




Article

Characterization and Optimization of a Conical Corona Reactor for Seed Treatment of Rapeseed

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Abstract: Plasma agriculture is a growing field that combines interdisciplinary areas with the aim of researching alternative solutions for increasing food production. In this field, plasma sources are used for the treatment of different agricultural goods in pre- and post-harvest. With the big variety of possible treatment targets, studied reactors must be carefully investigated and characterized for specific goals. Therefore, in the present study, a cone-shaped corona reactor working with argon was adapted for the treatment of small seeds, and its basic properties were investigated. The treatment of rapeseed using different voltage duty cycles led to an increase in surface wettability, possibly contributing to the accelerated germination (27% for 90% duty cycle). The discharge produced by the conical reactor was able to provide an environment abundant with reactive oxygen species that makes the process suitable for seeds treatment. However, operating in direct treatment configuration, large numbers of seeds placed in the reactor start impairing the discharge homogeneity.

Keywords: plasma agriculture; corona discharge; plasma source; non-thermal plasma (NTP); rapeseed; seed; germination acceleration



Citation: Nishime, T.M.C.; Werner, J.; Wannicke, N.; Mui, T.S.M.; Kostov, K.G.; Weltmann, K.-D.; Brust, H. Characterization and Optimization of a Conical Corona Reactor for Seed Treatment of Rapeseed. *Appl. Sci.* **2022**, *12*, 3292. <https://doi.org/10.3390/app12073292>

Academic Editor: Mohammed KOUBITI

Received: 23 February 2022

Accepted: 19 March 2022

Published: 24 March 2022

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1. Introduction

Non-thermal plasmas (NTP) are chemically active media composed of electrons, neutral particles, ionized atoms and molecules, photons, and reactive species [1]. NTPs can be generated in a reactor by means of an electrical discharge in a variety of gases (noble and molecular gases) and at different pressure ranges [2]. The possibility of producing cold plasma at atmospheric pressure cheapens the process broadening the spectrum of applications. Due to the reactive composition and low temperature, NTPs have been used and studied for application in a diversity of fields, such as material processing [3], surface cleaning [4], decontamination [5], medicine [6], and agriculture [7,8].

Plasma agriculture is a novel plasma application that has rapidly grown in the past 20 years [7]. A large number of published articles in this field focused on the plasma application for post-harvest [9], such as the treatment of fruits and vegetables for preservation and increased shelf life [10,11]. However, investigations on the use of cold plasmas in pre-harvest stages are gaining considerable attention. NTPs are being explored as a possible green alternative to the use of agrochemicals, given the substantial negative impact of the latter on the ecosystem [7]. Plasma has demonstrated promising effects that could lead to decreased infections by pathogens in seeds, growing plants and soil [12–14], improve crop abiotic stress tolerance [15], and increase plantation yield [16]. Effects of plasma treatment have been studied for a wide variety of plant species (such as soybean [17], wheat [18], barley [19], maize [20], tomato [21], radish [22], and pea [23]) and different plasma sources, including low pressure and atmospheric pressure devices, have been developed.

Oilseed crops are grown worldwide given their economic value and relevance for the production of edible oils and renewable energy [24]. Rapeseed is an essential oilseed crop being the second most produced oil crop in the world with a harvest of 75.9 million tons in 2017/2018 [25]. However, with climate change and the ongoing COVID-19 pandemic, the global production of rapeseed is prone to decline [25], which contrasts with the rapidly increasing consumption demand. Given the impractical solution of increasing the cultivation land area, it is important to find alternatives for securing and possibly increasing crop yield. Genetic improvement of rapeseed to develop climate-adapted cultivars can help to increase production under abiotic stress [26]. Another alternative for improving resistance to abiotic stress is the use of chemical priming of the seeds [26]. As a physical method, plasma treatment is being investigated for securing yield and enhancement of biotic stress resistance and thus, can be an advantageous option for the treatment of rapeseed.

Recently, diverse plasma sources have been reported in the literature for the treatment of rapeseed for different purposes. Li et al., 2018 [27], investigated the effects of a low-pressure radio frequency (RF) discharge treatment of rapeseed and observed enhancement of seed germination, plant growth, and yield. Islam et al., 2019 [28], studied the treatment response of exposed rapeseed to low-pressure dielectric barrier discharge (DBD). It was concluded that plasma stimulates germination and increases amylase activity [28]. Other studies investigated the decontamination of rapeseed seed surfaces using plasma [29,30]. Puligundla et al., 2017 [29], used a corona discharge plasma jet for the treatment of rapeseed and reported a maximum reduction of 2.2 log of total aerobic bacteria. In this study, the corona discharge had a dual effect, promoting germination and seedling growth in addition to reducing the microbial load [29]. Wannicke et al., 2020 [30], reported the indirect treatment of rapeseed seeds and other crop seeds using plasma-processed air produced in an atmospheric pressure microwave reactor. Artificially contaminated rapeseed seeds treated under multiple filling conditions presented a reduction in *Bacillus atrophaeus* spores of almost 2.7 log [30]. However, the same treatment condition affected the seed's viability, and a complete loss of germination was observed. In contrast, single filling only affected seed germination to a lower degree, while reaching a decontamination efficiency of 2.6 log units [30], indicating that plasma parameters need to be defined for appropriate treatment effects. A low-pressure RF capacitive coupled plasma (CCP) reactor was used by Ling et al., 2015 [31], for enhancing the germination of rapeseed under drought stress. According to the study, plasma treatment improved water uptake by the seeds and increased the accumulation of soluble sugars and proteins [31]. Kriz et al., 2017 [16], observed yield enhancement in field experiments of treated rapeseed using a low-pressure microwave discharge in combination with beneficial fungi spores.

On the one hand, plasma technology seems promising as an environmentally friendly alternative for agriculture, because the species produced are limited in space and time and do not encompass non-natural compounds. On the other hand, the diversity of plasma sources and the complexity of generated discharges lead to diverging results (improvement or impairment of germination, water uptake, growth, and others). Therefore, the reactor and discharge parameters have to be carefully studied and adjusted for securing or enhancing seed germination.

