ClO₂ Dipping Treatment Inhibits Gray Mold on Cut Rose Flowers During Storage

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This study investigated the antifungal effect of chlorine dioxide (ClO₂) dipping on Botrytis cinerea, the causal agent of gray mold, on cut rose flowers (Rosa hybrida L.). In vitro, the spore germination of gray mold was inhibited 100% by instant dipping with ClO₂ solution (5 to 10 μL·L⁻¹). In particular, ClO₂ at 5 μL·L⁻¹ was found to be ideal for hindering spore activity without causing any damage to the petals. This ClO₂ antifungal effect on cut flowers was investigated in a white cultivar ‘Beast’ with different treatments: dipping (one second), spraying (4.8 mL), or gassing (two hours) with 5 μL·L⁻¹ ClO₂. Six days after ClO₂ treatment, the incidence of gray mold in the artificially-inoculated flowers was 2.5% (dipping), 9.4% (spraying), or 8.4% (gassing), respectively, which were all significantly lower than the control incidence of 17.6%. Especially, ClO₂ dipping reduced the incidence of gray mold by up to 26.1% compared to the control in five other rose cultivars (‘Antique Curl’, ‘Green Beauty’, ‘Feel Lip’, ‘Pink Heart’, and ‘Venus Berry’). No petal discoloration was detected, and petal color values (chroma or hue) were maintained regardless of ClO₂ dipping. This result suggests that immediate ClO₂ dipping is applicable to inhibit gray mold on cut rose flowers at a level of 5 μL·L⁻¹ just before postharvest storage.

Key Words: antimicrobial, Botrytis cinerea, cut flowers, Rosa hybrida L., spore germination.

Introduction

Botrytis cinerea, the causal agent of gray mold, is a major determinant of cut rose flower quality and vase life after harvest (de Stigter and Broekhuysen, 1989). It is frequently caused by high relative humidity (RH) during postharvest storage and transportation, and it causes economic losses for growers, wholesalers and retailers (Williamson et al., 1995). The international trade volume of cut roses is increasing year by year, and domestic exports amounted to 1,590 thousand dollars in 2018 (Cheong et al., 2012; KATI, 2019). Cut roses produced in South American countries are mostly transported to the USA, while those produced in Kenya and Ethiopia are transported to Europe or Asian countries. Cut roses are usually transported by sea because of the lower costs (Harmannus et al., 2013). The shipping transportation of cut flowers usually takes days or weeks. With longer shipping times, packed cut roses are more vulnerable to gray mold because of the closed conditions in export containers with high RH (Fanourakis et al., 2013). Hoogerwerf et al. (1989) reported that the postharvest life of cut rose flowers during shipment was most strongly influenced by gray mold among various factors such as harvest time, air-exposure, ethylene, bacteria, and fungi. Moreover, gray mold is a major factor causing a quality decline in Korean roses exported to Japan (Park and Park, 2006). Gray mold increased with RH > 93%, which is unfavorable, and lesions could become necrotic and infect petals (Salinas et al., 1989; Williamson et al., 1995). The optimum temperature for conidia sporulation of gray mold is between 17 and 18°C. A higher or lower temperature will inhibit sporulation (Fanourakis et al., 2013). High level CO₂ fertilization and UV treatment during the cultivation period can reduce gray mold on plants (Svircev et al., 1984; West et al., 2000). Elevated levels of RH (> 93%) and plant debris during rose growth can increase the incidence of gray mold infection (Williamson et al., 2007). Ca⁺ deficiency also increases the incidence of gray mold during rose growth.
(Baas et al., 2000). Spraying fungicides such as ipro-
dine + thiram, tebuconazole + dichlofluanid, polyoxin
D, benzimidazole, dicarboximides, and dicarboxim can
inhibit gray mold (Lee et al., 2006; Roberts et al.,
2003). Nevertheless, a need remains for eco-friendly al-
ternative fungicides that do not generate environmental
pollution and leave little or no residue during disposal
(Gebhart and Kappauf, 1980; Roberts et al., 2003); new
eco-friendly alternatives are urgently needed (van
Jaarsveld, 2018).

