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REACTION OF FUNGI OF *Trichoderma* GENUS TO SELECTED ABIOTIC FACTORS

Joanna Dłużniewska
Department of Agricultural Environment Protection, Agriculture University of Cracow, Poland

ABSTRACT

The paper presents results of research into the effect of magnesium and zinc ions, Miedzian 50 WP and Topsin M 70 WP fungicides and Dispersive Afalon 450 SC and Racer 250 WP herbicides on the development and bioactivity of the following isolates of antagonistic fungi: *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai and *Trichoderma viride* Pers. ex Gray. There was defined an effect of the said factors in different concentrations on mycelium growth and germination of *Trichoderma* genus fungi spores and on their antagonistic activity towards fungi pathogenic for plants. It was observed that the factors studied affected the *Trichoderma* genus fungi tested, with reactions of fungi depending on the kind of factor, its concentration and on the fungus isolate. There were recorded some powerful fungistatic properties of zinc ions in concentrations of 1000 and 3000 ppm and Topsin M 70 WP, Dispersive Afalon 450 SC and Racer 250 WP in the dose of 100 ppm. Microbiological pea seed dressing against phytopathogens with *Trichoderma* genus fungi untreated with the abiotic factors tested significantly improved the health status of seedlings. Seed-dressing, on the other hand, with spores of the fungi studied grown on media including the said factors did not protect plants from pathogenic fungi infection.

Key words: *Trichoderma* genus, magnesium, zinc, fungicides, herbicides, mycelium development, antagonistic activity.

INTRODUCTION

Trichoderma genus is not only one of the most common, isolated from various habitats, soil fungi but also known to be secreting to the environment various secondary metabolites of a wide spectrum of effects on various fungal groups, especially pathogenic fungi. Reports by numerous authors show that *Trichoderma* spp. fungi are powerful antagonists of parasitic soil fungi of the following genera: *Pythium, Verticillium, Gaeumannomyces, Sclerotinia, Rhizoctonia* and *Fusarium* inflicting plants with root-rots of seedlings, root rot and wilt which lead to plant withering. For those reasons they are considered to be a biotic factor conditioning soil resistance

[9,22,23]. However the fungi, just like all the live organisms, depend on the effects of external factors which can modify their morphological characteristics as well as physiological functions. Such factors can include e.g. soil contamination with heavy metals, fungicides and herbicides. A toxic effect of metals on microorganisms growth and activity can result from binding of metals with covalent bonds with various biomolecules. Metals can also show a non-specific effect on many cellular structures and influence metabolic processes by enzymes blocking [2,19]. Also plant protection chemicals developed to affect other organisms (plants, insects, pathogenic fungi) are compounds of a high bioactivity and can affect the development of microorganisms and a course of bioprocesses in soil [1,7,20,26]. Fungicidal mechanisms of fungicides are related with disturbing physiological functions of fungi. Fungicides containing copper ions block enzymes active in the energy processes. On the other hand, benzimidazol fungicides (e.g. methylthiophene) inhibit mitotic division of the cell nucleus inactivating polymerisation of protein sub-units [2]. Although most chemical compounds accumulating in soil undergo decomposition and detoxification, there are reports of disturbed microbiological balance in soil. Applying high doses of benzimidazol fungicides against *Gaeumannomyces graminis* fungus increases the cereal infection with rhizoctonia root rot. This phenomenon is a result of deterioration of antagonistic microflora, mainly *Trichoderma* and *Penicillium* genera fungi [2]. Also herbicides, besides their herbicidal effect, influence the species composition and interactions between fungal species [20,31].

An essential and relatively poorly researched is an effect of chemical compounds introduced into the environment on non-pathogenic soil microflora and also on interactions between antagonists and fungi pathogenic for plants, which makes the present research into the effect of abiotic factors on antagonistic fungi of *Trichoderma* genus justifiable.

The present paper investigated selected isolates of the following fungi: *Trichoderma harzianum*, *Trichoderma pseudokoningii* and *Trichoderma viride* and metal ions, fungicides and herbicides and aimed at defining the effect of the said factors in different concentrations on:

- development of mycelium of the isolates studied,
- spore germination,
- bioactivity of *Trichoderma* genus fungi towards three pathogens *Botrytis cinerea, Fusarium solani and Rhizoctonia solani* based on lab tests and infection experiments.

