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*Research Article*

# Microbial Decontamination of Onion by Corona Discharge Air Plasma during Cold Storage

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Corona discharge air plasma (CDAP) is a nonthermal decontamination technology which is generating antimicrobial agents such as photons, electrons, positively and negatively charged ions, atoms, and free radicals. We investigated the effect of a corona discharge under atmospheric pressure on the sterilization of postharvest fungal pathogens on onion. The main antimicrobial reactive substance generated by CDAP was $O_3$. The active species such as nitric oxide (NO) and nitric dioxide ($NO_2$) were nearly detected in this experiment. CDAP treatment revealed different isolation frequencies depending on postharvest pathogens from diseased onions, showing less isolation frequency of *Fusarium* spp. and *Alternaria* sp. than that of *Botrytis* spp. when compared with untreatment onions during 10-month cold storage. CDAP treatment at 2~2.6 ppm of $O_3$ slightly stimulated the mycelial growth of *Alternaria* sp., while the treatment at 20~24 ppm of $O_3$ gradually inhibited mycelial growth by treatment time. However, *Botrytis* sp. showed different patterns of mycelial growth with CDAP treatment. Less than 4 hours' treatment of CDAP slightly inhibited the mycelial growth of *Botrytis* sp., while 8 hours' treatment of CDAP slightly stimulated the mycelial growth of *Botrytis* sp. not depending on the concentration of $O_3$. The inhibitory effect of CDAP on the conidial germination of *Alternaria* sp. and *Botrytis* sp. was examined with treatment time and intensity of CDAP. The conidial germination of *Alternaria* sp. treated with CDAP at the concentration of 13.7~14.4 ppm of $O_3$ was strongly inhibited by time, showing $y = 2.66x^2 - 85.139x + 4.88$ and $R^2 = 0.98$. When the conidia of *Alternaria* sp. were exposed for 2 hours with varying plasma $O_3$ concentration, the conidial germination was strongly inhibited as the concentration of $O_3$ increases, showing $y = -0.09x^2 + 6.905x - 0.764$ and $R^2 = 0.95$. The conidia of *Botrytis* sp. also showed similar patterns to CDAP. The inhibitory effect of CDAP on the germination of postharvest pathogens depends on treatment time and $O_3$ concentration.

## 1. Introduction

Onion, one of the widely consumed vegetables, is well known for various biological activities including antioxidant and antibacterial effects mediated by sulfur and phenolic compounds [1, 2]. Onion is commonly used as a spice in Korea. Onion is usually stored for several months in a cold, dry condition after curing process to cover the seasonal demands of market in Korea. Despite the cold storage to keep marketable quality, the onion losses are substantially occurred during the storage. The major losses are caused by plant pathogens without appropriate management of postharvest diseases [3]. *Botrytis* sp., *Fusarium oxysporum*, *Penicillium* sp., *Aspergillus awamori*, *Rhizopus oryzae*, and *Alternaria* sp. are well known to cause decay during onion storage in Korea [4, 5]. However, the application of agrochemical fungicides is limited because of public concerns over the human health and environmental risks over the agrochemical residues. Therefore, eco-friendly alternative measures should be considered to control the postharvest pathogens contaminated on onions bulbs.

Plasma is known as the state of ionized gas which contains energetic reactive species, such as electrons, photons, ions, free radicals, excited molecules, and atoms, and is considered as an emerging technology for the management of postharvest diseases. There are several methods to generate plasma, including gas discharge, photoionization, heat radiation, and radio frequencies. Among the methods, the common way to produce nonthermal plasma is gas discharge [6]. Corona discharge air plasma (CDAP) and dielectric barrier discharge are the most common approaches for nonthermal plasmas' generation under atmospheric pressure. They are known to produce chemically active species, oxygen ions, and charged species such as NO+, NO−, hydroxyl and hydroperoxyl radicals, hydrogen peroxide, nitrogen oxide species (NO, $NO_2$, etc.), atomic oxygen, and ozone [7]. The gases widely used to create plasma are air, pure Ar, mixture of $He/O_2$ and $Ar/O_2$, and pure $N_2$ [8]. These active species act as very strong oxidizers and are considered to contribute to the antimicrobial effects of gas plasma [9]. There are many reports that state that low-temperature atmospheric plasma can kill various kinds of microorganisms, such as fungi, bacteria, and yeast [10, 11]. The mechanisms of nonthermal plasma for the inactivation of microorganisms are suggested as surface erosion and oxidation of microbial cell membranes by reactive species [12] and DNA damage by UV radiation [13]. The potential of cold atmospheric plasmas for antimicrobial applications has been reported earlier. One atmosphere uniform glow discharge plasma has been used for the successful inactivation of *Escherichia coli* O157:H7, *Salmonella* sp., and *Listeria monocytogenes* on fresh produce surfaces [14]. As it constitutes one of the forms of atmospheric plasma, corona discharge plasma has also been shown to possess biocidal or biodecontamination effect [15, 16]. The predominant mechanism of biological action of corona discharges is believed to be oxidative damage produced by reactive oxygen species [17]. However, the effect of plasma sterilization depends on the kind of microorganisms, initial population of contaminated microorganisms, plasma treatment temperature, and relative humidity [18]. Sera and Sery [19] reported that the major sterilization factors in the nonthermal plasma food technology sector largely depend on the plasma source type or plasma characteristics. They reported the availability of nonthermal plasma for activation of seed germination, early growth of seedlings, microbial inactivation of seed/fruit surface, and possibility of increasing quantity of biological active compounds in sprouting seeds. In general, fungi are more profound than bacteria or viruses and have cell walls that lead to less susceptibility to external cell damage. In this study, we examined the effects of CDAP for the inactivation of postharvest pathogens contaminated on onion and investigated the inhibitory effect of CDAP on the mycelial growth and conidial germination of *Alternaria* sp. and *Botrytis* sp.