Chlorine dioxide (ClO₂), an eco-friendly biocide
(Karabulut et al., 2009; Macnish et al., 2010), has been
used to sanitize surfaces of fruits and vegetables (Aieta
and Berg, 1986; Chen and Zhu, 2011; Knee, 2000). In
China, America, and the EU, ClO₂ at 3 mg·kg⁻¹ or
below has been used to sanitize drinking water and sur-
faces of fruits and vegetables (Aieta and Berg, 1986;
Chen and Zhu, 2011). The US Food and Drug Adminis-
tration (FDA), China, and the EU allow the use of ClO₂
to wash fruits and vegetables (Chen and Zhu, 2011;
Karabulut et al., 2009; Macnish et al., 2008). The
Korean Ministry for Food, Agriculture, Forestry and
Fisheries (MIFAFF) has permitted the use of ClO₂ for
organic handling of materials and sanitization (gro-
cerries selection section 22, schedule 2, August 2010).
Toxicity studies in humans have revealed that daily in-
germination of 500 mL of ClO₂ at a concentration of 5 ppm
can be safely tolerated (Robert, 2016). In a postharvest
test of Gerbera ‘Jenny’ and Rosa ‘Beast’, the antibacte-
rial effect of ClO₂ was established after six days of treat-
ment, while the ClO₂ in the solution disappeared in two
days (Lee and Kim, 2014; Lee et al., 2014). This
antibiosis of ClO₂ (2–10 μL·L⁻¹) in the vase solution
resulted in extending the cut flower longevity of sev-
eral species: Alstroemeria, Antirrhinum, Gerbera,
Gypsophila, and Rosa (Koermer and Wldman, 2002;
Lee and Kim, 2014; Macnish et al., 2008). Regarding
shipping cut flowers, however, information on the in-
hibitory activity of ClO₂ against fungi such as gray
mold and relevant application techniques is very limit-
ed. Therefore, we determined the antimicrobial effect
of ClO₂ and the optimal application method and concen-
tration to minimize the incidence of gray mold in cut
rose flowers.

Materials and Methods

Plant materials, ClO₂, and Botrytis cinerea preparation

Cut rose flowers of six cultivars, ‘Antique Curl’,
‘Beast’, ‘Green Beauty’, ‘Feel Lip’, ‘Pink Heart’, and
‘Venus Berry’, were harvested at the commercial har-
vest phase (Kumar et al., 2008) from a greenhouse in
Paju, Gyeonggi-do, Korea. The cut flowers were trans-
ported within one hour to a laboratory at the University
of Seoul. In the laboratory, only stems with a diameter
of 7 to 9 mm were re-cut under distilled water (DW) to
40 cm shoot length. All leaves except the upper three
were removed. The stock solution of ClO₂ 100 μL·L⁻¹
was prepared by ClO₂ (CA-200; PurgoFarm Inc.,
Korea) with DW. The ClO₂ stock was diluted for each
concentration, which was measured using a test strip
(Insta-Test Strip; LaMotte Inc., USA). Naturally occur-
ing Botrytis cinerea was used in this study and it was
isolated in the rose production greenhouse. Its
pathogenicity was confirmed by re-inoculation on rose
petals followed by incubation on Potato Dextrose Agar
(DD, USA) at 28°C.

Experimental design and ClO₂ treatment

The study was comprised of three experiments to
find out (1) the antifungal effect of ClO₂ on B. cinerea
spore germination, (2) the antifungal effect of ClO₂ on
the gray mold incidence on ‘Beast’ roses with various
method and concentration, and (3) the antifungal effect of ClO₂
on gray mold incidence and petal discoloration in five
colored cultivars.

To confirm the antifungal effect of ClO₂ on B. cinerea
spore germination, we prepared B. cinerea conidia (1.0 × 10⁷ spores·mL⁻¹) in sterile DW (9 mL),
and then added 1 mL DW (control) or ClO₂ solutions
(0.5, 1, 2, 5, or 10 μL·L⁻¹). After a 24-hour incubation
at 20°C, we counted the germinated spores, and the ex-
periment was done with three replications.

