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Fungicidal Effect of Apokampic Discharge Plasma Jet on Wheat Seeds Infected with *Alternaria Sp.* and *Bipolaris Sorokiniana Shoemaker*

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***Abstract*—Atmospheric pressure plasma jets generated by apokamp discharge (APPJ AD) in ambient air was investigated for the improvement of growth parameters of spring wheat seeds («Irgina» cultivar) and for inhibition of potentially phytopathogenic fungi on seeds surface. In case of APPJ AD, active forms of oxygen and nitrogen (as well as the radiation of molecules) are formed in the air of atmospheric pressure, which increases their concentration in the streamer head by orders of magnitude, compared to traditional APPJ based on inert gases. Owing to this, we believe to concentrate action of active forms of oxygen and nitrogen in situ, on the biological target. In our case, this can intensify the processes of inactivation of microscopic mold fungi on the surface of seeds. We tested our hypothesis on wheat seeds infected with helminthosporiosis (*Bipolaris sorokiniana Shoemaker*) with a degree of infection of 50% and early blight (*Alternaria sp.*), whose infection rate was 10%. In the first case, the seeds were treated by apokamp plasma for 1 and 10 minutes. It provided a significant reduction in the degree of infection of seeds with helminthosporiosis by 1.7 and 2.3 times. In comparison with check variant, the treated seeds maintained high rates of viability and germination energy. In the second case, it was possible to completely inactivate alternariosis pathogens within 3 min of treatment. In addition, it is shown that the action of APPJ AD caused a statistically valid elongation of the roots of wheat seedlings. A hypothesis is proposed to explain the found effects. The potential advantages of APPJ AD in the task of seed surface treatment in comparison with other known atmospheric plasma techniques are discussed.**

Keywords—Alternaria sp., apokamp discharge, atmospheric plasma, Bipolaris sorokiniana Shoemaker, reactive oxygen species, phytopathogenic fungi

I. INTRODUCTION

The use of physical factors is attractive alternative to raise the yield of agricultural production while improving plant protection and storage. In comparison of conventional treatments based on chemicals, physical methods for seed invigoration reduce the use of fertilizers and also used for seed disinfection before sowing and during the storage [1, 2].

One of physical factors is atmospheric pressure plasma [3]. The most interesting techniques to practice are Atmospheric Pressure Plasma Jets (APPJs), plasma of Dielectric Barrier Discharges (DBDs) and atmospheric radio-frequency glow discharge plasma [4–7]. Operation at atmospheric pressure makes it easier to obtain and deliver plasma to biological objects.

The action of plasma is determined by its composition. Typically, atmospheric plasma is generated in gas or gas mixture, argon, helium, oxygen, nitrogen or air. Atmospheric plasma in air consists of a mixture of neutral atoms, charged particles, excited molecules, radicals and photons. The most interesting for biological applications are reactive oxygen and nitrogen species (RONS) such as nitric oxide and nitrogen dioxide (NO, NO_2), dinitrogen pentoxide (N_2O_5), ozone (O_3), hydroxyl ($HO^•$), superoxide ($^•O_2^-$), hydroperoxyl ($HO_2^•$), and UV photons [8–13].

Nowadays the positive effect of atmospheric plasma treatment on the germination of seeds of various crops, their viable rate, growth dynamics and crop quality has been experimentally established. Moreover, it is shown that the RONS produced within plasma are highly effective antimicrobial agents that are also capable of degrading a wide variety of toxic compounds, including mycotoxins [14–17]. The advantages of this treatment are the efficiency of inactivation of microorganisms, a relatively simple treatment procedure, complete or partial rejection of chemical growth stimulants and protectants, and a relatively short duration of treatment procedure.

In this paper, we study the effects of atmospheric plasma on spring wheat seeds contaminated with pathogenic fungi.

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Inasmuch as wheat is one of the world's leading food crops and is one of the most grown cereals, it was selected as the research material. Wheat is a staple source of nutrients for around 40% of the world's population [18]. According to statistics of the Food and Agriculture Organization (FAO) of the United Nations In 2018, global wheat production was led by China, India, and Russia [19].

