NON-THERMAL PLASMA INSTALLATION AS A PRE-TREATMENT METHOD OF BARLEY SEEDS (Hordeum vulgare L.)

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Abstract. Microbial organisms are key pathogens for plants, animals, and humans. The elimination of pathogenic microbes is an essential topic for researchers. Non-thermal atmospheric plasma (NTAP) can potentially inactivate pathogenic microorganisms. This paper draws the results of the biocidal effect study in pre-treatment of barley seeds by NTAP. The effect was observed in 7-day-old seedlings. The results of reducing the total microbial number (by 36.7% after 5 minutes of exposure) were obtained. Thus, treatment with non-thermal plasma can reduce the microbiological contamination of agricultural plant seeds.

Keywords: non-thermal plasma, seed disinfection, total microbial number, agricultural plant seeds, barley.

1. Introduction
In the agro-industrial complex, an essential task is to increase yields and improve the storability of grown raw materials and plant foods. According to the International Organization for Food and Agriculture (FAO at the UN), grain losses during storage are at least 10% and increase when the storage conditions for temperature and humidity deviate from optimal. Losses are mainly associated with pest damage and microbiological spoilage. In addition, with long-term storage of grain in adverse conditions, the ability to germinate is disturbed, and quality decreases.

One of the currently developing physical methods of pre-sowing treatment agricultural products is non-thermal atmospheric pressure plasma (NTAP) [1]. Pre-sowing seed treatment with NTAP can reduce microbiological contamination [2]. Previous studies have shown that the treatment of barley seeds with NTAP does not significantly affect germination capacity and the length of sprouts and roots. However, it reduces the infection with phytopathogenic fungi [3]. In addition, domestic studies [1, 4, 5] demonstrated the bactericidal effect of NTAP on several microorganisms: yeast, gram-negative, and gram-positive bacteria, and their spore forms. It is also worth noting the formation of free radicals due to plasma chemical reactions, which are strong oxidants [6, 7]. They cause membrane degradation and DNA disruption [1, 8].
This work aims to access the biocidal effect of NTAP on the surface microbiota of barley seeds and morphometric parameters of 7-day-old seedlings.

2. Materials and Methods
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 150 seeds. The seeds were pre-treated with NTAP, which uses a microwave discharge of a coaxial configuration. Argon was used as a plasma-forming gas. [3, 9]. The scheme of the experimental installation is shown in Figure 1.

![Figure 1. The scheme of the experimental installation](image)

A 20 cm long gas concentrator was additionally installed to reduce the flow temperature and maintain the concentration of active particles. The concentrator in the form of a truncated cone was made of stainless steel 0.4 mm thick.

Seeds were pre-treated with NTAP at three levels of time: 1, 5 and 10 minutes. The distance from the nozzle to the seeds was 13 cm. Argon consumption was 5 l/min, and the power of the microwave generator was 1.2 kW. The gas flow temperature at the seed placement level was measured using a thermal imager and was about 37°C.

Further, the control and treated seed groups were germinated on wet filtered papers in a thermostat at 20-21°C in accordance with Russian National Standard 12038-847. Each group contained 50 seeds. On the third day, the seed germination energy was determined. On the seventh day, laboratory germination, growth strength, sprout and root length, and wet and dry mass were determined. Then a phytosanitary examination of barley seeds was carried out. The degree of damage and the prevalence of diseases by Helminthosporium, Fusarium, and Penicillium were determined in accordance with Russian National Standard 12044-93.

3. Results and Discussion
The spring barley of the Vladimir variety has high adaptability to various cultivation conditions resistant to drought and soil acidity. The variety is moderately resistant to loose smut, highly susceptible to helminthosporiosis, moderately resistant to leaf rust and powdery mildew. Germination energy characterizes the ability of seeds to give uniform and even seedlings in the field, which guarantees good evenness and survival of plants. This indicator shows the percentage of germinated seeds of the total number of seeds planted in the experiment. Our experiments studied the effectiveness of presowing treatment with APNTP of spring barley seeds, which was determined by changes in morphological parameters and phytosanitary expertise. Following exposure to APNTP on the 7th day of germination, the length of the sprout and root did not change statistically significantly (Table 1). Our experiments decreased germination energy of barley seeds was observed during 5 and 10-minute
exposure to plasma. Next, the percentage of seeds that gave seedlings under standard conditions was determined - laboratory germination. Following exposure to APNTP, laboratory germination did not change statistically significantly. It was also found that pre-sowing plasma treatment of seeds before germination does not affect the growing strength of 7-day-old seedlings.