MATERIAL AND METHODS

The research material was made up of the following three antagonistic fungi isolates obtained from the collection of Department of Agricultural Environment Protection, Agriculture University of Kraków: *Trichoderma harzianum* Rifai, *Trichoderma pseudokoningii* Rifai, *Trichoderma viride* Pers. ex Gray. and pathogenic fungi species, including: *Botrytis cinerea* Pers., *Fusarium solani* Sacc., *Rhizoctonia solani* Kühn. The antagonistic isolates selected in earlier research showed most effective, while isolates of pathogenic fungi were obtained from diseased legumes. There was studied the effect of ions of magnesium ($MgSO_4$) and zinc ($ZnCl_2$), fungicides Miedzian 50 WP (50% copper oxychloride) and Topsin M 70 WP (70% methylthiophene) and herbicides Dispersive Afalon 450 SC (45% linuron) and Racer 250 WP (25% fluorochloride). Concentrations of ions were as follows: Mg 10, 50, 100, 250, 500 ppm and Zn 50, 100, 200, 300, 1000, 3000 ppm. Pesticide doses were defined as converted into active ingredient (a.i). Three solutions of chemical preparations were used: 1, 10, 100 ppm.

The investigations into the effect of abiotic factors on the fungal linear growth *in vitro* were carried out with the poisoned medium method [3]. A solid glucose-potato medium (PDA) was prepared with an addition of respective metal ions or pesticides. The media were inoculated with agar disc 5 mm in diameter overgrown with a two-week *Trichoderma* spp. mycelium. The control was made up of dishes filled up with medium with sterile water instead of the factors studied. The results obtained were expressed as a linear fungus growth inhibition coefficient calculated following Abbott's formula [3].

Germination capacity of *Trichoderma* genus fungi spores in the environment of the factors studied was evaluated with the method described by Burgieł [4]. In water solutions of metal ions or pesticides a suspension was prepared from spores sampled from two-week cultures. After 24 hours of incubation at 21°C the germination process was stopped by adding a drop of formalin. Next a degree of spores germination was estimated following the scale and, based on the results obtained, the index of spores germination was calculated [4].

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

The correlation between antagonistic fungi treated with the factors studied and *Botrytis cinerea, Fusarium solani, Rhizoctonia solani* pathogens were defined with the biotic series method following Mańka [17]. Inoculum of *Trichoderma* genus fungi was obtained from colonies growing for 2 weeks on the PDA medium with an adequate metal ion or fungicide in the concentration of 10 ppm of a.i. or herbicide in the concentration of 100 ppm of a.i. Inoculum of pathogens and inoculum of *Trichoderma* genus fungi for control combination were sampled from cultures grown on the medium with no factors studied added. All the above experiments were carried out in 4 replications.

In order to define the effect of metal ions and pesticides on the effectiveness of seed dressing in pea (*Pisum sativum* L. Iłówiecki cultivar) with *Trichoderma* spp. under disease threat posed by pathogenic fungi, a pot experiment was carried out with a modified method by Łacicowa [14]. Effective protection offered by microbiological seed dressing was investigated for 3 pathogens: *B. cinerea, F. solani, R. solani.* Inoculum of pathogenic fungi was made up of two-week cultures grown on PDA medium. Dressing surface-disinfected pea seeds involved the use of water suspension of spores ($2x10^5$ spores per cm^3) prepared from *Trichoderma* spp. cultures grown on the medium with metal ions or pesticides (fungicides in concentration of 10 ppm of a.i., herbicides in concentration of 100 ppm of a.i.) or on the medium with no factors studied. To compare the effectiveness of biological and chemical dressing, standard dressing Oxafun T (37.5% thiuram and 37.5% carboxine) was used in the dose of 2 g per 1 kg of seeds. In the control combination undressed seeds were being soaked in sterile distilled water. A combination was also made in which undressed seeds were sown into sterile quartz sand non-infected with pathogens. In each combination 15 seeds were sown in 4 replications. The experiment was carried out exposed to twelve-hour light at 21±1°C. 3 weeks since the experiment was set up the number of seedlings was defined and their health status - evaluated. To evaluate the infection of the root system and root crown, the following scale was adopted: 0 – healthy plants, 1 – root system 1-10% infected, 2 – root system 11-25% infected, 3 – root system 26-50% infected, 4 – heavy infection – broad necrosis on most roots, covering over 50% of their surface, seedlings withering. The results of the analysis were converted into infection index [4]. On the day the experiment was completed the fungi were re-isolated.