Fungi of the genus *Alternaria* (about 250 species), can produce a wide range of toxins is a significant and present threat to the health of humans. The *Alternaria* genus is commonly present in cereals, increasing risk in affected wheat. Prevention of contamination by *Alternaria* fungi is an urgent task. Pathogen *Bipolaris sorokiniana* (syn. *Drechslera prorokiniana* syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*) also causes great harm to agriculture. Globally, approximatly 25 million ha of wheat area is affected by spot blotch. *Bipolaris sorokiniana* is also believed to be the causative agent of common root rot, seedling rot, head rot, and the black dot of wheat and barley [19–21].

The peculiarity of our work is that the source of atmospheric plasma is a new type of discharge. It is so-called apokamp discharge. The aim of the work is to evaluate the suitability of a new plasma source for the tasks of fungicidal and stimulating treatment of wheat seeds.

II. MATERIALS AND METHODS

Figure 1 depicts the phenomenon of apokamp discharge and electrical circuit for discharge formation. The discharge was ignited in air of atmospheric pressure between stainless steel electrodes (1, 2), located at a distance of 0.5–1.2 mm. To obtain the discharge, a source of high-voltage pulses 5 was used (Fig. 1, b) and step-up transformer 6, which provided positive polarity voltage pulses with a frequency of $16 < f < 50$ kHz, pulse duration $\tau = 1.5$–2.5 μs and voltage amplitude up to 13 kV. Electrode (1) was connected to the high-voltage output of the transformer, and electrode (2) had a capacitive isolation with grounding ($C \sim 2$–5 pF).

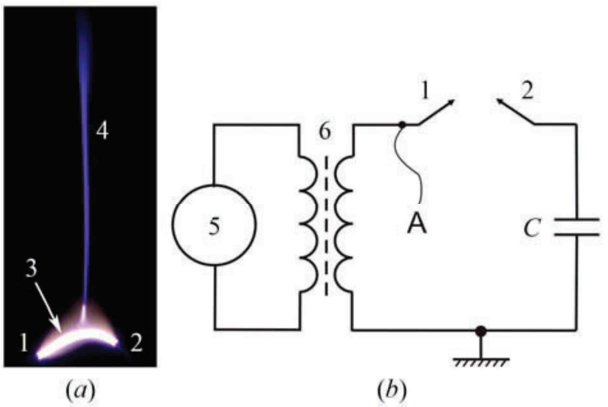

Fig. 1. The view (*a*) and power circuit (*b*) of apokamp discharge: *1* – high-voltage tip electrode of positive polarity; *2* – tip electrode, having a capacitive connection (*C*) to the ground; *3* – pulse discharge channel, carried out at a frequency of $f = 50$ kHz at the amplitude of the voltage pulses $U_p \sim 11$ kV; *4* – plasma plume (apokamp). The inter electrode distance $d = 8$ mm, the frame height (*a*) is 3.5 cm. The photo was taken with a Canon PowerShot SX 60 HS camera with a shutter speed of 1/8 c and a light sensitivity of ISO 1500.

Under the described excitation conditions, a pulsed high-voltage discharge (3) becomes the source of a luminous plasma plume (4), which was called apokamp (from the Greek words από – "from" and κάμπη – "bend"), because it occurs near the bend of channel (3) [22, 23].

As shown in (see references in [24]) apokamp is a positive streamer, similar to those obtained in the so-called atmospheric pressure plasma jets (APPJ) [27]. Therefore, it can be called the atmospheric pressure plasma jets generated by Apokamp Discharge (AD APPJ).

Our plasma source has a number of distinctive properties as compared with traditional APPJ. First of all, the obtaining an extended plasma plume does not require the use of expensive inert gases. Secondly, the gas pumping is not required, which simplifies the design of the plasma source [22, 23]. The apokamp plasma is a source of nitrogen oxides [13], which indirectly indicates the formation of RONS in the discharge. The latter can play a active role in inactivation of the surface of biological objects.

In traditional APPJ on inert gas plasma is first produced based on helium or argon, and only then the energy of the resulting plasma is transferred to the surrounding air molecules to form useful particles and emit nitrogen molecules and ions. This is associated with additional energy losses. In the apokamps, active forms of oxygen and nitrogen (as well as the radiation of molecules) are formed directly in the air of atmospheric pressure, which increases their concentration in the streamer head by orders of magnitude, compared to traditional APPJ based on inert gases [3]. Owing to this, we believe to increase the RONS concentration in situ, on the biological target. In our case, this can intensify the processes of inactivation of microscopic mold fungi on the surface of seeds, and, probably, enhance the plant growth parameters.

