ECOLOGICAL CHEMISTRY AND ENGINEERING A

Vol. 18, No. 11 2011

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IMPACT OF THE PULSED HIGH MAGNETIC FIELD ON *Fusarium culmorum* (W.G. Smith) Sacc.

WPŁYW IMPULSOWEGO, WYSOKIEGO POLA MAGNETYCZNEGO NA *Fusarium culmorum* (W.G. Smith) Sacc.

Abstract: The aim of the investigations was an assessment of the impact of the pulsed high magnetic field on a concentrated spore suspension of *F. culmorum* fungus, which is a dangerous pathogen of many crops. 5, 10, 15 and 20 pulses of 9 Tesla induction were applied. The pulsed magnetic field limited germination of the tested strain. A significant difference was found for 15 and 20 pulses. Mycelial linear growth was also reduced, as was found both for the inoculation and logarithmic phase of growth in Petri dish culturing, however, no limited mycelium sporulation was observed. There is a potential for the application of the tested physical method – the pulsed high magnetic field to limit the development and harmfulness of *F. culmorum*.

Keywords: pulsed magnetic field, *F. culmorum*

The magnetic field is one of physical factors which influence life processes of microorganisms [1]. Magnetic field may modify fungi sporulation, which is connected with disturbance of calcium ion flow playing an important role in this process [2, 3]. Albertini et al [4], who studied the effect of the constant high magnetic field on the observed physiological and morphological changes in *Fusarium culmorum*, ie weakening of mycelial growth, spore germination and a decrease in enzymatic activity. They described the occurring morphological changes as similar to those caused by the contact of these fungi with chemical pesticides. Similarly, while applying the constant magnetic field, Nagy and Fischl [5] registered even 83 % limited spore production in *F. oxysporum* species in comparison with the control. In their studies on *Saccharomyces cerevisae* Novak et al [6] observed that 20-minute exposure to 10 mT caused the death of some yeast cells, whereas the ones which survived did not lose the growth ability. On the other hand Ruiz-Gomez et al [7] did not register any significant effect on cell

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viability exposed to the constant magnetic field (0.35 and 2.45 mT) for 24 and 72 hours. The high constant magnetic field also reveals an antibacterial effect [8–11]. It was found that the magnetic field modifies bacteria sensitivity to antibiotics [12, 13]. Gaafar et al [14] found that antibacterial activity of the constant magnetic field depends on the applied exposure time. In some specific conditions, the constant magnetic field may be a factor stimulating bacteria [15, 16]. Studies conducted on the effect of the pulsed high magnetic field on microorganisms produced different results. Harte et al [17], who researched *Escherichia coli* and *S. cerevisiae* did not observe any inactivation of the microorganisms as a result of the pulsed magnetic field of 18 Tesla induction. On the other hand, San Martin et al [18] point to a potential *E. coli* deactivation by 50 pulses of 19 Tesla magnetic field, combined with other physical factors – ultrasounds, high hydrostatic pressure and the pulsed electric field. *In vitro* experiments conducted by Lipiec et al [19] on the effect of pulsed magnetic fields of 5, 10, 15 and 20 Tesla on potato pathogens *Ervinia carotovora*, *Streptomyces scabies* and *Alternaria solani* revealed a significant reduction of the number of formed colonies in comparison with the control, however bacteria revealed greater sensitivity than fungi. *In vivo* experiments demonstrated a statistically significant reduction of the number of surviving micro-organism colonies (bacteria and fungi) on germinating oat kernels subjected to the field activity in comparison with the control. It was also observed that the tested magnetic field caused a change of enzymatic processes. The same research team pointed to potential application of the pulsed magnetic field in the process of food stabilization for storage. The use of the oscillating magnetic field for microorganism deactivation is covered by an American patent [21].

Fusarium fungi occur commonly in nature and are polyphagous parasites infesting many plant species. Apart from that they play an important role in pathogenesis of many plant diseases and their metabolites may cause poisoning in people and animals. Therefore, they are the object of interest of phytopathologists all over the world. The imperfect stage of *Fusarium* is numbered among *Deutromycetes* type, *Hyphomycetales* order and *Tuberculariaceae* family. Their sexual forms are *Ascomycota* sac fungi. One of the commonest species occurring in Europe is *F. culmorum* classified to *Discolor* section [22].

The investigations aimed to assess the effect of the pulsed high magnetic field on concentrated suspension of *F. culmorum* fungus spores.

