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Benzalkonium chloride treatments improve water relations of cut roses

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ABSTRACT

8-hydroxyquinoline and benzalkonium chloride are broad-spectrum fungicides. In this study, cut roses (*Rosa hybrida*) were treated with 8-hydroxyquinoline (100 mg L^{-1}, 200 mg L^{-1} and 300 mg L^{-1}) and benzalkonium chloride (25 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1}). An analysis of microbial concentrations showed that a high concentration of 8-hydroxyquinoline (300 mg L^{-1}) could effectively control and kill fungi but had less effect or influence on bacterial reproduction, whereas benzalkonium chloride functions well in reducing the reproduction of both fungi and bacteria. Correlation analysis between the number of bacterial colony-forming units (CFUs) and water uptake revealed a significantly negative correlation between the log_{10} number of bacterial colonies and water uptake. When there were approximately 2.84×10^5 and 2.15×10^5 bacterial CFU mL^{-1} in the vase liquid, the water uptake of cut roses cv. Movie star and cv. Black lace decreased dramatically. It was observed that cut roses with higher water uptake had a better vase-holding quality after been treated with benzalkonium chloride (50 mg L^{-1}, 100 mg L^{-1}). Benzalkonium chloride (50 mg L^{-1}, 100 mg L^{-1}) was also found to be effective in extending the longevity for an additional 2–3 days, increasing water uptake, enlarging corolla diameter as well as extending blooming time.

1. Introduction

The production of cut roses as one type of export flower is prospering in China and therefore rapidly developing as an emerging industry. Research on the preservation and storage of cut roses is thus imminent as losses are up to 40% worldwide and even higher in China [22].

Halevy [7] reported that the most common cause of the termination of vase life in cut flowers is water stress. Bacteria enter and block stem xylem vessels and thereby reduce rates of water supply to flowers [4]. There is high level of bacteria present in the solutions used by growers, wholesalers, retailers and consumers to hydrate cut flowers [4,8]. Vascular occlusions and associated wilting in flower species such as *Dianthus* and *Rosa* usually develop when the number of bacteria in vase water reach 10^7–10^{11} colony forming units (CFUs) L^{-1} [21,27,28,33]. In most studies, different numbers of bacteria have often been added to the vase solution to analyse the effect of bacteria on stem blockage. However, the stems already contain some bacteria, and thus when added to the vase water, the bacteria that naturally reproduce using sucrose will thrive and water uptake will change. The rapid proliferation of microorganisms in vase water is thought to result in xylem blockage, water stress, and a subsequent reduction in cut flower longevity [30], and thus, various germicides have been suggested to prevent this problem [1,2,10–15]. In recent studies, 8-hydroxyquinoline (8-HQ) and its salts were the most commonly used broad-spectrum fungicides, including 8-hydroxyquinoline sulphate (8-HQS) and 8-hydroxyquinolinium citrate (8-HQC), which can inhibit the reproduction of more than 40 types of bacteria and fungi in cut flowers [22]. Benzalkonium chloride (BKC) is one of the most important quaternary ammonium compounds (QACs) with a surfactant ability that has considerable capacity to penetrate and adhere to porous surfaces. BKC is the active ingredient of many pharmaceutical formulations, cosmetics, commercial disinfectants, industrial sanitizers and food preservatives [25]. It is widely used as a clinical disinfectant and antiseptic in health care and domestic facilities, an antimicrobial preservative in drugs, an antiseptic for preoperative skin or for wounds, burns, etc., a disinfectant in processing lines and on surfaces in the food industry, and also as an antimicrobial agent in the treatment of common infections of the mouth and throat [17].

Benzalkonium chloride has a few records as a fungicide used on cut roses. Thus, this study was undertaken to investigate the effects of different concentrations of benzalkonium chloride on the vase-holding quality and number of microorganisms of cut roses in comparison with 8-hydroxyquinoline. In addition, the correlation between changes in the bacterial quantity in the vase solution and water uptake was determined, and the effect of water uptake on the vase-holding quality was analysed.

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2. Materials and methods

2.1. Material

The two rose varieties, "cv. Movie star" and "cv. Black lace", used in this experiment were obtained from the Shanghai Academy of Agricultural Sciences. 8-hydroxyquinoline (8-HQ) and sucrose (AR) agar medium and rose Bengal medium were sourced from Sinopharm Chemical Reagent Co. Ltd., while benzalkonium chloride (BKC, 45%, AS, WT/WT) was obtained from the China Research Institute of Daily Chemical Industry.

2.2. General processing

Cut roses were maintained under humid conditions at 15 °C, and the stems were placed in distilled water while the roses were transported to the laboratory. Stems with no visible damage were trimmed to a length of 40 cm with 2–3 leaves to prevent the formation of air embolisms. To enhance water uptake, the stems were trimmed to a chamfered surface, cleaned 3 times with distilled water and transferred to 2-L plastic vases, which contained 1 L of vase liquid, after an hour. The stems (10 cm of the stem) were immersed in the liquid.

2.3. Experimental design

To eliminate the interference of other factors, two rose varieties were used in this experiment, which comprised 7 treatments (Table 1), i.e., three concentrations of 8-HQ and BKC each and 1% sucrose. There were 10 roses in each treatment and each treatment was replicated 3 times.

