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Germination Energy, Germination Capacity and Microflora of *Allium cepa* L. Seeds after RF Plasma Conditioning

Joanna Pawłat [1,*], Agnieszka Starek-Wójcicka [2,*], Marek Kopacki [3,*], Piotr Terebun [1], Michał Kwiatkowski [1], Agnieszka Sujak [4], Simone Pascuzzi [5], Francesco Santoro [5] and Dariusz Andrejko [2]

1 Institiute of Electrical Engineering and Electrotechnologies, Lublin University of Technology, Nadbystrzycka 38a, 20-618 Lublin, Poland
2 Department of Biological Bases of Food and Feed Technologies, University of Life Sciences in Lublin, Głęboka 28, 20-612 Lublin, Poland
3 Department of Plant Protection, University of Life Sciences in Lublin, Leszczynskiego 7, 20-069 Lublin, Poland
4 Department of Environmental Engineering and Mechanical Engineering, University of Life Sciences in Poznan, ul. Wojska Polskiego 28, 60-637 Poznań, Poland
5 Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Via Amendola 11 165/A, 70126 Bari, Italy
* Correspondence: j.pawlat@pollub.pl (J.P.); agnieszka.starek@up.lublin.pl (A.S.-W.); marek.kopacki@up.lublin.pl (M.K.)

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Abstract: This paper presents the results of an experiment on the effect of the cold plasma (He+O_2 or He+Air) pre-sowing stimulation of seeds of the Wolska cultivar of onion on the process of their germination. Four groups of seeds characterized by different exposure times (60, 120, 240 and 480 s) were used. Untreated seeds were used as a control. The distance between the electrode and the tested material was 50 mm. Pre-sowing plasma stimulation improved germination parameters such as germination capacity and germination energy for all the tested groups relative to the control. The highest fractions of germinated seeds were observed for an exposure time of 120 s. Analysis of the data showed a statistically significant impact of RF plasma on the seed germination parameters of the onion. SEM analysis showed that the interaction with plasma produced tension in the cells, leading to a change in their shape. No visible damage to the onion seed cells was observed, apart from the effect of depletion of the upper wax layer. The best influence on pathogenic fungi was when the group of seeds underwent 240 and 480 s of exposure to plasma fumigation, especially using the He+Air RF plasma jet.

Keywords: atmospheric-pressure plasma; RF plasma jet reactor; Wolska onion; seed germination; antifungal activity

1. Introduction

Onion (*Allium cepa* L.) is one of the most important vegetables in terms of production worldwide. Unfortunately, the growth of the species is closely related to the availability of light and sunshine. Hence, onion can be produced only in some regions of the world. Between the Tropics, only short-day onion characterized by a short shelf life can be cultivated. Additionally, the length of the period of vegetative plant mass development depends on the date of sowing and weather conditions. It is important that onion plants have well-formed leaves before the formation of bulbs. If spring is delayed, sowing early onion cultivars is not advisable, as the shortened period of vegetative growth may induce the early formation of bulbs without them having sufficient amounts of leaf mass. This, in turn, may result in low yields [1–12].

Therefore, to increase the sowing value of seeds produced using methods that do not threaten the environment (without excessive use of chemical compounds in crop production), scarification and physical factors (i.e., laser radiation, a fixed or variable electric and magnetic field, ultrasound, ionizing radiation or microwave radiation) are used. The impact of these factors is reflected by the increased dynamics of germination, a faster vegetation season, higher and qualitatively better yields and enhancement of the functional components of the germinated seeds. This was proven in studies conducted at Chungbuk National University, where a combination of hydrostatic-pressure treatment (HPT) and germination efficiently enhanced the functional characteristics of rough rice [13]. Positive results were also obtained in Cuba, where extremely low-frequency non-uniform magnetic fields significantly increased the number of germinated seeds, as well as the growth and yield of bulbous plants [14]. Similarly, in investigations conducted in Poland, electromagnetic stimulation methods were shown to be promising and non-invasive tools in the enhancement of the seed germination process, in increasing the number of germinated seeds, as well as in improving the composition and amino acid content in seeds of different plants [15–19]. The application of AC in the field also promoted the germination of bean seeds (*Phaseolus vulgaris*) [20].

It is predicted that, among the numerous methods for improvement of the quality of seeds in horticultural practice, the most common application can be ascribed to non-equilibrium plasma. It is generated by means of electric discharges and is increasingly being used in biotechnological techniques as a medium for biochemical processes run at atmospheric pressure, ambient temperatures and without the production of environmentally harmful waste. The innovation of the application and generation of cold plasma may be associated with the possibility of controlling its parameters within wide limits via chemical composition of the working gas, pressure, electromagnetic field structure, discharge geometry and power supply parameters [21–25].

The world's literature demonstrates a rapid increase in research on the impact of low-temperature plasma on seeds.

The experiments show that cold plasma accelerates seed germination and influences the physiological and biochemical responses that are important in terms of the subsequent development and growth of the plant. After the application of plasma to wheat seeds (*Triticum aestivum*), longer roots, as well as aerial parts of the sprouts, were observed and compared with untreated samples. Interestingly, the length of the obtained sprouts was much unified, i.e., its distribution was narrower in treated samples than in the control. The sprouts and roots of the plasma-treated seeds showed statistically significant higher mass compared to the control [26]. A positive effect of plasma was also found for radish (*Raphanus sativus* L.). Experiments with usage of various feeding gases in dielectric barrier discharge (DBD) plasma treatment show that Air, O_2 and NO (10%)+N_2 enhanced plant growth, whereas N_2, He and Ar exerted a relatively small effect. Moreover, humidity was the key factor in air plasma irradiation, producing more effects in growth enhancement than upon usage of dry air [27].

Seed pre-treatment with low-temperature atmospheric-pressure plasma generated using a GlidArc reactor proved to be suitable for improving the germination of Thuringian Mallow (*Lavatera thuringiaca* L.). The best seed germination parameters were observed for plasma exposure times of 2 and 5 min [28]. Furthermore, 1 min cold atmospheric plasma (CAP) treatment of chickpea seeds (*Cicer arietinum* L.) produced an increased seed germination rate and germination speed, as well as better seed vigor. Additionally, a decrease in the mean germination time compared to control was observed [29].