## 2. Materials and Methods

*2.1. Corona Discharge Air Plasma Generation.* Corona discharge air plasma (CDAP) used in this experiment was purchased from Samdo Environment Co. Ltd., Kwangju, Korea. A schematic diagram of CDAP is shown in Figure 1. An air blower (Ventur Tekniska, Goteborg, Sweden) to generate remote or afterglow plasma stream from electrode point had 25 lpm of blower rate at the electrode tip. Power supply with the output voltage of 20 kV DC and the frequency of 60 Hz was used for the plasma. Plasma intensity was controlled by adjusting the electric current and frequency. The amounts of ionized gas of the plasma were determined by using a plasma-activated species ($O_3$, NO, and $NO_2$) detector. The amount of active species produced is shown in Figure 2 and Table 1, respectively. Major active species of corona discharge air plasma was ozone. $NO_2$ was poorly generated, and NO was not generated. The ionized gas of the plasma was flowed by channeling through a PVC flexible hose (1 m length; 70 mm in diameter) to a treatment chamber (0.35 $m^3$ in dimension) or generated in a cold storage room (50.4 $m^3$ in dimension).

*2.2. Isolation and Identification of Postharvest Pathogens of Onion.* To investigate the effect of CDAP on onion decay in low-temperature storage conditions, onion was stored at 0°C in a cold storage room (50.4 $m^3$ in dimension) for 10 months with corona discharge air plasma treatment at $O_3$ concentration of 5 ppm for 6 hours every day. The decayed onions were selected as plasma treated or untreated one after 10 months' storage. To isolate the causal fungi responsible for onion decay, the tissue (10 × 10 mm) of the boundary between the healthy and diseased areas on the decayed onion was aseptically taken. The tissues were sterilized with 70% ethanol and 1% NaOCl solution for 1 min., respectively. The tissue was washed with sterile distilled water and then dried on a sterile filter paper. Then, it was placed on a prepared water agar plate and incubated at 25°C. The grown mycelium was aseptically transferred to potato dextrose agar (PDA) and examined for single isolate under a microscope. The morphological characteristics of each isolated fungus were observed under an optical microscope. The frequency of isolated fungi was calculated by counting the number of individual pathogens among the total isolated.

*2.3. Pathogenicity Test of the Isolates.* Each isolate was cultured on PDA for 7 days and used for the pathogenicity test. The onion kept in the 0°C low-temperature storage was selected, and the skin and roots were removed. Then, the onion was washed with clean water and dried for 4 hours. Then, the onion was cut to half with a sterilized knife by an alcohol lamp and 70% ethanol. One side of the onion was injured with a needle, and the other side was prepared without injury. A wet paper towel with sterilized water was placed on the bottom of a plastic box (200 × 280 × 200 mm), and clean Petri dishes (35 × 10 mm; SPL Life Science Co., Pocheon, Korea) were placed on the paper towel. Six half onions (3 noninjured and 3 injured) were placed in Petri dishes on the bottom of a plastic box. Each isolate prepared to a Φ10 mm-sized mycelial disk was inoculated on the onion. Then, the plastic box was stored at 25°C to determine

