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THE INFLUENCE OF THE HEAVY METALS ON THE GROWTH OF PATHOGENIC FUNGI

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Abstract

The reaction of the isolates of the pathogenic species *Fusarium oxysporum* Schlecht. and *Pythium debaryanum* (R. Hesse) Nieuwl. to the presence of zinc, copper, lead and cadmium, which were added to the nutritive medium for the determination of their *in vitro* tolerance, measured by the inhibition of the growth of mycelium were studied. The experiments were performed in the laboratory conditions by adding the suspension of the zinc, copper, lead, and cadmium in three different concentrations on the nutritive medium. The influence of the heavy metals on the growth rate of the pathogenic fungi depended on the type of metal and their concentration. The lowest degree of influence was exhibited by the presence of zinc, whereas the highest concentration of cadmium exhibited the highest degree of influence. *Fusarium oxysporum*, the mycelium of which grew considerably slowly in the presence of all metals, exhibited the lowest degree of tolerance to heavy metals, whereas even the lowest concentrations of *Pythium debaryanum* are tolerant to the presence of all metals, even in case of the highest concentrations.

Key words: pathogenic fungi, nutritiv medium, tolerance to heavy metals, the growth of mycelium.

Introduction

The different metals are essentials to the biological activity. Nevertheless, in spite of being toxic or not, all metals can exibit toxicity at different levels. Heavy metals are toxic to all organisms when higher concentrations of them is present in the soil (Arnebrant 1987). The influence of them is unfavourable also to the microorganizms and microbial processes. The influence on the microbial processes in the soil is reflected by the influences on the discomposition of litter, enzyme activity and growth of plants (Whipps 2000), whereas it influences the fungal population by changing the number, composition and diversity of microorganisms (Rudawska 2000). The soil influences the presence of the fungi by its physical and chemical charcteristics (Carter 1987) and, mainly by the increased concentrations of heavy metals which can be present separately (Puhe 2003) or in a combination of several of them (Kieliszewska-Rokicka et al. 2000).

However, the interaction between the heavy metals and pathogenic fungi is conditioned by many factors, the

most significant one being the degree of tolerance of the fungi and their possibility of absorption of the metal suspensions. Nevertheless, under the influence of the adverse environmental effects the different types of the fungi became able to survive in the unfavourable conditions in the forms of scleroses, chlamydospores, or other (Agrios 1997).

The tolerance of some species of fungi to the presence of heavy metals is conditioned by the peculiarities of some species (Van West et al. 2003). It is necessary to be familiar with these peculiarities so that some species can be recommended as very favourable fungus for the future use of these fungi as the bioremediator in the polluted soils, or as the bioindicator (Khan et al. 2000).

Material and Method

The conditions for the growth of pure cultures – Laboratory studies were conducted in the laboratory of Institute of Forestry in Belgrade. The pure cultures of isolates were preserved on the nutritive medium at 23 ± 1°C in the dark.

The preparation of the media with the heavy metals – lead, copper, zinc or cadmium in the concentrations 0, 3, 33 and 100 ppm were prepared of the zinc, copper and cadmium added as sulphates ($ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $CdSO_4 \cdot 8H_2O$) and lead in the form of $Pb(NO_3)_2$, since the sulphate of lead is heavily soluble compound (Dunabeitia et al. 2004). The suspensions of these heavy metals were autoclaved separately in the glass dishes and upon autoclaving, when the temperature decreased at 50°C, they were combined with MEA medium (malt-extract agar, nutritive media which contained 20 g of malt (Sigma-Aldrich, USA) and 20 g of agar (Torlak, Belgrade, Serbia) in Petri dishes. Each Petri dishes contained the nutritive medium and suspensions of heavy metals in the concentrations 3, 33 and 100 ppm.

Inoculation of the prepared medium – When all media were poured, the fragments of fungi were sowed. The fragments (~1 cm) are extracted from the rubs of the colonies of fungus cultures with the sterile knife and used as inoculums in the experimental conditions. All concentrations were set in five repetitions. Petri dishes with the sowed fragments were put in the thermostat at the optimal temperature in the dark.

Measuring of the growth of mycelium – The radial growth of mycelium was monitored by the measurement of two cross-sectional diameters, and the obtained values were compared with the growth on the standard MEA medium. The initial diameter (~1 cm) was subtracted from the subsequent measurements.

Statistical analysis – All the experiments are set in five repetitions (4 colony radius values in each one). The average values and average errors were determined, whereas the statistically significant differences among the variances were determined by the analysis of variance (ANOVA) using SAS v.9.1.3., and averages were compared using Tukey test ($P < 0.05$).