The health of onion can be affected by many pathogens. Seed-borne pathogenic fungi (e.g., *Alternaria alternata*, *Aspergillus niger*, *Botrytis* sp., *Fusarium oxysporum* and *Penicillium* sp.) can delay germination, obliterate seedlings or diminish plant growth by harming the roots and vascular system, thus intercepting the transport of water and nutrients [30–32]. Before sowing, seeds are often subjected to chemicals; however, the application of plant-originated mixtures or the usage of alternative physical factors to fungicides to control seed-borne pathogenic fungi seem to be healthy choices in terms of organic farming [33]. Therefore, alternative seed treatments are demanded. Cold plasma used in seed pre-treatment is resourceful agricultural technology believed to stimulate seedlings and plant development. It has been proven that cold plasma treatment can liquidate bacteria and fungi [34–36]; therefore, it can be applied in seed sterilization. Many authors have used plasmas for disinfection in medicine and plant protection [37–40], which effectively limit pathogens colonizing seeds [39]. There are known experiments in which low-temperature plasma limited fungi such as *Alternaria alternata*, *Fusarium* sp., *Trichothecium roseum* and *Aspergillus flavus* [41]. Similar results were also obtained by Dasan et al. [42] in the reduction in *Aspergillus* fungi with the help of plasma on the surface of nuts. The destructive effect on fungi of the genus *Aspergillus* was caused by changes in the structure of the hypha and changes in DNA [43]. The high efficiency of the electromagnetic field and low-temperature plasma in reducing fungi of the genus *Fusarium*, *Stemphylliun* or *Alternaria alternata*, which colonize the seeds of leguminous plants, was also confirmed [44]. The strong influence of plasma on bacteria and on fungi of the genuses *Penicillium* and *Aspergillus* is also confirmed by Waskow et al. [45]. It is possible that in the future, the use of plasma will be able to replace the chemical treatment of seeds and may be useful in Integrated Pest Management [46,47], especially since some researchers show that plasma can stimulate the growth of beneficial fungi [48].

The aim of the presented study was to evaluate the effect of pre-sowing exposure in onion seeds (cv. Wolska) to non-thermal plasma generated in an RF reactor with a different gas mixture on the course of their germination. SEM analyses were conducted in order to explain the surface interaction between plasma and seeds. Another aim of the investigation was to determine the influence of cold plasma fumigation on the healthiness of seeds and to estimate the fungal communities colonizing onion seeds.

2. Materials and Methods

2.1. Cold Plasma Generator

A cylinder-shaped radio frequency (RF) plasma jet reactor with an internal rod-type high-voltage electrode with a 5 mm diameter was used. The outer diameter of the nozzle was 14 mm and the discharge gap was 1.5 mm [49,50]. An AG 1021 radio frequency generator (T&C Power Conversion, Rochester, NY, USA) was the power source used with a resonance-matching circuit. The load power was set at 45 W and its frequency at 14.32 MHz. The seeds were placed at a distance of 50 mm from the nozzle in an open batch container with a 60 mm diameter (Figure 1). Due to the relatively large distance from the discharge, the seed treatment was mainly carried out via the after-glow effect, where the active particles were carried along with the gas stream that spread over the seed surface, while minimizing the risk of uneven treatment related to their different shapes. The working gas was a mixture of helium and oxygen or air, regulated by separate rotameters at the same pressure (ratio: 3:2, total flow rate: 11.83 slm). The maximum gas temperature, measured using a DT-847U meter (Yu Ching Technology, Taipei, Taiwan) and a type-K thermocouple, was 52 °C and 50 °C for the helium-with-air and the helium-with-oxygen gas mixtures, respectively.

2.2. Germination Energy and Capacity

The experimental material consisted of Wolska seeds (*Allium cepa* L.) (seed purity: Standard ST). Seed germination was conducted in accordance with the ISTA (International Seed Testing Association) recommendations of 2017 [51]. The study used the between-paper method (BP), most appropriate for species with medium and large seeds between 2 mm and 1 cm in diameter, including many cereals, grain legumes and vegetables. The seeds were placed between two layers of filter paper and rolled in towels. The rolled towels were placed in the germinator in an upright position. The seeds were germinated after placing on blotting paper in 5 rows of 20 seeds in four repetitions. A total of 400 seeds were used in each experimental group. The seeds were covered with paper, and then, rolled into a roll. The experiment on seed germination was carried out in a CTC 256 climatic chamber (Memmert GmbH + Co.KG, Schwabach, Germany), at a temperature of $T = 20 \pm 1$ °C. Four groups of seeds characterized by different exposure times to plasma were used (60, 120, 240 and 480 s). In all the experiments, untreated seeds were used as a control.

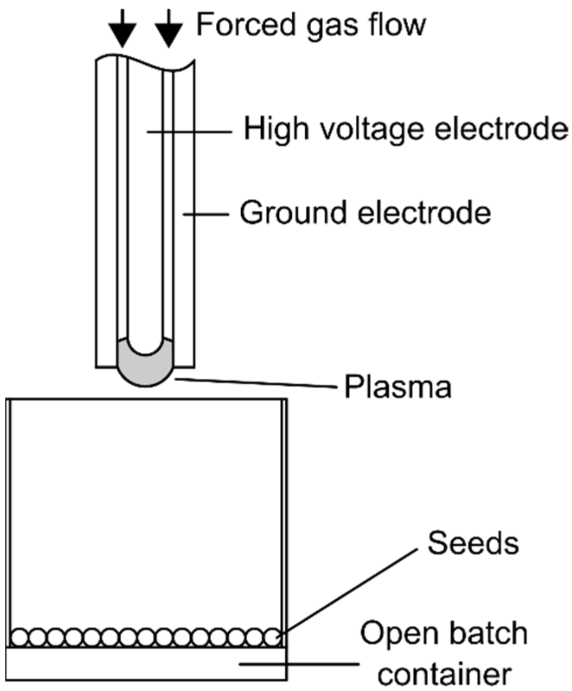


Figure 1. Experimental setup of plasma treatment.

The number of sprouts was determined every 24 h. The fraction of germinated seeds (number of sprouts) after 6 days of germination was defined as germination energy G_{EN}, while the fraction of germinated seeds after 12 days of germination was defined as germination capacity G_C. Both germination energy and germination capacity were expressed as a fraction of the germinated seeds G after a defined time t and calculated from the following equation:

$$G = \frac{n}{n_T} \cdot 100\% \quad (1)$$

where: n—the number of seeds germinated at time t and n_T—the total number of sown seeds.

