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ORIGINAL ARTICLE

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Inactivation of Microorganisms and Potato Tuber Moth Eggs and Pupae Using a Dielectric Barrier Discharge Plasma

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A dielectric barrier discharge plasma device (DBD) was built, characterized and operated. Ten species of gram-positive and gram-negative bacteria, as well as fungus and spores of *Bacillus cereus* and its vegetative cells, in addition to eggs and pupae of the potato tuber moth (PTM) were exposed to DBD plasma. A strong detrimental effect on the exposed species was observed in function to the exposure time at helium: air ratio 98:2, electrode gap 1.8 cm, amplitude of discharge voltage 6 kV and an effective power density 208 mW/cm^3.

The outcome of this work provides valuable data on the use of DBD plasma as an alternative non-heating sterilization method to kill or inhibit microbial growth and protecting potatoes from PTM infestation.

Key words: Dielectric barrier discharge, atmospheric pressure plasma in helium, non-thermal plasma, bacteria, bacterial spores, fungi inactivation, potato tuber moth

In the recent decades, the dielectric barrier discharge (DBD) plasma has achieved great scientific and technological advances, because of its unique properties that make it a useful tool in many different applications such as surface sterilization in food processing industries, destruction of pathogens, modification of packaging materials for enhancing self-life (Hati *et al.,* 2018). In this context, many researches were carried out, to understand the physical principle of the DBD that consists of uniform diffuse glows covering the entire electrode surfaces (Brandenburg, 2017, Maccaferri *et al.,* 2023).

The DBD plasma is characterized by its low temperature comparable with the room temperature, unremarkable heating effect on treaded materials like food or living tissues, low energy consumption, and not generating excessively chemically active particles or ionizing radiation, making it ideal for bacterial inactivation, sterilization and agricultural pests control (Sutar *et al.,* 2021; Harikrishna *et al.*, 2023).

For instance, DBD plasma has been used against the entophytic bacteria *Pseudomonas fluorescens* and *E*. *coli* (Croteau *et al.*, 2022; Ukhtiyah *et al.*, 2023). Moreover, inactivation for gram-positive and gram-negative bacteria was also obtained in a gap of air by igniting diffuse homogenous discharge of DBD (Motrescu *et al.,* 2015; Cong *et al.*, 2020).

The effects of air plasma treatments on the inactivation of pure bacterial culture (*Escherichia coli*) deposited onto the surface of agar plates were investigated. A significant reduction for *E*. *coli* activity was achieved within 60 s of plasma treatment. This result can be related to the presence of reactive species in the plasma volume, in particular, O and N radicals (Pedroni *et al.*, 2018).

The DBD was proven to be an effective method to control many insect pests such as the bruchid *Callosobruchus chinensis* and the red flour beetle, *Tribolium castaneum* (Pathan *et al.,* 2021; Zilli *et al.*, 2022). Moreover, the Effects of non-thermal plasma treatment on plant materials (vegetable leaves, or crops), seed germination and early growth of leguminous plants were also studied (Zhang *et al.,* 2018). It was proven that DBD plasma could be used as a new and effective treating method in surface microbial inactivation and seed stimulation useable in the agricultural and food industries (Sera *et al.*, 2021). For example, a positive effect on the germination and seedling growth of radish *Raphanus sativus* was observed when the seeds were treated with the DBD (Guragain *et al.*, 2022).

Thus, this work aims to use a DBD device in treatment of microorganisms including: bacterial species of *Salmonella typhi, Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Brucella melitensis* (gram-negative bacteria)*, Staphylococcus aureus*, *Listeria monocytogenes*, *Streptococcus pneumoniae*, and *Enterococcus faecalis,* (gram-positive bacteria), *Bacillus cereus strain as* spores*,* fungus species as *Candida albicans and Aspergillus niger*, and the potato tuber moth (PTM), *Phthorimaea operculella* (Lepidoptera: Gelechiidae) eggs and pupae under different exposure times.

