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C1 - Beam and plasma sources
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Fungicidal effect of apokampic discharge plasma jet on wheat seeds infected with *Alternaria sp*. and *Bipolaris sorokiniana*

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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, NO2), dinitrogen pentoxide (N2O5), ozone (O3), hydroxyl (HO•), superoxide (•O2–), hydroperoxyl (HO2•), 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.

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].

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 τ = 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 ~ 2-5 pF).

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].

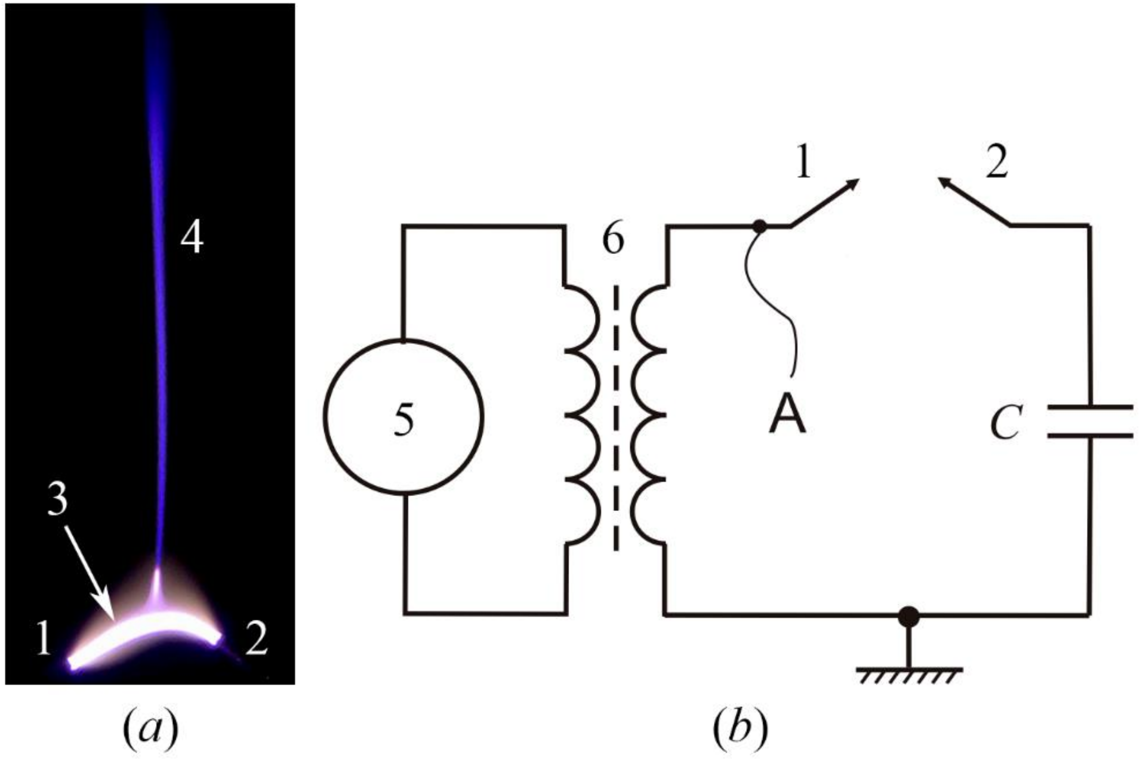
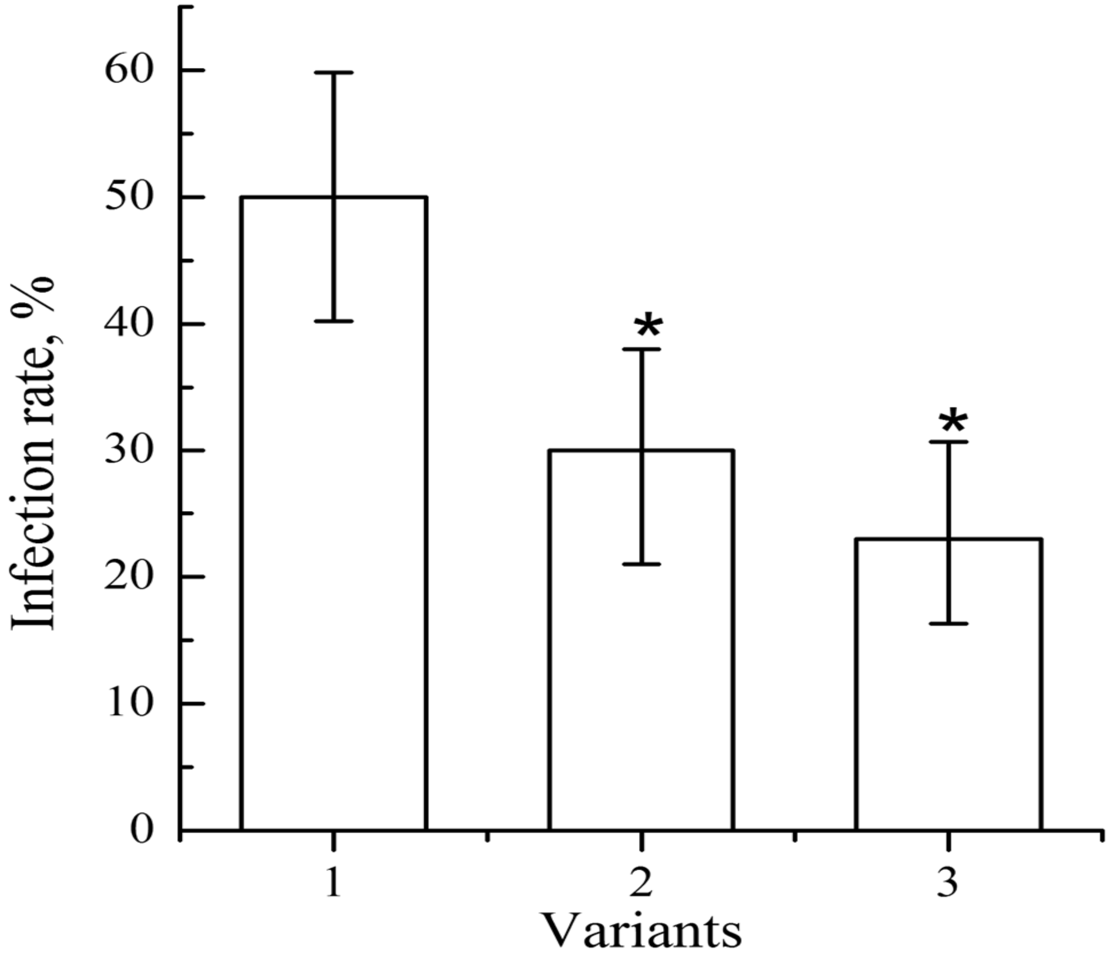