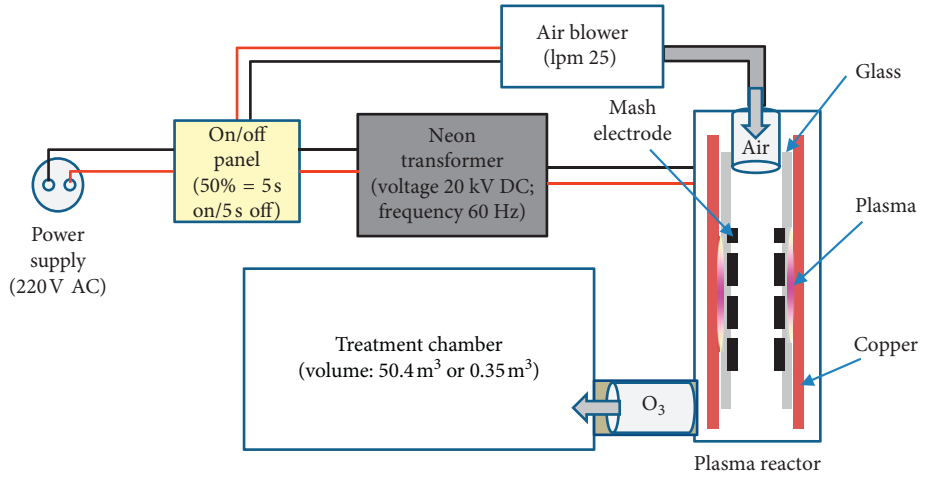


Figure 1: Schematic diagram of corona discharge air plasma (CDAP) system. 50.4 $m^3$ is the generated plasma in a low-temperature storage room. 0.35 $m^3$ is the scale of the device installed to check the degree of the inhibition of the microbial isolated from the onion.

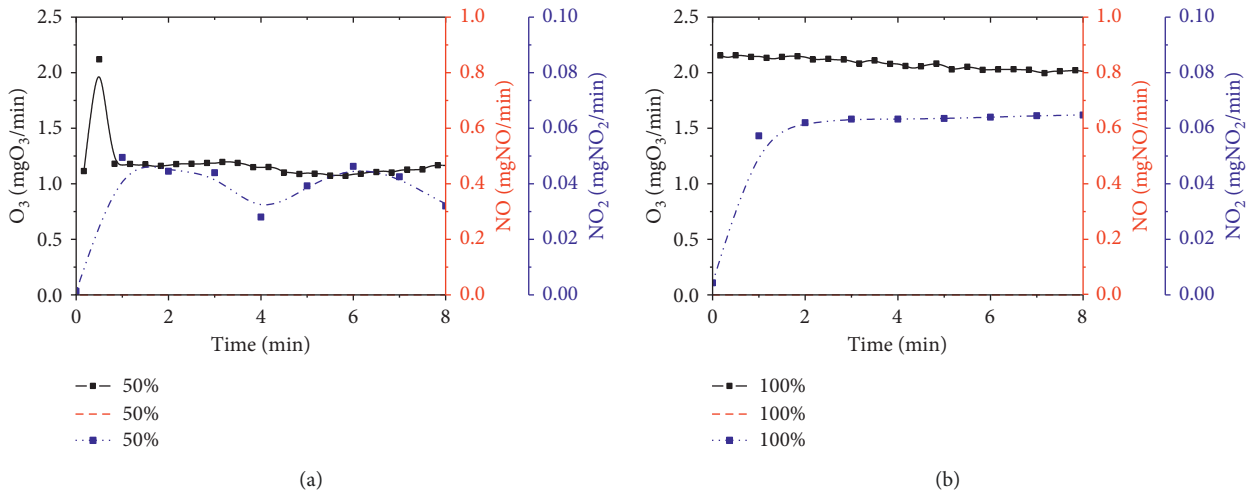


Figure 2: Active species generation amount of corona discharge air plasma.

Table 1: Measurement of active species generated by corona discharge air plasma.

| | Average generation rate at 25 lpm air, 8 min | | Average concentration at 25 lpm air, 8 min | |
|---|---|---|---|---|
| | 50% | 100% | 50% | 100% |
| $O_3$ | 1.18 (mg/min) | 2.07 (mg/min) | 22 (ppm v/v) | 38.7 (ppm v/v) |
| $NO_2$ | 0.033 (mg/min) | 0.058 (mg/min) | 0.644 (ppm v/v) | 1.13 (ppm v/v) |
| NO | 0 | 0 | 0 | 0 |

whether the disease develops. All isolates were tested for pathogenicity in three replicates over the second time.

*2.4. Inhibitory Effect of Corona Discharge Air Plasma on the Mycelial Growth of the Postharvest Pathogens.* We investigated the inhibitory effect of plasma treatment on the mycelium growth of two pathogens, *Alternaria* sp. 5RD1 and *Botrytis* sp. 2RG4, isolated from the diseased onion. They were inoculated on the PDA medium and cultured in a 25°C incubator for 7 days. The mycelial disk of the pathogens was prepared using a cork borer (Φ10 mm) and placed on a new PDA medium. Then, the PDA plate without lid was placed in an acrylic box equipped with a plasma device and treated for a certain period of time (1, 2, 4, 8, and 16 hours). After treatment, the PDA plate was cultured at 25°C to observe the mycelial growth for 7 days. The inhibitory effect was calculated by comparing the mycelial growth of the pathogens with and without plasma treatment.

