

**STIMULATION OF PATHOGENICITY
OF ENTOMOPATHOGENIC FUNGI WITH WATER
TREATED WITH LOW-TEMPERATURE,
LOW-PRESSURE GLOW PLASMA
OF LOW FREQUENCY**

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Key words: *Beauveria bassiana* (Bals.) Vuill, biopesticides, *Isaria fumosorosea* (Wize), plazmed water, *Tenebrio molitor* (Col., Tenebrionidae).

Abstract

Similarity of the structure and physicochemical properties of water treated with low-temperature, low-pressure, low-frequency glow plasma and water treated with static magnets of high induction suggests that like magnetically treated water, also plazmed water could stimulate the growth and pathogenicity of entomopathogenic fungi. It was found that distilled water and tap water treated with low-temperature, low-pressure, low-frequency glow plasma for 30 min stimulated pathogenicity of entomopathogenic fungi *Beauveria bassiana* (Bals.) Vuill. and *Isaria fumosorosea* (Wize). The total number of dead *Tenebrio molitor* larvae contacted with those stimulated biopesticides and the rate of their mortality significantly increased. Both kinds of water did not influence the linear growth of the fungi. Thus, plazmed water is suitable for the stimulation of pathogenicity of tested biopesticides.

STYMULOWANIE PATOGENICZNOŚCI ENTOMOPATOGENNYCH GRZYBÓW WODĄ PODDANĄ DZIAŁANIU NISKOTEMPERATUROWEJ, NISKOCIŚNIENIOWEJ PLAZMY JARZENIOWEJ O NISKIEJ CZĘSTOŚCI

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Słowa kluczowe: *Beauveria bassiana* (Bals.) Vuill, biopestycydy, *Isaria fumosorosea* (Wize), plazmowana woda, *Tenebrio molitor* (Col., Tenebrionidae).

Abstrakt

Podobieństwo struktury i właściwości fizykochemicznych wody poddanej działaniu niskotemperaturowej, niskociśnieniowej plazmy jarzeniowej o niskiej częstotliwości i wody poddanej działaniu statycznego pola magnetycznego o wysokiej indukcji sugeruje, że podobnie jak woda poddana działaniu magneŝów, woda plazmowana mogłaby stymulować wzrost i patogeniczność entomopatogennych grzybów. Woda destylowana i kranowa poddana działaniu niskotemperaturowej i niskociśnieniowej plazmy jarzeniowej przez 30 min stymulowała patogeniczność entomopatogennych grzybów *Beauveria bassiana* (Bals.) Vuill. i *Isaria fumosorosea* (Wize) wyrażoną całkowitą liczbą martwych larw *Tenebrio molitor*, które zetknęły się z tymi biopestycydami, oraz szybkością ginięcia. Oba rodzaje wody nie wpływały na liniowy wzrost grzybów. Plazmowana woda nadaje się do stymulowania badanych biopestycydów.

Introduction

Biopesticides, for instance, fungi, attracted attention already in XX century (GUL et al. 2014, ROBERTS and HAJEK 1992, SHAH and PELL 2003). These preparations offer alternative solutions providing meeting restrictive regulations for sustainable maintaining environment and ecological standards. Currently, several preparations containing biopesticides are available on the market. They are used for protection of land and horticulture plantations, orchards, lawns, forests as well as plantations of champignons and urban trees. For their high virulence (epizootics) (AUGUSTYNIUK-KRAM and KRAM 2012), among over 1000 biopesticides entomopathogenic fungi pay considerable attention. For their breeding, storage and applications their growth and pathogenicity are key properties (STRASSER et al. 2010, FERNANDES et al. 2012, KHAN et al. 2012).