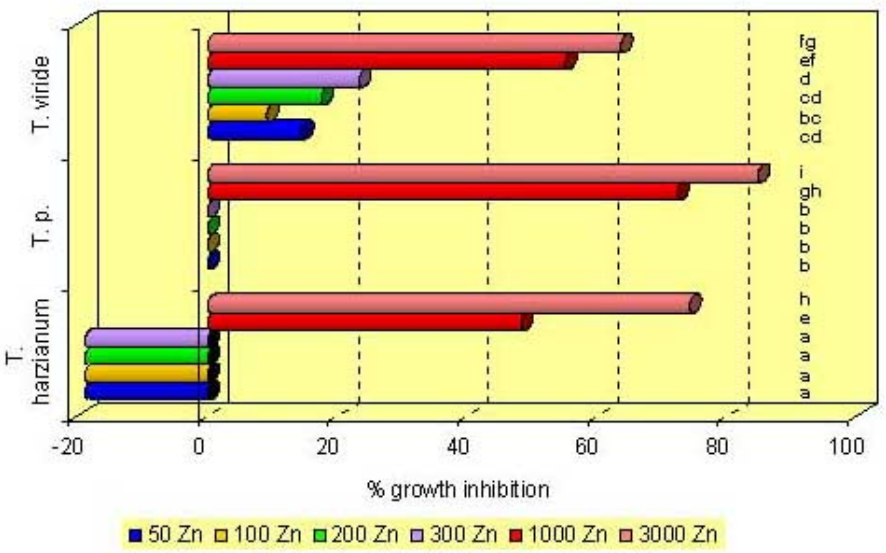
The results of the experiments were verified statistically with variance analysis assumed for three-factor experiments (factor A – seed dressing method, factor B – pathogen species, factor C – *Trichoderma* species). Significance of differences was verified with Duncan's test. Statistical calculations were made with Stat Skierniewice software.

RESULTS

The experiments showed that the effect of the abiotic factors studied on the linear growth of *Trichoderma* spp. depended on the kind of factor and its concentration and the fungus species.

None of the magnesium concentrations applied resulted in a significant inhibition of *Trichoderma* spp. colony growth. The research into the effect of zinc ions on a linear growth of *Trichoderma* spp. showed significant differences in the effect of metal ions on the isolates tested (Fig. 1). The highest growth inhibition by zinc ions was observed for *T. viride* fungus. A different reaction to the presence of metal in the medium was recorded in *T. harzianum* fungus. Zinc ions ranging from 50 to 300 ppm concentration stimulated *T. harzianum* colony growth by 18.9%. A significant growth inhibition of all the fungi was observed only after the application of high zinc concentrations (1000 and 3000 ppm).

Fig. 1. *Trichoderma* spp. growth inhibition when exposed to zinc ions (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)



The results of the investigations into the effect of selected pesticides on the *Trichoderma* genus fungus growth inhibition are given in Figs. 2-4. The most powerful fungistatic effect was observed for Topsin M preparation which, applied in the concentration of 100 ppm, totally inhibited the development of colonies of all the antagonistic fungi researched. Miedzian 50 preparation was the only one to show no fungistatic activity towards antagonistic fungi. A powerful fungistatic activity was observed for Afalon 450 and Racer 250 preparations in the dose of 100 ppm of a.i. as their *Trichoderma* spp. colony growth inhibition reached 67-89%. Out of all the fungi studied, *T. harzianum* isolate was most resistant to herbicides. The experiment which investigated the formation of germ tubes in the environment of selected abiotic factors showed that magnesium ions, irrespective of the concentration, significantly stimulated *T. harzianum* spores germination (Fig. 5). However zinc ions in each of the doses applied significantly inhibited spores germination of the fungi researched (Fig. 6). It was observed that all the fungicides and herbicides researched weakened antagonistic fungi spores germination (Figs. 7-9). Out of all the fungicidal preparations, the most powerful fungistatic effect was observed for Topsin M which in the concentration of 100 ppm completely inhibited spores germination in all the fungi tested. Also Afalon and Racer herbicides in the dose of 100 ppm significantly inhibited the development of germ tubes in the saprophytes researched.