Among the many possible ways of generating NTP at atmospheric pressure, corona discharge stands out as a well-known and easy option. It occurs in strongly non-uniform electric fields and is generated using asymmetric electrodes. The most frequent configuration comprises a pin-to-plate set of electrodes. In the present work, a pin-to-plate corona discharge reactor with a horn-like nozzle was studied. The original version of this reactor was previously reported elsewhere [32]. Filamentary corona discharges are usually very inhomogeneous. Mui et al., 2018 [32], reported an improvement in treatment uniformity of PET samples when changing the reactor nozzle size. The use of a conical nozzle led to a uniform treatment area underneath the reactor (up to 30 cm²) [32]. Here, this conical reactor was adapted for the treatment of seeds with small and round shapes. The funnel reactor was mounted on top of a glass Petri dish in which small seeds could be placed.

Thus, the system remained closed, and the seeds were prevented from being blown away. The new reactor configuration was characterized and its suitability for seed treatment was investigated using rapeseed. The discharge power could be easily controlled by the applied voltage duty cycle, which also contributed to gas temperature regulation. For this direct treatment reactor configuration, an influence of the number of seeds on the discharge parameters was observed. The effects of plasma treatment parameters on seed surface hydrophilicity and germination kinetics were studied.

2. Materials and Methods

2.1. Plasma Source

The reactor used in this work was a modified version of the corona plasma jet device with a conical nozzle reported by [32]. In contrast to the previous version, here, the plasma jet horn nozzle was placed directly on a glass Petri dish leaving no open gap between them, thus forming a virtually closed (but not air-tight) system. The schematic setup of the reactor is presented in Figure 1. It consists of an upside-down borosilicate glass funnel (80-mm-diameter and angle of 60°) mounted on top of an 80-mm-diameter glass Petri dish where seeds were placed for treatment. A tungsten pin electrode (1 mm thick and 10.3 mm long) was inserted in the straight part of the funnel, as shown in Figure 1. The pin passes through an insulating piece and is connected to a high voltage generator (AL-1400-HF-A, Amp-Line, West Nyack, NY, USA). Below the Petri dish, a 5.85-mm-thick glass plate was used as an additional dielectric layer under which a grounded electrode was located. Argon (4.0 slm) was introduced on the top part along with the pin electrode. When high voltage was applied, the electric field at the electrode's tip increased and discharge filaments (streamer corona discharge) were formed. The filaments tended to be homogeneously distributed throughout the horn volume and at higher voltage they stretched out, reaching the bottom of the Petri dish. The reactor was operated with a frequency of 10 kHz and the voltage was amplitude modulated (burst mode) into bursts with a repetition period of 100 ms. The applied voltage amplitude and/or signal duty cycle were varied during the experiments in Section 3.1.

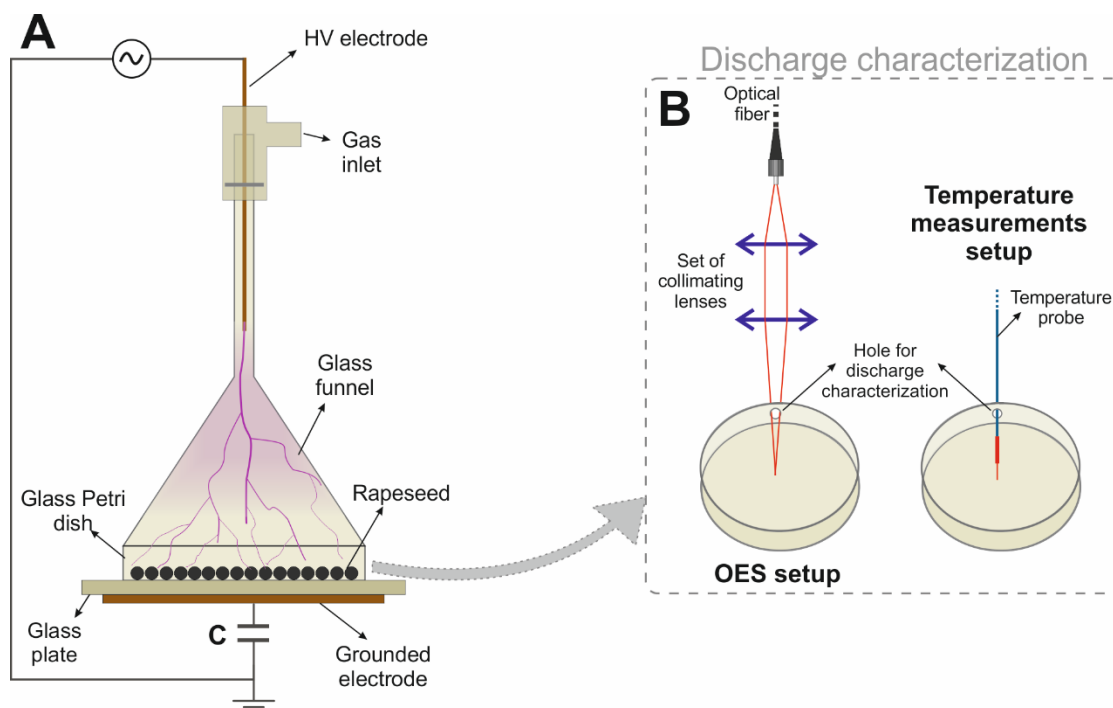


Figure 1. Experimental setup of the funnel reactor used in this study (A). Details of the discharge characterization setups for temperature and OES measurements are shown in (B).

2.2. Discharge Characterization

2.2.1. Electrical Characterization

The electrical characterization of the plasma source was performed by measuring the transferred charge at the grounded electrode underneath the glass plate. The charge was obtained by the voltage drop across a serial 1.0 nF mica capacitor (“C” in Figure 1). The voltage signals were monitored using a digital oscilloscope (DPO 2024, Tektronix, Beaverton, OR, USA), and the discharge power was calculated using the area of the formed Q-V Lissajous figure as shown in Equation (1):

$$P = \frac{E_c N}{T_r}, \quad (1)$$

where E_c corresponds to the Lissajous figure area for 1 cycle, N is the number of cycles in a burst, and T_r is the repetition period of the voltage bursts.