The results of measurement were analyzed statistically using StatSoft—Statistica 10.0 [52]. Statistical differences between groups were analyzed using one-way and two-way analysis of variance (ANOVA). The significance of the differences between mean values was determined using Tukey's test at a significance level $\alpha \leq 0.05$.

2.3. SEM Analysis

The seed surface was monitored using a TESCAN VEGA 3 LMU microscope (SEM) in the variable vacuum mode on a Peltier table. The pressure in the measuring chamber was 15 Pa and the temperature of the support amounted to minus 40 °C. Dry seeds (with moisture not exceeding 5%) were neither fixed nor sputtered.

2.4. Microbiological Analysis

The onion seeds were overspread at the bottom of Petri dish (100 mm in diameter), and exposed to plasma treatment with RF discharge. The control group without plasma treatment was used. For mycological analysis, 100 seeds from each group were collected (10 plates with 10 seeds on each plate) and transferred separately on the mineral medium after previous plasma exposure. The control seeds were placed on the mineral medium without any treatment. After 7 days, the obtained fungal colonies were transferred to potato dextrose medium (PDA, Difco) and identified as described previously in Kopacki and Wagner [53].

3. Results and Discussion

3.1. Germination Energy and Capacity

Non-thermal plasma generated at atmospheric pressure in the RF reactor affected the seed samples indirectly.

Figures 2 and 3 present the dynamics of the process of germination in onion seeds treated with plasma generated in RF using He+O_2 or He+Air as a running gas, respectively.

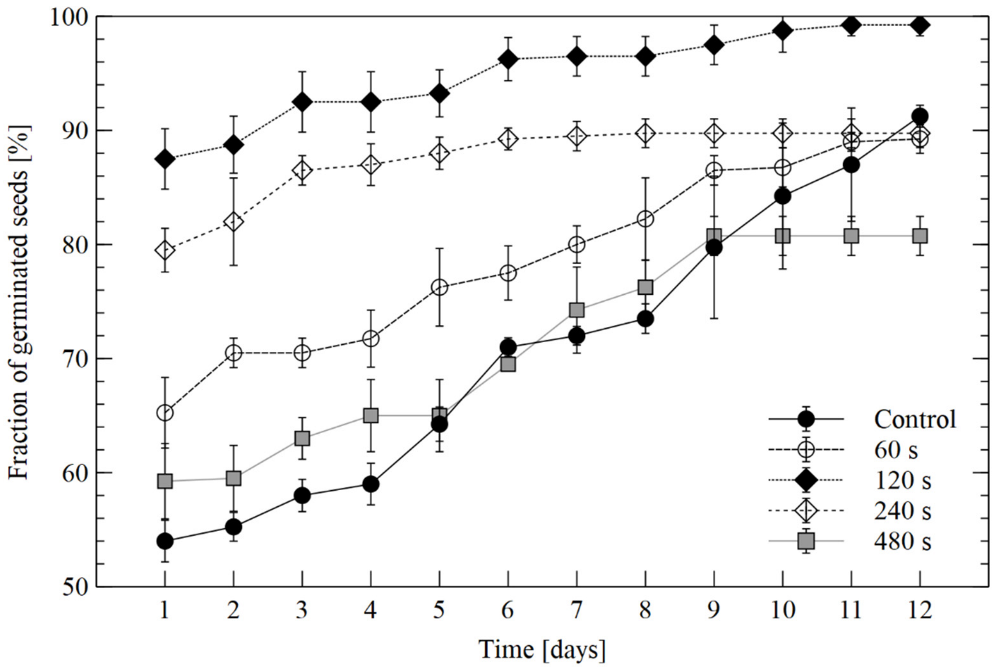


Figure 2. Fraction of germinated seeds of onion after pre-sowing treatment with He+O_2 RF plasma jet.

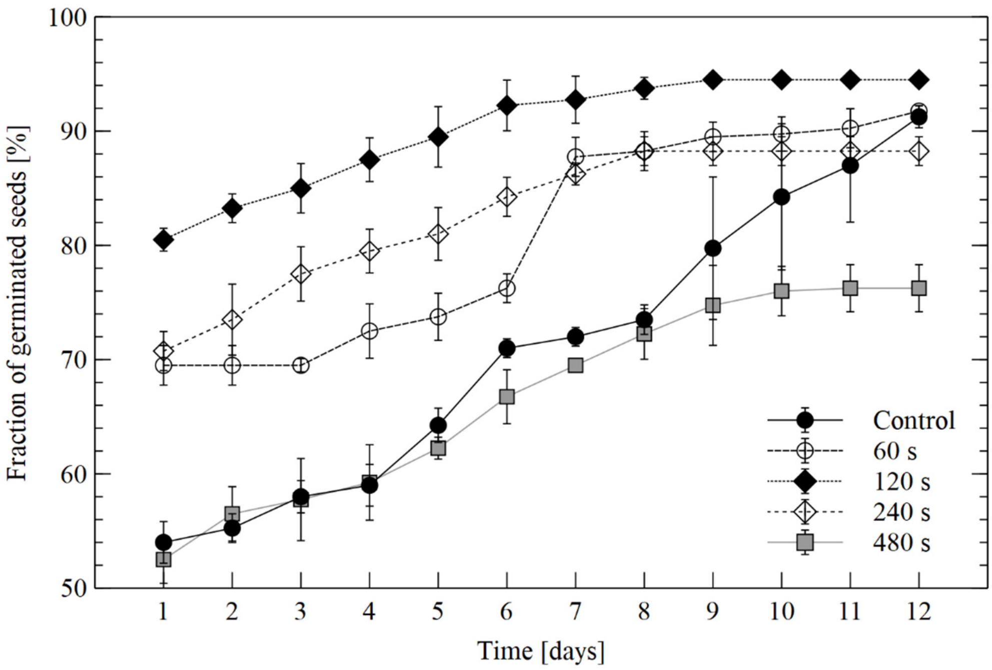


Figure 3. Fraction of germinated seeds of onion after pre-sowing treatment with He+Air RF plasma jet.