Material and methods

The apparatus used for the experiment had been constructed at the Department of Physics, University of Agriculture in Krakow. The battery of high-voltage capacitors with a total capacity of 1.5 mF was charged to the required voltage by the HV (*high-votage*) power supply with the maximum working voltage 5 kV and 0.3 kW power. Solenoid generating the magnetic field consists of a single layer copper Bitter coil with the external diameter of 30 mm and internal 12 mm. The number of coils is 12. The intercoil insulation is made of epoxy-glass laminate. The coil is placed inside a coven made of beryllium bronze to ensure an adequate mechanical strength. The condenser battery is discharged by means of a solenoid with the spark gap regulated by a step motor. The system is operated by a remote control panel situated, for safety reasons, in a separate room. The magnetic field generated in the solenoid has the character of strongly damped vibration of ca 3 kHz frequency and maximum induction amplitude of about 20T. Precise measurement of the parameters of the field is possible owing to an induction coil positioned inside the solenoid and coupled with digital oscilloscope.

F. culmorum strain used for the presented experiments was isolated from red cabbage in 1997 and purchased from the collection of the Plant Protection Institute in Poznan. The fungus was cultured on solid PDA medium until intensively sporulating mycelium developed.

A concentrated water suspension of the fungus spore ($3.7 \cdot 10^6$ pcs./cm^3) was prepared, 5 cm^3 of it was put into glass test tubes which were then subjected to 5-time, 10-time, 15-time and 20-time action of pulsed 9 Tesla induction magnetic field. The control was provided by the initial suspension, which was not subjected to the magnetic field action. Spore germination obtained after the magnetic field action was tested, therefore, the spore suspension was placed on concave microscope slides, on racks in covered containers filled with water to prevent evaporation. After 24 hours 25 photographs of each preparation were taken using Moticam 1000 camera and Nickon Eclipse E200 microscope with 40 times magnification. The photographs were computer analyzed using UTHSCA Image tool programme. The percentage of germinating spores and an average sprout length were computed.

After subjecting to the magnetic field action the suspension was also inoculated on the solid PDA medium in order to assess mycelial linear growth. The mycelial sporulation was also tested by collecting a 10 mm disc from each Petri dish by the end of culturing to prepare spore suspension in sterile distilled water. Spore concentrations in the suspensions were measured by means of Spekol 21 spectrophotometer made by Carl Zeiss Jena. Analytical wavelength (λ = 460 nm) was determined on the basis of absorption spectra analysis of spore mixture in water. In order to avoid errors due to spore sedimentation, all measurements were conducted at a fixed moment – 5 seconds after spore suspension pouring into the measuring cuvettes. All experiments were conducted in 7 replications. Measuring unreliability was estimated using the standard deviation considering the Student coefficient for the confidence level 0.997.

Results and discussion

The pulsed high magnetic field of 9 Tesla induction weakened germination of *F. culmorum* strain used in the experiments. The obtained results were presented in Figs. 1 and 2. In the control samples after 24 hours an average percentage of germinated spores exceeded 40 %. A significantly lower percentage of germinating spores ca 20 % was obtained at 15 and 20 pulses of the generated magnetic field. Spores subjected to 5-time and 10-time action of the magnetic field were germinating similarly to the control (on average 35 % germinating spores per sample). Sprout lengths measured in the samples were significantly shorter in comparison with the control only for 20 pulses

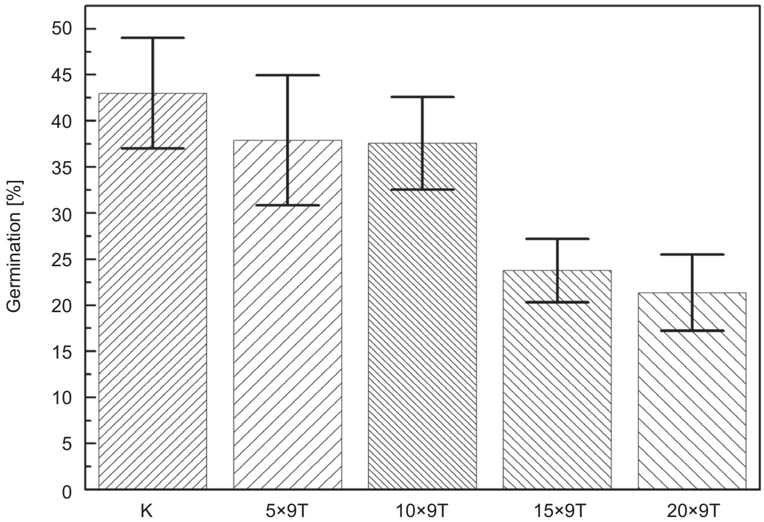

Fig. 1. Number of *F. culmorum* spores germinating after the action of pulsed magnetic field in comparison with the control

