Generation and characterization of atmospheric pressure dielectric barrier discharge air plasma and its antifungal potential: a case study on Alternaria alternata

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ABSTRACT
The present research evaluates the generation and characterization of 26 kHz-atmospheric pressure dielectric barrier discharge (DBD) air plasma (DBD-AP) and the possible antifungal action against the pathogenic fungus Alternaria alternata. The DBD-AP visual and emission spectra intensities increased with increasing the applied voltage. The generated plasma was mainly inhomogeneous due to the presence of streamers in the discharge gap and it showed diffuse with less gap distance or with increasing the applied voltage. The measured consumed power was 26.52 W for 10.3 kV and 17.4 mA at 3 mm gap. The emission spectra showed the presence of NO and N2 bands which were enhanced due to the increase of the applied voltage. Significant reduction in A. alternata growth accompanied by loss of fungal cells structure was observed with increasing the DBD-AP exposure time. The present article confirms the potential of DBD-AP to efficiently control the plant-pathogenic fungus A. alternata.

ARTICLE HISTORY
Received 24 October 2020
Revised 16 April 2021
Accepted 22 April 2021

KEYWORDS
Atmospheric pressure plasma; Alternaria alternata; pathogenetic fungi; dielectric barrier discharge; growth inhibition

1. Introduction
In recent years, high advanced technologies have been developed to harness different microorganisms for human benefits [1–3]. However, continuous increase in the number of infectious fungi that cause diseases to crop plants has been recorded, resulting in negative impacts on the global food security, as well as the direct massive economic losses [4,5]. Among many pathogenic fungi, mould is known as a conspicuous mass of fungal mycelia and fruiting structures belongs to genera Aspergillus, Alternaria, Penicillium, and Rhizopus which are associated with food spoilage and plant diseases. Alternaria spp. are considered as a serious crop pathogen that are often encountered during postharvest storage of fruits. Unfortunately, fungal infestations are difficult to neutralize since fungal cells and spores are characterized by being quite resistant. Therefore, postharvest chemical fungicides are applied by spraying or wax coating [6,7]. However, the use of chemical fungicides has resulted in health and environmental problems associated with chemical residues or the emergence of resistant isolates. In addition, the use of chemical fungicides has grown unpopular among most food consumers who prefer natural products [8,9]. Plasma can be classified thermodynamically into two main types: thermal and non-thermal plasmas. The temperature of electrons, ions, and neutral particles determine the thermal conditions of the generated plasma.

If the temperature of the electrons, ions, and neutral particles are equal, then this plasma is thermal, i.e. in a state of thermal equilibrium. However, if the temperature of the electrons is much higher than those of ions and neutral particles, the plasma is in a state of non-thermal equilibrium. In the latter type of plasma, the temperature of the neutral particles (gas) can reach room temperature and can be used safely with biological samples and human skin or tissues [10].

To combat against the growing challenge of food safety, consumer demanded several non-thermal technologies to be explored. In that context, cold plasma became a modern-day interest to researchers in agricultural science, biofuels, physics, as well as the food industry [1,4,5,11,12]. It is a gas which is partially or completely ionized according to the amount of supplied energy. Plasma contains electrons, ions and neutral particles (atoms and molecules), and active species such as radicals, active ions, ozone gas, in addition to ultraviolet radiation [1], which showed deadly effects against the infectious microbes and harmful bacteria [13]. Atmospheric pressure plasma has a confirmed impact on adjusting surface properties [14–16], depositing thin films on substrates [17,18], cleaning of surfaces [19], decontamination of biological materials [11], enhancement of biological molecules [1], and sterilization [16,20–30]. Plasma is considered to be of highly effective impact in a large number of applications due to
its ability to produce a large number of chemically reactive molecular fragments and reactive species such as O, OH and NO [1,31,32].

The reason behind the economic effectiveness of plasma technology is the ability of rapid sanitization at ambient temperature and pressure conditions, without affecting the crop quality nor enormous costs when using air as a working gas [33,34]. However, application of cold plasma to control the fungal growth remains of interest to the crop industry, since fungi constitutes a large group of plant-pathogenic organisms [35–37]. Recently, many researchers have succeeded to generate cold plasma at the atmospheric pressure in air. Many techniques were used to generate cold plasma such as jet plasma and dielectric barrier discharge (DBD) plasma, which in turn opened new horizons for plasma applications in many areas such as sterilization and surface treatments [38]. However, there is a research gap concerning the generation and impact of DBD air cold plasma (DBD-AP) against pathogenic fungi, such as *Alternaria alternata*. Therefore, the present study focussed on solving the problems of generating cold plasma in air without switching to arc discharge and to investigate the possible application to reduce the environmental and biological pollution in an effective and affordable means. DBD system was used to generate atmospheric pressure cold plasma. The electrical, optical and spectroscopically properties of the generated plasma were studied and analyzed in detail to get enough information on the physics of the generated plasma and to find out the optimal conditions for getting stable plasma in air. Moreover, the potential of the generated DBD-AP for the treatment of *A. alternata* spores was investigated. This would be useful in finding an alternative eco-friendly and economically feasible technology to the conventional sterilization that require the use of chemical fungicides.