Fig. 1. The view (a) and power circuit (b) of apokamp discharge: 1 – high-voltage tip electrode of positive polarity; 2 – tip electrode, having a capacitive connection (C) to the ground; 3 – pulse discharge channel, carried out at a frequency of f = 50 kHz at the amplitude of the voltage pulses Up ~ 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.

Results and discussion (1)



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.

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).

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 $p < 0.05$.

Results and discussion (2)

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)

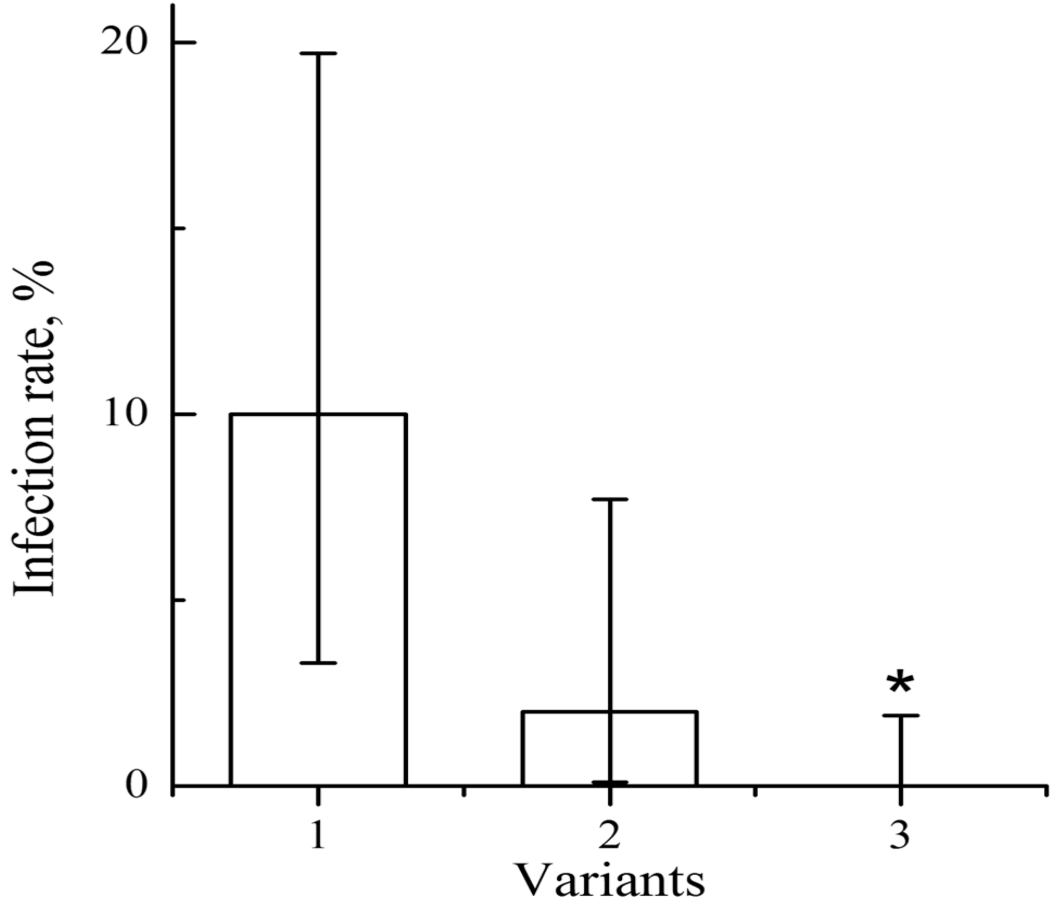


Fig. 3 Infection rate of wheat seeds with alternariosis: 1 – check varant; 2 – variant with 1-min apokamp treatment; 2 – 3-min apokamp treatment.

Results and discussion (3)

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 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.

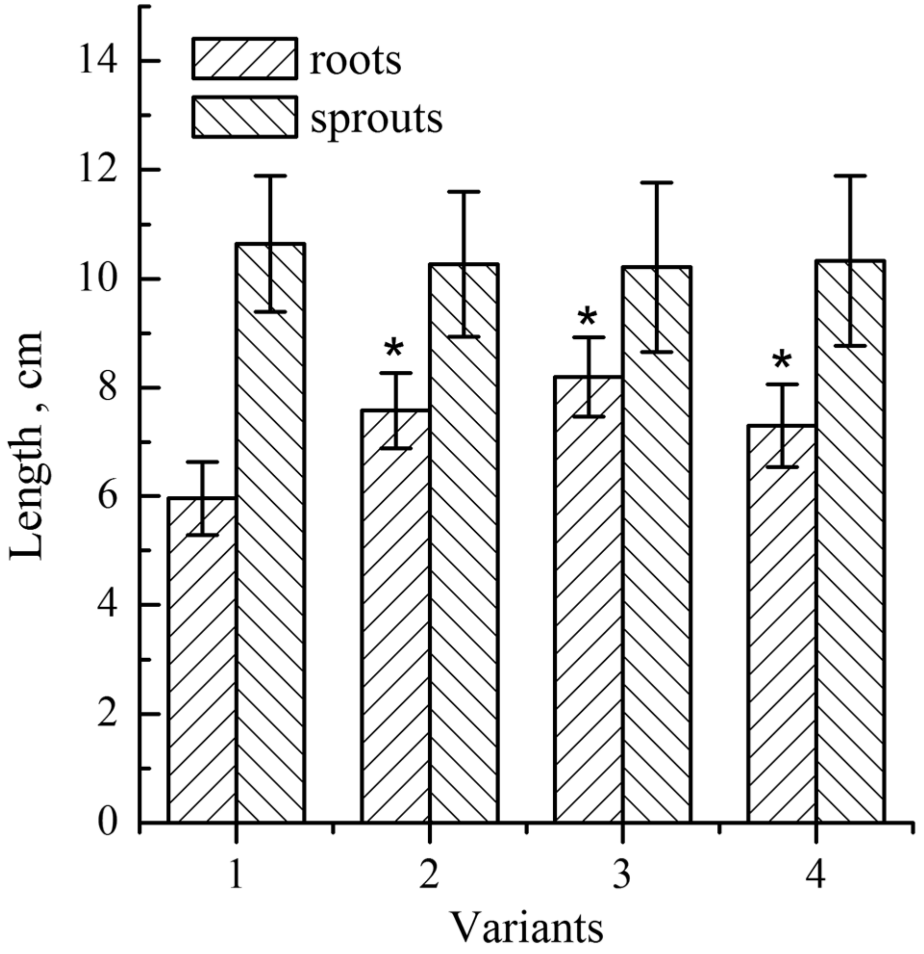


Fig. 4. Length of roots and sprouts of healthy wheat seeds on the seventh day after pre-sowing treatment and planting: 1 – check variant; 2 – 30-second treatment; 3 – 2-min treatment; 4 – 4-min treatment.

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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