RAD Conference Proceedings, vol. 5, pp. 75-77, 2021
ISSN 2466-4626 (online) | doi: 10.21175/RadProc.2021.14
www.rad-proceedings.org

THE EFFECT OF SEED TREATMENT WITH NON-THERMAL PLASMA

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Abstract. *The use of physical methods in agro-industrial production has recently become more widespread due to the desire to reduce the level of chemicals. The relevance of the work is also determined by the low level of use of plasma technologies in agricultural production. This work aims to study the effect of non-thermal plasma source discharges on barley of the Vladimir variety seed growth rates and the surface microbiota. During the work on the study of the effect of non-thermal plasma on the sowing qualities of spring barley, it was found that the plasma treatment, in general, does not have a negative or stimulating effect on the initial growth processes of barley seeds. Treatment of barley seeds leads to a decrease in the total microbial count of the surface microbiota. Thus, it has been experimentally established that the microwave plasmatron is suitable for processing seed material.*

Key words: *Non-thermal argon plasma, microwave plasmatron, crops, barley, sowing quality of seeds, phytosanitary safety*

1. INTRODUCTION

The study and application of non-thermal atmospheric plasma (NTAP) in the agro-industrial complex has high interest from researchers [1-3]. In addition, research on increasing global agricultural productivity is still relevant. This is due to the widespread use of chemicals that can negatively affect the ecosystem and humans. Therefore, new technologies are in demand in agriculture, including a non-thermal plasma [4-7]. In addition, it has been shown that NTAP plasma can also be used in the food industry because thermal processes, widely used for sanitization, can affect the organoleptic properties and nutritional value of products [8].

This work aims to study the effect of non-thermal plasma source discharges on the pre-sowing treatment of agricultural seeds (for example, barley) and the surface microbiota.

2. MATERIALS AND METHODS

Argonne microwave non-thermal plasma of atmospheric pressure was used in this work (Fig. 1). In the experiments, we used the barley seeds (*Hordeum vulgare L.*) of the Vladimir sort, harvested in 2018. Each investigated case of the experiment included 50 seeds in three replications. Petri dishes with seeds were placed under the plasma flow with different exposure times. After the treatment, the seeds were laid out on wet filter paper and kept at 20±0.5°C for 7 days.

Microorganisms of the surface microbiota of the seeds have been studied using conventional techniques (agar-plate method for total microbial count) and using the MALDI-ToF MS method.

The biocidal effect of plasma was assessed by the decrease in the number of microorganisms in washes from treated seeds relative to control samples. Sowing on Petri dishes was carried out 1 hour after the treatment of seeds with non-thermal plasma.

2.5 g of a seed sample was transferred into a sterile flask with 25 ml of saline (0.9% sodium chloride), incubated for 20 minutes and sown in 0.1 ml on the surface of solid nutrient media (3 Petri dishes for each sample).

Figure 1. General view of the universal hardware complex for obtaining of non-thermal plasma.

The Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM) was taken into account after cultivation at 28-30 ° C for 24-48 hours on the MAFAnM medium (CFU per 1 g of the sample).

Yeasts and molds were assessed by plating on Sabouraud's medium with chloramphenicol.

Phytopathogens were assessed by plating on potato sucrose agar.

Spore-forming microorganisms were evaluated on salt agar with polymyxin and 2,3,5-TTX.

The core of the non-thermal plasma source is a low-budget 2.45 GHz magnetron microwave generator with a high-voltage power unit [9]. The supply voltage of the magnetron is 4.45 kV. The discharge pin of the plasma source is powered by a rigid coaxial line from a waveguide-coaxial transition. The value of microwave power is regulated in the range from 1.2 to 2.5 kW by

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changing the location of the short-circuited contactless piston. The unused reflected power is absorbed in the water load, which is connected to the waveguide circulator.

The distance from the NTAP-plasma source to the processed object is 45 mm, the temperature is 40-50 C^{o}, and the argon consumption is 4.5 l/min.