2. Materials and methods

2.1. Plasma system

The generated cold plasma system at atmospheric pressure consisted of two parallel metal electrodes separated by an air gap (Figure 1). The upper electrode composed of a solid cylindrical disc made from brass with a 3.5 cm diameter, covered with an electrical insulation from Teflon, while its lower side was covered with an isolation disc of quartz (thickness 3 mm). A sinusoidal high-voltage waveform (0–40 kV, 26 kHz) was applied to the upper electrode. However, the bottom electrode composed of stainless steel disc with a diameter of 4.5 cm that was connected to the ground. A variable air gap (1–6 mm) was possible to be established between the two electrodes to form a dielectric barrier discharge when sufficient voltage was applied to the upper electrode. It is worth noting that the bottom electrode was used as stage to the treated sample. The current passed through the upper electrode was monitored using a current probe (Pearson Current Monitor, model: 6585). A high-voltage electrical probe (Tektronix P6015A-1:1000) was used to measure the applied voltage on the upper electrode. The measured current and voltage waveforms were recorded using a high-sensitive oscilloscope (3.5 GHz, Tektronix Oscilloscope DPO7354C).

2.2. Monitoring plasma parameters

A Nikon digital camera (D3200 with AF-S Micro NIKKOR 105 mm lens) was used to capture the visible plasma images. Moreover, a spectrometer (Princeton Instruments Acton SP2500) of length 0.5 m was used to measure the generated plasma emission spectra. The spectrometer is a tripled grating. The emission spectral signal was transmitted by a bundle of optical rating of 150, 1800, and 3600 g/mm that are blazed at 500,

![Figure 1. A schematic diagram showing the dielectric barrier discharge (DBD) used for air plasma generation.](image-url)
500 and 240 nm, respectively. The spectrometer has an optimal linear dispersion of 0.50 nm/mm. The emission spectral signal was transmitted by a bundle of optical fibres of length 3 m to the spectrometer equipped with a photomultiplier (PMT, model: PD471).

3. Results and discussion

Figure 2 shows the effect of applied voltage between the two electrodes with a gap distance of 3 mm on the generated current. All measurements presented in this work were carried out at an applied frequency of 26 kHz. The current and voltage were measured as the amplitude of the positive half of the waveform signals. The total current increased with increasing the applied voltage due to the presence of the dielectric material (quartz disc) in the discharge gap. The discharge current increases with voltage (the slope of the curve is positive) resembling the abnormal glow discharge which corresponds to higher power densities. The steady increase of the pulse current measured with the applied voltage, and this is a typical result of the DBD-AP. Figure 3 presents typical waveforms of voltage and current of the generated plasma. The current waveform shows the presence of spikes which indicate the formation of streamers in the DBD plasma. The plasma images have also confirmed the formation of the streamers in the 3 mm discharge gap (Figure 4). The number of streamers increased with increasing the discharge current or voltage. Figure 5 confirms that the discharge breakdown voltage, which is measured from the maximum Pk-to-Pk of the applied voltage just before breakdown, increases with increasing the discharge gap at constant atmospheric pressure in air. The onset of the breakdown can be detected by the change in the capacity of the discharge gap, by using a photodetector, or form the appearance of the spikes on the waveform of the discharge current. The results indicate that the discharge operates in the right side for the minimum value of Paschen curve with a constant aspect ratio, d/R, where d is the gap distance and R is the radius of the dielectric disc, which are consistent with previous studies; the breakdown voltage of the air is 30 kV/cm [39–41]. The number of discharge streamers increased with increasing the discharge voltage at constant discharge gap. However, the number of the streamers increased with decreasing the discharge gap (1–4 mm) at a constant applied voltage as illustrated in Figure 4. As the number of streamers increased, the discharge diffused and could form a homogenous discharge at a smaller gap and higher applied voltage as presented in Figure 6 for discharge at 9.1 kV applied voltage and 1 mm discharge gap.

The change of the DBD-AP formation at 3 mm was investigated at different applied voltages. Results showed growth of the horizontal plasma with the increase in the applied voltage as the plasma begins from a single point to be a light column (streamer) between the two electrodes (Figures 4 and 6). The number of streamers increased with increasing the applied voltage. The streamers get diffused with increasing the applied voltage at constant gap distance. The plasma volume increased with increasing the applied voltage and the loss in charger carriers increased. Therefore,

![Figure 2](image-url)  
**Figure 2.** The current–voltage curve at 3 mm gap and a frequency of 26 kHz.
more current was required to sustain the generated plasma as be presented in Figure 2.