In our recent patent and paper (JAWORSKA et al. 2014, 2016) a novel approach to breeding of *Beauveria bassiana* and *Isaria (Paecilomyces) fumosorosea* fungi was demonstrated. It involved stimulation of pathogenicity and growth of those entomopathogenic fungi with a magnetically treated water. These organisms are among those the most commonly used in the plant protection. Thus, the fungi were either cultured on the medium composed of glucose and potato dissolved in water treated with permanent magnets (MW) of ~ 0.5 T induction or their cultures were exposed to the magnetic field of such induction. In both cases, the magnetic field provided an increase in both the linear growth of the fungal colonies and their pathogenicity against the *Tenebrio molitor* (Col., Tenebrionidae) larvae. The observed effects depended on the fungus species – *I. fumosorosea* fungus better responded to both ways of treatment than did the *B. bassiana*, induction of applied magnets and the exposure time of either water or culture to magnets. The extent of evoked by the magnetic field changes in MW could be controlled by the Raman spectroscopy.

Recently (BIALOPIOTROWICZ et al. 2017), preparation and properties of water treated with low-pressure, low-frequency, low-temperature glow plasma (PW) were presented.

Spectral Raman analysis of PW showed that it was identical with MW. It suggested that like MW also PW might be useful in promoting linear growth and pathogenicity of entomopathogenic fungi.

This paper presents a study on a suitability of PW as the stimulant of the growth, and pathogenicity of *Beauveria bassiana* (Bals.) Vuill. and *Isaria fumosorosea* (Wize) entomopathogenic fungi.

Materials and Methods

Materials

Fungi

Beauveria bassiana and *Isaria fumosorosea* isolated from Polish soils were stored under no. 13 in the collection of Department of the Agricultural Environment Protection of the University of Agriculture in Cracow.

Water

Either distilled water or tap water from Bolesławiec of total hardness $129 \text{ mg l}^{-1} \text{ CaCO}_3$; pH 7.1; conductivity $334 \text{ }\mu\text{S cm}^{-1}$; Fe $< 50 \text{ }\mu\text{g l}^{-1}$; Mn $< 5 \text{ }\mu\text{g l}^{-1}$; dissolved oxygen 6.93 mg l^{-1} were used.

Methods

Plazming water

Either distilled or tap water (1000 ml) was placed in the chamber of the reactor (OSZCZĘDA et al. 2009) and exposed to plasma for 30 min. Plasma of 38°C was generated at $5 \cdot 10^{-3}$ mbar, 600 V, 50 mA and 280 GHz frequency. The produced water was stored at ambient temperature in 100 ml closed Teflon containers.

Culturing fungi

The procedure followed that described by Jaworska et al. (JAWORSKA et al. 2016). Thus, the solution of PDA (glucose and potato medium purchased from Biocorp. Warsaw, Poland) in 100 ml of either control distilled water (CDW), control tap water (CTW), plazmed distilled water (DPW) or plazmed tap water (TPW) was sterilized in an autoclave at 120°C for 30 min followed by its cooling to room temperature. That solution was poured into Petri dishes (10 dishes for each broth and 10 control dishes) and after 20 min since it solidified the fungi were inoculated on the medium. Dishes inoculated with fungi were incubated in 25°C. Every experiment was run in 10 replicates.

Test for linear growth and test for pathogenicity

The linear growth was measured with a caliper from the reversed side of the dish.

The test was performed against last development stage larvae of *Tenebrio molitor* from their own cultures. The tests were run in 10 replications involving always 10 larvae. The procedure followed the method published by JAWORSKA et al. (2016). Thus, larvae were completely sunk for 5 s in the fungus colony and transferred into Petri dishes lined with filter paper. Once a day up to the completion of the tests, the filter paper was moisturized with 1 mL of distilled water. Tests were performed after 2nd, 4th, 6th and 8th day of the experiment.

Statistics

The results of the linear growth were statistically elaborated with the single-factor ANOVA method. The means followed by the same letters are not significantly different at $p = 0.05$ according to the Duncan test. Statistical treatment of the results for pathogenicity employed analysis of variance preceded with the Freeman–Tukey transformation.