The cut flowers of the white rose cultivar ‘Beast’
were used in the second experiment. We inoculated
the petals by spraying with B. cinerea conidia at 1.0 × 10⁸ spores·mL⁻¹ (1.2 mL per flower) and then air-dried
them for two hours (Lee et al., 2006). The inoculated
petals were tested for the antifungal effect of ClO₂
(5 μL·L⁻¹) with three treatment methods: dipping (one second), spraying (4.8 mL), and gassing (two hours),
compared to a non-treated control. Water treatments by
dipping, spraying or gassing were not designed based on
previous studies: spraying and dipping water treatments
leave water droplets on petals, which can dis-
perse Botrytis cinerea conidia (Williamson et al., 2007).
Also, spraying of DW onto rose petals caused more
grey mold to grow compared to other fungicides such as
STS (silver thiosulfate) and aluminum sulfate (Choe,
2006). Especially, water dipping treatment at room tem-
perature after inoculation and drying for one hour re-
sulted in greater gray mold growth than the no-dipping
treatment, while transpiration increased and the water
uptake rate was lower (Lee et al., 2016). Gassing treat-
ment of water without ClO₂ in a closed place increased
relative humidity, causing grey mold growth on cut rose
flowers (Williamson et al., 2007). The gassing test was
conducted in a closed chamber (108.5 × 108.5 ×
108.5 cm) with commercial ClO₂ silica gel (PurgoFarm
Inc., Korea). After treatment, the vase life and gray
mold incidence of the flowers were measured in growth
chambers controlled as 20.5 ± 3°C air temperature,
70.1 ± 2% RH, and 8.2 ± 2 μmol·m⁻²·s⁻¹ PPFD with a
12h-photoperiod (Lee et al., 2016). Each flower stem
was placed in a vase containing 400 mL DW. The vase
life and incidence of gray mold on petals were measured with 10 replications. The incidence of gray mold on petals was calculated by the percentage of the number of diseased petals compared to total petals. We also tested the antifungal effect of ClO$_2$ against gray mold in five cut rose varieties with various petal colors, ‘Antique Curl’, ‘Green Beauty’, ‘Feel Lip’, ‘Pink Heart’, and ‘Venus Berry’. In this experiment, only dipping (one second) in ClO$_2$ (5 µL·L$^{-1}$) was performed. We inoculated the petals by spraying them with B. cinerea conidia at 1.0 × 10$^4$ spores·mL$^{-1}$ (1.2 mL per flower) and then air-dried them for two hours. Each flower stem was placed in a vase containing 400 mL DW and a completely randomized block design with 10 replications in growth chambers controlled as follows: 20.5 ± 3°C air temperature, 70.1 ± 2% RH, and 8.2 ± 2 µmol·m$^{-2}$·s$^{-1}$ PPFD with a 12h-photoperiod.

**Flower color analysis**

To investigate the ClO$_2$ effect on the discoloration in various colored petal cultivars, we measured the colors on the outermost center of outer perianth with a color meter (JX-777; MINOLTA, Japan) at day six after ClO$_2$ treatment (Berns, 2000). Based on the CIELAB values (L*: darkness-brightness, a*: green-red, and b*: blue-yellow), chroma (C*) and hue (H*) values were calculated using the reference formula (McGuire, 1992).

**Experimental design and statistical analysis**

Analysis of variance (ANOVA) was performed for results obtained using the Statistical Analysis System (SAS) software version 9.2 (SAS Institute Inc., Cary, NC, USA). Duncan’s new multiple range test and the Student’s t-test (at $P = 0.05$) were used to compare treatment means.

