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EFFECT OF GLOW DISCHARGE PLASMA ON GERMINATION AND FUNGAL LOAD OF SOME CEREAL SEEDS*

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Abstract. Glow discharge plasma was applied on barley and corn seeds in order to decrease the number of seed-borne fungi. It was found that the fungal loads of the seeds of both plant species decreased with the increasing exposure times of plasma. The initial germination and seed growth characteristics evolution together with the fungal load inactivation indicated that glow discharge could be applied as a pre-treatment of seeds in order to improve cereal crops.

Key words: barley, corn, Blotter method, pot trial, tillers, spikes, shoot length, root length.

1. INTRODUCTION

Fungi on cereal seeds may affect the crops quality and are responsible for most spoilage and germ damage during storage. Thus, they can infect food grains by producing toxins which cause diseases on human or animals using seeds as food [1]. *Aspergillus, Penicillium* and *Fusarium* species could be mentioned as major toxin producing fungi on cereal seeds [2, 3], barley and corn grains being good substrates for growth of such fungi. In order to prevent or decrease the damage caused by fungi, different physical or chemical methods can be used. Since some of them can lead to changes in the physicochemical properties of the seeds, decontamination of seeds without decreasing the seed or food quality is very important. Considerable interest is given recently to alternative control methods such as irradiation, microwave or plasma treatment. Studies on plasma used for decontamination/sterilization are currently conducted worldwide. The influence of

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a series of plasma parameters, such as gas, temperature, pressure and voltages of electric discharges on inactivation of microorganisms is intensively investigated [4–7]. It was found that plasma application is an effective sterilization technique and has recently been applied for the decontamination of seeds as an alternative to chemicals causing harm on human health and environment. It was also reported to have stimulating effect on the germination of seeds [8], with applications in agriculture, horticulture and forestry.

Despite of the large number of studies about plasma application technique, there is no investigation reported on germination and emergence of cereal seeds treated with glow discharge plasma and studied both by petri and pot trials. Therefore, the current investigation focused on the influence of glow discharge plasma on barley and corn seeds concerning the fungal load, initial germination and seed growth characteristics in order to control some seed-borne pathogens causing diseases and decreasing crop quality.

2. METHODOLOGY

Barley seeds (cultivar Tokak 157/37) obtained from the Agricultural Research and Application Center of Süleyman Demirel University (Isparta, Turkey) and corn seeds (*Zea mays everta* L.) obtained from the Black Sea region of Turkey were used in these experiments.

The plasma set-up used to treat seed samples is shown in Fig. 1. A sieve tray was mounted 32 cm above de anode at the ground potential. Each sample consisting on 500 seeds was then placed in a single layer in the tray. In order to maximize the seed area exposed to the plasma, the sieve tray had large openings. The vacuum chamber was pumped-down to 15 Pa and then a constant voltage was applied on the anode. The plasma was thus ignited in this residual gas containing the ambient gas at low pressure. The low pressure ambient air plasma filled the chamber uniformly. The samples were subjected to glow discharges at 100 W for barley seeds and 200 W for corn seeds for 2, 5, 10, 15 and 20 minutes. After the treatment, the seed tray was unmounted, the seeds were taken out, the tray was mounted back and a new sample was loaded. Untreated seeds were used as controls.

Standard blotter method was used to search the germination rates and fungal loads of the seeds [9]. For each test, 400 seed samples were used according to International Seed Testing Association (ISTA) procedures [10]. Thus, seeds were transferred to Petri plates (10 seeds per plate) with sterile water soaked blotters and incubated at 22 ± 2°C in 12 h light/12 h dark cycle for 7 days, in a climatized room. Seeds were then examined under stereomicroscope (12 – 50X magnification) for fungal growth. Germination rates were determined at the end of 8 days.