*2.5. Inhibitory Effect of Corona Discharge Air Plasma on the Spore Germination of Postharvest Pathogens. Alternaria* sp. and *Botrytis* sp. were inoculated on a potato dextrose agar (PDA) medium and cultured at 25°C for 10 days to form its spores. 20 ml of sterile water was added to the PDA plate, and the spores were suspended using a sterile loop. The

spore suspension (approximately $1 \times 10^6$ spores/ml) was prepared by passing through sterilized four-layered gauze to remove mycelial fragment and stored at 4°C before use. 100 $\mu$L of the spore suspension was inoculated on the surface of water agar for plasma treatment. Then, the water agar plate without lid was placed into a disinfected acrylic container connected with a plasma device by a hose. The plasma was treated at different time periods or $O_3$ concentration. After treatment, the plates were incubated for 16 hours at 25°C, and spore germination was observed under an optical microscope.

## 3. Results and Discussion

*3.1. Active Species Generated by Corona Discharge Air Plasma.* To measure the amount of plasma active species, the air flow of the internal suction pump was set to 25 lpm and the power setting value (time) was increased from 50% (5 s on/5 s off) to 100% (10 s on/10 s off). As shown in Figure 1 and Table 1, when $O_3$, $NO_2$, and NO detectors were used for the plasma active species, $O_3$ was presented for most of the active species. The average generation rate of $O_3$ was 1.18 mg/min at 50% (5 s on/5 s off) and 2.07 mg/min at 100% (10 s on/10 s off). The average generation rate of $NO_2$ was 0.033 mg/min at 50% (5 s on/5 s off) and 0.058 mg/min at 100% (10 s on/10 s off). NO was not detected. Plasma is known to vary in the ionized material produced by the process gasses used. Hertwig et al. [20] found that there was a significant difference in plasma emission intensity depending on the gas types (dry air, $N_2$, $O_2$, and $CO_2$). $N_2$ as a process gas showed the highest emission intensity compared to the other process gasses. They also reported that the use of $O_2$ as a flower gas with cold atmospheric pressure plasma produced a high ozone concentration in the treatment chamber. We also achieved a similar result that relatively high ozone concentration was detected with the use of the air as a flower gas of CDAP.

*3.2. Types of Fungi Isolated from Decayed Onions.* The decayed onions were separated as untreated or CDAP-treated one after 10-month storage at 0°C, and the causal agents were isolated. As a result of the isolates in Table 2 and Figure 3, a total of 77 fungi were isolated from CDAP-treated onion and total 103 molds from nontreated onion. When the fungi were classified by type, more *Fusarium* sp. were isolated from nontreated onions and more *Botrytis* sp. were isolated from CDAP-treated onions. The frequency of isolation by fungal type showed 24% of *Botrytis* sp., 70% of *Fusarium* sp., 2.0% of *Alternaria* sp., and 7.0% of unknown fungi in untreated (control) onions. On the other hand, it showed 39% of *Botrytis* sp., 26% of *Fusarium* sp., 0% of *Alternaria* sp., and 12% of unknown fungi in CDAP-treated onions. In general, *Fusarium* sp. is the dominant strain at 25°C, and *Botrytis* sp. is known to occur well below 15°C. Our results from Figure 2 suggest that the major active species on the antimicrobial effect of the CDAP-treated onion is ozone. Our result suggested that ozone seems to be more critical to the growth of *Fusarium* sp. than the other fungi isolated in this experiment. Ozone is known as a powerful oxidant and has been researched as a sanitizer in the food industry [21] and a removal agent of mycotoxins [22] or pesticide residues [23]. Ozone can also act as a host resistant inducer. Minas et al. [24] reported that the exposure of kiwifruits to ozone before inoculation of *Botrytis cinerea* in a cold storage room resulted in the reduction of disease incidence. They suggested that the treatment of ozone to kiwifruit induces resistance to *B. cinerea*.

*3.3. Pathogenicity and Characteristics of Fungi Isolated from Decayed Onions.* As a result of testing pathogenicity of isolates in Table 3, *Fusarium* sp. 3RC2 and *Fusarium* sp. 3RC1 revealed as strong pathogenic fungi on both wound and healthy onion. In addition, *Alternaria* sp. 5RD1 showed strong pathogenicity on wounded onion but weak pathogenicity on healthy onion. The unidentified fungus 2RC1 showed strong pathogenicity only in wounded onion. *Botrytis* sp. 2RG4 and 3RA2 and unidentified fungus 3RB2 showed intermediate pathogenicity only in wounded onion. The isolated fungi are mostly spore-forming fungi, which may be rapidly decayed by spore's germination during storage and distribution.