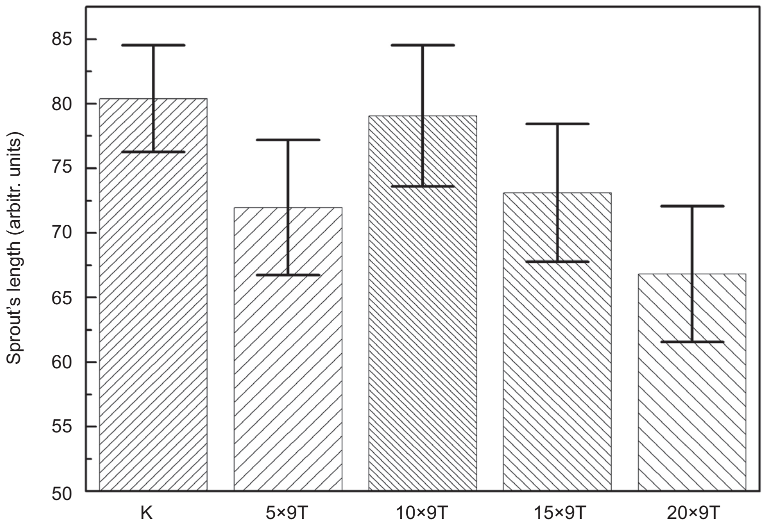

Fig. 2. Average length of *F. culmorum* sprout in the control and in sample after pulsed magnetic field action

of the 9 Tesla field. In the samples subjected to the action of 5, 10 and 15 pulses of the generated field the sprout length was slightly shorter in comparison with the control, but the registered differences were statistically insignificant.

The pulsed magnetic field also weakened the mycelia linear growth of the tested *F. culmorum* strain. Spores subjected to the action of magnetic field inoculated to the solid medium revealed poorer growth, the inoculation process was slower and at the 40th hour of culturing significantly smaller mycelium diameters were noted in comparison with the control. The obtained results were presented in Fig. 3. The mycelial growth rate observed at the logarithmic phase was also weaker in culturing of spores subjected to the action of the magnetic field. Limiting of the linear growth depended on the number

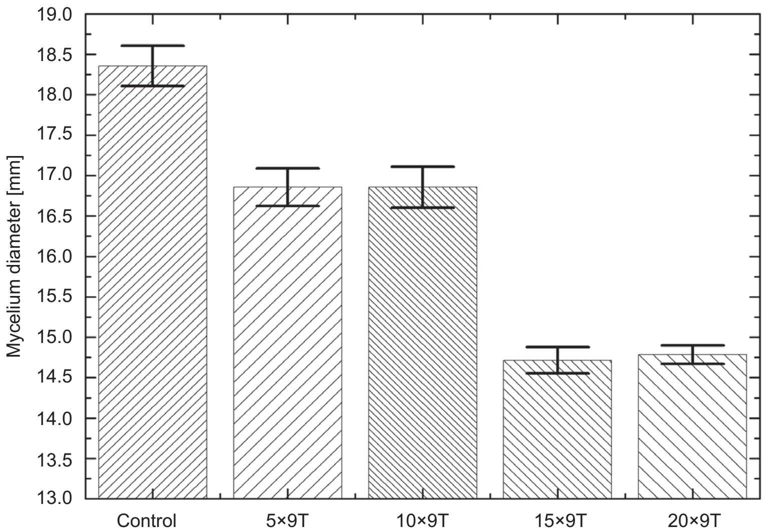

Fig. 3. *F. culmorum* inoculation to solid PDA medium expressed by mycelium diameter obtained 40 hours after suspension inoculation

