Fungicidal and bactericidal effect of plasma and radiowave treatment on biological and medical materials

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Résumé

A germicidal effect of low pressure rf plasma treatment on materials used in medical instruments manufacture has been investigated. An influence of rf plasma and rf electromagnetic field pre-treatments on level of fungal infection of some important agricultural plants has been studied.

Introduction

Plasma and radiowave treatment is widely used for activation and decontamination of surfaces. Nowadays non-thermal plasma is considered functionally, energetically and ecologically as the most efficient tool for pathogenic bacteria inactivation - due to high chemical activity of low-temperature plasma, short-time treatment and minimal thermolabile materials destruction [1–3]. Last years these methods have been successfully applied also in agriculture for seed's sowing quality improvement. It has been shown in a number of previous studies that plasma and electromagnetic field pre-treatments of seeds stimulate their germination and lead to suppression of fungal and bacterial pathogens that cause various plant diseases [4, 5]. A considerable part of the investigations have been performed using 13.56 MHz plasma, and the effect of electromagnetic fields on biological objects has been examined mainly in the microwave (300 MHz–300 GHz) and in the low frequency (50–100 Hz) regions. In this paper, low-pressure capacitively coupled 5.28 MHz discharge plasma as well as 5.28 MHz electromagnetic field treatments are used for plant seeds enhancement and surface decontamination.

Experimental set-up and conditions

Tested species were processed with 5.28 MHz air plasma at pressure 0.5 Torr and were exposed to rf electromagnetic field at atmospheric pressure under conditions of the prebreakdown mode of a capacitively coupled radio frequency discharge operation. The discharge was operated between two parallel round copper electrodes with diameter 120 mm. The distance between electrodes was 20 mm. The investigated samples were placed on the grounded electrode. The supplied full specific rf power was 0.5 W/cm^3. The experimental conditions for magnetic field treatment were as follows: the alternator frequency was 5.28 MHz, the root-mean-square value of magnetic and electric components of the electromagnetic field strength was 590 A/m and 12700 V/m accordingly. The exposure duration of treatments was 5, 10, 15, 20 and 30 min.

To study the peculiarities of microorganisms inactivation on different surfaces in plasma the culture crop emulsion was plotted on a surface of sterile medical products made of polymers, metals (stainless steel or copper) and capillary-porous (surgical suture) samples. The strains of Gram-positive bacteria – *Staphylococcus aureus ATCC 6538* and Gram-negative bacteria – *Escherichia coli ATCC 8739*, clinical isolates of *Staphylococcus - 37C1* and *Enterobacteriaceae EB 158*, as well as spore of *B. subtilis ATCC 6633* were chosen as tested species. Fat-free sterile samples were contaminated in a glass tube containing suspension of microorganisms with concentration of 10^9 CFU/ml prepared according to McFarland turbidity standards. The concentration of microorganisms on spore inoculated products was varied in the range of 10^5-10^7 CFU/ml depending on an effective area. A test of microorganisms survival for treated and control samples was obtained by perform several consecutive dilutions (1:10 in NaCl) of wash-outs from the surface of tested samples (by immersion into a sterile physiological solution). A portion of solution from the most suitable dilution were pipetted into the appropriate culture media and incubated at temperature 37°C for 24-48 hours before the examination.

The effectiveness of pre-sowing plasma and radiowave seed treatments was examined by means of evaluation of the laboratory/field germination ability, seed vitality and a level of fungal infection on sprouting seeds for processed and untreated (control) samples. Seeds of blue lupine (*Lupinus*

angustifolius), honey clover (*Melilotus albus*) and soy were chose for investigations. In the laboratory tests seeds were grown on a moist filter paper in Petri dishes that were kept for 5-7 days (before the first sprout occurrence) in a thermostat at 20-21°C. A plot with the area of 25 m^2 was used for the field test.

Results and discussions

The cultivable cell concentration on the tested surfaces decreased at least by 4-6 orders of magnitude after 20 min of plasma exposure, in dependence on material structure and microorganism genus (fig. 1).