Onion seeds start to germinate 24 h after sowing, which can be observed in Figures 2 and 3. Interestingly, pre-sowing seed stimulation with plasma resulted in a significant increase in the number of germinated seeds. The most effective time of exposure to the He+O_2 RF plasma jet (Figure 2) was 120 s, where a 1.6-fold increase in the number of germinated seeds was observed, followed by 240 s (1.5-fold), 60 s (1.2-fold) and 480 s (1.1-fold) during the first day of the experiment. In the case of stimulation with the He+Air RF plasma jet (Figure 3) the observed increases were a bit lower and amounted to 1.5, 1.33, 1.3 and 1.03, respectively, for the exposure times of 120, 240, 60 and 480 s. In the first 5 days, the number of germinated seeds increased with the increase in exposure time up to 120 s, and then, for 480 s, started to decrease.

Generally, the control sample showed a much bigger daily increase in the number of germinated seeds compared to plasma-stimulated seeds. The plasma effect was most pronounced during the first 5 days of the experiment, indicating a strong effect of plasma on the early development stages of onion seeds. For both gases used in plasma stimulation, the exposure time of 240 s was the most effective.

Tables 1 and 2 show the parameters describing the process of germination in the examined seeds.

Table 1. The results of germination energy parameters of Wolska onion seeds after pre-sowing treatment with cold plasma.

Time of Stimulation (s)	**Germination Energy G_{EN} (%)**	
	Control-71.0 ± 0.82 [a]	
	He+O_2	**He+Air**
60	77.50 ± 2.38 [c]	76.25 ± 1.26 [c]
120	96.25 ± 1.89 [f]	92.25 ± 2.22 [d]
240	89.25 ± 0.96 [d]	84.25 ± 1.71 [e]
480	69.5 ± 0.58 [ab]	66.75 ± 2.36 [b]

± standard deviation means with the same letter (a–f) are not significantly different at 0.05 significance level.

Table 2. The results on germination capacity parameters of Wolska onion seeds after pre-sowing treatment with cold plasma at different exposure times.

Time of Stimulation (s)	**Germination Capacity G_C (%)**	
	Control-91.25 ± 0.96 [a]	
	He+O_2	**He+Air**
60	89.25 ± 1.26 [ab]	91.75 ± 0.5 [ac]
120	99.25 ± 0.96 [f]	94.50 ± 0.58 [c]
240	89.75 ± 1.26 [ab]	88.25 ± 1.26 [b]
480	80.75 ± 1.71 [e]	76.25 ± 2.06 [d]

± standard deviation means with the same letter (a–f) are not significantly different at 0.05 significance level.

The treatment of onion seeds with cold plasma exerted an effect on the energy value and germination rate (Tables 1 and 2). The time of treatment and type of gas mixture used to generate the plasma were factors differentiating the analyzed parameters. The analysis of the germination energy value after stimulation with plasma, generated with the use of He+O_2, revealed that a positive effect was achieved after stimulation for 60, 120 and 240 s. The result was similar when plasma generated with the He+Air mixture was used. In turn, the germination capacity increased only after the 120 s exposure of the seeds to the action of plasma. The prolonged 480 s stimulation resulted in a decline in the value of the analyzed parameters in relation to the control. In this case, a reduction in the energy and germination capacity of the onion seeds was noted after treatment with both types of plasma, but the germination rate was statistically higher in the sample exposed to plasma generated with the use of the He+O_2 mixture than He+Air.

The highest energy and germination capacity were achieved in onion seeds subjected to the 120 s plasma stimulation, and higher values of these parameters were recorded in the treatment with plasma generated using the He+O_2 mixture. In comparison with the control, the germination energy and germination capacity values in this case increased by 25% and 8%, respectively.

3.2. SEM Analysis

Figure 4 shows exemplary SEM images of the surface of the onion seeds. The seeds possess an irregular, pyramidoid shape where one can distinguish the distinct foundation and slopes. The maximal linear size of the seeds varies between 2 and 3 mm, and some of them are more flat or more protuberated. SEM scans were made to visualize both the slopes (top view, Figure 4A) and the foundation (bottom view, Figure 4B). It is difficult to define the seeds' cell epidermal structure pattern [54]. Cells have an elliptical- or circular-angular shape and are rather flat (Figure 4C,D). Concerning the differences between the top and bottom cells, the latter differ with the degree of the coverage of the cuticle with wax (Figure 4C,E vs. Figure 4D,F).