**Results**

**Antifungal effect of ClO$_2$ on Botrytis cinerea**

In the spore germination experiments, DW and a low concentration of ClO$_2$ at 0.5 µL·L$^{-1}$ resulted in 100% Botrytis cinerea spore germination (Fig. 1). Treatment with 1 and 2 µL·L$^{-1}$ ClO$_2$ resulted in 4.6% and 7.3% germination of B. cinerea spores, respectively (inhibiting B. cinerea spores by 95.4% and 92.7%, respectively), whereas treatment with 5 and 10 µL·L$^{-1}$ ClO$_2$ resulted in no germination of B. cinerea spores (100% inhibition). Therefore, treatment with 1 µL·L$^{-1}$ ClO$_2$ inhibited B. cinerea spore germination by over 90%, while treatment with ClO$_2$ at 5 µL·L$^{-1}$ or more completely inhibited B. cinerea spore germination.

To investigate the inhibition effect on gray mold of 5 µL·L$^{-1}$ ClO$_2$ with different treatment methods, tests on cut rose flowers were conducted. As shown in Figure 2, dipping, spraying, and gassing treatment methods resulted in disease incidences (%) of 1.1%, 9.8%, and 8.4%. Dipping treatment was the most effective method to inhibit gray mold. With the control, spraying, and gassing treatments, vase life began to terminate at day 6 after treatment, while the dipping treatment maintained the quality of the cut roses (Fig. 3).

**Effect of ClO$_2$ dipping treatment on petal color**

To confirm the antifungal effect of ClO$_2$ dipping on the white rose ‘Beast’, we tested five other cultivars, ‘Antique Curl’, ‘Green Beauty’, ‘Feel Lip’, ‘Pink Heart’, and ‘Venus Berry’. The incidence of gray mold and petal color changes were investigated on the 6th day after treatment.
of storage after ClO₂ (Table 1). The incidence of gray mold was clearly decreased by ClO₂ dipping by up to 26.1% compared to the control in the five colored petal cultivars. Based on the CIELAB values (data were measured, but not tabulated), no petal discoloration was detected in any cultivars, and petal color values (chroma or hue) were almost all maintained regardless of ClO₂ dipping. Only in ‘Antique Curl’ did the chroma value significantly increase compared to that of the control (Table 1). Therefore, color changes and discoloration symptoms of cut rose petals after 5 μL·L⁻¹ ClO₂ dipping treatment were rarely observed.

### Discussion

With growing concern about the ecological problems caused by synthetic fungicides and their effects on human health, the development of eco-friendly practices has become a necessity (Ayoub et al., 2017). Therefore, the inhibition effect of environmentally-friendly ClO₂ on the incidence of gray mold was determined in cut rose flowers; ClO₂ completely inhibited the spore germination of *B. cinerea* at more than 5 μL·L⁻¹ (Fig. 1). Similar results were reported previously (Spotts and Peters, 1980), showing that treatment of *B. cinerea* conidia with 10 μg·mL⁻¹ ClO₂ for 0.5, 5, or 10 mins inhibited *B. cinerea* spore germination. It has been reported that mycelial fungi are sensitive to the destructive action of ClO₂ due to their low tolerance for nascent oxygen and to changes in the acidic medium in which they thrive following the liberation of organic acids (Robert, 2016).

The ClO₂ dipping treatment was more active on the inhibition of gray mold than spraying or gassing treatments (Fig. 3). The ClO₂ gassing treatment was reported to reduce bacteria and fungi by 85% (Burton et al., 2008). Aqueous ClO₂ treatment could also inhibit polyphenol oxidase and peroxidase, suppress bacteria, and extend the shelf life of asparagus lettuce, and apple ‘Golden Delicious’ (Chen et al., 2010; Fu et al., 2007). In this experiment, spraying with low concentrations of 5 μL·L⁻¹ ClO₂ did not have a great inhibition effect on

### Table 1. Effect of ClO₂ dipping treatment on the incidence (%) of gray mold and changes in petal color in several rose varieties of different colors.