2.2.2. Optical Emission Spectroscopy

Optical emission spectroscopy (OES) was used to analyze the excited species in the discharge produced by the conical-shaped reactor. A dual-channel spectrometer (AvaSpec-2048-2-USB2, Avantes, Apeldoorn, The Netherlands) with a spectral resolution of 0.7 nm was employed. The light emitted in the discharge was focused into a quartz optical fiber by means of a set of collimating lenses assembled in a way that the focal point was in the middle of the Petri dish (as shown in Figure 1). To avoid blocking the UV region, the emitted light was acquired through a hole (diameter of 1 mm) in the sidewall of the Petri dish. An irradiance calibration was performed prior to measurements.

2.2.3. Gas Temperature

A non-conductive fiber optic temperature sensor (TS2 and FOTOTEMP1-OEM, OPTOcon, Weidmann Technologies Deutschland GmbH, Dresden, Germany) was used to measure the gas temperature inside the reactor through the Petri dish hole (as shown in Figure 1). This GaAs-based sensor provides a voltage signal proportional to the temperature with a response time of less than 2 s. The increase in temperature was monitored using a digital oscilloscope (DPO 2024, Tektronix, OR, USA) for up to 5 min. Experimental deviation due to the positioning of the probe and to probe sensitivity (± 0.2 °C) was considered to be maximum ± 0.5 °C.

2.3. Seeds and Plasma Treatment

Rapeseed (*Brassica napus* L., Cv. Atora) seeds were obtained from NPZ Innovation GmbH (Holtsee, Germany). They are small (around 2 mm in diameter), round, and present a smooth surface morphology. To investigate the influence of the number of seeds on the produced discharge, the number of seeds was gradually increased from zero to around 1015 ± 2 (4.87 g) while the power density was measured. For the other experiments, 55 ± 2 seeds (around 0.26 g) were evenly distributed in the glass Petri dish prior to plasma treatment. The seeds were treated for 3 min using different voltage duty cycle values varying from 10% to 90%. Two control groups were considered: control “Ct” with untreated seeds and gas control “Ct_g”, where seeds were subjected to argon gas flow for 3 min with plasma off. Every treatment parameter was repeated 4 times ($n = 4$).

2.4. Seed Characterization

2.4.1. Temperature

The temperature at the seed surface was monitored using an infrared (IR) thermal camera (Testo 865, Testo, Titisee-Neustadt, Germany). Temperature assessment was made immediately after treatment on top of a wooden surface to avoid reflection interferences. The measurements were performed in three different seeds after the treatment of a group of 55 seeds.

2.4.2. Water Contact Angle

The surface of the seeds was analyzed with water contact angle measurements (WCA) using the sessile drop method. It was performed using a goniometer OCA 30 (DataPhysics Instruments GmbH, Filderstadt, Germany) with deionized water (a drop of 0.5 μL). Three seeds were analyzed per replicate, resulting in a set of 12 seeds for each parameter. The image evaluation and angle assessment were carried out with the software SCA 20 (DataPhysics Instruments GmbH, Filderstadt, Germany).

2.4.3. Germination Tests

The germination tests were performed immediately after plasma treatment. For each treatment replicate, 50 seeds were placed inside $12 \times 12 \text{ cm}^2$ square Petri dishes containing 4 layers of absorbent paper moistened with 12 mL of tap water. Germination took place in a climate chamber (Flohr Instruments, Nieuwegein, The Netherlands) with a controlled temperature of 25 °C under dark conditions. The germination process was monitored from 14 h until the seeds reached maximum germination (48 h). Seeds were considered germinated when the emerged radicle was longer than 1 mm. The germination percentage was calculated as described in [18].

2.5. Statistical Analysis

Significance between controls (C_t , C_g) and treatments for WCA measurements was proven using One-way analysis of variance (ANOVA) and the post-hoc test with multiple comparisons versus control groups (Bonferroni t-test) after checking for normal distribution of data. For the germination test, indices for maximum germination (G_{max}), mean germination time (t_{50}), and uniformity of germination (U_{25-75}), as well as statistical differences between treatments according to Student's T-test, were extracted from the germination assay using the Germinator package [33], as previously applied for wheat and barley [19].

3. Results and Discussion

3.1. Discharge Characterization

To understand the discharge generation by the conical reactor, the plasma source was operated with a sinusoidal voltage signal with different amplitude values (without amplitude modulation). Pictures of the discharge operating with different voltage values are shown in Figure 2. At low voltage values (around 8 kV p-p), plasma generation started as a corona discharge at the tip of the high voltage pin electrode. Raising the applied voltage increased the region of the high electric field, expanding the discharge outward until it transitioned to a glowing corona at around 10.5 kV p-p. Eventually (at around 13.5 kV p-p), the charged particles drifted far enough to sense the grounded electrode and the formation of streamers reaching the Petri dish took place. Starting at 14.0 kV p-p on, the generation of streamers was stable, and the discharge was distributed throughout the entire funnel volume. The discharge pictures in Figure 2a are displayed together with the power density for different applied voltage values. For the power density calculation, the funnel internal volume ($89.8 \pm 0.1 \text{ cm}^3$), in addition to the Petri dish volume ($50.0 \pm 0.1 \text{ cm}^3$), was used. A linear trend of the power density increase with the applied voltage was observed for all tested ranges. However, once the discharge transitioned to streamer mode, the transferred charge fluctuated substantially. This explains the higher standard deviation for the calculated power densities starting from 14.2 kV p-p in Figure 2a. Under these operation conditions, streamers reached the bottom surface of the Petri dish increasing the volume of produced plasma.