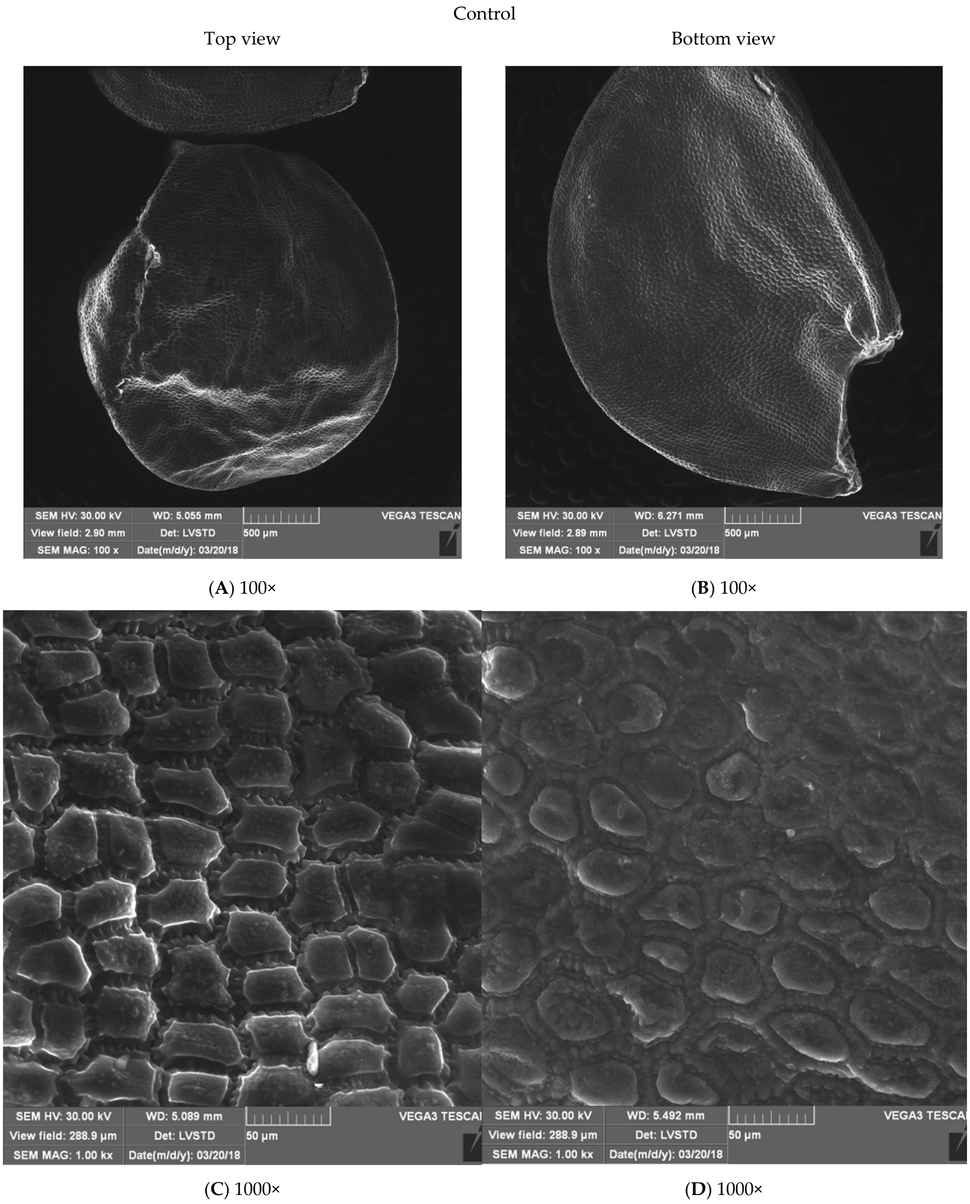
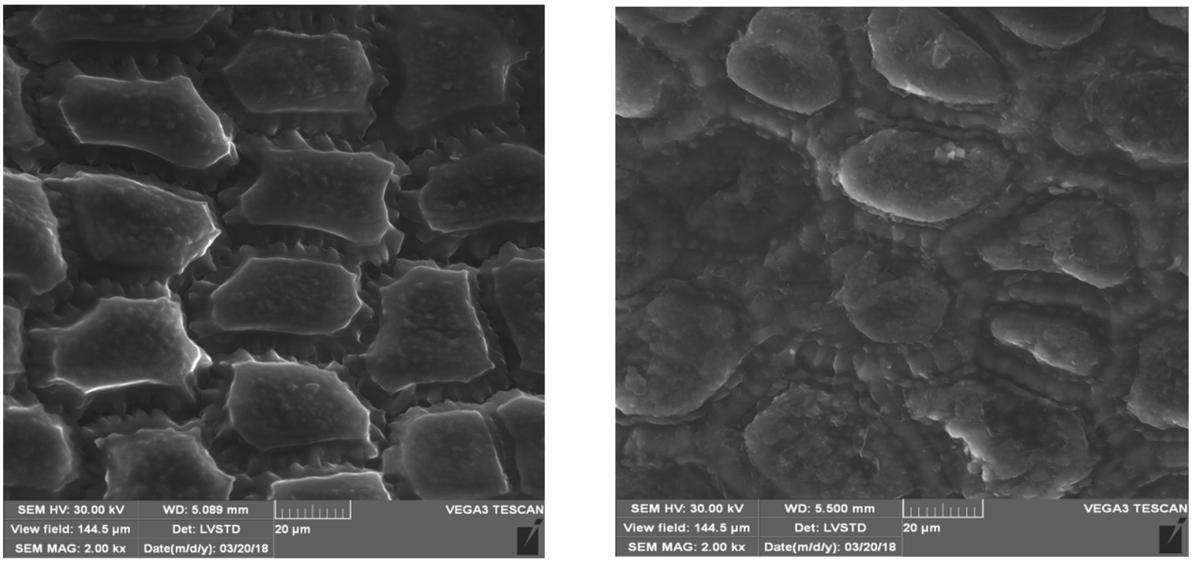


Figure 4. *Cont.*



(**E**) 2000× (**F**) 2000×

Figure 4. SEM images of onion seed surface at different magnifications; top and bottom view as indicated.

SEM scans of the surface of plasma-treated seeds show subtle anatomical changes are compared to untreated seeds. One can notice a discernible increase in the degree of surface corrugation, visible especially in the bottom view in the case of treatment of the seeds with plasma containing He+Air (Figure 5) or He+O_2 (Figure 6) (in both cases, 50 mm, 120 s). This effect could be explained by the change in the water content on the surface of the seed cover cells, on one hand, which is of little probability as the seeds are dry. On the other hand, the onion seed cells become more convex and more angular than oval, slightly resembling the surface of a raspberry fruit, which probably influences corrugation. One can suppose that interaction with plasma produces a kind of tension in the cells, leading to the change in the shape of the cells. Interestingly, the effect on the bottom part of the seeds seems to be much stronger. The effect of a change in the shape of the onion seed cells, such as cell proliferation and elongation, upon priming with 0.5% $ZnSO_4$ for 10 h has been previously reported by Saranya et al. [55]. The increase in the micro-roughness and sharpening of the pattern structure of the upper, epidermal layer of the seed coat was previously observed for *Lavatera thuringiaca* seeds treated with non-thermal plasma generated in a GlidArc reactor (N_2, atmospheric pressure) as well as with DBD non-thermal plasma (He+N_2, atmospheric pressure) [28,56]. A longer exposure time of *Lavatera* seeds to plasma affected the more inner parts of the cuticle and even damaged or fractured some parts of the cuticle. No visible damage to the seed cells was observed in the case of onion seeds, although a similar effect of depletion of the upper natural wax layer was observed. A general scheme reflecting the change in the onion cell's shape after the plasma treatment is presented in Figure 7.

3.3. Microbiological Analysis

Tables 3 and 4 show the analysis of the number of fungus colonies per 100 onion seeds subjected to plasma treatment versus the untreated control seeds. The species are divided into saprotrophic fungi: key regulators of nutrient cycling in terrestrial ecosystems and pathogenic fungi, the latter of which are potential contaminants of food as they may produce toxins and can trigger allergic reactions. Generally, the application of plasma results in a decrease in the number of fungus colonies, both saprotrophic and pathogenic. The only increase is observed in the number of *Chaetomium globusom* Kunze ex Fr colonies in the case of the application of He+O_2 plasma for 120s or He+Air plasma for 120 and 240 s.

