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ORIGINAL PAPER

Effect of Cold Atmospheric Pressure Plasma on the Wheat Seedlings Vigor and on the Inactivation of Microorganisms on the Seeds Surface

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Abstract Effects of a cold atmospheric pressure plasma (CAPP) treatment on the germination, production of biomass, vigor of seedlings, uptake of water of wheat seeds (*Triticum aestivum* L. cv. Eva) were investigated. The CAPP treatment influence on the inactivation of microorganisms occurring on the surface of wheat seeds was investigated also. The so-called Diffuse Coplanar Surface Barrier Discharge generating a cold plasma in ambient air with high power volume density of some 100 W/cm^3 was used for the treatment of seeds at exposure times in the range of 10–600 s. The optical emission spectroscopy and the electrical measurements were used for estimation of CAPP parameters. The obtained results indicate that the germination rate, dry weight and vigor of seedlings significantly increased for plasma treatment from 20 to 50 s. The plasma treatment of seeds led to an extensive increase in wettability and faster germination comparing with the untreated seeds. The growth inhibition effect of CAPP on the surface microflora of wheat seeds increased with the increase of the treatment time. The efficiency of the treatment of wheat seeds artificially contaminated with pure cultures of filamentous fungi decreased in the following order: *Fusarium nivale* > *F. culmorum* > *Trichothecium roseum* > *Aspergillus flavus* > *A. clavatus*.

Keywords Cold atmospheric pressure plasma · Wheat seed · Germination · Filamentous fungi · Inactivation

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Introduction

Because of the advantages, as the ability to large-scaling, low costs, no needed expensive vacuum systems and high processing speed, the cold atmospheric pressure (in the sense non-equilibrium) plasmas (CAPPs) have been used for a wide range of technological applications [1]. Currently, the plasma surface treatments are widely used to achieve desired changes on surfaces of various materials as polymers, metals, glass, etc. The plasma treatment influences properties of the surface, leading to a change in the surface energy, chemical composition and roughness of the surface. In recent years bio-applications of the CAPP for sterilization, bio-decontamination, preparing of the biocompatible surfaces, for direct treatment of hardly healing wounds [2–4] and many others, have become a hot topic. The low temperature of the heavy particles (ions, molecules) makes CAPP suitable for the surface treatment of sensitive biomaterials. On the other hand, the electron temperature is high enough to produce a variety of excited species, molecular and atomic radicals and UV radiation suitable to act effectively on the surface in contact with CAPP. The role of the individual active species and radicals, charged particles and UV radiation in the bio-decontamination and sterilization of surfaces as well as various another bio-medical applications is the subject of investigation of many research teams [3, 5, 6]. Recently, numerous works dealing with plasmachemical processes, possible reactions and mechanisms taking place in the contact of non-equilibrium plasma particles with the cells or the biological object have been published [7–12].

The cause of a low germination rate of seeds of various plant species is often connected with the seeds contamination with epiphytic and phytopathogenic bacteria and filamentous fungi. The negative effect of seeds contamination by microorganisms can be eliminated by disinfecting the seeds with fungicides for seed dressing or by different seed disinfectants [13–16]. In the last years also many physical methods have been used to influence a germinating power of seeds and reduce germinating time, as well as to interfere within a development, growth and yield of plants [17–20]. Many authors studied the stimulation of the seed germination and the plant growth by using plasma generated by corona discharges [21–23], low-pressure radio-frequency (RF) discharge [24], microwave [25] or other types of plasma sources [26–28], as well as by the electrostatic [25, 29] and magnetic fields [30, 31]. As reported by several authors [32, 33] the plasma treatment can improve also the wettability of seeds surface leading to germination enhancement and other growth parameters. Moreover, the plasma as the sterilizing agent can kill microorganisms on the seed surface.

It has been found that plasma is a capable sterilizing method to inactivate a wide range of microorganisms on the seed surface as well as on the stored food substrates [34, 35]. Its effect on various food contaminant microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Candida albicans*, *Aspergillus niger, A. flavus, Fusarium* spp. and other microorganisms has been studied on different materials [3, 8, 36, 37]. Atmospheric pressure plasma with TiO_2 were used together to inactive *Bacillus subtilis* spores, that have a very high degree of environmental resistance to ultraviolet (UV) photons and heat [35]. For the inactivation and/or elimination of two filamentous fungi, *Aspergillus* spp. and *Penicillium* spp. artificially contaminated on seed surface of grains and legumes by cold plasma treatment was studied by Selcuk et al. [38]. Risk factors entering the food chain emphasis were also placed to verify the impact of cold plasma to eliminate the selected representatives of epiphytic and toxicogenic filamentous fungi as potential producers of dangerous mycotoxins hazardous storage nutritional grains and cereals destined for human consumption and stock feed ingredient for livestock feed. The contamination of food substrates by aflatoxins produced for example by *Aspergillus parasiticus* is a serious problem

because of the potential threat to animal and human health [9]. The contamination of food commodities by toxinogenic fungal species, and consequently the presence of aflatoxins are unavoidable [39] and cause numerous acute or chronic toxicities [40]. According to Park et al. [41] the microwave-induced argon plasma at atmospheric pressure was used for degradation of some mycotoxins as aflatoxin B1, deoxynivalenol and nivalenol.

The objective of our work was to explore possible application of the cold plasma to inactive an epiphytic, phytopathogenic and toxicogenic microorganisms occurring on the surface of wheat seeds and to evaluate the effects of plasma on the germination rate, growth parameters, vigor of seedlings and dynamics of water uptake by seeds treated by plasma. As a plasma source we used a special type of dielectric barrier discharge with coplanar electrode system placed inside of ceramics dielectric. This so-called Diffuse Coplanar Surface Barrier Discharge (DCSBD) has been successfully used for increasing the surface energy, activation and surface cleaning of polymer, aluminium, silicon, wood and glass [42, 43]. It was used because of its robustness, safety at unintended contact, and possibility to operate in humid and dusty environment. We believe that for the potential industrial-scale seed treatment these unique properties of DCSBD provide significant technical advantages over traditional CAPP sources.