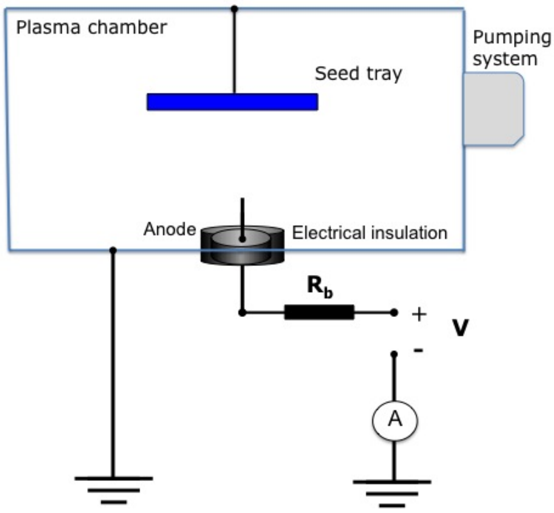


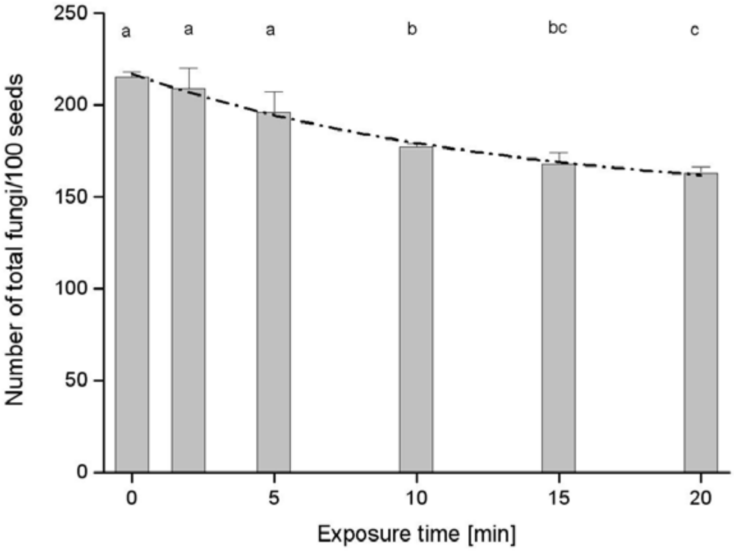
Fig. 1 – Schematic set up of plasma system.

At the same time, the samples were planted in plastic pots with sterile soil mixture in order to evaluate the seed growth characteristics. Thus, in each pot 10 barley seeds and respectively 5 corn seeds were sown using four replicate pots. Pots were kept under field conditions, irrigated with tap water as needed and sufficient amounts of fertilizers were applied. Numbers of tillers and spikes per pot and 1000 grain weight (g) were determined for barley seeds, whereas shoot length, root length, fresh and dry weights were determined for corn seeds.

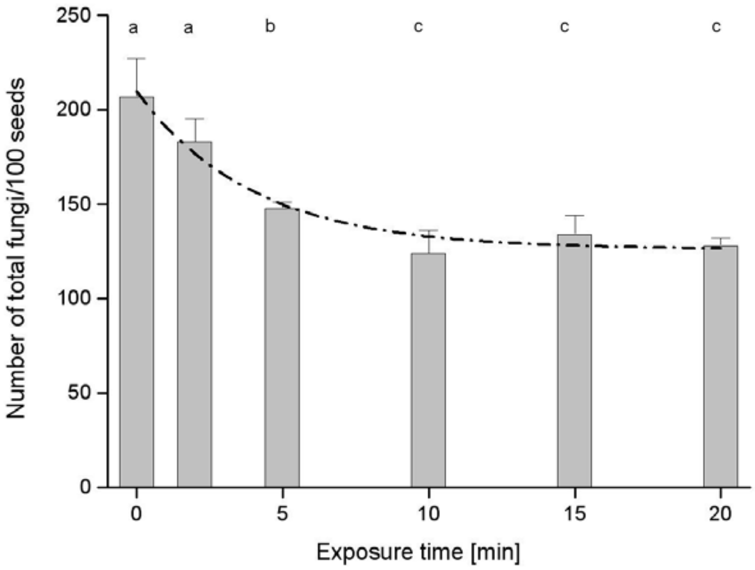
The results reported are expressed by means of values ± standard error of four replicates. Statistical evaluation was performed by Tukey test using IBM SPSS Statistics 20 program. The level of significance was considered at $P < 0.05$.