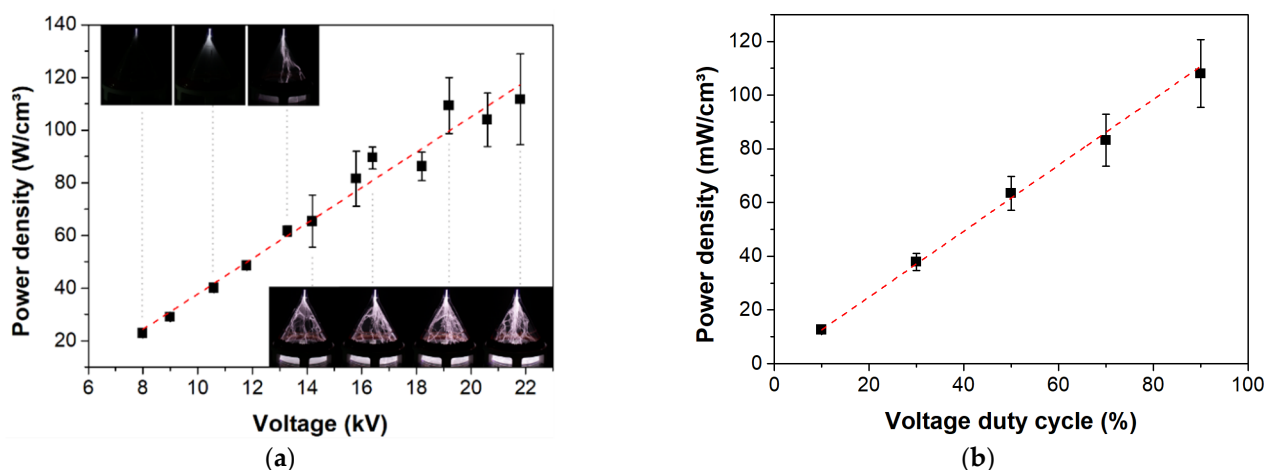


Figure 2. Power density values of the funnel reactor: (a) while varying the applied voltage with 100% voltage duty cycle and (b) when varying duty cycle and keeping the applied voltage at 20 kV p-p.

It has been demonstrated in previous works that operating a plasma source in burst mode allows a fine variation in the resulting mean discharge power [18]. This precise power control can be beneficial, especially for biological temperature-sensitive targets, such as seeds. Thus, the conical reactor was operated with different voltage duty cycle values for an applied voltage of 20.0 kV p-p (corresponding to around 105 mW/cm³ without bursting the voltage signal), and the data are presented in Figure 2b. A linear increase in power density can be observed. Thus, the discharge power density could be lowered to around 10 mW/cm³ using only 10% of the voltage duty cycle and keeping the amplitude voltage value. For some reactor configurations, such as plasma jets, power could also be regulated by the applied gas flow [34]. Mui et al. (2018) [32], studied the influence of the gas flow rate in a reactor with a similar configuration. The discharge power was not much affected by the gas flow rate variation when using a conical nozzle. Therefore, in the present study, the argon flow rate was fixed to 4.0 slm.

The discharge power adjustment by duty cycle variation led to a reduction in the discharge temperature [18]. Figure 3 presents the gas temperature for the voltage duty cycle values ranging from 10% to 90%. The temperature was monitored for 5 min of operation the time, including measurements prior to plasma ignition (around 20 °C). Within the measured time frame, the use of 90% duty cycles led to temperatures above 80 °C. For the same activity time (5 min), reducing the voltage duty cycle to 70%, 50%, 30%, and 10% decreased the gas temperature to around 68 °C, 54 °C, 41 °C, and 38 °C, respectively. Thus, controlling the discharge power can effectively lower the gas temperature preventing possible overheating when treating thermal sensitive targets.

Optical emission spectroscopy (OES) was used to identify excited species in the plasma. The discharge produced by the funnel reactor generated a large variety of excited species, as can be seen in the OES spectra presented in Figure 4a,c. Even though the funnel was closed by the Petri dish, excited species from air molecules (O₂ and N₂) were observed in the spectrum. Figure 4a shows a majority of N₂ second positive system bands between 300 nm and 450 nm. It also exhibited a weak NO(γ) emission between 230 nm and 280 nm and emission bands of OH at 308 nm, as depicted in Figure 4b. Figure 4c exhibits argon lines emitted between 690 nm and 930 nm. A small peak corresponding to atomic oxygen at 777 nm could also be observed. The excited oxygen and nitrogen species identified by OES can be directly related to the generation of reactive oxygen and nitrogen species (RONS) in the plasma region, which are important for several plasma processing techniques, including the field of plasma agriculture [35]. The identified excited species by OES are summarized in Figure 4d according to the relative intensity values for the different applied voltage duty cycles. It can be noticed that the OH band was the most intense from all analyzed species, followed by N₂, O, and NO. When increasing the voltage duty cycle, the intensity of those

species increased almost linearly. Even though the intensity values cannot be directly associated with the species concentration [36], they can be used as an indication. Thus, Figure 4d suggests that the generation of especially reactive oxygen species (ROS) was high using this reactor. Some ROS, such as the hydroxyl radical (OH) and singlet oxygen (O), are associated with fast surface modification of seeds leading to an increase in wettability [35]. As investigated in previous work using a DBD plasma source operating with argon and helium, a ROS-rich environment can be relevant for treatments of seeds, accelerating the germination process [18]. Moreover, wheat and barley displayed chemical modification of the caryopses surface, with altered surface wettability and water uptake [19]. The use of argon as a carrier gas facilitates plasma generation and leads to the production of ROS. An intensification of ROS generation could be easily obtained by introducing a small percentage of air to the carrier gas. Once, the use of pure air required operation using even higher voltage values. On the one hand, the conical corona reactor operating with argon used in the present study was a laboratory model and could only be used for the treatment of small numbers of seeds. On the other hand, the use of argon makes it difficult to apply this plasma source on an industrial scale and the conical configuration does not facilitate its upscaling. Therefore, this reactor configuration can only be used for small applications and basic science investigations.