Fig. 2. *Trichoderma harzianum* Rifai growth inhibition when exposed to fungicides and herbicides (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)

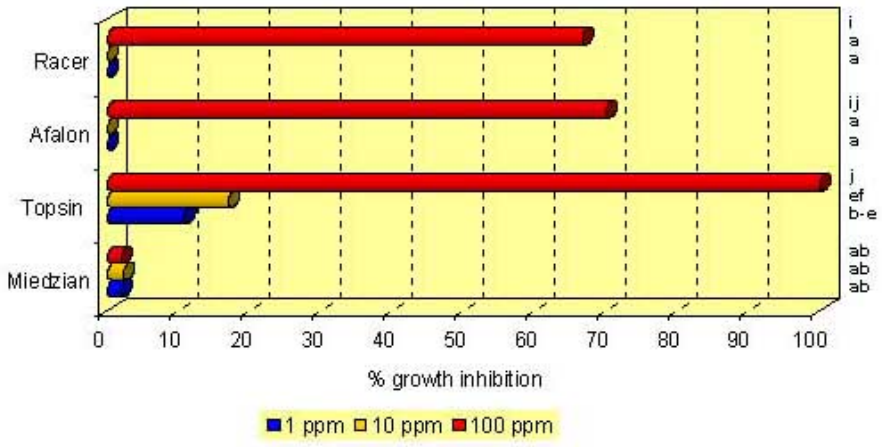


Fig. 3. *Trichoderma pseudokoningii* Rifai growth inhibition when exposed to fungicides and herbicide (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)

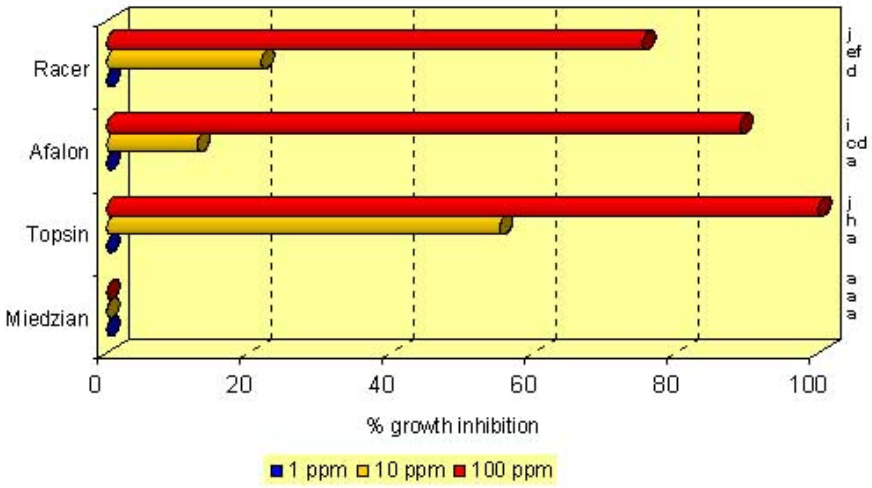


Fig. 4. *Trichoderma viride* Pers. ex Gray. growth inhibition when exposed to fungicides and herbicides (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)

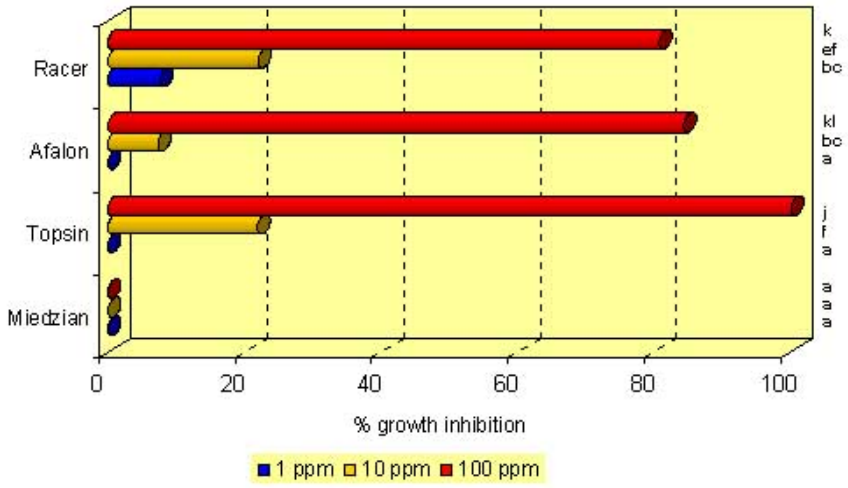


Fig. 5. *Trichoderma* spp. spores germination when exposed to magnesium ions (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)