Table 1. Parameters of 7-day-old barley seedlings after exposure to APNTP on seeds before germination

| Parameter               | Exposure time |           |           |           |
|-------------------------|--------------|-----------|-----------|-----------|
|                         | Control      | 1 min     | 5 min     | 10 min    |
| Sprout length           | 96 ± 4,00    | 93 ± 5,00 | 93 ± 4,00 | 92 ± 5,00 |
| Root length             | 132 ± 4,00   | 133 ± 4,00| 131 ± 4,00| 125 ± 4,00|
| Germination energy      | 96 ± 0,00    | 93 ± 3,06 | 89 ± 4,16 | 91 ± 2,31 |
| Laboratory germination  | 100 ± 0,00   | 97 ± 6,00 | 99 ± 2,00 | 99 ± 2,00 |
| Seed vigour             | 97 ± 2,00    | 95 ± 4,00 | 96 ± 4,00 | 97 ± 2,00 |

The percentage of water, wet and dry weight per 1 plant was determined (Table 2) for a more detailed study of the effect of plasma on the initial growth processes of spring barley. It was found that the percentage of water content in 7-day-old barley seedlings after exposure to APNTP on seeds before germination does not change statistically significantly. The study results showed that the fresh weight of seedlings after irradiation at 10 minutes decreased, but the dry weight did not change. Other paragraphs are indented (BodytextIndented style).

Table 2. Wet and dry weight of 7-day-old barley seedlings after exposure to APNTP on seeds before germination

| Exposure time | Weight per 1 plant, g | % of water in seedlings |
|--------------|-----------------------|-------------------------|
| Control      | 0,29 ± 0,02           | 0,0425 ± 0,0041         | 85,40 ± 1,42 |
| 1 min        | 0,28 ± 0,01           | 0,0425 ± 0,0008         | 84,69 ± 0,46 |
| 5 min        | 0,29 ± 0,01           | 0,0441 ± 0,0023         | 84,79 ± 0,33 |
| 10 min       | 0,26 ± 0,00           | 0,0379 ± 0,0054         | 85,29 ± 2,07 |

Next, the effect of APNTP on the phytosanitary state of barley seeds was studied. It was established that the seeds were infected by Penicillium, neither in control nor in the irradiated samples. The degree of damage by Fusarium, Helminthostorium, Aspergillus did not change statistically significantly (Table 3). The table presents the results of assessing the effect of plasma treatment on the prevalence of seed infections in spring barley. After exposure to APNTP on barley seeds, the prevalence of Fusarium root rot and fungi of the genera Helminthostorium, Aspergillus did not change statistically significantly.

Table 3. The degree of damage and prevalence of helminthosporiasis (Helminthosporium spp.) in 7-day-old barley seedlings after exposure to ANTP on seeds before germination

| Exposure time | Damage degree by helminthosporiasis, % | Prevalence of the disease, % |
|--------------|----------------------------------------|-----------------------------|
| Control      | 25,00 ± 5,00                          | 75,00 ± 12,00               |
| 1 min        | 24,00 ± 11,00                          | 68,00 ± 19,00               |
| 5 min        | 25,00 ± 9,00                           | 73,00 ± 24,00               |
| 10 min       | 22,00 ± 5,00                           | 70,00 ± 7,00                |

Thus, the obtained results of the study showed the absence of significant violations of the sowing qualities of barley seeds during presowing treatment with APNTP. The studied seed samples had a low initial infection with diseases (except for helminthosporiasis); therefore, no pronounced disinfecting effect was observed. However, the biocidal effect of argon ANTP was shown when exposed to barley seeds, determined by a decrease in the total microbial number of the surface microbiota (by 36.7% when exposed for 5 min) (Table 4).
Table 4. Dependence of the number of CFU QMA&OAMO of barley microbiota on the duration of exposure to APNTP on seeds.

| Exposure time | 1 day Mean ± standard deviation×10⁴ CFU/g | 2 day Mean ± standard deviation×10⁴ CFU/g |
|--------------|-------------------------------------------|------------------------------------------|
| Control      | 8.61 ± 0.51 (100 %)                       | 14.48 ± 0.70 (100 %)                    |
| 1 min        | 6.08 ± 0.59 (70.6 %)*                     | 10.56 ± 0.80 (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 %)                   |

* — significant differences (p ≤ 0.05) from control according to the Mann-Whitney U test.

As a result of studies on the impact of APNTP on barley seeds for 1 to 10 minutes, there was no change in their main morphometric parameters. However, exposure for 10 minutes reduced fresh weight in 7-day-old barley seedlings. Decrease in seed germination energy was observed after 5 and 10 min of plasma exposure, but laboratory germination did not change significantly. A decrease in seed germination energy was observed after 5 and 10 min of plasma exposure, but laboratory germination did not change significantly. The damage degree and the prevalence of seed diseases by various fungi were similar to control samples. A biocidal effect was shown, expressed in a decrease in the total microbial number of the surface microbiota of seeds.

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