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Original Research

The Effect of Manganese Ions on Development and Antagonism of *Trichoderma* Isolates

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Abstract

This work presents results on the effect of manganese ions on the development and biological activities of the fungal antagonists *Trichoderma harzianum, Trichoderma pseudokoningii* and *Trichoderma viride*. The effects of manganese on linear growth of *Trichoderma* spp. depend on the metal concentration and the fungal species. *T. harzianum* proved the most sensitive fungus. On the other hand, none of the applied manganese ion concentrations inhibited mycelial growth of *T. viride*. At the highest concentration of 800 ppm, manganese ions caused a weakening of the conidial germination of *T. harzianum* and *T. viride*. An increase in manganese ion concentrations had a negative influence on the effect of *T. harzianum* on *Botrytis cinerea* and *Rhizoctonia solani.*

Keywords: *Trichoderma* spp., manganese ions, mycelial growth, spore germination, biological activity

Introduction

Manganese is an element common in the Earth's crust. The presence of manganese depends both on its content in the bedrock and on the soil-forming process, as well as on climatic conditions, topographic features and plant cover. Average contents of this metal for various kinds and types of soil range between 100 and 1300 ppm [1].

Quantitative and qualitative changes in soil microorganisms, often leading to changes in soil biological activity, are observed in soil subjected to the effect of heavy metals. Metal ions influence fungal growth rate, sporulation and enzymatic activities. Many elements described as heavy metals play important roles as biogenic ions necessary for proper functioning of microorganisms; however, they may be toxic at higher concentrations [2-4].

Trichoderma species are common soil fungi. Their role in agrocenoses involves limiting dangerous pathogens which cause seedling rot, root rot and withering that lead to dieback. It has been demonstrated that *Trichoderma* spp. are good antagonists of fungi from the genera *Pythium*, *Fusarium*, *Rhizoctonia* and *Sclerotinia*. Antagonism of *Trichoderma* spp. is based on diverse mechanisms such as antibiosis, mycoparasitism and competition. In these mechanisms the basic role is ascribed to fast mycelial growth, sporulation and the production of antibiotics and extracellular enzymes [5-10]. Reports show that biotic and abiotic factors of the environment may influence the effectiveness of *Trichoderma* spp. against phytopathogens [4, 11].

Due to the important role of antagonistic fungi in the soil environment, investigations were conducted on the effect of manganese ions on growth and biological activities of *Trichoderma* isolates. The aim of this work was to determine the influence of manganese ions at various concentrations on the following features of the examined fungi: growth rate and mycelium development, conidial germination and antagonistic activities towards three pathogens: *Botrytis cinerea*, *Fusarium solani* and *Rhizoctonia solani*.

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Material and Methods

The research material was made up of three antagonistic fungal isolates obtained from the collection of the Department of Agricultural Environment Protection at the Agricultural University of Kraków: *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai and *Trichoderma viride* Pers. ex Gray, and pathogenic fungal species including *Botrytis cinerea* Pers., *Fusarium solani* Sacc., and *Rhizoctonia solani* Kühn. The antagonistic isolates were selected previously based on their effectiveness, while isolates of pathogenic fungi were obtained from diseased legumes. The effect of manganese ions ($MnSO_4x5H_2O$) was studied at the following concentrations: 100, 200, 400, 600, 800 ppm ($mg \cdot kg^{-1}$).

The *in vitro* effect of manganese ions on fungal linear growth was examined with the poisoned medium method [12]. A solid glucose-potato medium (PDA) was prepared with the addition of manganese ions. The media were inoculated with agar discs (5 mm in diameter) overgrown with two-week-old mycelium of *Trichoderma*. The control was made up in a medium without manganese ions. The results obtained were expressed as the inhibition coefficient of linear fungal growth, calculated according to Abbott's formula [12].

Germination capacity of *Trichoderma* conidia in the presence of manganese ions was evaluated with the method described by Burgieł [13]. In solutions of manganese in water, a suspension was prepared from conidia sampled from two-week-old cultures. The germination process was stopped by adding a drop of formalin after 48 hours of incubation at 21°C. The degree of conidial germination was estimated according to a scale, and the index of conidial germination was calculated based on the results obtained [13].

The results of the experiments were verified statistically with variance analysis assumed for two-factor experiments (factor A – concentration of the manganese ions, factor B – *Trichoderma* species). Significance of differences was verified with Duncan's test.