So, we believe that AP APPJ will provide us with several advantages in the preparation and delivery of active forms of oxygen and nitrogen to seeds surface of wheat.

The object of the study was the seeds of soft spring wheat (*Triticum aestivum L.*, «Irgina» cultivar). Seeds contaminated with helminthosporiosis (*Bipolaris sorokiniana Shoemaker*) and alternariosis (*Alternaria sp.*) were used, as well as healthy seeds for comparison.

During treatment the seeds were placed on a dielectric grid in 1–2 layers, placing it over the apokamp (4) (Fig. 1, a). To prevent overheating of the seeds, the grid was placed at a distance of at least 3 cm from the discharge channel (3). At the processing site the air temperature was 45 ± 8 ºC. The average diameter of the mesh cell was about half the length of the seed. During processing, the seeds were mixed. Thus, the apokamp (plasma plume) acted directly on the surface of the seeds through the pores in the grid. Processing time varied from tens of seconds to several minutes.

Standard methods were used to determine the growth qualities of grain and the level of infection. Phytoexpertize of seeds was carried out using the rolled paper towel method (GOST 12044-93) [25]. The infection and germination of seeds at the 7th day after treatment were tested. For seed germination, two layers of filter paper moistened by sterile water were used.

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The size of the filter paper strips is 10 cm × 110 cm. The seeds were laid out with tweezers in one line through 1 cm at a distance of 2–3 cm from the upper and side edges of the paper with the embryos down. From above, the seeds were covered with a strip of moistened filter paper, rolled into non-dry rolls and placed vertically in vessels and placed in a thermostat. Seed germination was carried out at a temperature of 22–25ºC.

After 3 days, the sprouted seeds were counted to determine the viable rate (GOST 12038-84). The seeds were placed in Petri dishes and germinated in a thermostat at +23 ºC.

Evaluation of the statistical significance of infection rate, viable rate and germination energy of wheat seeds was carried out by comparing the sample shares with the Student's criterion for 95% of the significance level for probabilities of 25–75% inclusive, or with the Fischer criterion for other probability values.

III. RESULTS AND DISCUSSION

Three sets of experiments were conducted. In the first one, we used seeds infected with helminthosporiosis, with viable rate of 96%, germination energy of 100% and infection rate of 50%. The apokamp exposure time was 1 and 10 minutes. Treatment provided a significant reduction of seeds infection rate by 1.7 and 2.3 times, respectively (Fig. 2).

In addition, compared with the check variant, the treated seeds maintained high rates of viability and germination energy (Table 1). This is important because plasma action is multi-factorial and it is necessary to be sure that plant growth indicators at least will not decrease.

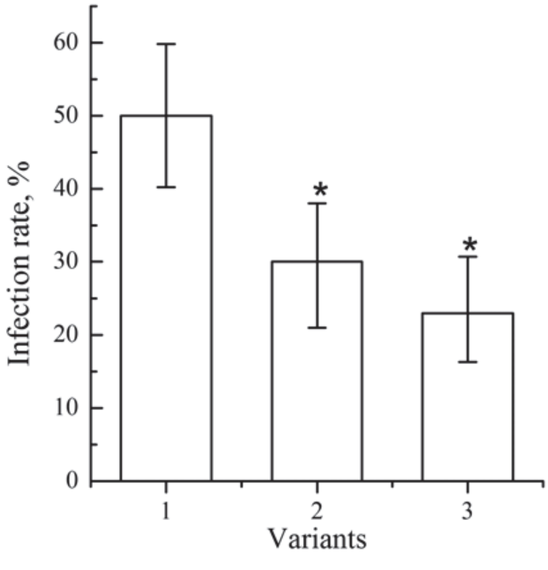

Fig. 2. Infection rate of wheat seeds with helminthosporiosis: 1 – check varant (without plasma treatment); 2 – variant with 1-min apokamp treatment; 2 – 10-min apokamp treatment. Here and further, the '*' sign marks significant differences between control and experience at $p < 0.05$.