Results

The average daily growth of the fungi on the control medium ranged from 4.67 mm.day^{-1} (*P. debaryanum*) to 10.5 mm/day (*F. oxysporum*). The presence of heavy metals influenced the change growth rate of both fungi, depending on the type of metals and their concentration. The effects of heavy metals on the growth of the fungus species in the pure cultures are presented in the fig. 1–4.

Tolerance to the presence of zinc

Both of fungi that were studied exhibited significant tolerance to the presence of zinc in all concentrations (Fig. 1). The rate of growth of *P. debaryanum* in all concentrations is balanced with the growth on the control medium. This metal influenced the growth of *F. oxysporum* by decelerating the rate of growth in comparison with the control medium, but it depends on the concentration of it.

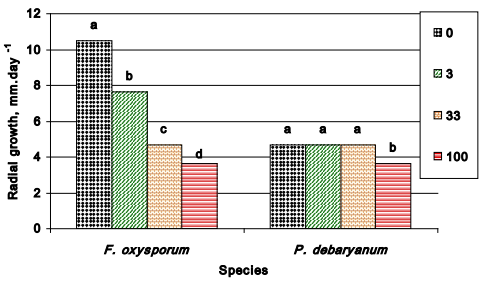

Fig. 1. The rate of growth of mycelium of the pathogenic fungi on MEA medium with the different concentration of zinc in the medium.
For each fungus values fallowed by the same letter are not significantly different (P < 0.05).

Tolerance to the presence of copper

The copper influenced the studied species of fungi in a different way (Fig. 2). The lowest concentrations stimulated the growth of the species *F. oxysporum* and *P. debaryanum*, whereas the highest concentrations caused the significantly slower growth (P < 0.05).

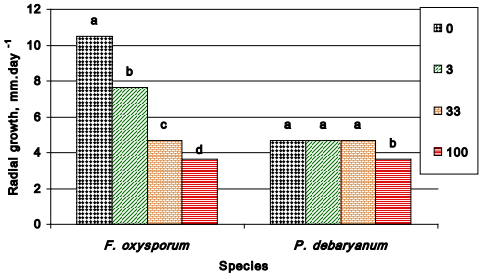

Fig. 2. The rate of growth of mycelium of pathogenic fungi on MEA with different concentrations of copper in the medium.
For each fungus values fallowed by the same letter are not significantly different (P < 0.05).

Tolerance to the presence of lead

The lead influenced the growth of *F. oxysporum* by decelerating the rate of growth in comparison with the control medium, but it depends on the concentration of it. The rate of growth of *P. debaryanum* in highest concentrations is balanced with the growth on the control medium, and the smallest concentration of this metal stimulated the growth (Fig. 3).

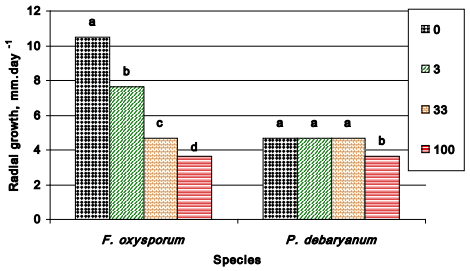

Fig. 3. The growth rate of mycelium of pathogenic fungi on MEA medium with the different concentrations of lead in the medium.
For each fungus values fallowed by the same letter are not significantly different ($P<0.05$).

Tolerance to the presence of cadmium

The fungi exhbited the lowest degree of tolerance to cadmium, since the presence of it caused the total inhibition of growth of *F. oxysporum* in the highest concetration. The lowest concentrations stimulated the growth of the species *P. debaryanum*, whereas the highest concentrations caused the significantly slower growth (Fig. 4).

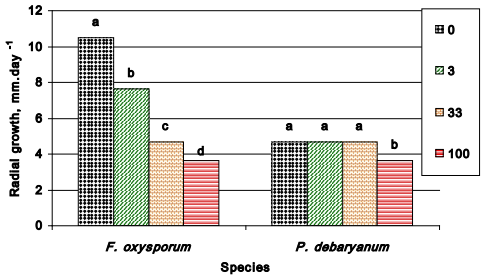

Fig. 4. The rate of growth of mycelium of mycorrhizal fungi on MEA medium with the different concentrations of cadmium in the medium.
For each fungus values fallowed by the same letter are not significantly different ($P<0.05$).

Discussion

In our experiment the average daily growth of both fungi with the presence of the heavy metals altered, which depended upon the type of metal and concentration of it. The most adverse effect was exhibited by cadmium in the concentration 100 ppm.