To estimate the energy dissipated during one period of the applied voltage, the area in charge–voltage was calculated the enclosed characteristic curve of the generated plasma, “Lissajous figure”; the (Q–V) charge–voltage characteristics. A capacitance C of 15 nF has been used as explained in some previous studies [42,43]. An example of the Lissajous figure (charge–voltage curve) which used in estimating the DBD-AP power and energy consumed by measuring its enclosed area as presented in Figure 7. The consumed power for 3 mm gap was investigated at 10.3 kV and 17.4 mA. The current and voltage was measured for the positive cycle of the waveform. The calculated power consumption was 26.52 W, which is calculated by multiplying the consumed energy and the applied frequency. The generated plasma was inhomogeneous and unstable; therefore, an average of 64 time was recorded for each measurement with 5 times of repetition. The consumed power increased with increasing the applied voltage. This increase in the consumed power is due to the increase in the charge carriers loses due the volume enlargement of the DBD-AP with increasing the discharge applied voltage. The current density of plasma discharge increased with further increase in the applied voltage, when the discharge covers the electrodes surface. Thus, the electron density also increased with the increasing of current density. This increase led to increase of the loss in the charge carrier through mainly the diffusion and recombination processes, which results in an increase in the consumed power to compensate the charge carried losses [44].

The plasma emission spectra were measured at 3 mm discharge gap. The fibre was placed at the middle of the gap. Figure 8 shows the DBD emission spectra in the wavelength range between 200 and 800 nm using 1800 g/mm grating. The nitrogen second positive system emission spectra bands (C3Πu → B3Πg) of its maximum intensity at its 0–0 transition (337.2 nm) are dominant and have the highest intensities [45]. The UV range of the spectra from 220 to 300 nm (using
3600 ng/mm blazed at 240 nm) proves the formation of NO radicals. The development of the emission spectra, from the DBD-AP in the range from 200 to 450 nm, as a function of increasing the applied voltage from 9.10 to 11.27 kV is shown in Figure 9. The NO and N2 emission spectra increase with increasing the applied voltage. The electric discharge gained its energy from the applied electric field. Then, it transfers its energy to the ambient species to cause excitation ionization in the discharge gap. The de-excitation of the excited species emits the detected spectra. The N2 + first negative system (B2 Σ+ u → X3 Σ+ g) emission spectra at 391 nm peak band indicates that the generated DBD-AP has included high electron temperature. The presence of NO radicals and N2 + first negative system (B3 Π g → A3 Σ+) means that the generated plasma has a high level of non-equilibrium. High-temperature electrons initiate excitation to higher levels, ionizations and dissociations which is important in decontamination and fungal germination reduction. The formation of the nitrogen second positive system in the UV range and the first positive system in the visible range indicates that the meta-stable state N2 A3 Σ+ u is formed which has a long-life time and energy of 6 eV [46]. This state has an important role in initiating the formation of the reactive species in an atmospheric air plasma which has a high collision rate [47]. Therefore, BDB-AP was applied on A. alternata for different times, and its impact on spore germination was evaluated.

*Alternaria alternata* is a plant-pathogen responsible for causing black spot diseases in many plants, such as grains and legumes causing great losses to these
seeds, especially during storage under relatively high moisture content and moderate temperatures. To check the effect of the generated plasma on the growth of *A. alternata*, the fungus was incubated at 28°C, on potato dextrose agar, culture medium for 7 days. Spores were collected from the growing edges of the resulting fungal colonies and divided into three groups. Two groups were exposed to the generated plasma for 3 min at 10.5 and 11.3 kV of applied voltage. The DBD-AP was operated also at 3 mm gap distance. The third group was taken as a reference (without plasma exposure). The spores were then placed again on the culture medium for another 7 days of incubation to track the resulting mycelium growth. There was a clear effect of DBD-AP
Figure 9. Emission spectrum from 200 to 450 nm taken by the spectrometer (0.5 m) with (grating 3600 G/mm blazed at 240 nm) at (3 mm) at (26 kHz). All data were normalized to the intensity of the peak 337.2 nm at 1.27 kV.