Results and Discussion

Comparison of the time dependent changes in the Raman spectra of MW and PW showed that production of PW proceeded much faster than production of MW. Moreover, construction of the generating plasma device (OSZCZĘDA et al. 2009) provided a massive production of PW. Therefore, the use of PW for the stimulation of entomopathogenic fungi seemed advantageous over the use of MW. However, identical Raman spectra of MW and PW (BIAŁOPIOTROWICZ et al. 2017) did not rationalize assumption that both waters would have the same macrostructure and, hence, the same functional properties, particularly these suitable for stimulation the fungi growth and entomopathogenicity of the fungi. Indeed, it has appeared that the functional properties of MW and PW were not identical. Figure 1 demonstrates that both fungi grew practically identically on the media prepared with either CDW, CTW or PDW and on the medium prepared with PTW a slight inhibition of the growth could be observed. This behavior contrasted with the formerly reported stimulating effect of MW upon the growth of those fungi (JAWORSKA et al. 2014, 2016).

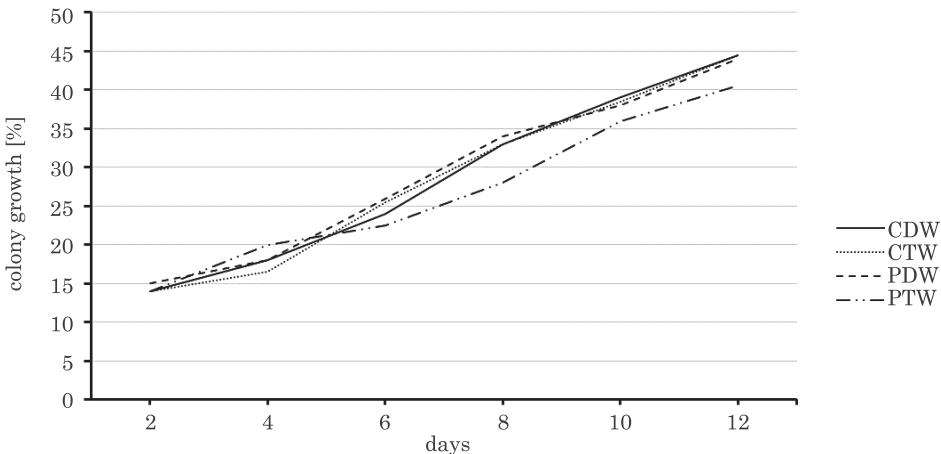


Fig. 1. The lack of the stimulation of the linear growth of *B. bassiana* and *I. fumosorosea* with CDW, CTW, PDW and PTW within 8 day experiments. The course of the curves are identical for both fungi

However, the pathogenicity of *I. fumosorosea* and *B. bassiana* was nicely stimulated by PW and, as shown in Table 1, *I. fumosorosea* was more sensitive to stimulation than *B. bassiana*.

The same difference was noted in the sensitivity of those fungi to stimulation when MW was applied (JAWORSKA et al. 2014, 2016).

Table 1

Observed within 8 day experiments mortality [%] of *Tenebrio molitor* larvae caused by entomopathogenic fungi stimulated in various aqueous media^{a,b}

Water ^c	Fungus											
	<i>B. bassiana</i>						<i>I. fumosorosea</i>					
	days						days					
	3	4	5	6	7	8	3	4	5	6	7	8
CDW	0 ^a	0 ^a	0 ^a	20 ^a	52 ^a	76 ^a	0 ^a	20 ^a	64 ^a	92 ^a	96 ^a	100 ^a
CTW	0 ^a	0 ^a	8 ^{ab}	35 ^a	56 ^a	72 ^a	0 ^a	16 ^a	48 ^a	96 ^a	100 ^a	100 ^a
DPW	0 ^a	4 ^a	20 ^b	48 ^a	75 ^a	81 ^{ab}	4 ^a	40 ^{ab}	64 ^a	96 ^a	100 ^a	100 ^a
TPW	0 ^a	4 ^a	4 ^{ab}	51 ^a	84 ^a	100 ^b	8 ^a	64 ^b	96 ^b	100 ^a	100 ^a	100 ^a

^a means of five repetitions with five larvae in each experiment. The presented data are related to the mortality of 100 larvae of *Tenebrio molitor*

^b the means followed by the same letters in particular columns are not significantly different at $p = 0.05$.