Methods

Characteristics of Plasma

In our experiments the plasma treatment of plant seeds was carried out using the DCSBD planar source of CAPP, its detailed characteristic and properties were reported previously [42–45]. A schematic draw of the experimental set-up and of the DCSBD electrode system is shown in Fig. 1. Two systems of parallel strip-like electrodes (1.8 mm wide, 0.1 mm

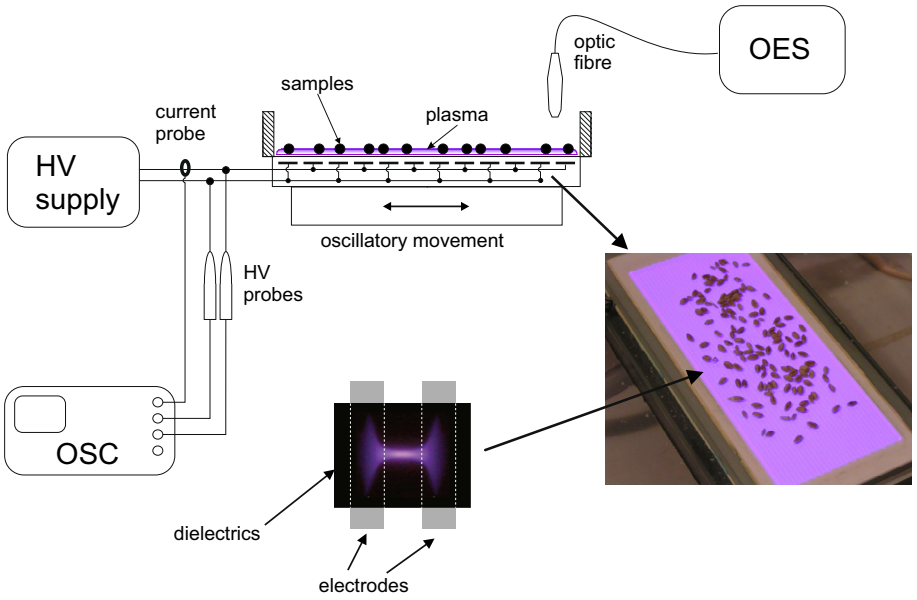

Fig. 1 Experimental set-up and the schematic draw of DCSBD electrode system

thick), 230 mm long, 0.4 mm strip to strip; prepared from silver were embedded in 96 % alumina. The thickness of the ceramic layer between the plasma and electrodes was 0.4 mm. The discharge was powered by sinusoidal high frequency, high voltage (14 kHz, approximately 20 kV peak to peak), supplied by HV Plasma Power Supply. The electrical parameters of discharge were monitored by Pearson current monitor Model 4100 and two high voltage probes Tektronix P6015A (1000:1). The signals from all three electrical probes were recorded by the digitizing oscilloscope Tektronix TDS 2014B. The total power consumed by the discharge plasma was calculated from the measured current $i(t)$ and voltage $u(t)$ waveforms $P = \frac{1}{T}\int_0^T u(t) \cdot i(t)dt$ (Fig. 2).

To achieve homogeneous treatment of the seeds on each side, the plasma source (DCSBD) was placed and firmly fixed to the laboratory orbital shaker (PSU-10i, f. Biosan). If the rotation speed of shaker was properly set, seeds began to rotate in the plasma field on the ceramics surface. The total plasma processing time is thus the total time spent in the plasma field.

Such a discharge electrode arrangement and energization were found to generate visually almost uniform plasmas in ambient air at atmospheric pressure that is illustrated by Fig. 1. The visually diffuse plasma that is almost uniformly coating the alumina surface consists, in fact, from a diffuse surface discharge plasma generated on the electrode system surface over metal electrodes and a filamentary streamer plasma on the surface over the gap between electrodes. This is because the discharge consists of numerous *H*-shaped elementary discharges developing with a high density and running on the electrode system surface along the embedded strip electrodes.

Non-equilibrium, non-thermal character of DCSBD air plasma burning at atmospheric pressure was investigated by optical emission spectroscopy (OES) measurements. This diagnostic methods is suitable to establish the rotational and vibrational temperatures in plasma and by detailed analyse of excited molecular and atomic states help to further finding the mechanisms playing a role in plasma contact with the surface of seeds. AvaSpec-2048 Thermo-Electric Cooled Spectrometer in spectral range 300–400 nm and

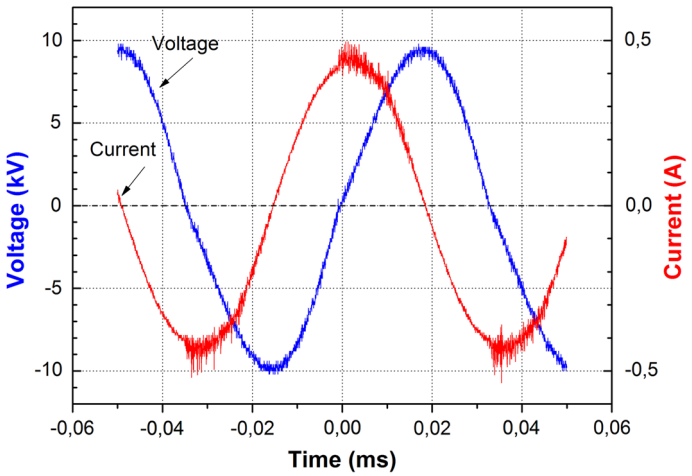

Fig. 2 Typical time development of voltage and current waveforms in DCSBD ambient air plasma burning at atmospheric pressure at input power of 400 W