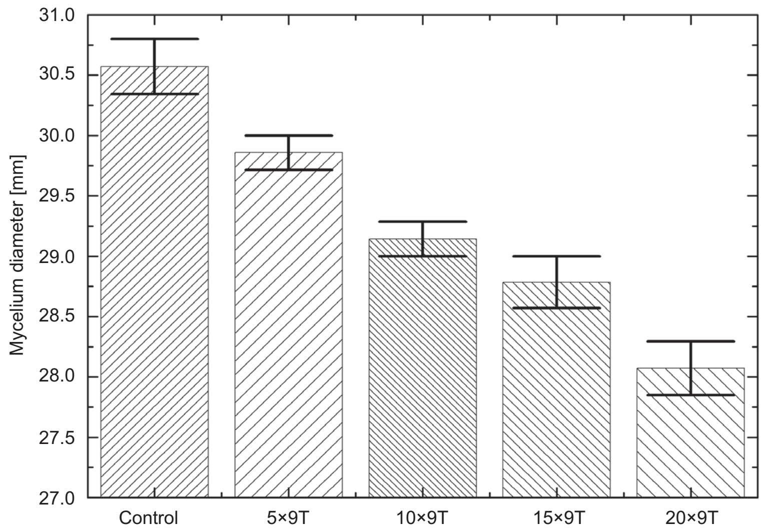

Fig. 4. *F. culmorum* mycelium diameter on solid PDA medium obtained at logarithmic phase of fungus growth

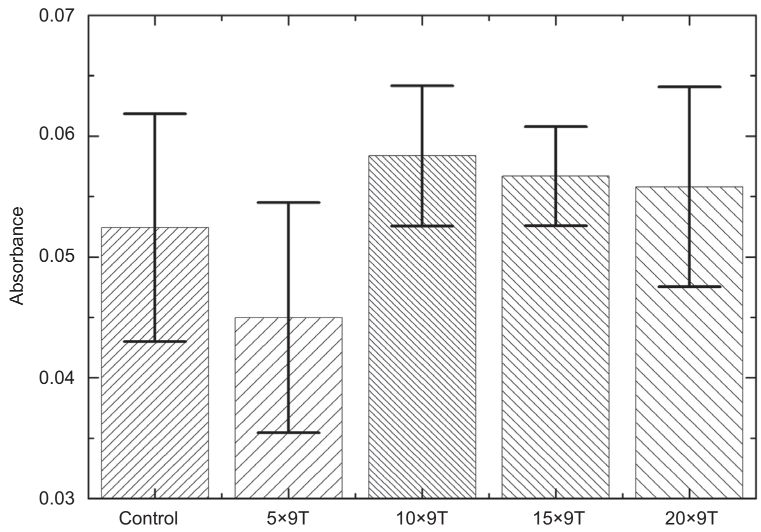

Fig. 5. Effect of pulsed magnetic field on *F. culmorum* sporulation obtained in consecutive culturing

of applied magnetic field pulses. Inhibition of growth was more apparent with growing number of pulses. The results were shown in Fig. 4. The fungus sporulation analyzed after completion of the culturing was uniform. The fluctuations fell within the range of the measuring error (Fig. 5).

Conclusions

1. The pulsed magnetic field limits germination of the tested *F. culmorum* strain. A significant difference was assessed for 15 and 20 pulses of 9 Tesla field.

2. Inoculation and linear growth of the mycelium were weakened in comparison with the control after the action of the pulsed magnetic field.

3. No reduced mycelial sporulation was registered for the tested fungus strain.

4. There is a potential for the application of the tested physical method – the pulsed high magnetic field – for limiting the development and harmfulness of *F. culmorum*.

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Abstrakt: Celem przeprowadzonych badań była ocena wpływ impulsowego, wysokiego pola magnetycznego na skoncentrowaną zawiesinę zarodników grzyba *F. culmorum*, groźnego patogena wielu roślin uprawnych. Zastosowano 5, 10, 15 i 20 impulsów pola magnetycznego o indukcji 9 Tesli. Impulsowe pole magnetyczne ograniczyło kiełkowanie badanego szczepu. Istotną różnicę stwierdzono dla 15 i 20 impulsów. Wzrost liniowy grzybni również ulegał ograniczeniu, co stwierdzono zarówno dla inokulacji, jak i fazy logarytmicznej wzrostu w hodowli szalkowej. Nie zaobserwowano jedynie ograniczenia sporulacji grzybni. Istnieje możliwość wykorzystania testowanej metody fizycznej – impulsowego wysokiego pola magnetycznego – do ograniczania rozwoju i szkodliwości *F. culmorum.*

Słowa kluczowe: impulsowe pole magnetyczne, *F. culmorum*