3. RESULTS AND DISCUSSION

The control and treated samples of the two seed types were investigated by Blotter test method to search the fungal loads and germination rates of the seeds. The fungal loads of the seeds decreased for both barley and corn seeds after glow discharge treatment, depending on exposure time (Fig. 2). Thus, for barley seeds the number of fungi was insignificantly affected up to 5 min ($P > 0.05$), but the treatment of 10 min led to a decrease of ~15% ($P < 0.05$) from the initial number of fungi while after 20 min the decrease was up to 25% ($P < 0.05$). Conversely, for corn seeds the fungal load showed drastic reductions of ~30% after treatment of 5 min and ~ 40% after 10 min ($P < 0.05$). Similarly, Filatova *et al*. [8] showed that plasma processing had a fungicidal effect on grain and legume seeds, the initial fungal infection being reduced by 3–5%.



(a)



(b)

Fig. 2 – Total number of fungi on Petri dishes with (a) barley seeds and (b) corn seeds exposed to glow discharge plasma. The different letters within each graph indicate significantly different results ($P < 0.05$).

However, both barley and corn seeds showed similar pattern of microbial inactivation curve with the exposure time to glow discharge plasma in accordance with a biphasic model (eq. 1). The biphasic model is based on the hypothesis that two subgroups having different resistance to stress coexist in a microbial population describing a biphasic log-linear decrease in the population [11].

$$N = N_0\left[f + \left(1 - f\right)\cdot \mathrm{e}^{-\frac{t}{D}}\right], \tag{1}$$

where N is the number of survivors at a given time, N_0 is the initial number of fungi, f is the fraction of the initial population more resistant to the treatment, t [min] is the treatment time, and D [min] is the time constant indicating the treatment time when the fraction of the initial population more sensitive $(1 - f)$ to the treatment decreased by e times.

Table 1

Parameters of inactivation microbial curves for seeds exposed to glow discharge plasma

Seed type	f	D [min]
Barley	0.68 ± 0.04	13 ± 3
Corn	0.61 ± 0.03	4 ± 1

This kind of biphasic model has the advantage that allows estimating the minimal counts achievable by a decontamination technique and indicates that no additional inactivation would be achieved by prolonging of treatment beyond the time when the sensitive population is inactivated, but the samples may be deteriorated [12]. Based on experimental findings, it can be said that the barley seeds had a microbial population of ~30% sensitive to treatment compared with corn seeds that had a sensitive microbial population of ~ 40% (Table 1). This aspect could be explained by the difference in plasma power and seed types, namely the substrate on which fungi have grown. The higher plasma power for corn seeds led to greater inactivation of fungal load at shorter times than lower power for barley seeds. Similar findings have been reported by Sen and Mutlu [13] on inactivation of *Escherichia coli* by non-thermal plasma treatment. Moreover, the fungal decontamination of seeds can be strongly related to the type, size, shape, surface, wrinkle or crevices of the seeds and the minimum contact area of the samples with the holder [3, 8, 14]. Selcuk *et al.* [3] clearly demonstrated the importance of the surface structure and hull composition of the seeds in relation to effectiveness of the plasma used. At the same time, other factors which could influence the efficacy of the treatment include the density of the fungal loading, exposure time and spatial location [15]. The sensitivity of microorganism type is another factor that should be considered, mostly that previous works [3, 8] reported differences in the response level of seed fungal infection to the respective plasma treatments. In general, the highly energetic species as photons, electrons, ions, free radicals, excited or non-excited molecules and atoms generated in plasma or their combination are able of damaging cellular organic molecules (*i.e.*, nucleic acids, aminoacids, lipids) and can thus cause the microorganism inactivation. For instance, the microorganisms in plasma are exposed to an intense bombardment by the reactive oxygen species (ROS) and reactive nitrogen species (RNS), such as O•, O_2, O_3, HO, NO, NO_2, N_2, etc., that have been widely associated to a direct oxidative effects on the outer surface of microbial cells [16]. Recently, by means of optical emission spectroscopy, Filatova *et al.* [8] identified the chemical species generated in plasma treatment (in air, at atmospheric pressure) of grain and legume seeds considering that atomic oxygen and hydroxyl radicals are the most probable inactivation agents of microbial load while the nitrogen plays an important role in the intensification of the biological processes in seeds. However, the physiological and biochemical mechanisms for fungal inactivation by plasma treatment are not yet fully understood.