resolution 20 px/nm, respectively, was used for the OES measurements of DCSBD plasma. The light emitted from the plasma was led through the optical system consisting of aperture and lens of focal point 25 mm, and focused on the entrance of optical fiber. The spectra were integrated for 1000 ms. Spectrum was processed with program Avasoft Avantes. In this range, the 2nd positive system of molecular nitrogen ($N_2(C^3\Pi_n - B^3\Pi_g)$) can be detected in all discharges contained nitrogen, particularly in non-isothermal plasma. In Fig. 3a, it can be seen the measured spectrum of DCSBD plasma in air between electrodes at input power 400 W. Peaks were identified using Spectrum Analayzer 1.7 [46] and database of emission spectra of diatomic molecules NIST. Rotational and vibrational temperature were determined from comparison of measured spectrum and spectra simulated in SPECAIR 2.2 [47]. Vibrational band 0–1 was used for the calculation of rotational temperature. Spectrum calculated with $T_{rot} = 370 \pm 30$ K correlates with the measured spectrum (Fig. 3b). Vibrational temperature was determined from simulated spectra, which were normalized at transition 0–1. Vibrational temperature was determined as

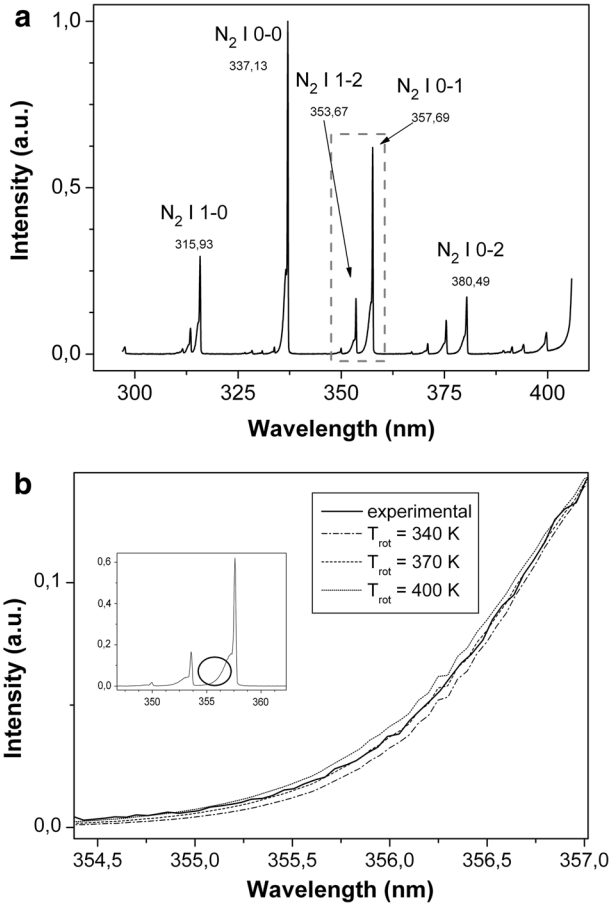

Fig. 3 Typical emission spectra of DCSBD plasma: vibrational states in the 2nd PG of nitrogen used to evaluation of vibrational temperature in plasma (**a**). Rotation transition used to evaluate the rotational temperature of N_2 molecule (**b**)

$T_{vib} = 3300 \pm 100$ K, by comparison of intensities at 1–2 transition corresponding to different vibrational temperature.

Plasma Treatment of Seeds

Three-year-old wheat seeds (*Triticum aestivum* L. cv. Eva) which were used in this study, were obtained from the Sedos Co. Krakovany, Slovakia. The seeds were stored at 10 °C in the dark.

Plasma treatment of seeds was done at input power 400 W, when the whole DCSBD electrode is covered with plasma layer and all seeds are treated homogeneously. Considering the efficiency of the power supply, the dimensions of the plasma field (200 mm × 80 mm) and the plasma layer thickness (0.3 mm), the corresponding plasma volume power density was determined to be approximately 70 $W \cdot cm^{-3}$.

As depicted in Fig. 1, the treated seeds (about 100–300 pcs) were placed in the plasma layer on ceramics and movement of seeds on its surface was carried out mechanically to ensure their uniform treatment. The plasma treatment times were in the range of 10–80 s in the experiments with seeds germination and growing conditions. In the experiments with microorganisms inactivation the plasma treatment times were in the range of 30–300 s. The seeds had been taken out from the plasma field after treatment and exposed to the atmosphere for 24 h before the biological experiments started. DCSBD electrode was cooled by oil with high dielectric permittivity which also functions as insulation. Also in the case of long-term continuous operation the oil cooling system maintains the temperature of ceramics not more than about 50–55 °C. In addition, during the treatment of seeds the reactor is in continuous rotational motion, therefore the seeds rotate in the plasma not lying motionless on ceramics, on the contrary, the temperature of seeds is reduced by this motion.