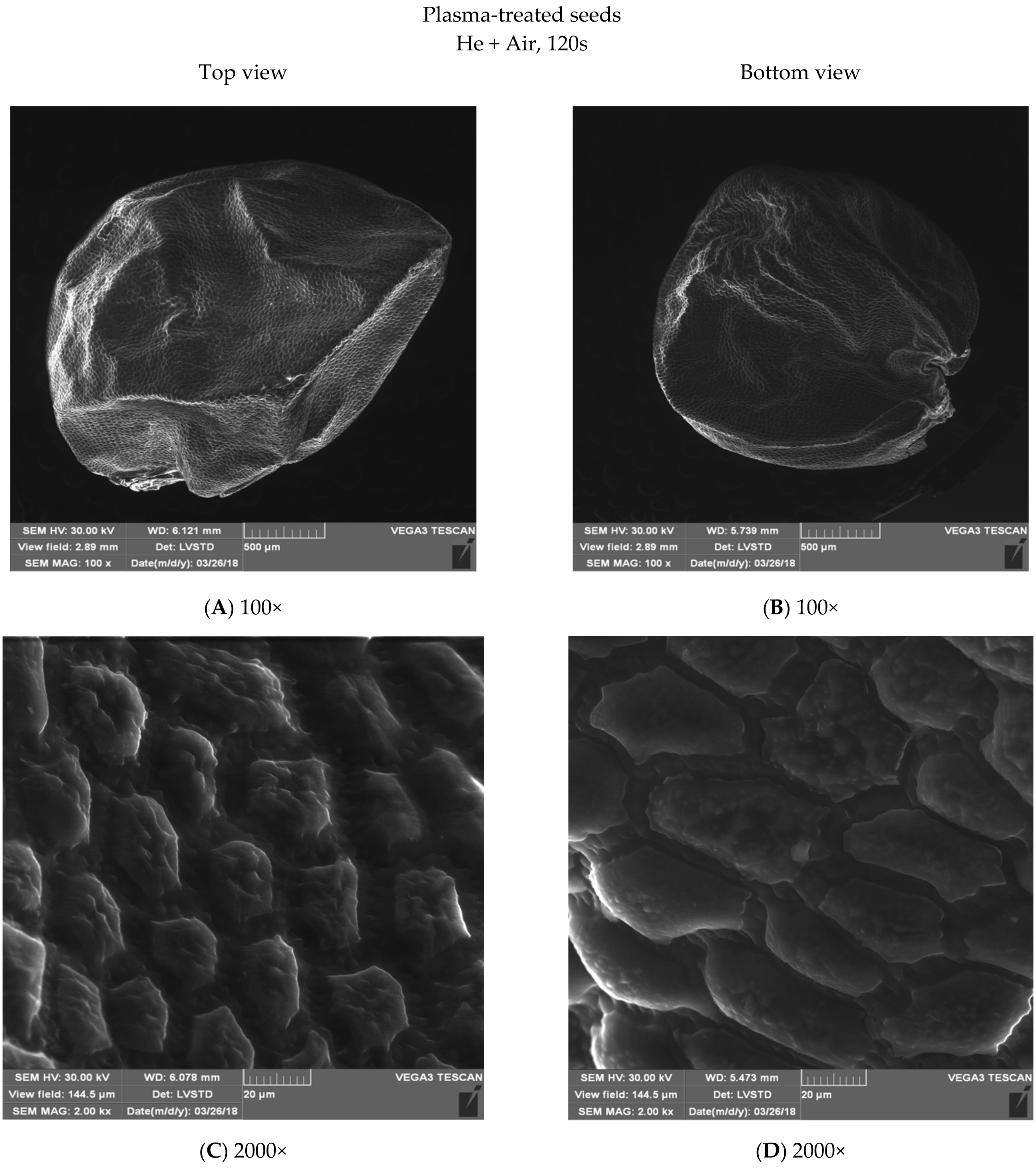


Figure 5. SEM images of He+Air cold plasma-treated onion cells, exposure time: 120 s, distance between seeds and electrodes: 50 mm. Magnification as indicated.

The results of the investigation showed that the exposure time of plasma fumigation had an effect on the decrease in populations of onion seed pathogens. Differences between the number of fungi on the monitored Petri dishes were observed. Among the pathogenic fungi, the most abundant species was *Fusarium oxysporum* (16 on 100 seeds from the control group and 6 for 60 s, He+O_2) followed by *Alternaria alternata* (19 on 100 seeds from control group and 11 for 120 s, He+ Air) and *Botrytis cinerea* (8 on 100 seeds for control group and 9 on 100 seeds for 480 s, He+O_2). Plasma application results in the complete elimination of pathogenic fungi such as *Fusarium equiseti* Link. and *Phoma glomerata.*