The germination and growth of barley seeds exposed to glow discharge plasma are displayed in Table 2. Plasma treatment retarded the germination of barley seeds at the beginning of the germination period. At the end of 8^{th} day the germination rate was slightly diminished by the plasma application especially in the longer exposure times, but no more than 7% after 20 min. However, the plasma treatment did not significantly affect ($P > 0.05$) the growth of barley plants. Similarly, Sera *et al.* [17] found that plasma inhibitory effect on germination and initial growth of buckwheat seeds increased with increasing time exposure. Contrarily, Filatova *et al*. [8] reported that laboratory and field germination of steadfast seeds (soy, honey clover and catgut) treated with plasma increased by 10 – 20% as a result of seed coat scarification during the plasma treatment. However, the same research group [8] showed that treatment with $t > 15$ min caused the germination suppression for seeds of some grain and legumes.

Table 2

Germination rate and growth characteristics of barley seeds exposed to glow discharge plasma

Exposure time [min]	Germination rate [%]	Tillers/pot	Spikes/pot	1000 grain weight [g]
0	95 ± 3^a	21 ± 4^b	15 ± 2^c	37 ± 2^d
2	94 ± 2^a	20 ± 1^b	20 ± 6^c	38 ± 9^d
5	91 ± 2^a	21 ± 5^b	12 ± 3^c	33 ± 5^d
10	87 ± 5^a	22 ± 1^b	11 ± 3^c	33 ± 10^d
15	90 ± 2^a	24 ± 5^b	12 ± 4^c	31 ± 9^d
20	88 ± 7^a	22 ± 4^b	14 ± 2^c	34 ± 5^d

Values within each column with different superscripts are significantly different ($P < 0.05$).

In contrast, germination rates of corn seeds were unaffected by the plasma application, while growth of them showed slight improvement (Table 3). Both shoot and root lengths slightly increased especially with 5 min exposure. The fresh weight of corn plants increased after plasma treatment showing significant value for 20 min exposure. Similar findings of significant increase of some growth parameters in comparison to the control plants were reported for maize seeds exposed to low temperature plasma for 1 min as result of plasma induced changes in some biochemical parameters, though the anatomy and morphology of primary roots were insignificantly affected [18]. On the other hand, Selcuk *et al.* [3] showed that no significant difference in germination percentage, shoot weight, shoot height and root length was observed for wheat and bean seeds treated with 5, 10 or 15 min of air or SF_6 plasma gasses compared to control plants.

Table 3

Germination rate and growth characteristics of corn seeds exposed to glow discharge plasma

Exposure time [min]	Germination rate [%]	Shoot length [mm]	Root length [mm]	Fresh weight [g]
0	98 ± 2^{a}	78 ± 4^{b}	21 ± 6^{d}	28 ± 8^{e}
2	99 ± 1^{a}	87 ± 8^{bc}	22 ± 7^{d}	27 ± 13^{e}
5	90 ± 2^{a}	93 ± 2^{c}	26 ± 3^{d}	40 ± 13^{ef}
10	98 ± 2^{a}	82 ± 8^{bc}	25 ± 5^{d}	42 ± 13^{ef}
15	93 ± 5^{a}	84 ± 5^{bc}	21 ± 7^{d}	42 ± 16^{ef}
20	95 ± 5^{a}	79 ± 6^{b}	24 ± 5^{d}	66 ± 15^{f}

Values within each column with different superscripts are significantly different ($P < 0.05$).

The dependence of the treatment results on seed type was also proven for wheat and oat seeds exposed to non-thermal plasma treatment by different responses in relation to the surface erosion, number of germinated caryopses, length of shoot, and root seedlings [19]. Moreover, Sera *et al.* [17] reported that the germination and early growth characteristics of buckwheat seeds were strongly dependent on the type of plasma discharge and the duration of plasma exposition.

4. CONCLUSION

The inactivation curve of fungal load followed the same biphasic model for both barley and corn seeds. However, the response level of fungal contaminated seeds to glow discharge plasma treatment was connected with seed type, plasma power and exposure time. The growth of barley plants was unaffected by the glow discharge. The germination rate of corn seeds was also unaffected while the growth of corn plants showed slight improvement by the plasma application. Therefore, the glow discharge plasma could be applied as a pre-treatment of seeds in order to improve the cereal crops.

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