Seed Germination and Growth Conditions

Control—untreated (0 s) and treated seeds (10–80 s), 24-h after plasma treatment were sown in experimental pots containing soil substrate (sand/peat/pearlite 1:1:1 v/v/v) with 100 seeds per variant in three repetitions. The water level was adjusted at 2 days intervals with water to avoid changes due to evaporation. The plants were cultivated in controlled growth conditions: 26 °C in the light and 18 °C in the dark, 12 h light/12 h dark photoperiod with a photon flux density of 120 $\mu mol/m^2/s$ and 60 % air humidity. Germination rate and growth parameters (fresh and dry weight of seedlings) were determined 10 days after sowing. For determination of a fresh and dry weight, the twenty plants from each treatment in three repetitions including control were harvested and a soil was washed out of the roots. These plant samples were rinsed using distilled water, dried at 80–104 °C for 3 days, and weighed. After weighing the dry mass of plants was evaluated. After 10 days of cultivation, the germination percentage was calculated using the equation:

$$\text{Final germination percentage} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds planted}}$$

and seedlings vigor I and II were calculated according to the fromula Abdul-Baki and Anderson [48] in the following modification:

$$\text{Seed vigor} = \frac{\text{Fresh or dry weight of seedlings in mg} \times \text{germination precentage}}{100}$$

Plasma Effect on Water Uptake

Three replications each of 100 treated (10–200 s) and untreated seeds (0 s) were kept into the glass flasks with 50 ml of distilled water and allowed to imbibe at laboratory temperature 25 °C. Immediately after the initial wetting and at 2-h interval the seeds were blotted dry, weighed on an electronic balance (Sartorius BL-210S, Germany) to the nearest 0.1 mg and returned into the glass flasks with distilled water. After 8-h of imbibition the procedure was repeated. The amount of water taken up was determined as actual increase in seed weight.

Water content per seed was measured and expressed according to the following equation: Water content $(\mathrm{WC})\left(\mathrm{mg\,seed}^{-1}\right) = (\mathrm{FW} - \mathrm{DW})/100$, where FW is fresh weight and DW is dry weight.

Plasma Effect on Microorganisms

The standard agar plate count method was used to determine the total number of cultivalable natural microflora on the surface of wheat seeds as colony forming units (CFU) on the surface of wheat seeds [16, 49]. 20 g of the air-dried wheat seeds were treated with CAPP at the dose rate of 0 (control) and 30–600 s, and suspended into 200 ml of sterile saline, mixed by vortexing for 30 min, and left to sediment seeds for 5 min. Solutions above the seeds were diluted through serial dilution with sterile saline (10^{-1}–10^{-7}). Two hundred microliter aliquots of diluted samples were spread on surface of sterile Petri agar plate (diameter 90 mm) with nutrient medium (Mueller–Hinton agar-bacteria; malt extract agar with chloramphenicol – yeasts and filamentous fungi) in triplicate sets. After cultivation (bacteria 2-days at 30 °C and fungi 7-days at 25 °C) the number of colonies was counted and the concentration of microorganisms as CFU/g of seeds was calculated (mean of three experiments). Taxonomic identification of bacteria was done according to Betina et al. [50]. Identification of isolates filamentous fungi was done microscopically (Axio Imager A1, Carl Zeis, Germany) on the basis of morphology of fructifying structures [51].

Effect of CAPP on wheat seeds artificially infected with pure cultures of filamentous fungi *Fusarium nivale, F. culmorum, Trichothecium roseum, Aspergillus flavus* and *A. clavatus* (isolates from surfaces of untreated wheat seeds) was determined on dead wheat seeds. 50 g of wheat seeds were killed by autoclaving at 126 °C for 15 min. Dead seeds were then infected with 7.5 ml of a spore suspension (concentration-1 $\times$ 10^5 ml^{-1}) of the filamentous fungi from 21-days old strains in 0.1 % (v/v) aqueous Tween 80 [52] by intensive shaking on the rotary shaker at 25 °C during 20 min. Infected wheat seeds were given to sterile Petri dishes (diameter 140 mm), in order to partially evaporate water adsorbed to the surface of seeds when it infects. After 24-h, 15 g of infected wheat seeds were treated by CAPP at the dose rate of 0 (control), or 30–300 s. Individual samples of treated wheat seeds, as well as untreated controls, were placed into the surface nutrient medium (malt extract agar) in Petri dishes (diameter 185 mm)-50 seeds/dish in triplicate sets. After incubation (3–5 days) at 25 °C we subsumed each individual wheat grain into the scales from 0° to 5°, according to the intensity of fungal attack of surface area of each wheat grain (the score 0 indicated absence of fungal attack; the score 5-fungal attack >60 %). The infection degree (ID) was evaluated from obtained values using Townsend-Heubergeŕs formula according to Puntner [53]:

$$ID = \frac{\sum (nv) \times 100}{NV}$$

where: n = degree of infection rated on a scale of 0–5, v = number of seeds in a category, N = the highest degree of infection rate, V = total number of seeds screened.

The efficacy of CAPP wheat seeds treatment was calculated according to Rekanović et al. [54]:

$$\%\, efficacy = \frac{control - treatment}{control} \times 100$$

(control-infection degree of untreated wheat seeds; treatment-infection degree of CAPP-treated wheat seeds).

Statistical Analysis

Each data point was the mean of three replicates. All data obtained were subjected to a one-way analysis of variance (ANOVA), and the mean differences were compared by lowest standard deviations (LSD) test. Comparisons with $P < 0.05$ were considered significantly different. In the figures, the spread of values is shown as error bars representing standard errors of the means.

Results

Experimental results confirm that the seeds treated with plasma took more water than untreated seeds. Increasing exposure dose of plasma also increased water uptake in seeds. Water uptake after 2-h ranged from 6.41 to 9.60 mg, and after 8-h from 12.53 to 16.07 mg per seed in comparison to the control (Fig. 4). Water uptake by seeds was more profound after 2-h of imbibition than after 8-h. The experiments showed that treatment of wheat seeds with CAPP also increased the germination capacity and stimulated the growth of