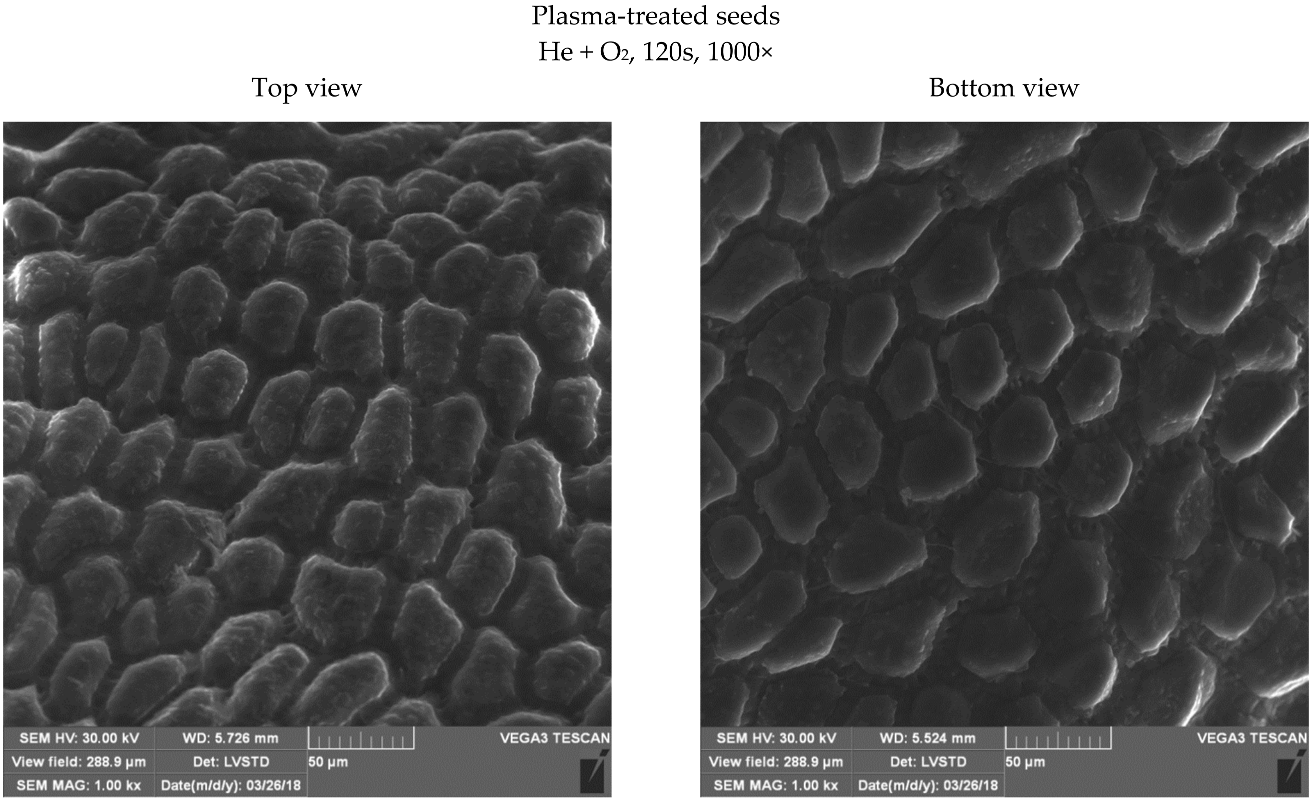


Figure 6. SEM images of He+O_2 cold plasma-treated onion cells, exposure time: 120 s, distance between seeds and electrodes: 50 mm. Magnification as indicated.

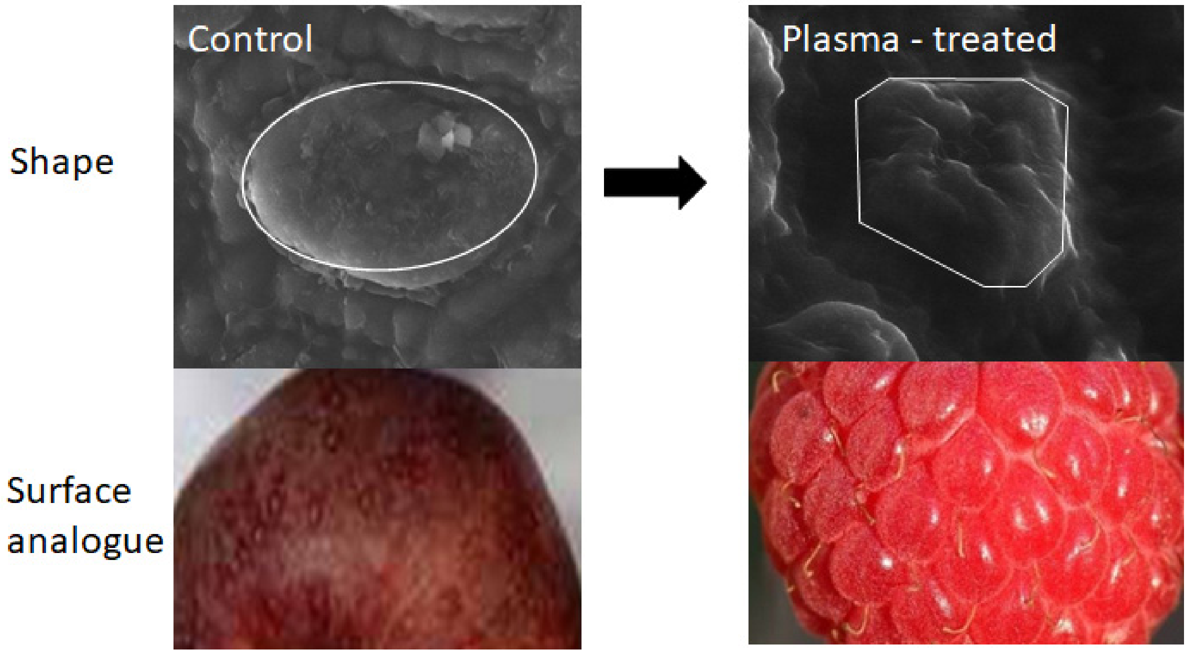


Figure 7. Scheme reflecting general effect of plasma treatment on the cell shape and surface of onion seeds.

Table 3. Number of saprotrophic and pathogenic fungi colonizing onion seeds after pre-sowing treatment with He+O_2 plasma jet; results indicate number of fungus colonies per 100 onion seeds.

Fungus Species	**Exposure Time (s)**				
	Control	**60 s**	**120 s**	**240 s**	**480 s**
	Saprotrophic fungi				
Aspergillus niger van Tieghem	10 ± 0.75	6 ± 0.70	5 ± 0.97	2 ± 0.42	3 ± 0.48
Chaetomium globusom Kunze ex Fr.	6 ± 0.53	n.d.	11 ± 0.88	n.d.	n.d.
Epicoccum nigrum Link	2 ± 0.53	n.d.	n.d.	n.d.	n.d.
Gliocladium catenulatum Gill. et Abb.	4 ± 0.58	n.d.	n.d.	n.d.	n.d.
Penicillium spp.	16 ± 0.74	11 ± 0.99	4 ± 0.51	14 ± 0.97	10 ± 1.05
Rhizopus stolonifera Ehrenb.	6 ± 1.62	n.d.	n.d.	n.d.	n.d
Stemphylium botryotinum	1 ± 0.63	4 ± 0.52	n.d.	n.d.	n.d.
Trichoderma harzianum Rifai	9 ± 1.06	2 ± 0.42	13 ± 0.82	4 ± 0.7	8 ± 0.63
Trichoderma koningii Oud.	5 ± 1.76	n.d.	n.d.	n.d.	n.d.
	Pathogenic fungi				
Alternaria alternata (Fr.) Keiss	19 ± 1.26	6 ± 0.84	5 ± 0.84	2 ± 0.63	3 ± 0.67
Botrytis cinerea Pers. ex Fries.	8 ± 1.19	5 ± 0.52	n.d.	n.d.	9 ± 0.99
Botrytis alli Munn.	8 ± 0.48	n.d.	n.d.	n.d.	n.d.
Fusarium avenaceum (Fr.) Sacc.	5 ± 1.05	n.d.	n.d.	n.d.	n.d.
Fusarium oxysporum Schlecht.	16 ± 1.78	6 ± 0.96	3 ± 0.67	4 ± 0.84	4 ± 0.69
Fusarium equiseti Link.	4 ± 0.84	n.d.	n.d.	n.d.	n.d.
Phoma glomerata	1 ± 0.31	n.d.	n.d.	n.d.	n.d.

n.d.—not detected.