*3.4. Inhibitory Effect of Plasma on the Mycelial Growth and Spore Germination of Isolates.* We investigated whether CDAP treatment by time and intensity inhibits the mycelial growth of *Alternaria* sp. 5RD1 and *Botrytis* sp. 3RG4 on the PDA medium. Treatment of CDAP at 10% intensity stimulated the mycelial growth of the isolate, showing the mycelial growth rate of −3.36% in 1 hour, −7.21% in 4 hours, and −2.51% in 16 hours, respectively. Treatment of CDAP at 50% intensity showed the mycelia growth rate of −4.33% in 1 hour, −1.44% in 4 hours, and 5.86% in 16 hours. The higher the concentration of the plasma treatment or the longer the plasma treatment time, the more inhibitory effect on the mycelial growth of *Alternaria* sp. 5RD1 was observed (Figure 4(a)).

CDAP treatment to *Botrytis* sp. 3RG4 inhibited the mycelial growth up to 6.57% in 1 hour, 10.22% in 2 hours, and 7.66% in 4 hours at 10% plasma intensity, but treatment of CDAP with increased time slightly stimulated the mycelial growth of *Botrytis* sp. 3RG4, showing the mycelial growth rate of −3.65% and −5.15% in 8 hours and 16 hours, respectively. Treatment of CDAP at 50% intensity showed a similar mycelial growth pattern with 10% plasma intensity (Figure 4(b)). Our results showed that the CDAP treatment effect on the mycelial growth of *Alternaria* sp. 5RD1 and *Botrytis* sp. 3RG4 isolated from decayed onions is little significant or insignificant.

We investigated whether CDAP treated by time and plasma intensity influences on the spore germination of *Alternaria* sp. 5RD1 and *Botrytis* sp. 3RG4. The inhibitory effect of CDAP at 45% intensity ($O_3$ concentration: 13.7~14.4 ppm) on the spore germination of *Alternaria* sp. 5RD1 is shown in Figure 5. The inhibition rate of spore germination of the isolate was 72.6% for 1 hour and 92.3% for 2-hours exposure. Regression analysis showed high

Table 2: Comparison of isolation frequency of fungi isolated from nontreated or CDAP-treated onions.

| Treatment | Frequency of isolation (%) | | | | Total isolates |
|---|---|---|---|---|---|
| | *Botrytis* spp. | *Fusarium* spp. | *Alternaria* spp. | Unknown | |
| Control | 24 | 70 | 2.0 | 7.0 | 103 |
| CDAP | 39 | 26 | 0 | 12 | 77 |

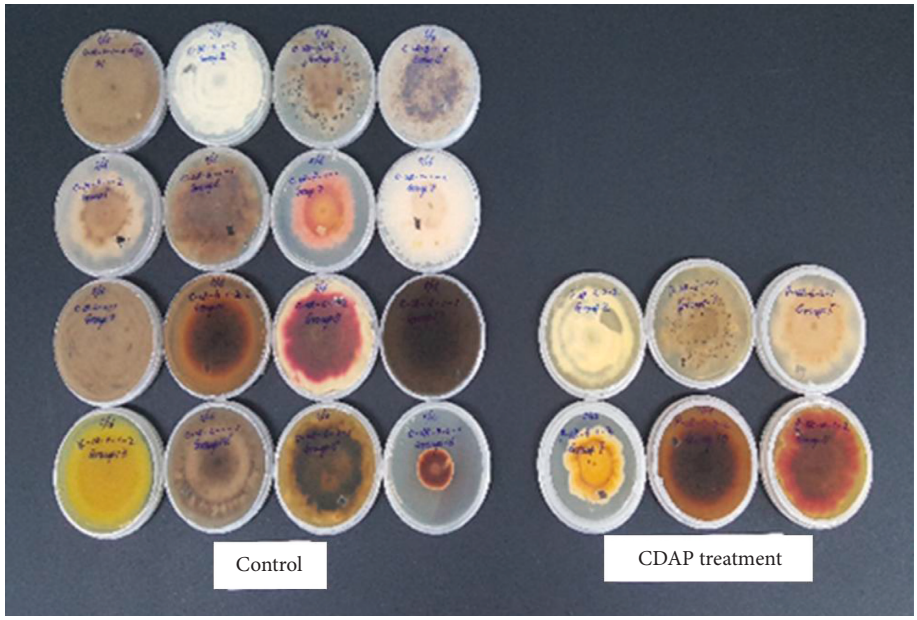


Figure 3: Colors of representative fungal isolates grown on potato dextrose agar isolated from nontreated or CDAP-treated onions.