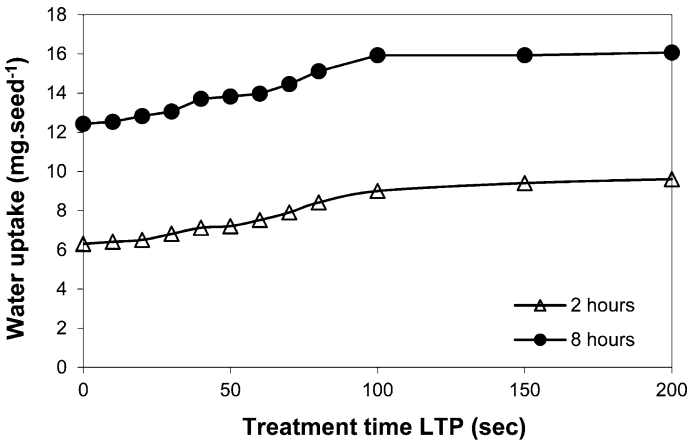

Fig. 4 Changes in water uptake at 25 °C for a cold plasma treated (10–200 s) and untreated- control (0) wheat seeds

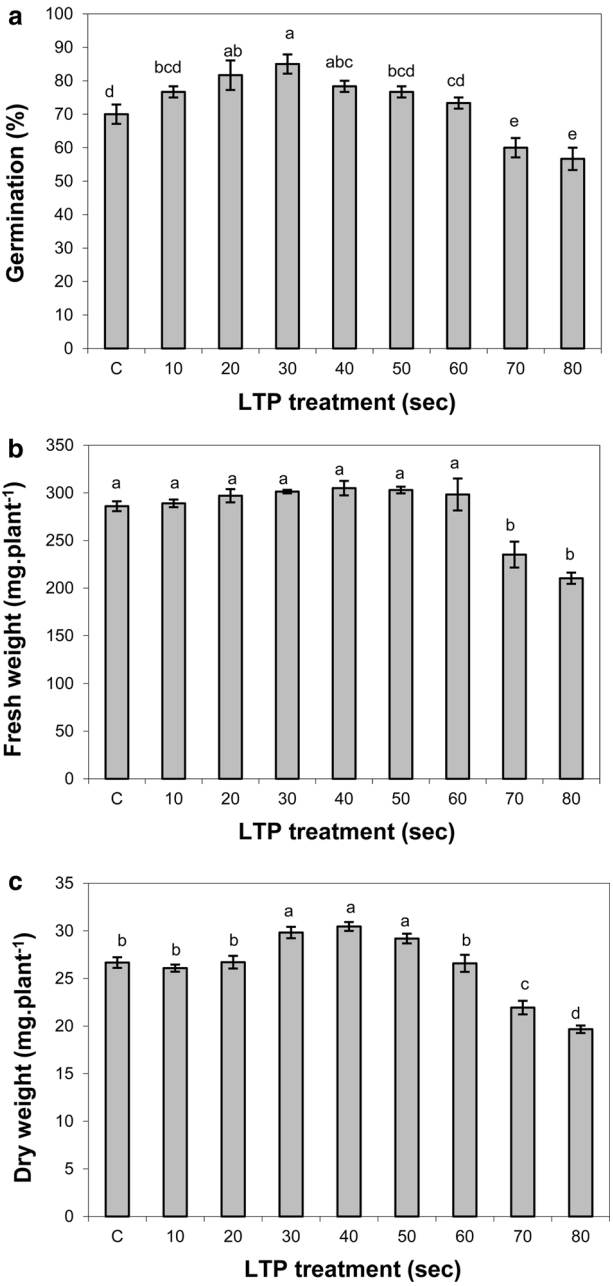

Fig. 5 Effect of a cold plasma exposure at different time interval (10–80 s) on germination (**a**), fresh weight (**b**) and dry weight (**c**) of 10-days old wheat seedlings

seedlings. Under influence of CAPP in the time range of 20–50 s the germination rate (Fig. 5a) and seedling dry weight (Fig. 5c) significantly increased. Fresh weight of seedlings grown from treated seeds was not significantly affected (Fig. 5b). The highest

positive responses were found when the seeds were treated for 30 s. An intensity of 30 s CAPP produced significant ($P < 0.05$) increases in germination rate (21 %) in dry weight (12 %) and vigor index I and II (28 and 36 %) respectively, compared to the control (without treatment). Higher exposure time of plasma for 70 and 80 s significantly inhibited all measured growth parameters in comparison with untreated seeds: germination rate decreased by 14 and 24 %, fresh and dry weight by 18 and 26 %, and vigor index by 29 and 44 % (Figs. 5, 6).

The plate count method that was used to determine the total number of cultivable cells of bacteria, yeasts and filamentous fungi on the surface of wheat seeds as CFU satisfactorily monitored the effect of CAPP treatment. It showed that seeds of *Triticum aestivum* were contaminated with bacteria and filamentous fungi. The concentration of surface natural microflora in non-treated wheat seeds ranged from 6.0×10^2 (filamentous fungi) to 5.52×10^4 (bacteria) CFU/g; none yeasts were presented (Table 1). Predominant there were bacterial isolates of the genera *Bacillus* sp., *Micrococcus* sp. (both G^+ bacteria), *Aeromonas* sp., *Morganella* sp., *Serratia* sp. (G^- bacteria). The most frequent fungal species were identified *Aspergillus clavatus*, *A. flavus*, *A. niger*, *Fusarium nivale*, *F.*