On the media with the other metals the growth of mycelium of both species of fungi was reported in the lowest concentration, and even the stimulating influence on some species of fungi was observed. The stimulating influence of lead on the growth of *P. debaryanum*, was reported. The presence of all metals in the concentration 33 ppm enabled the growth of fungi. The fungi exhibited the least tolerance to the presence of metals in the highest concentration 100 ppm. The most adverse effect was exhibited by cadmium in this concentration, since the growth of mycelia of fungi *F. oxysporum* is inhibited. Similar results were obtained in the researches conducted by Mittra et al. (2004) and Rai et al. (1995). These scientists studied the influence of lead, copper, zinc and cadmium in the media on the growth of *F. oxysporum*, and the greatest influence on the inhibition of growth was exhibited by cadmium, whereas the least influence was exhibited by zinc. In contrast to these results, Babich and Stotzky (1977) showed in their study of the influence of cadmium on the mycelium *F. oxysporum* that it was tolerant to the presence of cadmium, stating that the positive molecular cell reaction in the exposure to the heavy metals was influenced by the presence of enzymes (Antal et al. 2000, Edel et al. 2000).

Based on the results of our researches, *P. debaryanum* exhibited the highest degree of tolerance to the presence of the heavy metals, since this was the only species which grew on all metals and in all concentrations.

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References

Agrios G. N. 1997. Plant pathology, Academic Press, San Diego, USA. 616 p.

Antal Z., Manczinger L., Szakacs G., Tengerdy R. P., Ferenczy L. 2000. Colony growth. *in vitro* antagonism and secretion of extracellular enzymes in cold-tolerant strains of *Trichoderma* species. Mycological Research, 104 (5): 545–549.

Arnebrant K., Baath E., Nordgren A. 1987. Copper tolerance of microfungi isolated from polluted and unpolluted forest soil, Mycologia, 79 (6): 890–95.

Babich H., Stotzky G. 1977. Effect of Cadmium on Fungi and on Interactions Between Fungi and Bacteria in Soil: Influence of Clay Minerals and pH. Applied and Environmental Microbiology, 33 (5): 1059–1066.

Carter M. R. 1987. Seedling growth and mineral nutrition of Scots pine under acidic to calcareous soil conditions. Soil science, 144 (3): 175–180.

Dunabeitia M. K., Hormilla S., Garcia-Plazaola J. I., Txaterina K., Arteche U., Becerril J. M. 2004. Differential responses of three fungal species to environmental factors and their role in the mycorrhization of *Pinus radiata* D.Don. Mycorrhiza, 14: 11–18.

Edel V., Gautheron N., Alabouvette C. 2000. Ribosomal DNA-targeted oligonucleotide probe and PCR assay specific for *Fusarium oxysporum*. Mycological Research, 104 (5): 518–526.

Khan A. G., Kuek C., Chaudhry T. M., Khoo C. S., Hayes W. J. 2000. Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere, 41: 197–207.

Kieliszewska-Rokicka B., Kurczynska E. U., Leski T. 2000. Physiological activity of ectomycorrhizas in a moderately polluted forest (Ratanica catchment, Southern Poland). Dendrobiology, 45: 47–59.

Mittra B., Gnosh P., Henry S. L., Mishra J., Das T. K., Gnosh S., Babu C. R., Mohanty P. 2004. Novel mode of resistance to Fusarium infection by a mild dose pre-exposure of cadmium in wheat, Plant Physiology and Biochemistry, 42 (10): 781–787.

Puhe J. 2003. Growth and development of the root system of Norway spruce (*Picea abies*) in forest stands-a rewiew. Forest ecology and management, 175 (1–3): 253–73.

Rai B., Chandra A., Singh S. K. 1995. Effects of heavy metals on growth of some pathogenic and non-pathogenic soil microflora. Indian Botanical Society, Madras, 74 (1–4): 35–39.

Rudawska M., Kieliszewska-Rokicka B., Leski T. 2000. Effect of aluminium on *Pinus sylvestris* seedlings mycorrhizal with aluminium-tolerant and aluminium-sensitive strains of *Suillus luteus*. Dendrobiology, 45: 89–96.

Van West P., Appiah A. A., Gow A. R. 2003. Advances in research on oomycete root pathogens. Physiological and Molecular Plant Pathology, 62: 99–113.

Whipps J. M. 2000. Microbial interactions and biocontrol in the rhizosphere. J. of Experimental Botany, 52: 487–511.