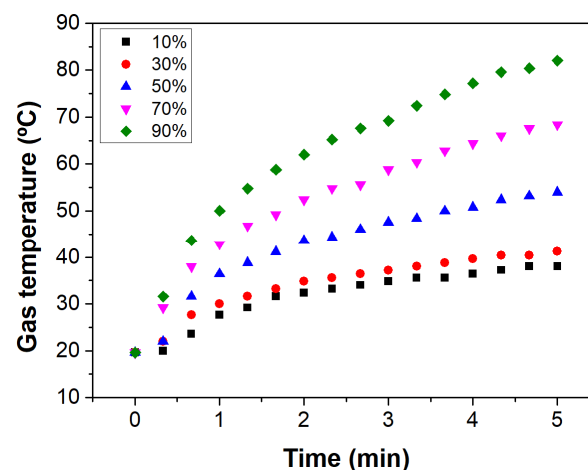


Figure 3. Temperature raised up to 5 min measured inside the reactor at the bottom central part of the glass Petri dish. A maximum error of ± 0.5 °C for all data was considered.

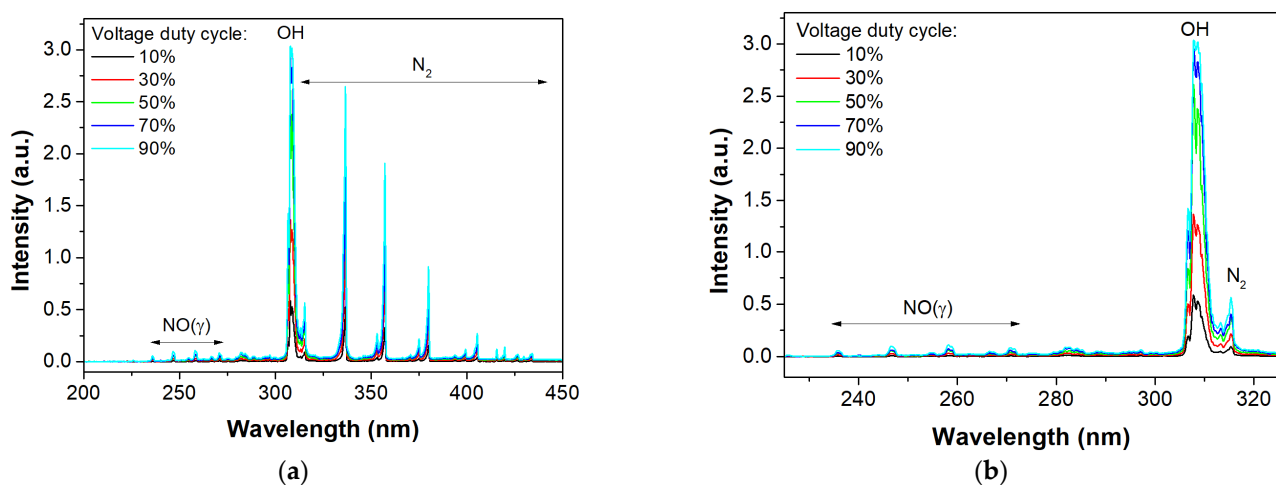


Figure 4. Cont.

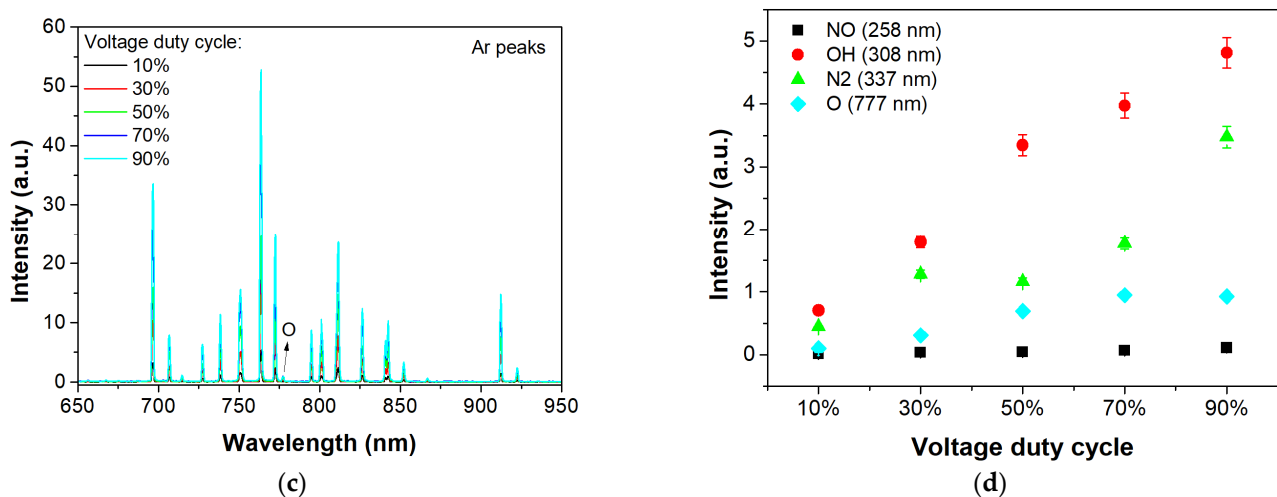


Figure 4. Optical emission spectra of the discharge generated by the funnel reactor for different voltage duty cycles. (a) Spectra for the wavelength range from 200 to 450 nm (mainly UV region) and (b) detailed spectra highlighting the emission bands of NO and OH from 225 nm to 325 nm. The visible region of the spectra is shown in (c) from 650 nm to 950 nm. (d) Overview of the OES peaks for different values of voltage duty cycle.

3.2. Treatment of Rapeseed

The effects of plasma treatment on seed properties and germination, as well as the influence of seeds on the discharge homogeneity, were investigated using seeds of rapeseed as a test model. First, the reactor was tested with different filling conditions to verify the effect on the discharge power. Figure 5 exhibits the power density values for different numbers of seeds treated (ranging from an empty reactor to a completely filled Petri dish). For this experiment, the voltage duty cycle was kept at 50% and new seeds were used for each batch of filling conditions. Complete filling of the Petri dish was achieved with approximately 1015 seeds (4.97 g). A small number of seeds (0.64 g, around 130 ± 2 seeds) did not affect the discharge power, and thus, the power density value did not differ from the empty reactor condition (60 mW/cm^3). Further increasing the seed number led to large fluctuations of power density values between 35 mW/cm^3 and 115 mW/cm^3 . The large standard deviations for power values observed from around 300 (1.59 g, 330 ± 2 seeds) to 1000 seeds (4.97 g, 1015 ± 2 seeds) suggest an impact on the discharge caused by the filling condition. As a result, substantial inhomogeneity of the treatment with larger numbers of seeds should be expected. Therefore, to maintain the discharge uniformity, further treatments performed for this study comprised only 55 seeds of rapeseed for each batch.