The correlations between antagonistic fungi treated with the factors studied and *Botrytis cinerea, Fusarium solani* and *Rhizoctonia solani* were defined with the biotic series method following Mańka [14]. Inocula of *Trichoderma* isolates were obtained from colonies grown for 2 weeks on PDA medium with manganese ions. Inocula of pathogens and inocula of *Trichoderma* isolates for control experiments were sampled from cultures grown on the medium without metal ions. *Trichoderma* was inoculated to the centre of a Petri plate with PDA medium at a distance of 2 cm apart from the pathogenic fungus. After 10 days of incubation, each combination was assessed on a scale regarding three parameters: extent to which one fungal colony was surrounded by the other, inhibition zone and colony diminishing. The highest mark on the 8-point-scale denoted a complete lack of fungal growth. A "+" sign (positive effect) was used in the case of *Trichoderma* domination, a "–" sign (negative effect) for the domination of the pathogenic fungus, and "0" was given if no prevalence of any colony could be observed. Obtained signs provided jointly an individual biotic effect (IBE) illustrating the influence of individual *Trichoderma* isolates on the growth of the pathogen.

All the above experiments were carried out in 4 replicates.

Results

Based on the experiments it was found that the effect of manganese ion upon linear growth of *Trichoderma spp*. depended on both the metal concentration and the fungal species (Table 1). The *T. harzianum* isolate was the most sensitive, its colony growth rate declined significantly already at of 400 ppm of manganese. Inhibition of mycelial growth at the highest concentration of manganese ions was 27.2% (Fig. 1). The growth rate of *T. pseudokoningii* decreased significantly when the medium was supplemented with manganese at 600 and 800 ppm (Table 1). Inhibition of *T. pseudokoningii* growth at the highest tested manganese concentration reached 10.9% (Fig. 1). On the other hand, the growth rate of *T. viride* was unaffected by the influence of manganese ions (Table 1), none of the applied concentrations caused an inhibition of mycelial growth.

Manganese ions at the highest concentration of 800 ppm caused a weakening of conidial germination in the case of *T. harzianum* and *T. viride* (Table 2). A different response was observed for *T. pseudokoningii*, where manganese ions acted positively on conidial germination at 600 and 800 ppm.

The effect of manganese ions on the interaction between *Trichoderma* isolates and plant pathogens was determined on the basis of individual biotic effect (IBE). On the applied assessment, scale "0" denotes uniform development of both fungal colonies, whereas the highest degree on the scale (+8) denotes that the *Trichoderma* colony completely inhibited pathogen development [14].

In the control experiment, the tested *Trichoderma* isolates revealed the strongest antagonistic properties

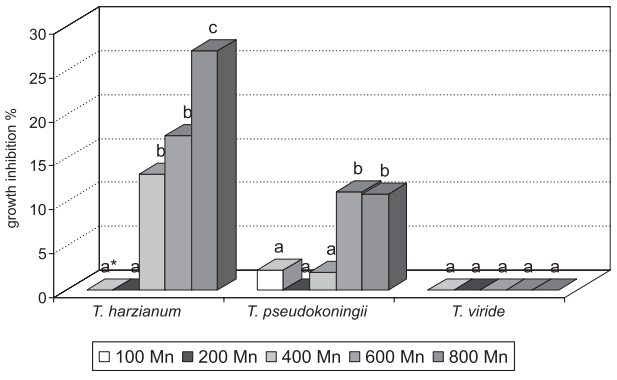

Fig. 1. Inhibition of the mycelial growth of *Trichoderma* spp. exposed to manganese ions (*columns marked with different letters differed significantly according to Duncan's test at p = 0.05).

Table 1. Growth rate of *Trichoderma* spp. exposed to manganese ions (*means marked with different letters differed significantly according to Duncan's test at p = 0.05).

Concentration of Mn ions [ppm]	Growth rate [mm/day]		
	Trichoderma harzianum	*Trichoderma pseudokoningii*	*Trichoderma viride*
100	99.5 cd*	102.3 de	99.8 de
200	98.8 cd	106.7 e	100.2 de
400	84.1 b	102.6 de	99.4 cd
600	80.4 b	92.4 c	99.0 cd
800	70.6 a	92.6 c	98.9 cd
Control (without ions)	100.4 de	106.9 e	99.7 de

Table 2. Conidial germination of *Trichoderma* spp. exposed to manganese ions (*means marked with different letters differed significantly according to Duncan's test at p = 0.05).

