METHOD OF FUNGAL WHEAT SEEDS DISEASE INHIBITION USING DIRECT EXPOSURE TO AIR COLD PLASMA

BOGDAN-GEORGE RUSU¹, VLADUT POSTOLACHE¹, IRINA-GABRIELA CARA¹,
VALENTIN POHOATA², ILARION MIHAILA³, IONUT TOPALA², GERARD JITAREANU¹

¹Ion Ionescu de la Brad University of Agricultural Sciences and Veterinary Medicine of Iași, Department of Pedotechnics, Faculty of Agriculture, 3 Sadoveanu Alley, Iassy 700490, Romania
E-mail: bgrusu@uaiasi.ro
²Alexandru Ioan Cuza University of Iași, Faculty of Physics, Iași Plasma Advanced Research Center (IPARC), Bd. Carol I No. 11, Iași, 700506, Romania,
E-mail: vpohoata@uaic.ro
³Alexandru Ioan Cuza University of Iași, Integrated Center of Environmental Science Studies in the North-Eastern Development Region (CERNESIM), Bd. Carol I No. 11, Iași, 700506, Romania

Received September 18, 2017

Abstract. Applications of physics-derived technologies in agriculture are more and more popular and cold plasma seeds treatment is a modern eco-agricultural technology that has been found to stimulate plants growth. It is based a mixture of active agents, generated in the volume of cold plasmas, which can modify the vitality of seeds, without causing gene mutations. This kind of treatment has also been reported to improve the growth and yield of wheat. Since cold plasma treatment can improve the growth of the plant, it may have an additional impact on its disease resistance. This feature was demonstrated in this work. In order to study the inhibition of fungal disease, we exposed to atmospheric pressure plasma, for 3 minutes, a number of 50 wheat seeds contaminated with Rhizopus nigricans. After the exposure, the seeds were allowed to germinate and we observed an earlier development of the fungal disease and at higher density for the control seeds group in comparison with the plasma treated seeds group. Additionally, the atmospheric pressure plasma had also a cleaning effect on the seeds surface, as it was observed using SEM imaging. Gas phase plasma chemistry analysed by FTIR and UV-VIS spectroscopy indicates that the main plasma products at the reactor outlet are O₃ and N₂O. Not detectable level of nitrogen oxide (NO) or nitrogen dioxide (NO₂) was found. DRIFT spectroscopy of seeds indicates no major chemical structural changes at the surface of seeds after DBD plasma treatment and plasma does not affect the outer pericarp of seeds. Even after a longer time of plasma treatment (90 minutes) the maximum seeds surface temperature was 40.2 °C. The seeds nitrogen and moisture content, measured 24 hours after plasma treatment, increase for short treatment time and tend to saturation after 30 minutes.

Key words: inactivation of fungal diseases, atmospheric pressure plasma, plasma seed treatment.

1. INTRODUCTION

Recently, many kinds of plasma based devices, especially at atmospheric pressure, have been developed for medical and agricultural applications. Some
plasma sources are already applied in medicine for the inactivation and sterilization of specific microorganisms [1–10]. Also in industry of agriculture, the inactivation of microorganisms and acceleration of seed germination and crop growth by using plasma-processing methods have attracted much attention [11–18]. Some plasma processing methods possess many advantages, such as a low-temperature treatment and short processing duration. Many studies on application of plasmas in agriculture and medicine have been reported [11, 19–22]. The inactivation of bacteria on plant seeds and other agricultural products by plasmas also has been reported in these studies [23, 24]. The effect of atmospheric and low-pressure plasmas on pathogenic fungi on the seed has been investigated. Non-equilibrium (low-temperature) atmospheric-pressure plasmas, as well as low-pressure plasmas, were applied in fungal disease inactivation, and showed promise as a very effective system that causes minimal damage to crops, foods, seeds, humans, and the environment.

2. MATERIALS AND METHODS

2.1. PLASMA GENERATION AND MONITORING

The plasma reactor consists of a dielectric barrier discharge (DBD) operating at atmospheric pressure in open air, with a parallel plate geometry. On a circular stainless steel plate (grounded electrode) a glass made Petri dish was placed and acts as dielectric (Fig. 1). The power electrode is made from adhesive aluminium taped on a poly(methyl methacrylate) disc (3 mm thick and 5 cm diameter). The total dielectric gap (air and glass) was kept constant to 1 cm, during all experiments.