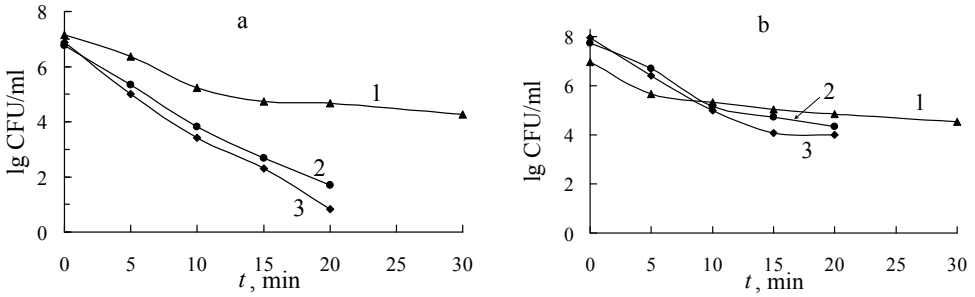

Fig. 1: Concentration of survived cells of microorganisms on metallic (a) and capillary-porous (b) samples in dependence on time of rf air plasma irradiation: 1– spore of *B. subtilis* ATCC 6633, 2 and 3– Gram- positive and Gram-negative bacteria correspondingly.

In the case of samples with a solid surface (polymer, metal) micro-organisms suspension assigned rather evenly on their surface, easily accessible for plasma irradiation. The reason for decrease of plasma sterilizing effect in the case of a porous sample treatment is connected apparently with peculiarities of plasma interaction with porous materials: an initial concentration of microorganisms on capillary-porous samples depended essentially on their surface texture and was not homogeneous upon their surface. It has been shown that Gram-positive bacteria cultures independently of their nature (the museum strains or clinical isolates) demonstrated a greater resistance to the plasma treatment than Gram-negative ones. It may be caused by the basic difference in the form of their cell wall structure: the cell walls of Gram-positive bacteria are made up of twenty times as much peptidoglycan than Gram-negative bacteria that formed together with other proteins a thick outer matrix serve membrane transport regulation and cell expansion of bacteria.

Plasma and radiowave seed pre-treatment during 10-15 min led to decrease (6-14%) of the level of fungal infection caused by *Fusarium oxysporum*, *Alternaria brassicae*, *Stemphylium botryosum* (fig.2).

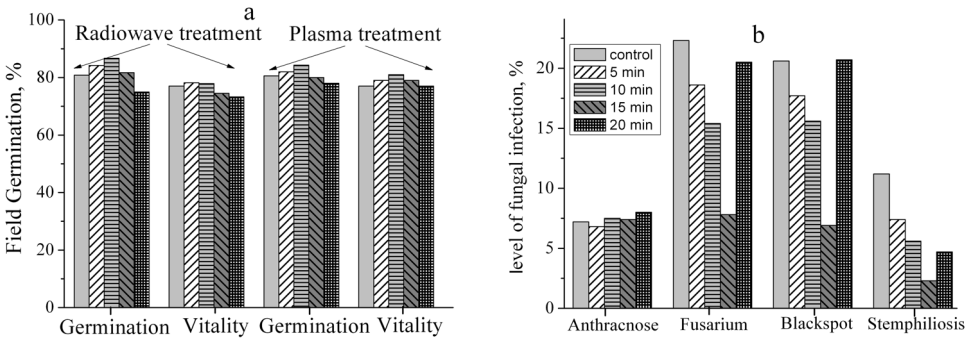

Fig. 2: Field germination capacity of control and treated seeds (a) and a level of fungal infection of blue lupine (*Lupinus angustifolius* L.) (b) as a result of radiowave treatment for different exposure durations.

The same results were obtained for another tested species. It was observed that the increase of treatment duration over 20 min could lead to some oppression of seeds and did not improve plant resistance to fungal infection.

References

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