TABLE I. LABORATORY VIABLE RATE AND GERMINATION ENERGY OF WHEAT SEEDS INFECTED WITH HELMINTHOSPORIOSIS (%)

Variant	Viable rate, %	Germination energy, %
Check	96 (+3.6/–7.4)	100 (–1.9)
1 min	91 (+6.3/–9.4)	92 (+5.8/–9.1)
10 min	92 (+5.9/–9.1)	92 (+5.8/–9.1)

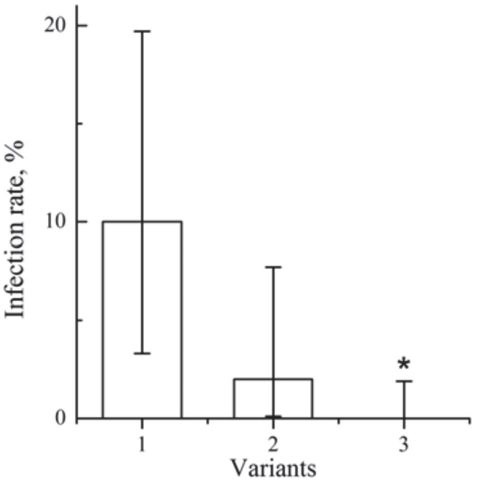

Fig. 3. Infection rate of wheat seeds with alternariosis: 1 – check varant; 2 – variant with 1-min apokamp treatment; 2 – 3-min apokamp treatment.

In the second group of experiments, seeds infected with alternariosis were used, with germination rates of 76% and initial infection rate of 10% (Fig. 3).

After 1 and 3 minutes of plasma treatment, the germination rate of wheat seeds did not significantly differ from the check variant. The 1-min treatment resulted in a 5-fold reduction in seed contamination. The 3-min treatment provided complete disinfection of the seeds.

In addition, on the 7th day after treatment, the growth indicators of wheat seedlings (root and sprout length) were taken into account. A statistically significant increase in the average length of sprouts compared to check variant was revealed (at a significance level of $p < 0.05$). Compared to check, in case of 1 and 3-min plasma treatment the roots length was 52 and 35% longer, respectively. But the length of sprouts in experimental and check variants had no significant differences.

Will this effect persist in case of healthy seeds plasma treatment? To answer this question, a third set of experiments was conducted. The results are shown in Fig. 4. It can be seen that apokamp treatment also increases the root length of healthy wheat seeds, and does not affect the length of rootlets.

Let's try to explain the revealed effects. Our hypothesis is as follows. First, ultraviolet radiation and oxygen-containing chemically active species of apokamp plasma plume head directly provide inactivation of pathogens. Secondly, nitrogen-containing chemically active species can stimulate the growth of the root development of seeds. But to check this later, it is necessary to measure the exact composition of the chemically

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active particles that are born in the head of the positive streamer (apokamp) under experimental conditions. Another approach would be to estimate of the specified characteristics by numerical simulation.

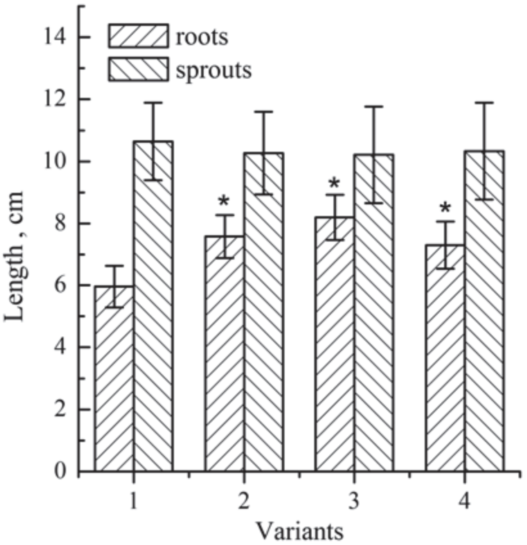

Fig. 4. Length of roots and sprouts of healthy wheat seeds on the seventh day after pre-sowing treatment and planting: 1 – check variant; 2 – 30-second treatment; 3 – 2-min treatment; 4 – 4-min treatment.

IV. CONCLUSION

It was proposed for the first time to use a plasma jet of apokamp discharge for disinfection of plant seeds from fungi pathogens. On the example of processing wheat seeds infected with helminthosporiosis (*Bipolaris sorokiniana Shoemaker*) and early blight (*Alternaria sp.*), the effect of inactivation of these pathogens was statistically significant. In addition it was shown that apokamp treatment stimulates the growth of roots of infected and healthy seeds. A hypothesis is proposed that links the found effects with the composition of the apokamp plasma plume. These features of AD APPJ (as a type of atmospheric plasma) have potential advantages in comparison with other techniques of using atmospheric plasma in the processing of biological objects. The obtained results could be the scientific basis for a new research directions.

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