When dealing with direct treatments, it is important to consider that the substrate dielectric properties will affect the system leading to changes in the equivalent circuit model [37]. In the case of seeds, a capacitor and a resistor in parallel, whose values are excitation frequency-dependent, can represent the equivalent electrical circuit [37]. Therefore, the number of seeds placed inside a direct treatment reactor will impact the gap capacitance, resulting in variation in the discharge power. A large number of seeds inside the Petri dish resembles a packed-bed reactor. In such reactors, the discharge gap is completely filled with beads and the electric field at the voids (small space between the closely packed beads) is greatly enhanced [38,39]. Van Laer et al., 2017 [39] reported an influence of the beads' dielectric constant on the electron density in the voids. In addition, the electrons' density is lower for higher dielectric constant values (dielectric permittivity (ϵ) > 25 depending on the applied voltage). Kovalyshyn et al. (2020) [40] investigated the electrical properties of rapeseed seeds and reported a frequency-dependent ϵ ranging from 8.5 to 13 (for 1000 kHz and 1 kHz, respectively). Thus, the increase in power density observed in Figure 5 for larger numbers of seeds can be associated with an intensification of the electric field in the small regions (voids) between the seeds caused by surface charge

accumulation. Since the space in the conical reactor is not fully packed with seeds as in a packed bed reactor, the seeds can drift during treatment. Therefore, the voids between the rapeseed will have variable sizes leading to inconsistent discharge power with large measurement uncertainty. To avoid operating the reactor in the heterogeneous plasma density regions, for further seed treatments, the number of seeds placed in the reactor was kept with 55 seeds per batch.

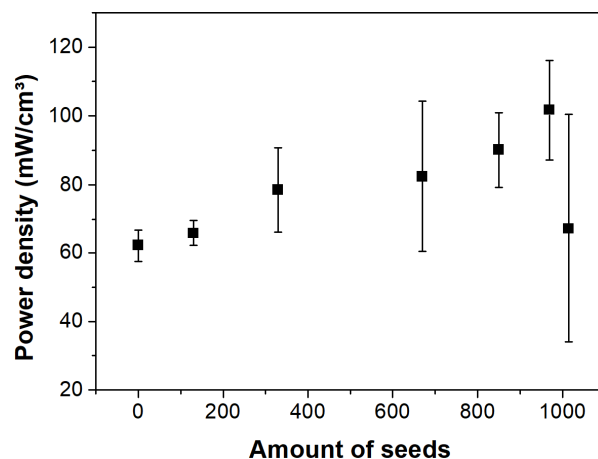


Figure 5. Influence of the number of seeds on the power density for zero (empty reactor) to around 1015 seeds (Petri dish bottom completely filled).

Another important aspect that should be considered when treating any kind of plant material is the temperature. As discussed in Section 3.1, raising the duty cycle leads to an increase in produced ROS. Yet, the duty cycle also affects the discharge temperature. Since most seeds should not be manipulated at high temperatures to prevent damage (for long time exposure), the temperature evolution caused by duty cycle variation needs to be investigated. In a study about the effects of seed germination conditions on the germination of *Pinus bungeana* seeds, Guo et al. (2020) [41], reported the importance of temperature. Increasing the temperature to which seeds are exposed benefits the seed germination process until an optimum value, after which it decreases promptly [41]. Figure 6 exhibits temperature curves assessed for different voltage duty cycles after 3 min of plasma exposure: for the gas temperature and the temperature measured at the seed coat. Three minutes of treatment was chosen as a middle point from the gas temperature assessment (Figure 3). For this exposure time, the gas temperature rose linearly with an increase in duty cycle reaching up to 70 °C for D = 90%. Differently, the estimated temperature at the seed coat increased initially with a similar pace but saturated for higher duty cycles, reaching a maximum of 50 °C for 90% of the voltage duty cycle. Therefore, even though the overall temperature remarkably rose inside the reactor after 3 min of plasma treatment, the seeds were not excessively heated.

When plasma is applied to a material's surface, its characteristics are modified [42]. Following the advance of plasma agriculture studies, the modifications caused by plasma on the surface of seeds have been investigated [43]. Several studies reported chemical modification of the seed coat after exposure to plasma. Gómez-Ramírez et al., 2017 [44], reported that plasma treatment can oxidize the seed coat and attach new nitrogen radicals. Brust et al., 2021 [19], observed an increase in oxygen-carbon ratio in wheat and barley seeds after treatment using a DBD reactor operating with argon. Some studies also reported physical changes after plasma treatment. Hosseini et al., 2018 [45], reported the formation of cracks in the seed coat caused by treatment using low-pressure RF nitrogen plasma. As a result of physical and chemical changes in the seed coat, its wettability may also change. This can be accessed via contact angle measurements. Studies, such as Li et al., 2016 [46], and Teerakawanich et al., 2018 [47], had already reported reductions in apparent contact angle in seeds after treatment using cold atmospheric discharges. Ling et al. (2015) [31],

reported a maximum reduction of 30.4% in apparent contact angle in rapeseed treated with low-pressure plasma. Other studies also observed a reduction in the apparent contact angle in treated rapeseed seeds, such as a reduction of 20% in [16] and a maximum reduction of 41.57% in [27]. Wannicke et al. (2020) [30], reported no significant reduction in the water contact angle in rapeseed treated indirectly using plasma processed air.