3. RESULTS AND DISCUSSION

In the first experiment, non-thermal plasma was generated using a multiple resonant microwave discharger. The inner conductor was a metal tube for supplying a plasma-forming gas – argon. The axial movement of the inner conductor is provided by a choke joint, which allows the regulating of the position of the inner discharger relative to the external tip of the coaxial line. A resonant microwave discharge pin in the form of a 6-pin removable tip was developed.

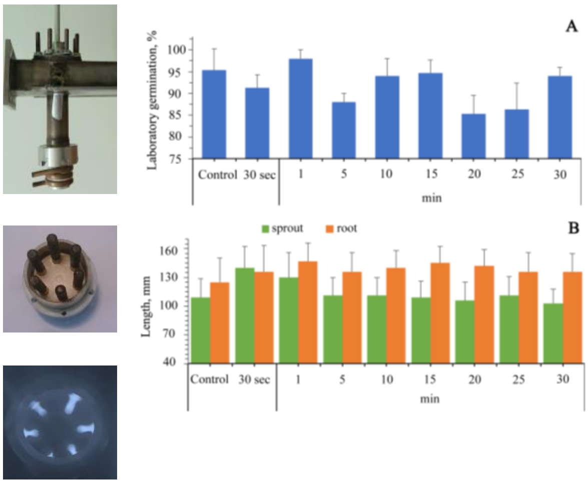

Figure 2. The effect of plasma exposure on (A) the laboratory germination of seeds, (B) the length of sprouts and roots when using the six-pin discharger and a teflon disk.

The exposure of non-thermal plasma on seeds did not lead to a statistical change in the sprouts' length and roots of 7-day-old barley seedlings (Fig. 2). However, after exposure for 5 and 20 minutes, the laboratory germination significantly decreased to the control value. In other cases, no changes were detected.

After the first tests of the non-thermal plasma source, its deficiencies, that could harm the sowing qualities of seeds after their irradiation by plasma, were observed. Because of design specifics during prolonged work time, the discharger's overheating caused a breakdown in the coaxial line. Probably, one of the reasons for this was insufficient heat rejection. To solve this problem, an octagonal spark pin was formed with improved heat removal from the discharge zone due to recirculating blowing zones' formation. With the new geometry of the discharger, it was possible to distribute the load more uniformly, produce the stable discharge and achieve the installation's stability, including during prolonged work operation.

The exposure of seeds with non-thermal plasma after device modification did not negatively affect the sowing characteristics of barley seeds (Fig. 3). The laboratory germination of the seeds did not exceed the control value. It was also found that the plasma exposure does not affect the root and shoot length on the 7th-day of ontogenesis.

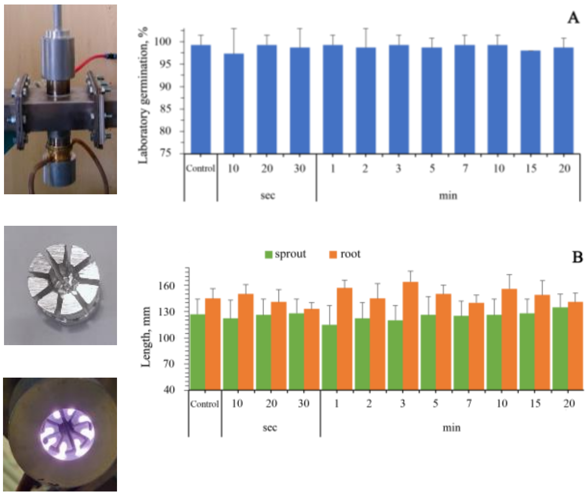

Figure 3. The effect of plasma exposure on (A) the laboratory germination of seeds, (B) the length of sprouts and roots when using the new eight-pin discharger

Thus, during the work on the study of the effect of non-thermal plasma on the sowing qualities of spring barley of the Vladimir variety, it was found that the plasma treatment, in general, does not have a negative or stimulating effect on the initial growth processes of barley seeds. The biocidal effect of non-thermal argon plasma was found during the treatment of spring barley seeds to reduce their infection with diseases (phytopathogens) [10, 11], as well as on the culture of gram-positive bacteria (*Lactobacillus* isolated from walnuts) [12].

