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SENSITIVITY OF FUNGAL AND STREPTOMYCETE STRAINS TO TRIFLURALIN AND MAGNETITE NANOPARTICLES

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Abstract

The aim of this study was to estimate the sensitivity of microorganisms, isolated from soil long-term polluted with obsolete pesticides, to magnetite (Fe₃O₄) nanoparticles and fluorinated dinitroaniline herbicide trifluralin, and to evaluate the inhibition activity of these substances. The response of fungi and streptomycete strains to the presence of magnetite nanoparticles in culture media is individual to each microorganism. For the most of studied microorganisms, the addition of trifluralin to culture media had a growth inhibition effect. An exception was the strain *Streptomyces sp.* 0412, which growth was stimulated in the presence of xenobiotic. The negative effect of pesticide was reduced, when before the addition to the culture medium the trifluralin was mixed and incubated with magnetite nanoparticles for 1 hour.

Keywords: *fungi, growth inhibition, magnetite nanoparticles, streptomycetes, trifluralin*

Introduction

Pesticides are extensively used in agriculture as a part of pest control strategies. The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem, and thus, may affect human health by entering in the food chain. Some pesticides stimulate the growth of microorganisms, but other pesticides have depressive effects or no effects on microorganisms. Pesticides interact with soil organisms and their metabolic activities and may alter the physiological and biochemical behavior of soil microbes (Nazarco 2008; Hussain et al. 2009; Lo et al. 2010; Ivantsova et al. 2015; Chowdhury et al. 2008).

Trifluralin is the synthetic fluorinated dinitroaniline herbicide. Trifluralin has been used in agriculture since 1963 for control of a variety of weeds in agronomic and horticultural crops. Adverse impacts of trifluralin on soil microbial diversity and activities have been described by many researchers (Hang et al. 2001; Fernandez et al. 2003; Nowak et al. 2008; Hussain et al. 2009).

Iron-containing systems are an effective remediation technology for contaminated environments with halogenated organic compounds (Moor et al. 2010; Tor et al. 2000; Klupinski & Chin 2003). The recent rapid development of the nanotechnology has generated a considerable number of studies dedicated to the usage of iron-based nanoparticles, which due to unique properties and high surface area, enhances many

of the advantages of traditional iron remediation (Thompson & Bezbaruah 2008; Bai & Wang 2009).

Many recent studies reveal positive impact of nanoparticles on growth and production of secondary products by different microorganisms (Xiu et al. 2010; Kirschling et al. 2010; Chatterjee et al. 2011; Barzan et al. 2014; Darwish et al. 2015). However, information regarding the impacts of engineered nanoparticles on soil microbial communities is currently limited (Dinesh & Hamza 2012; Pawlet et al. 2013; Simonin & Richaume 2015) and appears conflicting.

The aim of our study was to estimate the sensitivity of microorganisms to magnetite (Fe_3O_4) nanoparticles and trifluralin, and to evaluate the inhibition activity of these substances.

Materials and methods

5 strains of micromycetes (1LD, 4D, 5D, 8D, *Penicillium viride*) and 5 strains of streptomycetes (*Streptomyces sp.* 0112, *Streptomyces sp.* 0312, *Streptomyces sp.* 0412, *Streptomyces sp.* 0512, *Streptomyces sp.* 0612) isolated from soil for long-term polluted by obsolete pesticides were used as test-microorganisms.

In our experiment, we used the solution of trifluralin in concentration of 100 mg/L (Variant 1), the solution of magnetite (Fe_3O_4) nanoparticles (diameter of nanoparticles was 20-25 nm) in concentration of 100 mg/L (Variant 2) and the mixture of these solutions in concentration of 100 mg/L each (Variant 3). The mixture of solutions of Fe_3O_4 nanoparticles and trifluralin was incubated for 1 hour before using. As a control, the solid Czapek medium (pH 5.5-5.7 for micromycetes and pH 7.0-7.2 for actinomycetes) have been used.

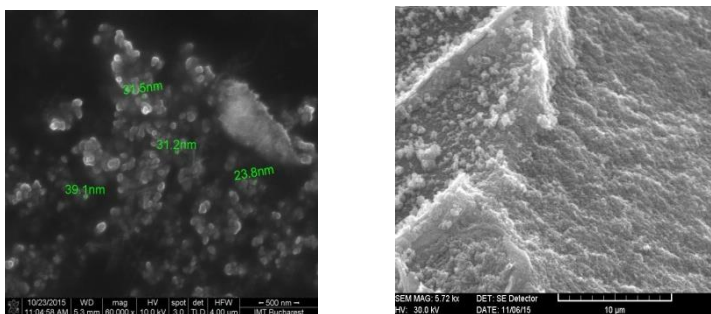


Figure 1. The scanning electron microscope photos of magnetite nanoparticles

For assessing the inhibition activity of the magnetite and trifluralin the agar diffusion method was used. Initially the strains were inseeded in lawn, after that agar disks of micromycetes / streptomycetes colony mass was prepared by using sterile borers. Disks were then aseptically transferred to Czapek plates having magnetite or trifluralin or the mixture of trifluraline and magnetite solutions.

Diameter of growth zones of the strains of fungi / streptomycetes was measured at the 8th day. Inhibition activity (IA, %) of trifluralin and Fe_3O_4 nanoparticles was calculated in percent of inhibition of growth, compared to the control, according to the method proposed by Pandey et al. 1982.

Results and discussions

The inhibition activity values (%) and sensitivity of fungal strains to the magnetite nanoparticles and trifluralin were represented in Table 1. The obtained results showed that the strain 1LD did not react to the presence of iron oxide nanoparticles in the culture medium, i.e. the diameter of growth zones was at the control level. On 4D and 5D fungal strains magnetite nanoparticles had a weak inhibitory action – their growth was inhibited only on 0.47 and 2.55% respectively, and the growth of micromycetes 8D and *P. viride* was even stimulated by nanoparticles of iron oxide.

