Beside *Bacillus*, *Coccus* was also observed in aluminum sulfate treatment group. 10% of the microbial contamination of all narcissus aluminum sulfate vase solutions belonged to *Staphylococcus* sp. (Jowkar, 2006). Beside Bacillus, *Burkholderia* sp. and *Pseudomonas* sp. were also found in NaOCl vase solution treatments. However, *Burkholderia* sp. and *Pseudomonas* sp. were not found in other treatments. Similar to most treatments, tap water and sterilized distilled water were also contaminated with *Bacillus* bacteria.

In general, flora and microbial population of agricultural products are determined by the product's physiological state and the mixture of microorganisms covering the product such as bacteria, yeasts and fungi. When cut flowers are placed in vase solution, bacteria from cut flower stem and flower surface transfer into vase solution. Vase water, vase solution, contaminated vases, containers, or vessels can be other possible sources of microbial contamination. The initial microbial load of the flower and its container can explain the difference between microbial contamination of our studied rose flowers and the other studies. Besides that sanitation of the postharvest handling facilities can play an important role in vase solution microbial contamination both in microorganism diversity and population.

Conclusion

Our results indicated that nano silver is as effective as other conventional biocides such as HQC and Calcium hypochlorite in controlling vase solution microbial proliferation. Beside microbial proliferation control, this compound significantly improved vase life of cut 'Cherry Brandy' rose flowers. It also has ethylene antagonistic effects and could delay senescence and also have other beneficial effects on climacteric cut flowers. After nano silver particles, aluminum sulfate could be recommended if vase solution is replaced every 4 days. This is because aluminum sulfate substantially controlled vase solution microbial proliferation and it only allowed proliferation of *Bacillus subtilis* [one of the most effective microorganisms for biological control of *Botrytis cinerea* (the principal causes of pre- and postharvest losses in greenhouses produced roses)] when it lost its efficiency.

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