Table 3: Pathogenicity and characteristics of fungi isolated from decayed onions.

| Strains | Pathogenicity | | Characteristics in potato dextrose agar |
|---|---|---|---|
| | Injured onion | Fresh onion | |
| *Fusarium* 3RC1 | +++ | ++ | White mycelium, rod-shaped conidiospore, good mycelium growth |
| *Fusarium* 3RC2 | +++ | +++ | White mycelium, rod-shaped conidiospore, good mycelium growth |
| *Alternaria* 5RD1 | +++ | + | Conidiospore, medium mycelium growth |
| Unknown 2RC1 | +++ | − | Nonspore formation, poor mycelium growth |
| *Botrytis* 2RG4 | ++ | − | Small-type conidiospore, good mycelium growth |
| *Botrytis* 3RA2 | ++ | − | Grey mycelium, small-type conidiospore, good mycelium growth |
| Unknown 3RB2 | ++ | − | Nonspore formation, medium mycelium growth |
| *Botrytis* 1RA2 | + | − | Grey mycelium, small-type conidiospore, poor mycelium growth |
| Unknown P2RB1 | + | − | Nonspore formation, poor mycelium growth |
| Unknown P5RF2 | + | − | Nonspore formation, poor mycelium growth |

+++ and ++: good mycelium growth; +: medium mycelium growth; −: poor mycelium growth.

significance at $y = 2.66x^2 - 85.139x + 4.88$ and $R^2 = 0.98$ (Figure 5(a)). When *Alternaria* spores were exposed with various intensities (various ozone concentrations) of CDAP for 2 hours exposure, the inhibition rate of spore germination was 21.7% for ozone concentration of 4.37 ppm, 72.7% for 10.85 ppm, and 95.41% for 19.45 ppm. Regression analysis showed high significance at $y = -0.09x^2 + 6.905x - 0.764$ and $R^2 = 0.95$ (Figure 5(b)).

The inhibitory effect of CDAP at 10% intensity ($O_3$ concentration: 1.5~2.9 ppm) on the spore germination of