Figure 10. *Alternaria alternata* germinating spores: Panel A Unexposed (control); Panel B the fungus exposed for six minutes and panel C after 8 min of exposure to plasma with operating parameters (10 kV, 26 kHz at 3 mm gap distance).

on the treated samples of the pathogenic fungus compared to the untreated group as shown in Figure 10. The untreated fungus showed extensive mycelial growth and spores, which were decreased significantly by exposure to the plasma for 6 and 8 min. The negative impact of cold plasma on the living microbial eukaryotic cells was previously reported. The main effect was attributed to the significant reduction of the pH in the medium [1]. The decrease in the pH after exposure to plasma was attributed to oxidation by reactive species, which leads to carboxylic acids formation [48]. In addition, longer treatment times with cold plasma allow more extension of the plasma in the sample which enables it to conserve its quantitative amount of free radicals leading to more pronounced negative impact [49]. Another factor influences the microbial cells is the shock waves generated which create cavitation bubbles [50], which negatively affect the cell physiology and results in growth retardation. Moreover, exposure of living cells to high-intensity electric fields across the cell membrane for long duration results in electroporation and damage of the cell wall. Therefore, at high doses of plasma, cell membrane permeability increases and oxygen free radicals can easily pass into the cell causing severe oxidative damage [1]. Overall, long pretreatment times result in cell injury due to oxygen free radicals and lipid peroxidation which eventually lead to cell death. Thus, increasing of exposure time showed more reduction in germinating spores of *A. alternata*.

The variation in *A. alternata* spores’ germination percentages after DBD-AP treatment was investigated for exposure time ranging from 0 to 8 min (Table 1). The plasma operating parameters were fixed at 3 mm gap, 10 kV, 26 kHz. The treatment with the generated
Table 1. The effect of the used DBD-AP on Alternaria alternata spore germination at different exposure times.

| Exposure time (min.) | Percentage of microbial germination in different replicates | Mean ± SD |
|----------------------|------------------------------------------------------------|-----------|
| 0                    | 91 ± 8 93 ± 90 94 ± 94 90.8 ± 3.1                          | 90.8 ± 3.1 |
| 1                    | 86 ± 8 85 ± 84 89 ± 89 85.4 ± 2.3                          | 85.4 ± 2.3 |
| 2                    | 79 ± 8 78 ± 77 83 ± 83 79.8 ± 2.6                          | 79.8 ± 2.6 |
| 3                    | 70 ± 8 59 ± 60 71 ± 70 64.2 ± 5.8                          | 64.2 ± 5.8 |
| 4                    | 58 ± 7 51 ± 45 55 ± 55 49.6 ± 7.6                          | 49.6 ± 7.6 |
| 5                    | 27 ± 5 34 ± 33 42 ± 42 33.4 ± 5.5                          | 33.4 ± 5.5 |
| 6                    | 22 ± 5 19 ± 23 29 ± 29 23.0 ± 3.7                          | 23.0 ± 3.7 |
| 7                    | 17 ± 4 15 ± 12 18 ± 16 15.2 ± 2.4                          | 15.2 ± 2.4 |
| 8                    | 11 ± 5 4 ± 6 9 ± 9 7.0 ± 2.9                               | 7.0 ± 2.9 |

DBD-AP revealed a significant reduction in fungal spore germination (from 90.8% to 7.0%) specially at longer exposure time. Such reduction in the fungal spore germination was observed when exposed to DBD-AP has been previously examined [51,52]. The results presented in this study are in agreement with Panngom et al. [53], who reported that the extent of decrease in the germination levels of fungal spores depends on the rate and level of exposure to plasma. Other studies using scanning electron microscopy revealed considerable morphological alterations in the fungal spores due to plasma treatment. These morphological alterations appeared in the form of rupturing, flattening, shrinkage, surface wrinkling and loss of integrity [36,54]. It is hypothesized that plasma treatment results in the destruction of both proteins associated with fungal spores’ cell wall, allowing the leakage of intracellular components as well as the DNA in fungal spore [55]. However, confirming the negative impact of DBD-AP on A. alternata reported in the present study is a new finding that requires future detailed examination on the mechanism of action of the applied plasma.

4. Conclusion

In this study, cold plasma was generated at atmospheric pressure using an electrical barrier discharge (DBD-AP). The electrical, optical, structural and spectroscopic properties of the generated plasma were studied and optimized to be used as a sterilizer for the plant-pathogenic fungus A. alternata isolated from grains and legumes. The results showed that the size of the plasma and its intensity increases with the increase of the applied voltage, and the generated plasma approaches homogeneity at small discharge gaps. Streamers become apparently at larger gap distances. In addition, the DBD-AP get diffused with increasing the applied voltage. The emission spectra showed the presence of the nitrogen first negative system, nitrogen second positive system and NO spectra. The emission spectra intensity increased with increasing the applied voltage. The generated plasma showed a potential sterilization effect on A. Alternata. The fungal germination rate significantly reduced due to the increase in the plasma exposure time. The present study provides a new approach to control the pathogenic fungi using eco-friendly technique of DBD-AP.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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