3. Determination

3.1. The determination of the vase-holding quality

The factors used to determine the vase-holding quality of the cut roses included the vase life, maximum corolla diameter, time to achieve maximum corolla diameter, blooming period [23] and bent stem severity. Stem bending was scored based on the angle between the corolla and the stem, with 0 representing 0ÿ, 1 for (0, 45°), 2 for (45°, 90°) and 3 for (90°, 180°).

3.2. Colony counting of bacteria and fungi in the processing liquid

Bacterial and fungal colonies were measured using the bacterial plate count method with 3 dilutions and 3 replicates each. The quantity of the microorganisms was first determined when the stems were placed in the distilled water and then daily after they were transferred to the vase liquid (Microbiological examination of food hygiene–Enumeration of moulds and yeasts of China. Microbiological examination of food hygiene–Enumeration of bacteria of China) [18].

Table 1
The different compositions of the vase liquid.

Treatment	Compositions of vase liquid
Control	1% sucrose + distilled water
P1	1% sucrose + distilled water + 100 mg L^{-1} 8-HQ
P2	1% sucrose + distilled water + 200 mg L^{-1} 8-HQ
P3	1% sucrose + distilled water + 300 mg L^{-1} 8-HQ
P4	1% sucrose + distilled water + 25 mg L^{-1} BKC
P5	1% sucrose + distilled water + 50 mg L^{-1} BKC
P6	1% sucrose + distilled water + 100 mg L^{-1} BKC

3.3. Cut rose water absorption

The water absorption of the cut roses was determined as: $_{\triangle}W = W_1 - W_n$, where $_{\triangle}W$ = cut rose water uptake; W_1 = initial weight (vase + processing liquid); and W_n = daily weight (vase + processing liquid) [32]. To prevent evapotranspiration, the vases were covered with plastic wrap.

3.4. Statistical analysis

All experiments were conducted twice and arranged in a completely randomized design. The data were analysed using ANOVA, and the means were separated using Duncan's multiple range test at a 5% probability level to examine significant differences between groups using Statistical Package for the Social Sciences (SPSS) 17.0 software. The figures were drawn using Origin 8.0.

4. Results and analysis

4.1. Effects of various vase solutions on the vase-holding quality of "cv. Movie star" and "cv. Black lace"

The mean separation revealed significant differences amongst the vase lives of the cut roses treated with different concentrations of benzalkonium chloride (BKC). However, there were no significant differences amongst the different concentrations of the 8-hydroxyquinoline (8-HQ) treatments and the control for both varieties used in the experiment. The maximum corolla diameter of "cv. Movie star" was largest in the BKC treatments P5 and P6, which were significantly different from BKC treatment P4 and 8-HQ treatments P1 and P3. There were no significant differences between 8-HQ treatment P2 and the control. The control and 8-HQ treatments P1 and P3 achieved the maximum corolla diameter last. This was significantly different from treatments P2 and P4, where the same maximum corolla diameter was achieved at an earlier time, and from treatments P5 and P6, where the maximum corolla diameter was achieved first. The cut roses bloomed for longer under the BKC treatments P4 and P5, which differed significantly from the blooming time of the control, P6 and P2, while the shortest blooming time was recorded in P3 and P1. The largest corolla diameter of "cv. Black lace" was measured in the BKC treatments P4 and P5, which differed significantly from P6. The smallest corolla diameter was measured in 8-HQ treatment P3, which differed significantly from the other 8-HQ treatment concentrations and the control. The time to achieve the maximum corolla diameter was the highest in the 8-HQ treatments, which did not differ significantly amongst themselves, and was the lowest in the BKC treatments, which were not significantly different amongst themselves but were significantly different from the control and the 8-HQ treatments (Tables 2 and 3).

Table 2
Effects of the vase solution on the vase-holding quality of "cv. Movie star".

Treatment	Vase life (d)	Maximum corolla diameter (cm)	Time to achieve maximum corolla diameter (d)	Blooming days (d)
Control	14.1 ± 0.6 b	6.1 ± 1.2 c	8.0 ± 0.5 a	6.0 ± 1.0 ab
P1	14.5 ± 1.1 b	6.4 ± 0.9 b	8.0 ± 0.4 a	5.2 ± 1.1 c
P2	14.0 ± 0.4 b	6.1 ± 0.3 c	6.8 ± 0.2 b	6.0 ± 0.3 ab
P3	13.0 ± 1.4 b	6.4 ± 0.2 b	8.0 ± 0.7 a	6.0 ± 0.6 bc
P4	16.1 ± 0.7 a	6.5 ± 0.4 b	6.0 ± 0.2 b	8.0 ± 0.3 a
P5	17.0 ± 0.5 a	6.9 ± 0.8 a	5.0 ± 0.4 bc	8.0 ± 0.3 a
P6	17.3 ± 0.5 a	6.9 ± 1.0 a	4.3 ± 0.3 c	7.4 ± 0.5 ab

Note: Different letters indicate significant differences ($P < 0.05$). Data shown in the table are the average value ± SE (standard error).