![Fig. 1 – Sketch of the DBD plasma reactor.](image)

Sinusoidal voltage was applied using a power line transformer. During the treatment process, the electrical parameters were maintained constant, voltage amplitude 10 kV at 50 Hz frequency and the treatment time was 3 minutes. The discharge current was monitored using a Pearson 6585 probe connected to a line...
triggered digital oscilloscope (Tektronix TDS 5034B). The DBD discharge mode is a filamentary one [25–27], stochastically distributed on the electrode surface, characterized by a multitudes of positively and negatively current peaks at each alternating half-period sine (Fig. 2). The positive peaks package is known in literature as primary discharge and the negative peaks package corresponds to secondary discharge. The averaged amplitude of the peaks current for both discharges is around 10 mA with maximum amplitude of 38 mA.

The Petri dish acting as dielectric for the plasma set-up may be also used to host various types of seeds. We report here results regarding the air plasma treatment of wheat seeds. A total quantity of 50 wheat seeds, contaminated with *Rhizopus nigricans*, was arranged in a single layer and then exposed for 3 min to plasma.

The plasma UV-VIS emission spectra was acquired using high resolution optical spectrometer (Jobin Yvon Triax 550), equipped with a Peltier cooled CCD detector (Horiba Jobin Yvon Symphony). The global plasma emitted light was guided to spectrometer by an optical fiber ended with an additional collimating lens system.

Additional information about gas phase plasma chemistry were obtained by infrared spectroscopy. The DBD plasma reactor was covered by a gas collecting bottle connected to a gas cell (optical path 150 mm) used for infrared spectroscopy. Ambient air enter the plasma reactor, fed with wheat seeds, from bottom and gaseous plasma products are extracted from top, through the cell gas. The pressure gradient is ensured by a dry pump at a low flow rate regime (2 slpm). The FTIR spectrometer (Bomem MB 104) was operated in transmittance mode with 3 scans per spectrum and a spectral resolution of 1 cm$^{-1}$. Kinetic measurements, launched simultaneously with plasma ignition, were performed.
2.2. SEEDS PHYSICO-CHEMICAL CHARACTERIZATION

In order to check the morphological modifications of treated seeds, the surface topography was analysed by Scanning Electron Microscopy (SEM) technique, using a (FEI Quanta 450) microscope operating at 30 kV acceleration voltage. The chemical modifications of the treated seeds was analysed by Diffuse Reflectance Infrared Fourier Transform Spectroscopy (DRIFTS) technique using Interspec 200-X apparatus coupled with a diffuse reflectance accessory (model PIKE Technologies EasiDiff).

The nitrogen content was measured based on Kjeldahl method (Kjeldatherm, GERHARDT, Germany) according to the ICC Standard Method 105 (AOAC Official Method 979.09 Protein in Grain). All the samples were analyzed in triplicate and the treated seeds were kept for 24 h in controllable environment (25°C and humidity 33%) before analysis. The untreated and treated seeds were milled to obtain flour particles. One gram of each sample was weighed into the digestion tube and 20 mL of concentrated sulfuric acid (Merk, Germany) and catalyst tablets Kjeltabs were added. The digestion parameters used were as follows: initial power 50% for 40 min, increased to 100% and held 50 min and then decreased to 70% power and held 70 min.

After the digested samples were cooled at room temperature, water steam distillation is performed by adding 100 mL H₂O and 63 mL of 40% w/v sodium hydroxide solution at 100% power for 240 s. About 3–4 drops of the mixed indicator are added to the distillate, which is then titrated with 0.05 mol/L sulphuric acid until the colour changes from green to grey/violet.

The amount of nitrogen was calculated using the following equation:

$$N\% = \frac{1.4007 \times c \times (V - V_b)}{E}$$

Where:
- $c$ – H⁺ ion concentration of the standard acid solution;
- $V$ – consumption of standard acid solution of sample (mL);
- $V_b$ – consumption of standard acid solution of blank sample (mL);
- $E$ – initial sample weight (g).

The seeds percent of wettability was analysed using Unimeter Digital Moisture Meter XL. A number of 50 seeds at different treatment time was analysed. The seeds was kept at a temperature of 25°C and a humidity of 33% for 24 hours after the plasma treatment.

2.3. EVALUATION OF FUNGAL DISEASE

*Rhizopus nigricans* is the fungus commonly known as bread mould and is a very common fungal disease and may live or be found in indoor as well as outdoor
environments. The seeds used in this experiment was natural infested with this fungus. For fungal disease evolution, a number of 50 treated and untreated seeds was placed in Petri dish with distilled water, for seeds humidity saturation. Every Petri dish sample was photographed every day at the same time for comparison of fungus evolution between treated and untreated seeds.