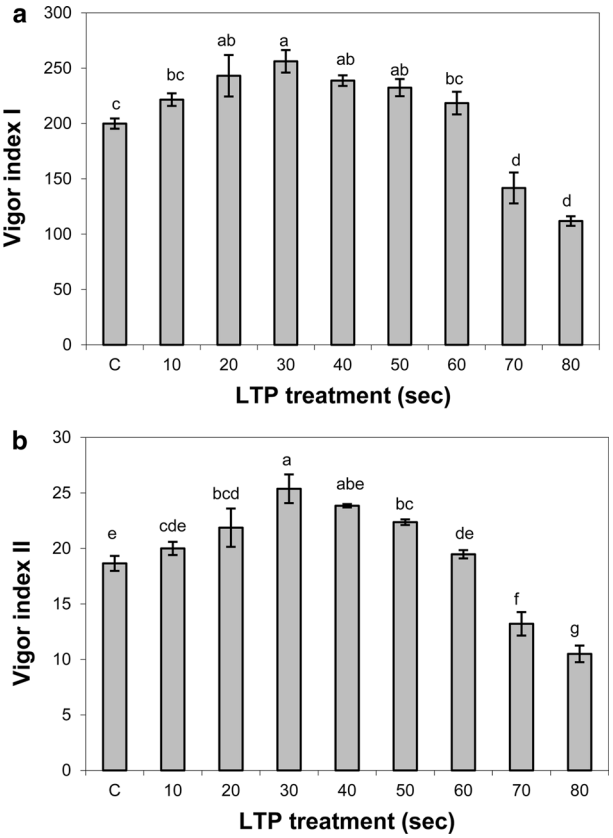

Fig. 6 Effect of a CAPP exposure at different time interval (10–80 s) on vigor index I (**a**) and vigor index II (**b**) of 10-days old wheat seedlings. Vigor index I = germination % × seedling fresh weight in mg/100. Vigor index II = germination % × seedling dry weight in mg/100

Table 1 Reduction of surface microbial contamination on wheat seeds by CAPP

Treatment time (s)	Epiphytic microorganisms (CFU/g seeds)		
	Bacteria	Yeasts	Filamentous fungi
0 (control)	5.52×10^4	None	6.00×10^2
60	1.80×10^4	None	1.00×10^2
120	7.40×10^3	None	0
240	3.16×10^3	None	0
600	1.43×10^3	None	0

CFU colony forming units

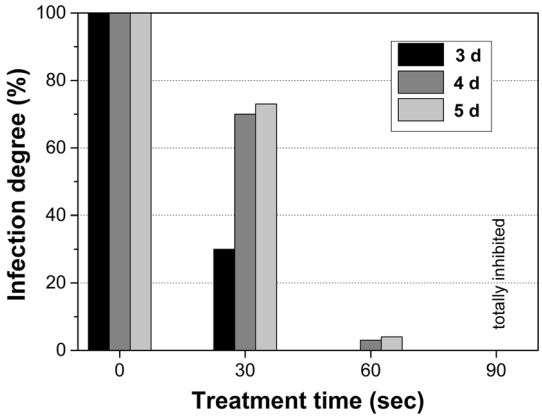

Fig. 7 Growth inhibition of seed-born pathogen *Fusarium nivale* on wheat seeds after CAPP treatment

culmorum, *Trichothecium roseum*, and *Penicillium* spp. CAPP treatment of seeds reduced microbial contamination proportionally to the duration of its operation. The initial number of bacteria on wheat seeds 5.52×10^4 CFU/g before CAPP treatment, decreased 3.8 times after CAPP treatment in duration 600 s. Approximately a 1-log reduction in the population of bacteria occured on CAPP treated seeds was observed for the duration 120 s. After that time the reduction effect of CAPP on bacteria remained stable. On the other hand total devitalization of the initial number of filamentous fungi (6.00×10^2) was determined after 120 s CAPP treatment seeds.

The effect of CAPP treatment on wheat seeds artificially infected with pure cultures of filamentous fungi *F. nivale*, *F. culmorum*, *T. roseum*, *A. flavus* and *A. clavatus* was different. We found that representatives of the genus *Fusarium* spp., important phytopathogenic seed-borne pathogens were the most sensitive to CAPP treatment of wheat seeds. Total growth inhibition of *F. nivale* (Fig. 7) and also *F. culmorum* was already observed after 60 s CAPP seeds treatment during 3-days incubation. The results in this study further confirmed, that killing effect on conidia of both fusaria was observed after 90 s CAPP seeds treatment. Exposure in CAPP even for 180 s significantly reduced growth of toxinogenic *A. flavus* and total growth inhibition with letal effect on conidia was observed after 240 s CAPP seeds treatment (Fig. 8). The growth inhibition effect of CAPP treatment wheat seeds on epiphytic *T. roseum* was higher than effect on toxinogenic *A. flavus* (Figs. 8, 9). Comparison of the CAPP effect on three fungal species studied is evident from Fig. 10. We determined approximately 70 % growth inhibition of *T. roseum* during 60 s CAPP seeds treatment after 3-days incubation, and total growth inhibition of

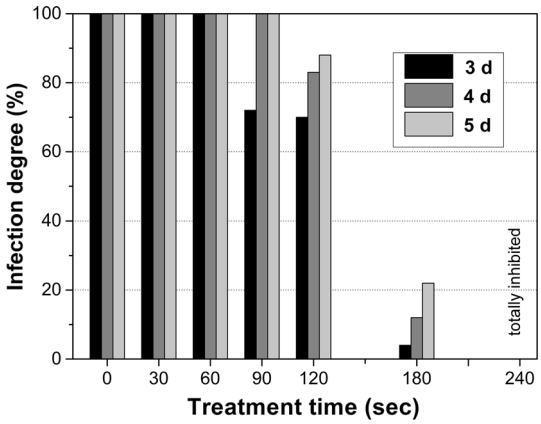

Fig. 8 Growth inhibition of toxicogenic *Aspergillus flavus* on wheat seeds after CAPP treatment