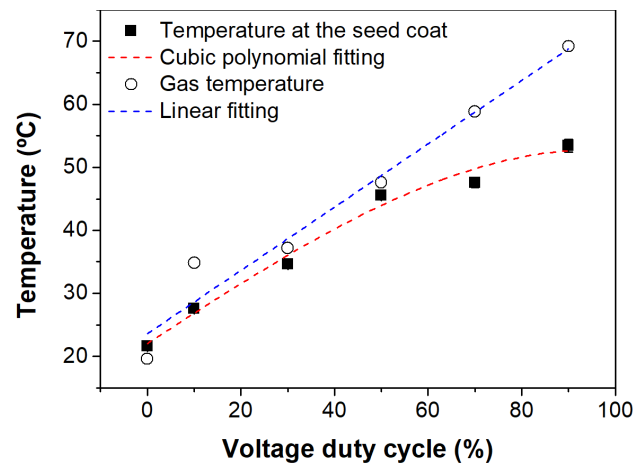


Figure 6. Comparison of the temperature measured inside of the empty reactor (center bottom of the Petri dish) after 3 min and the temperature obtained on the seed surface after treatments for 3 min using different duty cycle values.

Figure 7 presents the WCA measurements of rapeseed seeds treated with the funnel reactor using different voltage duty cycles. Seeds used in this study displayed a hydrophobic surface with a WCA of around 110°. After plasma treatment for 3 min, the seed surface wettability increased for all used voltage duty cycles. The seed coat became hydrophilic, and the contact angle value decreased for higher duty cycle percentages. For $D = 10\%$, the water contact angle dropped to around 65° (reduction of 37.7%) and this value reduced even more for $D = 90\%$, where a contact angle of 40° (reduction of 64.2%) was obtained. Interestingly, only a small wettability recovery of around 20% was observed after one week (data not shown), where the fastest recovery period happened in the first two days. Suggesting that plasma modifications persisted on the seed coat for longer periods as investigated in [19]. For higher duty cycle values, the period of plasma on was increased. As indicated in Figure 4d, the increase in voltage duty cycle might also lead to a higher production of RONS. Therefore, seeds treated with higher duty cycle values were not only exposed to plasma for longer periods but also might be exposed to higher amounts of reactive species.

The reduction in water contact angle on the seed coat is often associated with an improvement in water uptake by the seed. The ruptures caused by plasma treatment on the seed coat of artichoke seeds reported by Hosseini et al., 2018 [45], were pointed out as one of the responsible aspects that led to increased water uptake. Other studies also reported cracking of the seed coat and investigated its correlation with the increased water uptake and consequent improvement in germination [48,49]. In addition to morphological changes at the seed coat, another factor that contributes to improved germination is the surface activation by incorporation of radicals via etching [44].

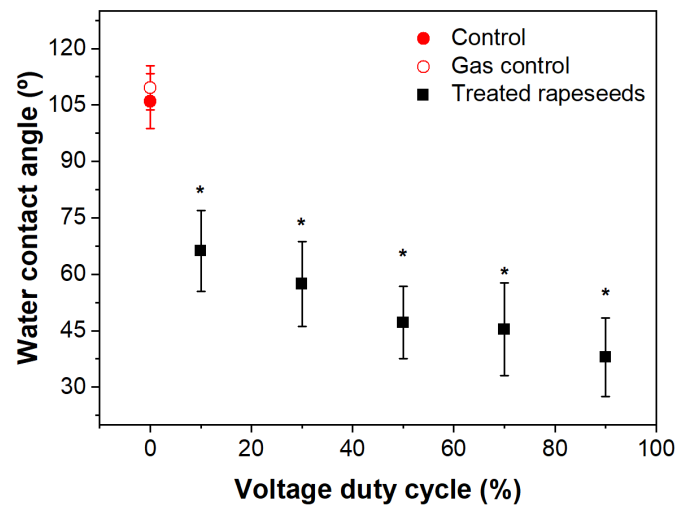


Figure 7. Water contact angle of rapeseed treated with the funnel reactor for 3 min using different voltage duty cycle values. (*) Represents a statistically significant difference ($p < 0.001$).

In this study, the germination rate of rapeseed treated with the funnel reactor using different duty cycles was investigated. Figure 8 shows the germination curves for gas control and treated seeds with different duty cycles ($D = 10\%$, 50% , and 90%). Acceleration in germination below 22 h of observation time could be detected for all treatment conditions ($D = 10\%$, 50% , and 90%). A maximum increase in germination was observed after 17 h of germination time, where an increase of around 20% was obtained for $D = 10\%$, 50% , and 70% and an increase of around 27% for $D = 90\%$. This moderate increase in germination caused by plasma treatment contrasts with the considerable decay in WCA for all duty cycle values. Such discrepancy was also reported and discussed in previous works [18,19]. It indicates that other factors besides improvement in surface wettability play a role when improving seed germination. Some reactive species, such as hydrogen peroxide and nitric oxide, might impact metabolic processes in plants [50]. Such species can be generated by further reactions between plasma-produced ROS and water and be taken up during the imbibition process [18].

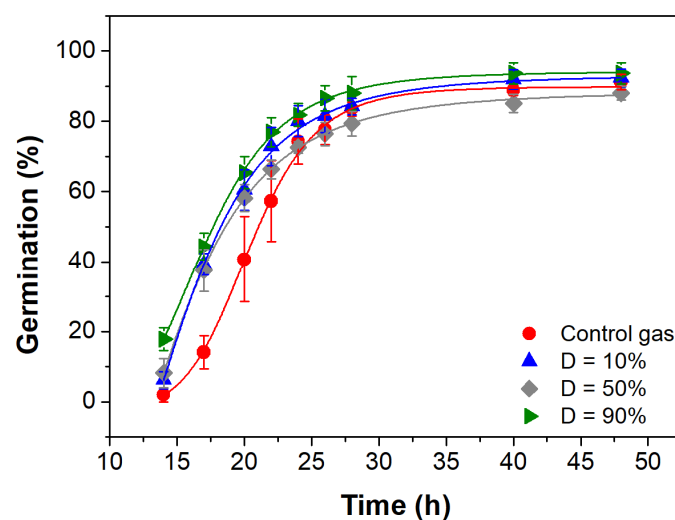


Figure 8. Germination acceleration of treated rapeseed (3 min) using different duty cycles. Germination curves were fitted with the Hill function.