| Cultivar          | Treatment (5 μL·L⁻¹ ClO₂) | Incidence (%) of gray mold | Petal color | Chroma | Hue |
|-------------------|---------------------------|----------------------------|-------------|--------|-----|
| ‘Antique Curl’    | Non-treated (control)     | 33.8                       | 22.2        | 85.4   |
|                   | ClO₂ dipping              | 22.6*                      | 32.2*       | 84.7   |
| ‘Green Beauty’    | Non-treated               | 27.0                       | 36.4        | 83.3   |
|                   | ClO₂ dipping              | 19.5                       | 34.1        | 81.0   |
| ‘Pink Heart’      | Non-treated               | 17.9                       | 22.2        | 89.3   |
|                   | ClO₂ dipping              | 12.0***                    | 20.0        | 24.9   |
| ‘Feel Lip’        | Non-treated               | 50.7                       | 55.8        | 214.8  |
|                   | ClO₂ dipping              | 24.6***                    | 53.0        | 356.2  |
| ‘Venus Berry’     | Non-treated               | 36.3                       | 13.8        | 75.7   |
|                   | ClO₂ dipping              | 17.7**                     | 13.3        | 68.1   |

* Chroma indicates the hypotenuse of a right triangle formed by joining point (0, 0), (a*, b*), and (a*, 0).

Hue indicates the angle between the hypotenuse and 0° on the a* axis. Asterisks indicate significant differences between the non-treated control and ClO₂ dipping in the t-test at P<0.05 (n = 10 for the incidence of gray mold; n = 5 for petal color).
gray mold. ClO2 gassing treatment at 300–1000 μL·L−1 in Black fig (*Ficus carica L.* ‘Bursia Siyahi’) also inhibited bacteria and *B. cinerea* (Karabulut et al., 2009). In the case of chlorine, 1.4–9.6 g chlorine gas (Cl2) treatment inhibited gray mold in table grapes (‘Flame Seedless’, ‘Thompson Seedless’, ‘Ribier’) (Zoffoli et al., 1999). However, gassing treatment with 5 μL·L−1 ClO2 barely inhibited gray mold, implying that this concentration must be too low.

Flower color is an evaluation indicator of the postharvest quality of cut rose flowers (Roberts et al., 2003). In this study, the 5 μL·L−1 ClO2 dipping treatment inhibited the incidence of gray mold on a white rose cultivar ‘Beast’; we then tested this treatment on other colored petal cultivars. As a result, the antifungal effects of ClO2 dipping on gray mold did not cause any apparent damage to petals, especially discoloration. Usually, to achieve a strong fungicidal effect, a high concentration of fungicide to suppress the pathogen, and this may cause severe damage to plants (Ayoub et al., 2017). ClO2 dipping treatment on the flowers of 10 cut rose cultivars did not affect color, cause tissue damage, and affect the volatility of petals (Lee and Kim, 2019). Electrolyte leakage (%) indicates the degree of tissue damage, and is the basis for estimating tissue stability (Lee et al., 2016). The petals of five rose cultivars after ClO2 dipping did not show any significant difference in electrolyte leakage compared to control ones (Lee and Kim, 2019). In the case of fresh-cut asparagus lettuce (sensitive species), washing with 100 mg·L−1 ClO2 for 20 minutes could maintain its visible quality, odor, aroma, and texture (Chen et al., 2010). Nevertheless, it is crucial to find the optimal concentration and treatment time for each cultivar because ClO2 is still a potent oxide (Lee et al., 2006).

In this study, we were able to observe the antimicrobial effect of ClO2 on the incidence of gray mold in post-harvest rose flowers with a new ‘dipping’ treatment method. Only one-second ClO2 dipping (5 μL·L−1) was enough to inhibit spore germination of *B. cinerea* in ‘Beast’ roses. Meanwhile, the other treatment methods of spraying and gassing were not as effective as dipping. The flower dipping treatment was applied to five other cultivars of different colors, and the antifungal effect on gray mold was verified. The discoloration of petals did not appear in any varieties. However, the mode of action of the antifungal effect of ClO2 dipping was not elucidated in this experiment (It can be considered to be due to oxidation of ClO2 and a simple washing action). We chose a simple experimental design with limited levels to determine the applicability of ClO2 dipping. Therefore, further studies should investigate additional information on the physi-morphological changes in gray mold after ClO2 dipping treatment with various treatment concentrations, times and combinations.

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