3. RESULTS AND DISCUSSION

3.1. OPTICAL DIAGNOSIS OF DBD PLASMA VOLUME

The DBD discharge operate in ambient air at atmospheric pressure and all the observed spectral bands correspond to excited molecular nitrogen (Fig. 3). The air DBD generates almost no emission spectra in the range of 200 to 300 nm, where nitrogen oxide (NO) spectra should be detected. Also atomic lines of oxygen (777 nm) and hydrogen-alpha (656 nm) were not detected in this OES assessment even for enhanced spectrometer acquisition condition (20 second integration time).

![Optical emission spectrum of DBD plasma at atmospheric pressure in ambient air, during seeds exposure.](image-url)

The rotational temperature of molecular plasma species is close or equal to the gas temperature. It was found in similar studies that the rotational temperature
is 418 K which corresponds to 145°C and the vibrational temperature 3143 K or 2870°C, supporting the non-equilibrium character of some atmospheric pressure plasma sources. The seeds surface temperature is reaching about 44°C after 3 minutes of treatment [28]. For our plasma set-up, during seeds exposure the rotational temperature was calculated from N\textsubscript{2} transition (1–5 at 426.9 nm Fig. 3) and was found around 410 K, as revealed by LifBase software simulation band profile. Also, the vibrational temperature is around 2331 K, as returned from Boltzmann plot analysis of N\textsubscript{2} vibrational bands intensities at (0–1) 357.69 nm, (1–0) 315.93 nm and (2–1) 313.60 nm. For our plasma treatment method, the seeds surface temperature, measured with Proscan 530 infrared thermometer, increased from room temperature to 40.2°C after 90 minutes of plasma treatment.

Concerning the volume chemistry, notice that just before plasma ignition (Fig. 4), the ambient air contains small amount of water (H\textsubscript{2}O) and carbon dioxide (CO\textsubscript{2}); their level is assigned to 100% in transmittance spectra. In the first seconds after plasma ignition, the CO\textsubscript{2} level increase due to desorption processes that take place at the seeds and electrodes surface. High energy electrons can dissociate the CO\textsubscript{2} and H\textsubscript{2}O and new reactive species are produced as CO, O, OH and H [29]. However, these reactive species are not detected by optical emission spectroscopy and infrared spectroscopy due to the low level of plasma ionization degree. After

![Fig. 4 – Kinetic gas phase analysis by infrared measurements (transmittance values of all spectra, except the initial spectrum, are intentionally shifted for clarity reasons).](image)
half of minute of plasma ignition, the ozone (O₃) level increase due to reaction of molecular oxygen with the produced atomic oxygen. During the plasma discharge, the O₃ level increase more (Fig. 4), detrimental to CO₂ that diminishes, less than the ambient level. Nevertheless, CO₂ reforming reaction from CO in humid air mixture [30] determines the equilibrium of CO₂ level. The H₂O increase constantly during plasma discharge mainly due to water desorption from seeds and electrodes surface. Additional to mentioned reactive species, low level of nitrous oxide (N₂O) was detected as gas phase product. Nitrogen oxide (NO) and nitrogen dioxide (NO₂) were not detected in the gas phase by infrared measurement indicating a very low level of these products. The gas phase analysis indicates that the main products of plasma discharge at the outlet reactor are O₃ and N₂O. After approximately 3 minutes of plasma discharge the concentration of O₃ and N₂O remains constant and similar results can be found in [31].

3.2. MORPHOLOGICAL AND CHEMICAL MODIFICATIONS OF TREATED SEEDS

This experiment was focused to demonstrate the applications of DBD plasma at atmospheric pressure in agriculture, more exactly to find the good treatment parameters for almost completely inactivation of seeds disease. In addition to inactivation of fungal seeds disease, the atmospheric pressure plasma also clean the surface of seeds, effect which was observed in SEM image (Fig. 5a and Fig. 5b). The DBD plasma does not affect the outer pericarp of seeds (Fig. 5c and Fig. 5d), but affect the granular structure from the interior seeds, where the endosperm granule are not spherical after the plasma treatment (Fig. 5e and Fig. 5f). This effect can be attributed to the micro-discharge which can take place inside of the seeds by electrical charging of the endosperm granule during the treatment.