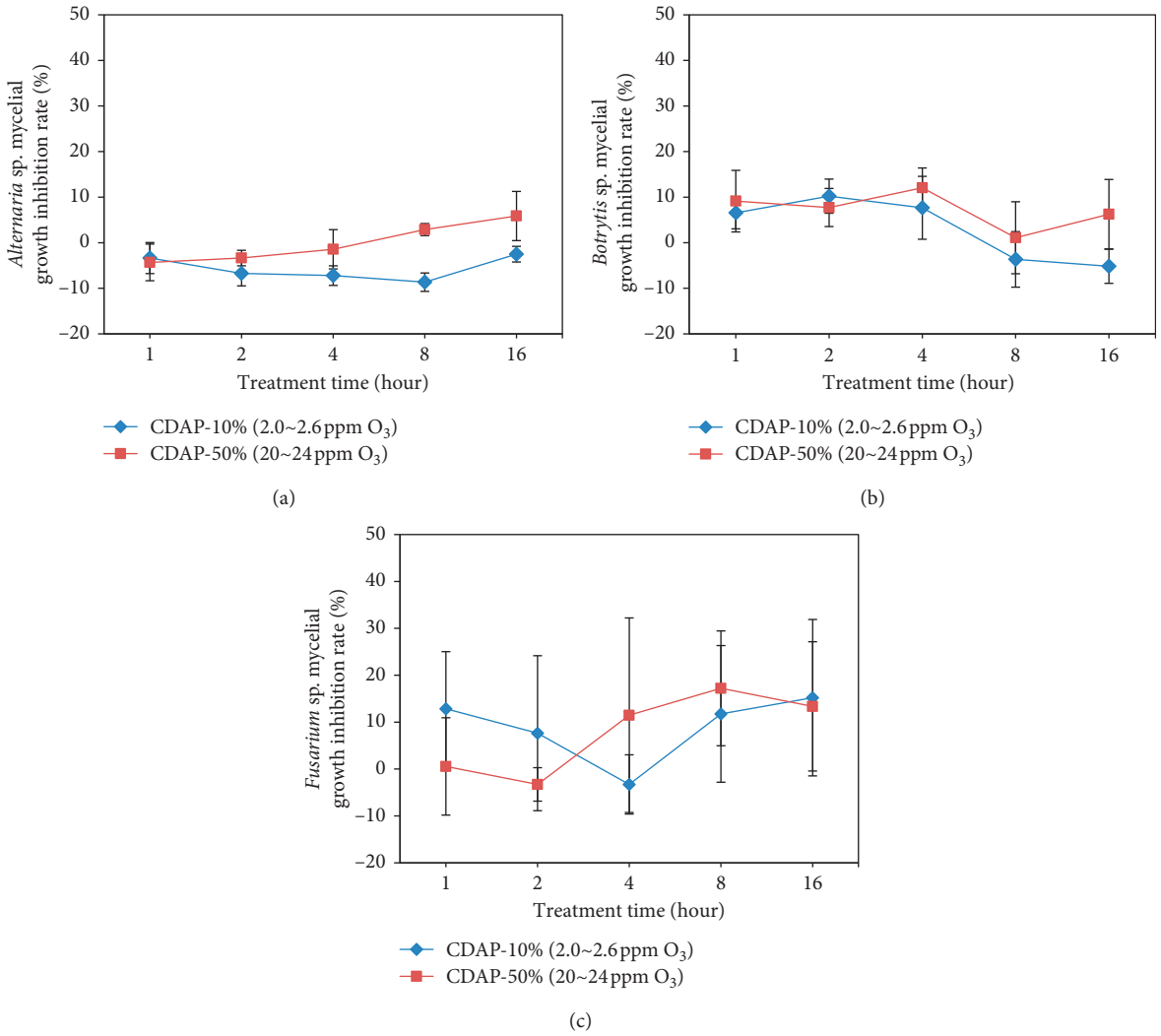


Figure 4: Effect of CDAP treatment time on mycelial growth of *Alternaria* and *Botrytis* sp. (a) Mycelial growth of *Alternaria* sp. 5RD1 after culturing at 25°C for 4 days after CDAP treatment, (b) mycelial growth of *Botrytis* sp. 2RG4 after culturing at 25°C for 3 days after CDAP treatment, and (c) mycelial growth of *Fusarium* sp. 3RC2 after culturing at 25°C for 3 days after CDAP treatment. The CDAP-10% and CDAP-50% mean that the plasma operation time is operation on during 1 second/operation off during 1 second (concentration of $O_3$ is 2.0~2.6 ppm) at CDAP-10% and operation on during 5 second/operation off during 5 second (concentration of $O_3$ is 20~24 ppm) at CDAP-50% per hour for 16 hours.

*Botrytis* sp. 3RG4 is shown in Figure 6. The inhibition rate of spore germination was 70.7% for 4 hours and 98.5% for 8 hours of exposure, respectively. Regression analysis showed high significance at $y = -0.684x^2 + 18.307x - 4.809$ and $R^2 = 0.95$ (Figure 6(a)). When the spore of *Botrytis* sp. 3RG4 was treated with various intensities (various ozone concentrations) of CDAP for 2 hours exposure, the inhibition rate of spore germination was 23.7% for ozone concentration of 2.1 ppm, 56.9% for 3.39 ppm, and 97.53% for 6.0 ppm, respectively. Regression analysis showed high significance at $y = -0.635x^2 + 19.347x - 4.772$ and $R^2 = 0.97$ (Figure 6(b)).

The inhibitory effect of fungal spores in Figures 4–6 was much better than that of mycelium. The inhibitory effect of fungus mycelium on ozone produced in the CDAP treatment was not effective in *Fusarium* sp. (Figure 4(c)). *Alternaria* sp. and *Botrytis cinerea* showed some inhibitory effect at high $O_3$ concentration, but it promoted the growth of fungus mycelium at low $O_3$ concentration (Figures 4(a) and 4(b)). However, the inhibitory effect of fungi spores on ozone showed more than 80% inhibition rate at ozone concentration of 13~14 ppm with 2 hours treatment in *Alternaria* sp. spores (Figure 6(b)) isolated from onion and at ozone concentration of 6 ppm with 2 hours treatment for *Botrytis cinerea* spores (Figure 5(b)). These results indicate that the effect of plasma treatment varies depending on the kind of fungal species and propagules. Gabler et al. [25] also reported that ozone treatment effectively controlled the postharvest grey mold disease on grapes, while poorly controlled the decay of grapes caused by *Alternaria* and *Penicillium* sp. in semicommercial experiments. Minas et al. [24] reported that continuous treatment of *B. cinerea* cultures grown on potato dextrose agar with gaseous ozone showed a direct inhibitory effect, but removal of the pathogen from the ozone-enriched environment led to resume the growth within 48 hours. They also