^c CDW – control distilled water; CTW – control tap water; DPW – plazmed distilled water; TPW – plazmed tap water

Regardless PW stimulated or non-stimulated, *B. bassiana* did not kill any larva within 3 first days of experiment. First larvae were killed by stimulated fungus just in the fourth day. PW stimulated *I. fumosorosea* appeared to be more toxic for the larvae. First larvae died in contact with PW stimulated fungus already in the third day. An insight in the time dependent mortality of larvae (Table 1) both fungi performed better when stimulated with TPW.

Although non-stimulated, *I. fumosorosea* also killed all *T. molitor* larvae. The kinetics of the pathogenic effect (Figure 2a and Figure 2b) shows benefits of the stimulation of the fungi with plazmed water. *I. fumosorosea* stimulated with TPW provided a significant pathogenic effect already in the fifth day of experiment that is by one day earlier that did non-stimulated fungus. The total annihilation of the test larvae by *B. bassiana* stimulated with TPW was possible in the 8th day of experiment whereas non-stimulated fungus in that day left around 25% of the alive larvae population.

Above findings indicated an advantage in the stimulation of the fungi with PW over their stimulation with MW. PW prepared by 30 min plazming performed more efficiently than MW treated for 2 h with static neodyme magnets of 0.1 and 0.2 T induction (JAWORSKA et al. 2016).

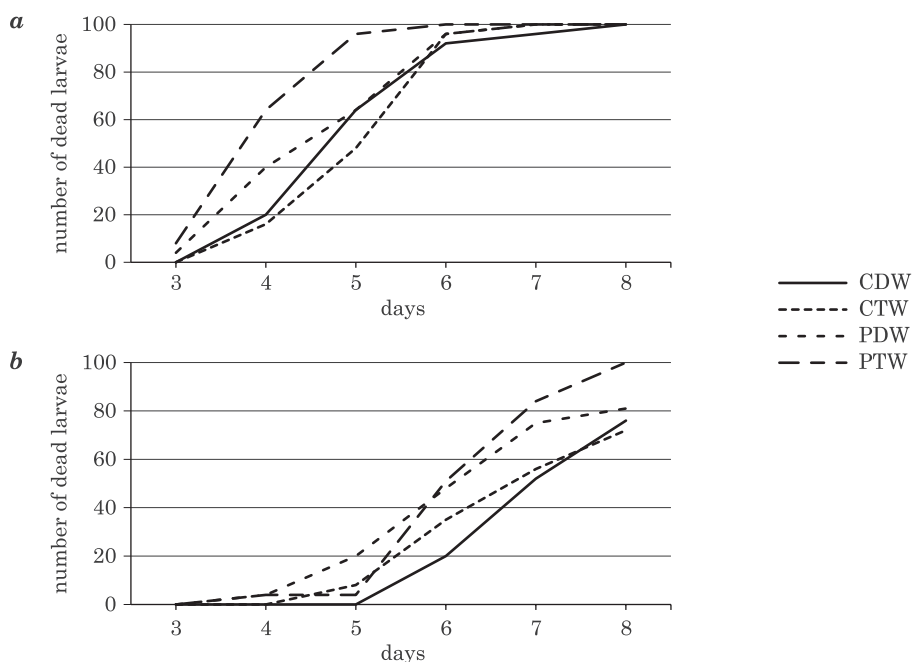


Fig. 2. Kinetics of the development of pathogenicity: *I. fumosorosea* (a) and *B. bassiana* (b) and against *T. molitor* larvae (mortality in %)

Conclusions

1. Low-temperature, low-pressure, low-frequency glow plasma treated water stimulates pathogenicity of entomopathogenic *Beauveria bassiana* and *Isaria fumosorosea*.

2. Low-temperature, low-pressure, low-frequency glow plasma treated water does not stimulate the linear growth of entomopathogenic *Beauveria bassiana* and *Isaria fumosorosea*.

3. *Isaria fumosorosea* is more sensitive to the stimulation of their pathogenicity than *Beauveria bassiana*.

4. Low-temperature, low-pressure, low-frequency glow plasma treated water provides more efficient stimulation of the fungi pathogenicity than formerly presented water treated with static neodyme magnets.

5. Plazmed tap water provides higher efficiency of the stimulation than plazmed distilled water.

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