Infrared vibrational analysis of the untreated wheat seeds (Fig. 6) show a typical spectra assign to a mixture of carbohydrates and proteins [34]. Specific to proteins, the amide-I (CONH) band (1630–1750 cm⁻¹) is associated with the C = O stretching vibration and amide-II (~1580 cm⁻¹) is related to N–H in-plane bending vibration and the tiny peaks of amide-III (~1300–1450 cm⁻¹) are associated to the nature of nitrogen side chains in relation with hydrogen bonding. Specific to carbohydrates, the broad band from 3200 to 3600 cm⁻¹ corresponds to hydroxyl O-H stretching mode and this strong band covers any N-H stretching peaks assigned to protein structure. Additionally for carbohydrates, in the fingerprint region (~900–1200 cm⁻¹) two strong peaks are found associated to C–O stretching [32, 33]. The two peaks (~2800–3000 cm⁻¹) corresponds to C–H stretching and may be assigned also to the small amount of fats from wheat seeds. The vibrational analysis indicates no major chemical structural changes at the surface of seeds after DBD plasma treatment.
Fig. 5 – SEM image of epidermis tissue (a), (b), cross section (c), (d), (e), (f) of treated and untreated wheat seed.
The nitrogen content for untreated seeds was found around 1.46% and the results of nitrogen content measurements of plasma treated seeds are shown in Fig. 7. For short time of plasma treatment (0–40 min), the nitrogen content increase faster, around value of 1.50%. Instead for the longer treatment time (60–90 min), the nitrogen content reach a saturation value around 1.52%.

The increasing of nitrogen content, as revealed by the above results, may be explain by chemical reactions of nitrogen reactive species with first layers of biomolecules present in the outer pericarp of seeds. Increasing the exposure time
will allow more and more surface reactions until all reactions sites are occupied, equilibrium is reached and surface nitrogen content is no longer increasing.

After plasma treatment, the seeds were kept for 24 h in controllable environment (25°C and humidity 33%). For filling the water surface absorption of the treated seeds we measured the seeds moisture content using Unimeter Digital Moisture Meter XL. For short time of plasma treatment (0–40 min), the surface moisture content increase faster, around value of 13.9% instead for the longer treatment time, the moisture content decrease at untreated seeds value around 13.3% (Fig. 8). This increase of moisture content for shorter treatment time can be attributed of water adsorption molecule from the atmosphere. For the longer treatment time, the free radicals formed at the surface seeds during the plasma treatment can formed chemical bonds with other molecules presented in plasma, N₂ for example. This effect can be attributed to decreasing of seeds moisture content for longer treatment time.

![Fig. 8 – Seeds moisture content vs. plasma treatment.](image)

3.3. Fungal disease inactivation

Concerning the inactivation of fungal disease, 50 wheat seeds contaminated with *Rhizopus nigricans* were treated by DBD plasma for 3 minutes and 5 days after, the seeds are placed in Petri dishes and germination initiation was started (Fig. 9). At the control sample with untreated seeds, the fungal disease started to
develop faster, in comparison with the Petri dish containing treated seeds. After the 8 days of germination, the *Rhizopus nigricans* fungal disease is much higher compared with the plasma treated seeds.

![Fig. 9 – Evolution of fungal wheat disease for treated and untreated seeds during germination.](image)

4. CONCLUSIONS

In this study, we presented the effect of dielectric barrier discharge plasma that operates in ambient air on wheat seeds contaminated with *Rhizopus nigricans* fungal disease. In terms of electrical and optical characteristic of plasma, discharge are filamentary, but randomly distributed on the treated surface. The major gas phase chemical species generated in discharge are excited molecular nitrogen and ozone ($O_3$) and low level of nitrous oxide ($N_2O$). Nitrogen oxide (NO) and nitrogen dioxide (NO$_2$) were not detected in the gas phase by infrared measurement.

Treated seeds analysis indicates that DBD plasma does not affect its outer pericarp and the vibrational analysis by DRIFTS technique indicates no major chemical structural changes at the surface of seeds. Temperature measurements either of the plasma gas or the seeds surface indicates that the outer pericarp is not affected by thermal effects induced by plasma treatment.
The plasma treatment can induce surface activation on the outer pericarp of seeds and can later increase the water and nitrogen content, facts which lead to improved the germination process.

Concerning of fungal disease inactivation, the plasma at atmospheric pressure reduce the development of *Rhizopus nigricans* fungal after 3 minutes of treatment, leaving the seeds to germinate further. In conclusion, we propose that the air plasma treatment is a relatively non expensive method and very useful in agriculture industry for fungal disease inactivation, surface cleaning, without altering the basic properties of seeds.

Acknowledgements. One of the authors (I. Topala) thanks to Alexandru Ioan Cuza University of Iasi (UAIC) for supporting the work under the grant GI-2015-06, within the internal grant competition for young researchers.

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