Seed germination tests can provide a variety of information, such as maximum germination capacity (G_{max}), mean germination speed (t_{50}), and germination uniformity (U_{25-75}) [19]. The indices can be extracted from the germination curve acquired for different

times until maximum germination is reached. The seeds of rapeseed used in this study presented high maximum germination (G_{\max}) of around 90%. All the values extracted from the germination curves are displayed in Table 1. For all conditions, maximum germination was achieved within 48 h and the seeds treated with a duty cycle of 70% exhibited a significantly higher G_{\max} (around 96%) when compared to gas control (Table 1). It is important to highlight that plasma treatment did not negatively affect the maximum germination value of rapeseed for any of the tested treatment conditions. Puligundla et al., 2017 [29], also investigated the effects of a corona discharge on rapeseed. An impact of treatment time on the maximum germination was detected, where an improvement was observed for shorter treatment times (1 and 2 min) and a significant reduction for 3 min treatment. This contrasts with the obtained results for the conical reactor for 3 min treatment time and is possibly a consequence of the shorter gap size and different high voltage electrode configurations implemented by [29]. Germination impairment of rapeseed treated indirectly with multiple filling conditions of plasma processed air (microwave discharge) for decontamination purposes was also reported by [30]. On the other hand, single filling conditions did not completely harm the seeds which emphasize the importance of precisely investigating various parameters for different plasma sources.

Table 1. Effect of plasma treatment on the germination of rapeseed treated with different duty cycles. Values of maximum germination (G_{\max}), mean germination time (t_{50}), and uniformity of germination (U_{25-75}) were extracted from the fitted germination curves (Hill function). Bold numbers significantly deviated from control group (Ct) * and gas control (Ct_g) ** with $p < 0.05$.

Treatment	G_{\max} (%)	t_{50} (h)	U_{25-75} (h)
Ct	92 ± 2	21.3 ± 0.6	5.8 ± 0.2
Ct_g	91 ± 1	20.9 ± 0.7	4.7 ± 0.4
D = 10%	92 ± 1	18.6 ± 0.2 */**	5.5 ± 0.4
D = 30%	90 ± 1	19.6 ± 0.5	5.4 ± 0.6
D = 50%	88 ± 1	18.6 ± 0.3 */**	5.9 ± 0.7
D = 70%	95.6 ± 0.4 **	18.8 ± 0.2 */**	6.0 ± 0.5
D = 90%	94 ± 2	18.0 ± 0.1 */**	5.9 ± 0.2 **

The germination acceleration is also indicated in Table 1, where a significant decrease in t_{50} value was observed for D = 10%, 50%, 70%, and 90% when compared to both control groups. The t_{50} value is the time when 50% of the viable seeds are germinated. Therefore, a reduction in this value indicates a faster germination rate. Germination acceleration caused by non-thermal plasmas has been observed for other plant species, such as wheat [18], barley [19], radish [22], chickpea [51], and beans [52]. In the case of rapeseed, Kriz et al., 2017 [16], and Li et al., 2018 [27], also observed faster germination for treatments using low-pressure discharges. In addition, Ling et al., 2015 [31], reported an acceleration in the germination of rapeseed under drought stress after treatment using a low-pressure CCP reactor.

Another important parameter that can be assessed via a fitted germination curve is the germination uniformity (U_{25-75}). Li et al., 2018 [27], reported an improvement in the germination uniformity of rapeseed in a discharge power-dependent manner after treatment using a low-pressure RF discharge. Here, the uniformity of rapeseed germination was not affected by plasma (Table 1).

4. Conclusions

A corona discharge reactor with a horn-like nozzle was adapted for the treatment of small seeds. The discharge generated by this new configuration was characterized and its effects on rapeseed were investigated.

Rapeseed seeds exposed to the plasma produced by the funnel-shaped reactor exhibited accelerated germination. Observed improvements concerning seed germination showed to be dependent on the discharge power. The ROS-rich plasma environment

produced by this reactor led to the incorporation of oxygen radicals at the seed coat contributing to the pronounced increase in surface wettability. Given the direct treatment approach, the number of seeds that can be put in between the electrodes was revealed to be an important parameter regarding process homogeneity and reproducibility.

The conical configuration of the reactor presented in this study is designed for the treatment of small numbers of seeds and basic science investigations. However, considering the positive results on seed germination and surface wettability improvement, a large-scale corona reactor (possibly working with air) can be easily manufactured for certain future field applications.

Given the large variety of plasma sources already studied for applications in agriculture, each plasma system intended for seed treatment has to be individually investigated and optimized. The set of parameters determined to obtain specific results is unique for each plasma source. In addition, in direct systems, such as the funnel reactor, the biological target strongly affects the plasma treatment (homogeneity and expected results). Therefore, the precise adjustment of discharge parameters is of utmost importance to reach positive results, and also the process has to be carefully investigated for seeds from other plant species.

Author Contributions: Conceptualization, T.M.C.N., T.S.M.M. and H.B.; methodology, T.M.C.N., T.S.M.M. and J.W.; validation, T.M.C.N. and J.W.; formal analysis, T.M.C.N.; investigation, T.M.C.N. and J.W.; data curation, T.M.C.N. and N.W.; writing—original draft preparation, T.M.C.N.; writing—review and editing, T.M.C.N., N.W., K.G.K. and H.B.; supervision, H.B. and K.G.K.; project administration, K.G.K., H.B. and K.-D.W.; funding acquisition, K.G.K., N.W., H.B. and K.-D.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research was partly funded by the Federal Ministry of Education and Research of Germany (BMBF) within the framework of the project “Physics for Food” (No. 03 WIR 2805 A and 03 WIR 2808 D). Some authors (T.S.M.M. and K.G.K.) acknowledge financial support from the Coordination for the Improvement of Higher Personnel Education—CAPES, Brazil, under the program PrInt.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful to Michael Timm and Udo Nehmzow for technical assistance in the construction of the plasma reactor.

Conflicts of Interest: The authors declare no conflict of interest.

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