Concentration of Mn ions [ppm]	Index of conidial germination [%]		
	Trichoderma harzianum	*Trichoderma pseudokoningii*	*Trichoderma viride*
100	7.6 c-e*	7.7 c-f	8.4 e-g
200	7.2 bc	7.5 c-e	8.6 g
400	7.8 c-g	6.2 a	8.1 d-g
600	7.4 cd	8.0 d-g	7.8 c-g
800	6.6 ab	8.0 d-g	7.1 bc
Control (without ions)	7.6 c-e	7.1 bc	8.5 fg

Table 3. Individual biotic effect (IBE) of *Trichoderma* spp. on phytopathogens when exposed to manganese ions.

Concentration of Mn ions [ppm]	*T. harzianum*			*T. pseudokoningii*			*T. viride*		
	*B. c.	F. s.	R. s.	*B. c.	F. s.	R. s.	*B. c.	F. s.	R. s.
100	+5	+6	+6	+6	+7	+7	+6	+6	+6
200	+3	+6	+6	+6	+7	+6	+6	+6	+7
400	+2	+7	+6	+6	+7	+6	+6	+7	+7
600	+4	+6	+5	+5	+7	+5	+6	+6	+7
800	+1	+7	+4	+6	+7	+7	+6	+7	+7
Control (without ions)	+5	+7	+6	+4	+7	+6	+5	+7	+7

*B. c. – *Botrytis cinerea*, F. s. – *Fusarium solani*, R. s. – *Rhizoctonia solani*

towards *F. solani* and *R. solani*, while they most poorly limited the growth of *B. cinerea* (Table 3).

Increase in manganese ion concentration had a negative influence on the effect of *T. harzianum* on *B. cinerea* and *R. solani*. It was testified by IBE decline even by 4 points in the case of *B. cinerea*. On the other hand, manganese ions stimulated the antagonistic effect of *T. pseudokoningii* towards *B. cinerea.*

Discussion of Results

Our investigations revealed that manganese ions influence the growth, conidial germination and antagonistic activities of the examined *Trichoderma* isolates. The responses of *Trichoderma* spp. to manganese ions depended on both the metal concentration and the fungal isolate.

A diverse effect of manganese ions on fungi was also observed by Przeździecki et al. [15]. They found that manganese ions limited sporulation of *Fusarium culmorum* and *F. avenaceum*. The biogenic elements magnesium, manganese and copper had a positive effected on growth of *Aspergillus flavus*, *Botrytis cinerea* and *Beauveria bassiana* [16-18]. Studies on the effect of manganese ions at concentrations between 0 and 500 ppm on mycorrhizal fungi such as *Cenococcum graniforme*, *Pisolithus tinctorius*, *Suillus luteus* and *Thelephora terrestris* revealed that these fungi tolerated the presence of manganese ions in the medium [19]. On the other hand, application of manganese ions at concentrations between 25 and 50 $g \cdot l^{-1}$ apparently inhibited the growth of the phytopathogenic fungi *Rhizoctonia solani*, *Botrytis cinerea* and *Sclerotinia sclerotiorum*, whereas *Alternaria alternata*, *Fusarium oxysporum* and *F. culmorum* proved more tolerant to these concentrations [20].

Fungi of the genus *Trichoderma* also respond to the presence of metals in the environment. A variable effect of mineral feeding on *T. viride* was reported by Sierota [21] who observed stimulation of conidiation by manganese ions. Kredics et al. [22, 23] found that metal ions; including manganese; have an important influence on mycelial growth of *T. aureoviride*, *T. harzianum* and *T. viride* strains. Metal ions may also influence the enzymatic activities of *Trichoderma* spp., which can cause changes in the antagonistic effect of these fungi on plant pathogens. It was noticed that manganese ions led to changes in the quantities of extracellular enzymes produced by *Trichoderma* spp.: endoxylanase activities declined under the influence of manganese ions, whereas the same ions enhanced cellobiohydrolase activities [24]. Furthermore, mutants tolerant to manganese could also be isolated in this study.

Certain commercial fungicides, e. g. Maneb and Mancozeb, contain manganese. Fungicid Dithane M-45 WP (active ingredient 80% Mancozeb) at concentration 100 ppm significantly inhibiting of mycelial growth of *Trichoderma* strains [25]. Therefore, data about the effects of manganese on *Trichoderma* strains are of special importance from the point of view of integrated pest management, i. e. if *Trichoderma* as a biocontrol agent is planned to be applied in combination with manganese-containing fungicides.

The presented results reveal that while affecting the growth and germination of *Trichoderma* conidia, manganese ions may cause changes in the antagonistic effect of these mycoparasites on plant pathogens.

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