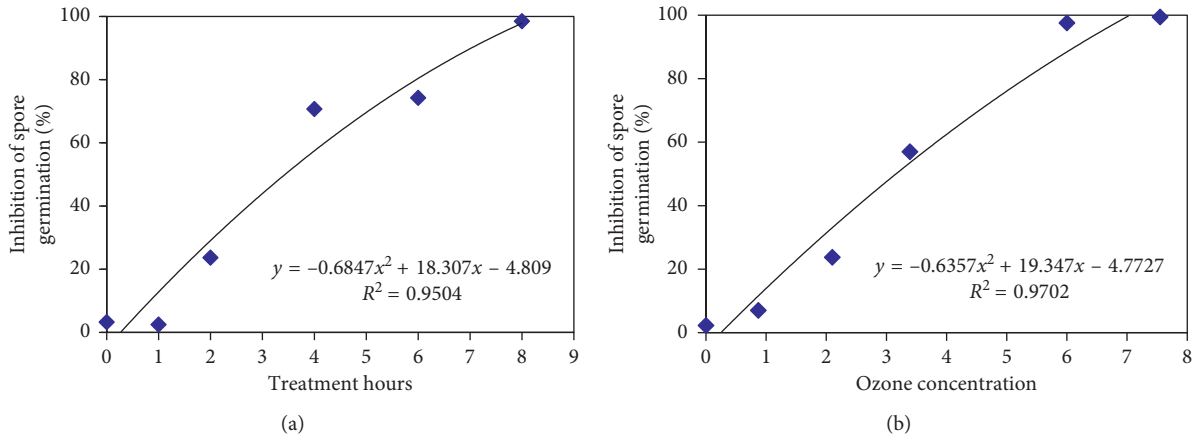


Figure 5: The effect of different plasma treatment time and concentration on the inhibition of germination of *Botrytis sp.* spore. (a) Plasma treatment of *Botrytis sp.* spores with different treatment time periods at constant $O_3$ concentration (CDAP-10%, $O_3$: 1.5~2.9 ppm) and (b) plasma treatment of *Botrytis sp.* spores with different $O_3$ concentrations at constant treatment time (2 hours).

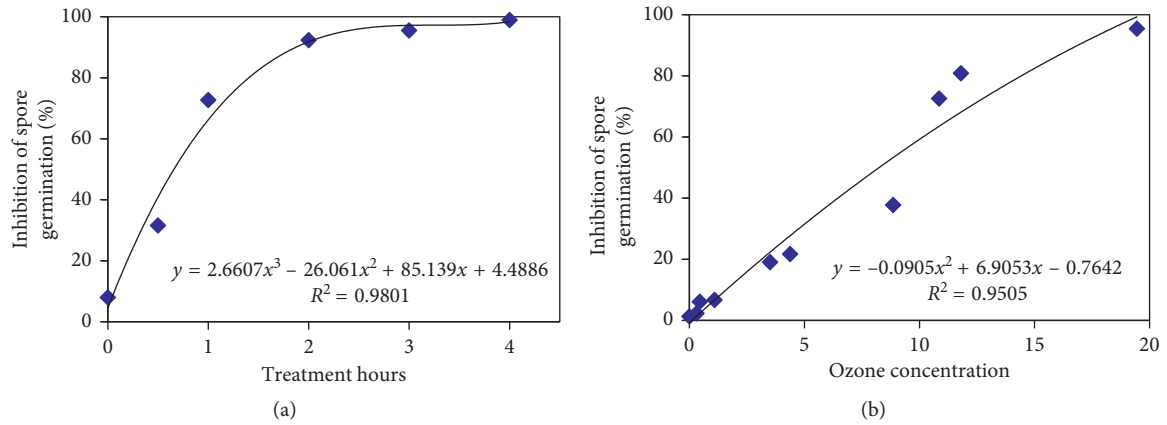


Figure 6: The effect of different plasma treatment time and concentration on the inhibition of germination of *Alternaria sp.* spore. (a) Plasma treatment of *Alternaria sp.* spores with different treatment time at constant $O_3$ concentration ($O_3$: 13.7–14.4 ppm) and (b) plasma treatment of *Alternaria sp.* spores with different $O_3$ concentrations at constant treatment time (2 hours).

reported that gaseous ozone treatment of the fungal spores for more than 8 hours significantly reduced the spores' viability. Ryu et al. [26] measured the extent of spore survival and the shape of spores by treating plasma with spore of the bread mold. The fungus has less sterile effect by the plasma treatment than the bacteria, because the fungus has a cell wall composed of a carbohydrate called $\beta$-glucan. In the case of bread molds, $\beta$-carotene, which acts as an antioxidant, is present in large amounts in the spores and may have the effect of protecting it from oxidation by plasma. Many molds have a variety of pigments, and these pigments can also act as antioxidants in many cases, so that, the fungus may be more resistant than bacteria to plasma treatment. Our data in this study showed that CDAP treatment significantly triggered fungal spore death depending on time. These data suggest that CDAP is a promising tool to inactivate the fungal spores. However, the optimization of CDAP processing conditions should be evaluated by the kind of microorganisms, type of products, temperature and humidity in a storage, etc.

## 4. Conclusions

The main antimicrobial reactive substance generated by the CDAP method was ozone. Ozone produced by CDAP treatment may be effective in inactivating fungal spores, whereas inactivation of fungal mycelium was not effective. Data presented in this report demonstrated that the low concentration exposure of CDAP had the potential of inactivating fungal spores. In addition, CDAP treatment triggered significantly fungal spore death that depends on time. These data suggested that plasma represents a novel technology with the capacity of inactivating fungal spore in plants. The inhibitory effect of CDAP on the germination of postharvest pathogens depends on treatment time and $O_3$ concentration.

## Data Availability

No data were used to support this study.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

## Authors' Contributions

Y.S.B. and H.J.C. conceived and designed the study; E.H.C. and Y.S.B. carried out the experiments; I.S.S., J.H.L., and J. W.C. analysed the data; and E.H.C. and Y.S.B. wrote the manuscript. All authors read and approved the final manuscript.

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