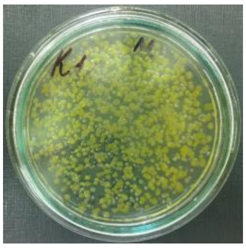

Figure 4. Appearance of inoculation of an untreated sample on QMAFAnM medium (3 days)

Table 1. Dependence of the number of CFU QMAFAnM of barley microbiota on the duration of exposure to non-thermal plasma on seeds

Duration of exposure to non-thermal plasma	1 day mean ± std. dev.	2 day mean ± std. dev.
Control	8.61±0.51 (100 %)	14.48±0.7 (100 %)
1 min	**6.08±0.59** **(70.6 %)**	**10.56±0.8** **(72.9 %)**
5 min	**7.21±0.73** **(83.7 %)**	**9.16±0.67** **(63.3 %)**
10 min	11.43±4.99 (132.8%)	13.63±4.12 (94.1%)

Note. Statistically significant differences ($p \leq 0.05$) from the control by the Mann-Whitney test are shown in bold.

The aim of the second test is to assess the low-temperature plasma biocidal effect on the surface microbiota of barley seeds [11]. The distance from the beam to the subject under study is 55 mm; the argon flow rate is 5 L / min. The treatment duration for barley seeds was 1, 5 and 10 minutes. Studies on the isolation and identification of microorganisms grown on nutrient media in Petri dishes were carried out using conventional techniques and the MALDI-ToF MS method using an Autoflex speed (Bruker Daltonics, Germany) mass spectrometer [11].

Under natural conditions, a mixed microbiota is found on the surface of a barley grain (Fig. 4). Taxonomic identification of the detected microorganisms was performed, such as fungi, yeast, and bacteria. Inoculation on different substrates identified two yeast types of the *Rhodutorula* and *Clavispora* genera; molds of the *Pinicillum* and *Rhizopus* genera; *Bacillus subtilis* and *Bacillus mesentericus (pumilus)* spore-forming bacteria; *Pseudomonas herbicola*, *Arthrobacter pascens* and *Micrococcus luteus* epiphytic microorganisms; *Rhodococcus fascians* phytopathogenic microorganisms. *Bacillus cereus* was not found. Treatment of seeds of agricultural plants with non-thermal argon plasma can reduce their microbiological contamination (Tab. 1).

Treatment of barley seeds leads to a decrease in the total microbial contamination due to the Quantity of Mesophilic Aerobic and Facultative Anaerobic Microorganisms (QMAFAnM) depending on the duration of exposure (e.g., reducing by 36.7 % when treated for 5 minutes). With an increase of up to 10 min in the treatment duration of barley seeds with non-thermal plasma, a tendency to an increase in the amount of MAFAnM is observed relative to the control. This can be explained by the acceleration of spore germination due to heating during prolonged plasma treatment. The ratio of species of surface microbiota of barley seeds does not change significantly due to treatment with non-thermal argon plasma. However, *Rhodococcus fascians* phytopathogenic bacteria are not found in the treated samples.

4. CONCLUSION

An upgraded microwave plasmatron was developed to work with a new type of arrester. The upgraded plasmatron is characterized by greater efficiency and increased stability, namely: it works with 4- and 8-sided resonant dischargers with an optimal length for stable maintenance of the discharge and improved cooling due to the formation of recirculation blowing zones, which leads to a more uniform distribution of the load, stable discharge and allows to increase the time of stable operation of the installation.

It has been experimentally established that the modernized design of the microwave plasmatron is suitable for processing seed material because it generates a sufficient plasma flow and ensures the most stable maintenance of the discharge for a long time and its self-formation. No significant violations of the sowing qualities of seeds were found. At the same time, the total microbial count of the surface microbiota decreased.

The practical significance lies in the fact that the developed installation for the generation of non-thermal atmospheric plasma allows for long-term laboratory research in introducing plasma technologies in crop production, microbiology, agricultural production, and the food industry.

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