MATERIALS AND METHODS

Construction of the DBD Device

A plasma device type of DBD was constructed according to (Bures *et al.,* 2006). The parameters of the constructed DBD device in proper operational conditions are presented in Table 1.

Bacterial Strains and Growth Conditions

Both Gram-negative and Gram-positive bacteria, two fungal species and spores were challenged with the DBD plasma. All isolates used in the current study belonged to the pure culture collection of our Microbiology and Immunology Division.

The bacterial strains as *S. typhi, E. coli*, *P. aeruginosa*, *P. vulgaris*, *B. melitensis*, *S. aureus*, *L. monocytogenes*, *S. pneumoniae*, *E. faecalis* and *B. cereus* were cultured on 2×YT agar plates (Difco, BD, Sparks, USA) and incubated under aerobic conditions at 37 °C for 24 h. The fungus strains as *C. albicans and A. niger* were cultured on Potato Dextrose agar (PDA, Titan Biotech, India) and incubated under aerobic conditions at 30 °C for 5 days. Afterwards, a single colony was inoculated into Luria Bertani medium (LB Broth, Bio

Basic Inc., Canada) and grown at 37 °C for 24 h with shaking at 150 rpm.

The bacterial and fungal cells were harvested by centrifugation at 5400 rpm for 10 min at 4 °C and washed three times with sterilized phosphate-buffered saline (PBS) then the final pellets were re-suspended in PBS.

The microorganisms' cells were adjusted to 1 x 10^9 CFU/ml by measuring optical density (OD) at 600 nm with a spectrophotometer (OD600, BioQuest, CE2502, England), viable count of the bacterial suspensions were checked by culturing 10 µl of them on Luria Bertani medium (LB agar, Bio Basic Inc., Canada). The standard Nichrome wire loops are available in 6 sizes from 1 µl to 10 µl which mean this volume is enough for culturing in plates. The plates were incubated at 37 °C for 24 h and the number of CFU determined and results were expressed in Log_{10} CFU/ml.

The sterile Whatman qualitative filter paper discs (Grade 113, Zelpa, Belgium) with a reported pore size of 30 µm and a thickness of 0.33 mm were used. The filter paper discs were impregnated with the final suspensions (1 × 10^9 CFU/ml) of each bacterial and fungal sample. The impregnated paper discs were treated for each strain by exposing each paper disc to DBD plasma from 5 to 60 min as indicated in Table 2 respectively. The treated paper discs were then placed in a sterile PBS and left for an hour. Then all treated filter paper discs with controls were re-cultured on 2×YT plates for the bacterial samples and on PDA agar for the fungal samples. The results were determined by the bacterial growth or growth inhibition on the media plates.

Treatment of *P. operculella* Eggs and Pupae

Newly emerged *P. operculella* adults were collected and confined in 800 ml transparent plastic jars (10-12 pairs in each jar). A band of filter paper was added to the bottom of each jar for oviposition and 10% sucrose solution was presented as a food source. Eggs on filter paper bands were removed, counted by using a stereoscopic microscope.

Eggs aged 48 h (n ≈ 2360) deposited on sterile filter paper were exposed to plasma for durations of 30 and 60 min. The control eggs were placed during the exposure time inside the discharge chamber outside the discharge. After treatment, eggs were incubated at 25 °C and 60% relative humidity (RH) until hatching (≈ 5 days), after which the percentage of hatched and unhatched eggs was determined. Egg mortality values were adjusted for control mortality according to Schneider-Orelli's formula (Krosche *et al.*, 1996):

$$M\,\% = (b - k\ /100 - k\ ) \times 100$$

Where *M* % is a percent corrected mortality, *b* is a percent mortality (unhatched eggs) in the treatment, and *k* is a percent of mortality in the control. The experiment was replicated 3 times with ≈ 780 eggs per replicate.