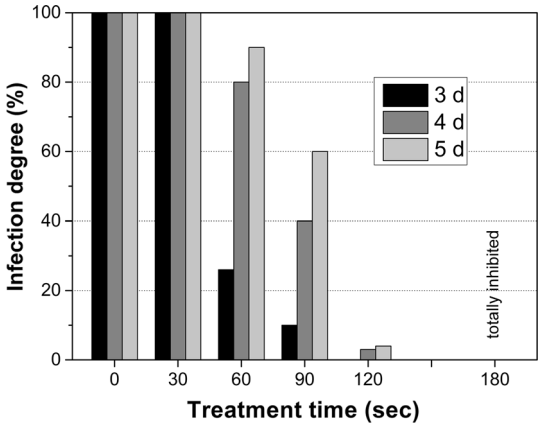

Fig. 9 Growth inhibition of epiphytic *Trichothecium roseum on wheat seeds* after CAPP treatment

this fungus with lethal effect on conidia was observed after 180 s of CAPP treatment wheat seeds (Fig. 9). The least effective it was CAPP treatment of infected wheat seeds by epiphytic *A. clavatus*. We found that exposure of CAPP to even 300 s with infected seeds did not cause all-out devitalization of conidia *A. clavatus.* Fifty percentage reducing of the number of *Fusarium* spp. was achieved after 30 s of CAPP treatment, in the case of *Aspergillus* spp. it was after more than 2 min. The efficacy of CAPP on selected filamentous micromycetes on the surface of wheat seeds decreased in the following order: *F. nivale* > *F. culmorum* > *T. roseum* > *A. flavus* > *A. clavatus*.

Discussion

The seed germination rate and the early stage of a seedling growth after application of the different plasma sources were investigated in a number of plant species. Under influence of plasma generated by DCSBD at atmospheric pressure not only the germination rate was stimulated, but also the achieved higher production of biomass increased in comparison with control seedlings. There was a growth improvement in wheat plant species when the

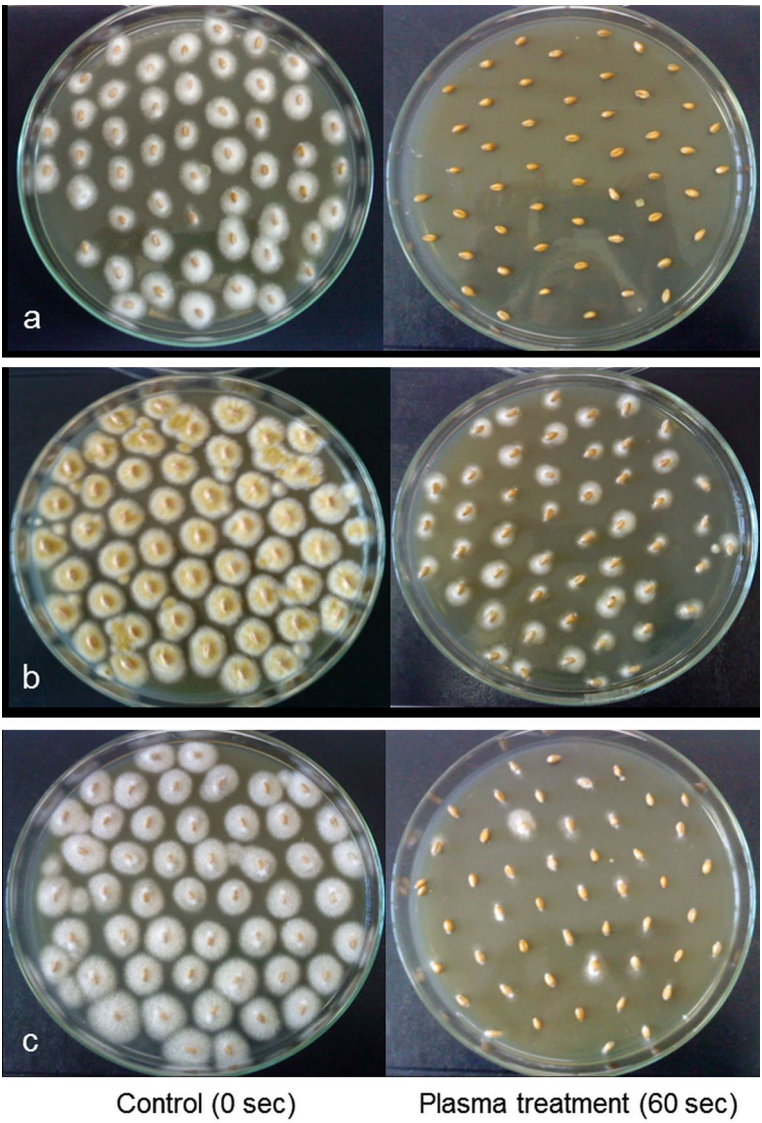

Fig. 10 Growth inhibition of *Fusarium nivale* (**a**), *Aspergillus flavus* (**b**), *Trichothecium roseum* (**c**) on wheat seeds after CAPP treatment for 0 and 60 s and 3-days of incubation

seeds were treated with low and medium levels of the plasma exposure time that is at 20 and 50 s, respectively. Based on our actually results (unpublished data) of many agricultural crops we resulted that the germination rate of plasma-treated seeds was different in individual plant species. The positive or negative effect depended not only on plasma exposure time but also on the natural germination capacity of individual plant species and also on the size of seeds and their surface morphology, seed coat hardness and thickness of endosperm. The positive effect of plasma on the germination rate and biomass production (mainly dry weight) favorably affect the overall viability and vigor index of 10-days old

wheat plants. Fischer et al. [55] ascertained that sunflower seedlings exposed to magnetic field showed significant increases in total fresh weights, whereas dry weights and germination rates remained unaffected, but in treated wheat exhibited the higher total fresh weights and higher germination rates what corresponds with our results. One possible explanation of an increase in germination and growth development under CAPP treatments ($P < 0.05$) is based on the fact that the disturbance due to the application of plasma on the tissue structures leads to the transport of essential substances through the channels induced on the cell membranes [56].