Table 1. The sensibility of fungal strains to magnetite nanoparticles, trifluralin, and mixture of trifluralin and magnetite nanoparticles, on the 8th day of growth

| Fungal strain | Inhibition activity (%) | | |
|------------------|--|-----------------------------|--|
| | Medium Czapek + Fe ₃ O ₄ nanoparticles | Medium Czapek + trifluralin | Medium Czapek + trifluralin + Fe ₃ O ₄ nanoparticles |
| 1LD | 0.00 ± 0.02 | 5.29 ± 0.25 | 1.18 ± 0.04 |
| 4D | 0.47 ± 0.02 | 16.36 ± 0.21 | 10.28 ± 0.23 |
| 5D | 2.55 ± 0.05 | 7.08 ± 0.68 | -0.47 ± 0.01 |
| 8D | -0.39 ± 0.02 | 6.61 ± 0.15 | 5.06 ± 0.11 |
| <i>P. viride</i> | -2.86 ± 0.52 | 10.48 ± 1.45 | -2.86 ± 0.37 |

Experimental variant, where micromycetes were grown on the medium with addition of trifluralin, showed that all strains were sensitive, in particular the micromycetes 4D and *P. viride*, where the growth inhibition percent was 16.36 and 10.48% respectively. Experimental variant, when the mixture of trifluralin and nanoparticles solutions was used, showed that inhibitory action of trifluralin was reduced by iron oxide nanoparticles, as in the case of 1LD, 4D, and 8D fungi strain, or even it was removed completely, as in the case micromycetes 5D and *P. viride*, where the diameter of growth zones exceeded the control.

Also, it should be mentioned that the solution of iron oxide nanoparticles had a stimulating effect on the formation and maturation of the fungal spores (Figure 2).

In Table 2 there were presented the values of IA, obtained by measuring the diameter of streptomycete colonies in the control and experimental variants at the 8th day of growth. The streptomycete strains had an individual reaction to the solution of magnetite nanoparticles added to the culture medium. The strain *Streptomyces sp.* 0412 has reacted by an active increase in the presence of iron oxide nanoparticles, the diameter of growth zones exceeded the control by 18% (Table 2).

The streptomycetes proved to be more resistant to trifluralin than fungi, and in the case of the strain *Streptomyces sp.* 0412 we obtained even a growth stimulation in the presence of trifluralin. When the streptomycetes were cultivated in the presence of the mixture of trifluralin and magnetite nanoparticles, in general, it was observed the same pattern – that iron oxide nanoparticles diminishes the negative effect of the xenobiotic.

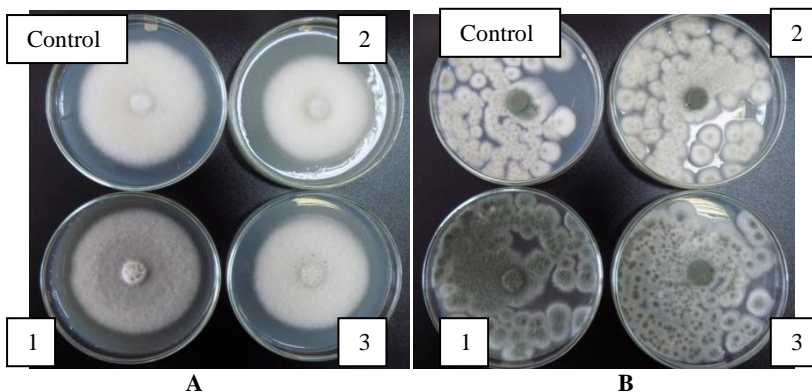


Figure 2. The growth of micromycetes *P.viride* (A) and 4D (B) on Czapek medium with addition of Fe₃O₄ nanoparticles (1), trifluralin (2) and the mixture of trifluralin and Fe₃O₄ nanoparticles (3)

Table 2. The sensibility of streptomycete strains to magnetite nanoparticles, trifluralin, and mixture of trifluralin and magnetite nanoparticles, on the 8th day of growth

| Streptomycetes strain | Inhibition activity (%) | | |
|------------------------------|--|-----------------------------|--|
| | Medium Czapek + Fe ₃ O ₄ nanoparticles | Medium Czapek + trifluralin | Medium Czapek + trifluralin + Fe ₃ O ₄ nanoparticles |
| <i>Streptomyces sp.</i> 0112 | -5.56 ± 0.21 | 0.00 ± 0.06 | -4.17 ± 0.28 |
| <i>Streptomyces sp.</i> 0312 | 0.00 ± 0.14 | 1.52 ± 0.06 | 0.00 ± 0.05 |
| <i>Streptomyces sp.</i> 0412 | -18.57 ± 2.63 | -5.71 ± 0.45 | -8.57 ± 0.67 |
| <i>Streptomyces sp.</i> 0512 | 3.70 ± 0.17 | 3.70 ± 0.13 | 3.70 ± 0.17 |
| <i>Streptomyces sp.</i> 0612 | 1.28 ± 0.05 | 7.69 ± 0.28 | 1.28 ± 0.06 |

Conclusions

Each fungal and streptomycetes strain had an individual reaction to the solution of magnetite nanoparticles added to the culture media. Most of the studied microorganisms were found to be sensitive to the trifluralin. The presence of xenobiotic in the medium had a growth inhibition effect, with the exception of strain *Streptomyces sp.* 0412, for which the stimulation was determined. In all cases, when the mixture of trifluralin and magnetite nanoparticles was added, the same phenomenon was repeated – namely, the negative effect of trifluralin was reduced.

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