Pupae of *P. operculella* at the age of 3 days old (n = 270) were exposed to discharge plasma for durations 30 and 60 min. The control was left inside the discharge chamber outside the discharge. The treated and untreated pupae were held at 25 °C until adult moth's emergence. The percentage of emerged moth was determined. The bioassay was replicated 3 times, with 90 pupae per replicate.

Statistical analysis was performed using State-view program at the 5% level (P ≤ 0.05). Numerical data are expressed as mean ± standard deviation (SD). One-way analysis of (ANOVA) variance followed by Fisher's protected least significant difference test, (PLSD) were carried out for all possible mean comparisons.

RESULTS

Effects of DBD plasma on bacterial, fungal and spores culture

Under conditions mentioned in Table 1 (with dg=1.8 cm, He:Air=98:2, effective power density=208 mW/cm^3), gram–negative and gram-positive bacteria, fungus and spores, PTM eggs and pupae were treated.

Our results revealed that, a total absence of growth at all times of exposure was recorded for *P. aeruginosa* and *E. faecalis*. The growth of *E. coli* and *P. vulgaris* was inhibited after treatment with DBD at time 15 min; no growth was observed after exposure to 30 min for *S. typhi*, *B. melitensis*, *S. aureus*, *L. monocytogenes* and *S. pneumonia.* Growth of *C. albicans* fungus was negatively affected at 30 min of exposure to DBD, while growth of *A. niger*, *B. cereus* spores and vegetative cells germination were inhibited at 60 min of DBD treatment

(Table 2).

Effects of DBD plasma on PTM eggs and pupae

There was a high proportion of eggs that did not hatch due to DBD treatment (F = 113.5; df = 2, 17; P = 0.0001), the mean percentage of eggs hatch in the control was 80.8% and decreased to 65.7 and 55.5% in 30 and 60 min treatments, respectively (See Table 3).

The DBD treatment has a significant negative impact on PTM adult emergence (F = 4325; df = 2, 89; P = 0.0001). The percentage of emerged adults from 3-d-old pupae subjected to DBD sharply decreased with the increase of exposure time from 0 to 30 and 60 min, respectively. Adult emergence was completely inhibited after exposure to 60 min (Table 4).

Table 1: Electrical and operational parameters of constructed DBD device working on helium: air mixture.

Parameters	Value
Amplitude of discharge voltage,U_0	6 kV
Amplitude of discharge current	40 mA
Effective Power: P_{rms}	120 W
Effective discharge power density	208 mW/cm^3
Gap distance, d_g	1.8 cm
Working gas at atmospheric pressure	98 He: 2 Air
Working frequency, f	5 kHz
Surface area	320 cm^2 (16 ×20 cm)
Flow rate of helium	20 sccm (20 cm^3/min)
Dielectric matter	Garolite G-7 (0.8 mm)
Dielectric constant of G-7	ε=4.2
Electrode matter	Al alloy
Temperature of cooling water	5 ^{o}C
Ambient temperature of discharge	14 ^{o}C

Table 2: Bacterial, fungal and spores growth (+) or inactivation (-) after treatment with DBD plasma at different exposure times for 1 × 10^9 CFU/ml.

Treatment time of microorganisms, min	5	10	15	30	45	60
Gram-negative bacteria						
Pseudomonas aeruginosa	-	-	-	-	-	-
Escherichia coli	+	+	-	-	-	-
Proteus vulgaris	+	+	-	-	-	-
Salmonella typhi	+	+	+	-	-	-
Brucella melitensis	+	+	+	-	-	-
Gram-positive bacteria						
Enterococcus faecalis	-	-	-	-	-	-
Staphylococcus aureus	+	+	+	-	-	-
Listeria monocytogenes	+	+	+	-	-	-
Streptococcus pneumoniae	+	+	+	-	-	-
Fungus						
Candida albicans	+	+	+	-	-	-
Aspergillus niger	+	+	+	+	+	-
Bacillus cereus						
Spores	+	+	+	+	+	-
Vegetative cells	+	+	+	+	+	-

Table 3: Mean percentage ± SD of eggs hatch of potato tuber moth subjected to DBD plasma.