Based on our actually and presented results considerable variations were observed in individual crop species (barley, maize, pea, lettuce, pepper, tomato and radish) especially at higher CAPP levels in response to a germination percentage. These results are in agreement with Volin et al. [18] who found that high levels of cold plasma can significantly inhibit seed germination and retard the seedling growth compared with the control. Also Muraji et al. [57] reported that only relatively low frequencies of magnetic fields stimulated growth of corn roots, whereas higher frequencies above 240 Hz inhibited growth. Seed germination is initiated by water imbibition that results in the enhancement of key enzymes involved in the catabolism of seed storage reserves. Seed germination starts with imbibition and ends with radicle protrusion [58]. The amount of water to be imbibed for seed germination depends on species. The results with wheat seed showed, that the water content increased with CAPP exposure time and uptake of water was more intensive after 2-h than 8-h imbibition. The water imbibed by the treated plant seeds activates hydrolytic amylase enzyme and facilitates metabolism of a stored starch and also a protein in beans seed [59]. Thus, water absorption (imbibition) is the most important event to ensure a nutrient supply to the germinating embryo and to generate energy for the commencement of active germination and seedling growth [60]. The mechanism of CAPP action on plant seeds is not well known. According to some authors the plasma induces not only structural changes on the seed surface [18, 24] but also changes in some biochemical parameters [19, 26, 61]. Based on our previous results [62] the effect of CAPP on maize seeds showed an increase in activity of some antioxidative enzymes SOD, CAT and G-POX in roots what is connected with the production of ROS by germinating seeds due to CAPP stress. Moreover, we determined an increase of dehydrogenase activity in embryos of the treated maize seeds in comparison with untreated seeds, what could depend on the intensive respiratory activity at an early stage of the germination process. Similarly, the results on CAPP treatment of pea showed the faster germination and hormonal activities related to plant signaling and development during early growth of pea seedlings [63].

Several studies with plasma biodecontamination mostly concentrated on the artificial non-living surfaces. On these surfaces D-value (decimal reduction dose-time of plasma treatment required to reduce an initial number of microorganisms by 1-log) to inactive/destroy a great variety of microorganisms ranged from 1 s to 30 min [41]. All plants are hosts to one or more microorganisms, which include the bacteria and fungi. Natural concentrations of microorganisms can vary between 10^2 and 10^6 CFU/g as described Kobayashi and Palumbo [64]. As microbial isolates usually found on wheat seeds are according to Wachowska et al., [65], *Pseudomonas*, *Azotobacter*, *Fusarium, Alternaria*, *Rhizopus*, *Penicillium*, *Aspergillus*, *Cladosporium*, *Fusarium* and 25 other filamentous fungi [66] from which we found in our experiments five bacterial isolates and seven fungal isolates on surface of wheat seeds. It appears that the effect of the plasma on the cells depends on the type of microorganism, and results showed that the bacteria are more resistant microorganisms than filamentous fungi. A significant reduction was achieved at an atmospheric-plasma plume in helium flow with the *Bacillus subtilis* spores [34]. There

are some Gram-positive bacteria form resistant bacterial endospores [67], as is genus *Bacillus*, which was major present in the epiphytic microflora was also found on the wheat seeds. We have found that even the highest CAPP exposure time of 600 s did not cause the total devitalization of bacteria. In comparison with bacteria the total devitalization of filamentous fungi was observed already after 120 s CAPP seeds treatment. Similarly a significant reduction for both species fungi, *Aspergillus* spp. and *Penicillium* sp. artificially contaminated on different seeds surfaces was achieved within 15 min of SF_6 plasma treatment time by Selcuk et al. [38]. In our results the exposure in CAPP even 180 s significantly reduced the growth of toxicogenic *Aspergillus flavus.* 100 % growth inhibition of *A. flavus* with lethal effect on conidia was observed after 240 s of CAPP treatment wheat seeds. Relatively short time required to achieved these results are due to the high power density plasma, what is one of the advantage of DCSBD in comparison with another plasma sources. Mechanism of plasma action on bacteria and fungi spores is studied by many authors. According to Jung et al. [35] the atmospheric pressure plasma and photocatalyst metal oxide titanium dioxide (TiO_2) as well as a low-temperature, high pressure, non-equilibrium plasmas according to Laroussi [2], are very effective at generating reactive oxygen radicals, which is known to be a dominant factor in bacterial and fungi spores inactivation. Gaseous discharges are known to produce antimicrobially active dissociation species of molecular oxygen such as ozone, atomic oxygen, hydroxyl, nitric oxide and super oxide radicals as well as other free radicals [5] which have the dominant role and cause damage by reacting with macromolecules, such as membrane lipids, proteins and nucleic acids. Free radicals can also cause surface erosion and localized lesions in the cell membrane [3, 67–70]. Nowadays, many organic fungicides have been widely used in plant protection as seed treatments on cereal seeds for elimination of seed-borne pathogens. Introduction of alternative environmentally and economically advantageous methods of adjustment of seed would allow to reduce the quantity of pesticides and contribute to the reduction of unwanted residues of xenobiotics in the plants and environment.

Conclusion

The results presented in this report have shown that the short treatment time of seeds by using a DCSBD plasma source is perspective way for the stimulation and protection of plants. The big advantage of the CAPP treatment of seeds is that the plasma source is capable working in wet and dusty environments and continuous mode. There was determinated a significantly effect of plasma treatment on the germination rate and growth parameters of wheat seedlings. CAPP treatment leads to a significant reduction of epiphytic bacteria, phytopathogenic and toxinogenic filamentous fungi. The availability and efficiency of the preparation make the method of pre-sowing treatment of seeds in CAPP attractive for agricultural praxis. In the future it will be necessary to compare the efficiency of the plasma treatment with other ways of finishing processes using pesticides or combined treatment of seed by plasma + fungicide in relation to the reduction of the number of epiphytic and phytopathogenic microorganisms occurring on the surface of the field crops.

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