Concerning saprotrophic fungi, the highest number of colonies was observed for *Penicillium* sp. (16 on 100 seeds from the control group and 14 on 100 seeds for 240 s, He+O_2) followed by *Chaetomium globosum* (15 on 100 seeds for 120 s, He+Air) and by *Trichoderma koningii* (15 on 100 for 120 s, He+Air).

It is worth noting that fungi that are considered antagonists and that could be potentially applied in biological protection were more abundant in groups after plasma treatment (He+Air) for 120 s.

Botrytis cinerea, *Fusarium oxysporum* and *Alternaria alternate* predominated among fungi which are regarded as pathogenic, especially in control group. The most dangerous for onion, *Botrytis alli*, was isolated infrequently from seeds of the control group (Tables 3 and 4).

In our investigations, the use of cold plasma reduced the severity of the fungal disease in the group with 480 s treatment, He+Air.

Table 4. Number of saprotrophic and pathogenic fungi colonizing onion seeds after pre-sowing treatment with He + Air plasma jet; results indicate number of fungus colonies per 100 onion seeds.

Fungus Species	Exposure Time (s)				
	Control	60 s	120 s	240 s	480 s
	Saprotrophic fungi				
Aspergillus niger van Tieghem	10 ± 0.75	2 ± 0.63	n.d.	n.d.	2 ± 0.42
Chaetomium globusom Kunze ex Fr.	6 ± 0.53	5 ± 0.53	15 ± 0.85	11 ± 0.99	n.d.
Epicoccum nigrum Link	2 ± 0.53	n.d.	10 ± 1.25	n.d.	n.d.
Gliocladium catenulatum Gill. et Abb.	4 ± 0.58	4 ± 0.84	n.d.	n.d.	n.d.
Penicillium spp.	16 ± 0.74	14 ± 1.17	10 ± 1.15	2 ± 0.63	2 ± 0.42
Rhizopus stolonifer Ehrenb.	6 ± 1.62	n.d.	3 ± 0.48	n.d.	1 ± 0.31
Stemphylium botryotinum	1 ± 0.63	n.d.	n.d.	n.d.	n.d.
Trichoderma harzianum Rifai	9 ± 1.06	5 ± 0.71	12 ± 1.75	12 ± 0.92	2 ± 0.63
Trichoderma koningii Oud.	5 ± 1.76	3 ± 0.48	15 ± 1.18	11 ± 1.1	2 ± 0.42
	Pathogenic fungi				
Alternaria alternata (Fr.) Keiss	19 ± 1.26	9 ± 0.57	11 ± 0.57	3 ± 0.48	2 ± 0.42
Botrytis cinerea Pers. ex Fries.	8 ± 1.19	3 ± 0.48	n.d.	n.d.	n.d.
Botrytis alli Munn.	8 ± 0.48	n.d.	3 ± 0.48	2 ± 0.42	3 ± 0.67
Fusarium avenaceum (Fr.) Sacc.	5 ± 1.05	10 ± 1.5	2 ± 0.63	2 ± 0.63	2 ± 0.63
Fusarium oxysporum Schlecht.	16 ± 1.78	2 ± 0.63	5 ± 0.53	2 ± 0.42	2 ± 0.42
Fusarium equiseti Link.	4 ± 0.84	n.d.	n.d.	n.d.	n.d.
Phoma glomerata	1 ± 0.31	n.d.	n.d.	n.d.	n.d.

n.d.—not detected.

It has been proven that reactive forms of oxygen, such as ozone, raise the level of abscisic acid (ABA), the main stimulant that triggers the stomata to close, which may be associated with plant defense responses [57]. The increase in the metabolism of reactive oxygen species activates the innate ability of plants to cope with negative external factors [58]. In addition, oxygen and nitrogen that come into contact during plasma application decompose into active forms and, as RONS, may damage the microbial cell membrane [59,60], inactivating or only preventing reproduction [61]. The results stated in Tables 3 and 4 indicate that formed ROS are more effective than RNS in reducing the population of onion seed pathogens, resulting in better germination of onion seeds.

In spite of the fact that longer plasma exposure time results in better antifungal activity, it also causes damages to seeds. Thus, shorter plasma exposure gives better effects in terms of germination, even if fungal decontamination is incomplete.

4. Conclusions

Short plasma treatment positively affected the energy value and germination rate of onion seeds. The best results in terms of energy and germination capacity were obtained for the 120 s plasma treatment and the He+O_2 gas mixture, causing a 25% and 8% increase in the germination energy and germination capacity, respectively. For the longest tested treatment (480 s) deterioration of the germination parameters was observed.

Slight changes in the microstructure of the seeds' surface after plasma treatment were visible during SEM analysis: the cuticle layer with wax was subtly thinned and the level of corrugation increased.

The predominating fungus species were *Aspergillus niger*, *Alternaria alternata*, *Fusarium oxysporum.* and *Penicillium* spp. Among the fungi, the most dangerous for onion was *Botrytis* sp. More fungus colonies were observed in the control group than in the plasma-treated group. Plasma application resulted in the complete elimination of pathogenic fungi such as *Fusarium equiseti* L. and *Phoma glomerata*. Generally, atmospheric-pressure plasma resulted in a decrease in the number of pathogenic and saprotrophic fungus colonies, except *Chaetomium globuso* Kunze ex Fr. Such a pre-sowing treatment could play a protective role against the microbial infestation of seeds, and then, seedlings.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en15207687/s1.

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