Exposure period min	No. of eggs	Mean % eggs hatch	Corrected % of unhatched eggs
0	751	80.8 ± 4.1a	–
30	795	65.7 ± 3.8b	29.8
60	814	55.5 ± 3.9c	31.3

Mean in column followed by the same letter are not significantly different (P < 0.05, Fisher PLSD). Mean of 3 replicates, 780 eggs per replicate.

Table 4: Mean percentage ± SD of adult emergence of potato tuber moth subjected as pupae to DBD plasma.

Exposure period min	No. of pupae	Mean % emerged moths
0	90	85.9 ± 4.9a
30	90	26.7 ± 5.9b
60	90	0.0 ± 0.0c

Mean in column followed by the same letter are not significantly different (P < 0.05, Fisher PLSD). Mean of 3 replicates, 90 pupae per replicate.

DISCUSSION

Our DBD device was built and characterized, in order to obtain stable plasma of glow discharge in helium at atmospheric pressure, for treating microorganisms, eggs and pupae of the PTM under conditions indicated in Table 1.

The inactivation of gram-negative and gram-positive bacteria after exposure to DBD plasma was detected for *P. aeruginosa* and *E. faecalis* at 5 min; *E. coli* and *P. vulgaris* at 15 min; others bacteria species and fungus of *C. albicans* at 30 min (Table 2). Several mechanisms could be involved to explain the detrimental effects of DBD plasma on microorganisms. One mechanism pretends that plasma effects on bacteria are attributed to existence of very important role of charged particles in tearing off the outer membrane of the bacterial cells (Ricchiuto *et al.*, 2021). Therefore, the sensitivity to DBD between gram-negative and gram-positive bacteria found in our results could be attributed to the fact that gram-negative contains a layer of murein (peptidoglycan) thinner than that found in gram-positive bacteria and therefore gram-positive bacteria, which have a thicker murein layer, are more resistant to DBD plasma (Motrescu *et al.*, 2015; Figueroa-Pinochet *et al.*, 2022).

Another mechanism considers UV radiation effect on bacteria; several researches (Ghomi *et al.,* 2012; Bourke *et al.*, 2017) indicated that UV radiation could play a role in the killing mechanism of bacteria (UV radiation disables cells if their wavelength range is located between 220 nm and 280 nm). However, the generated plasma used in our work could have a weak effect in killing bacteria due to the existence of two wavelengths in our recorded spectrum (313.6-391.44 nm, data not shown), which belongs to the middle UV-B region, they are 313.6 nm and 315.9 nm lines.

The third mechanism takes into account that, the reactive oxygen and nitrogen species (RONS) in DBD devices working on atmospheric air might play a dominant role in the process of disrupting bacteria (Pedroni *et al.*, 2018). For example, ROS are supposed to be capable to oxidize the cell membrane, and then damage the protein and DNA inside the cells.

Our study showed that *A. niger* fungus, *B. cereus* spores and vegetative cells were resistant to DBD plasma (60 min of exposure) as shown in Table 2. This could be attributed to the operational parameters of our DBD treatment at 6 kV as amplitude of discharge voltage. Since, Zahi *et al.* (2023) found that 4 min was adequate for the reduction of survival *Penicillium expansum* spores in kiwi-fruit juice after DBD plasma treatment at 18 kV.

CONCLUSION

Encouraging results were obtained in inactivation of

a set of 10 bacterial species by using DBD plasma. For instance, the exposure period to DBD did not exceed 5 min for *S. typhi* (gram-negative bacteria) and *L. monocytogenes* (gram-positive bacteria).

Thus, DBD plasma could be used to eliminate or destruct microorganism from the contaminated surfaces (sterilization) or for microbiological decontamination such as the pathogenic bacteria *B*. *cereus*.

Treatment results by DBD against *P*. *operculella* are interesting since 100% of pupae mortality was recorded after exposure to 60 min, noting that infested potato tubers could contain PTM eggs and pupae.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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