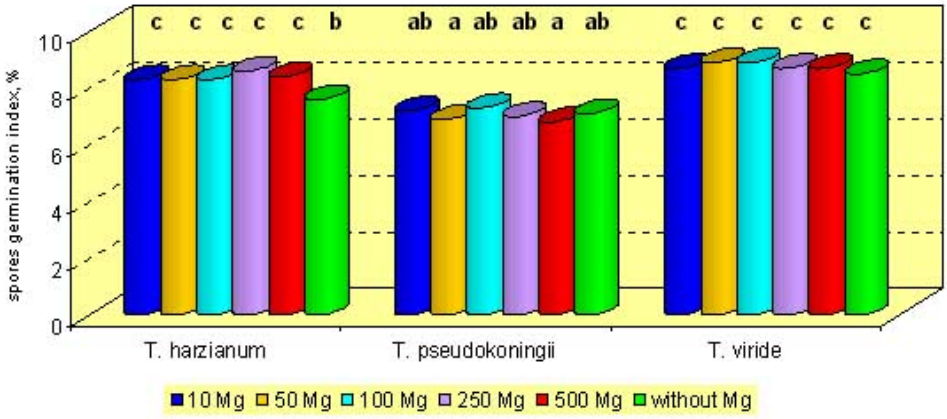


Fig. 6. *Trichoderma* spp. spores germination when exposed to zinc ions (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)

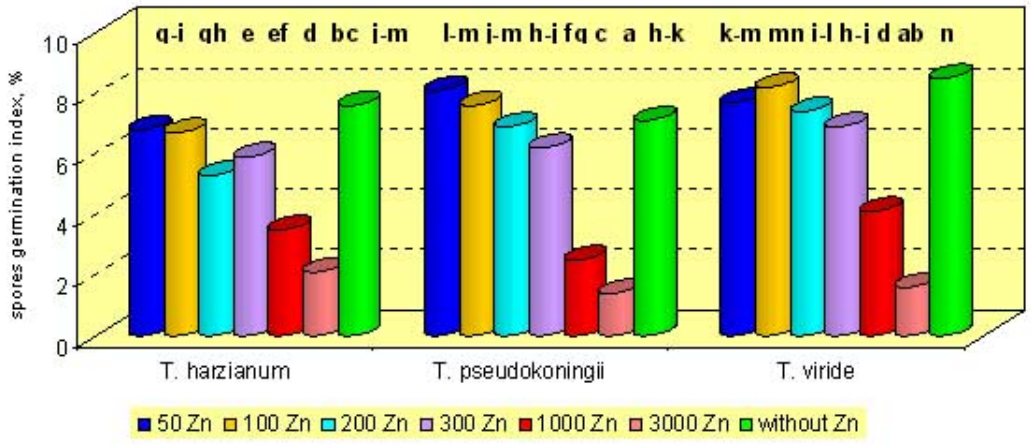


Fig. 7. *Trichoderma harzianum* Rifai spores germination when exposed to fungicides and herbicides (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)

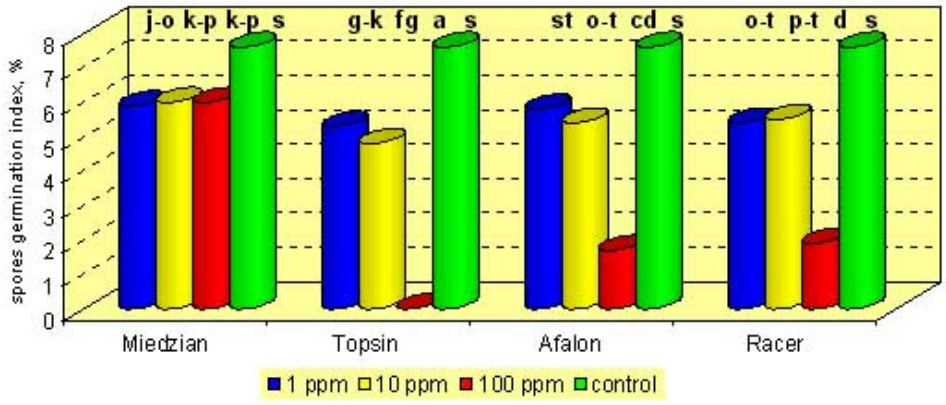


Fig. 8. *Trichoderma pseudokoningii* Rifai spores germination when exposed to fungicides and herbicides (columns marked with different letters differ significantly according to Duncan's test at p = 0.05)

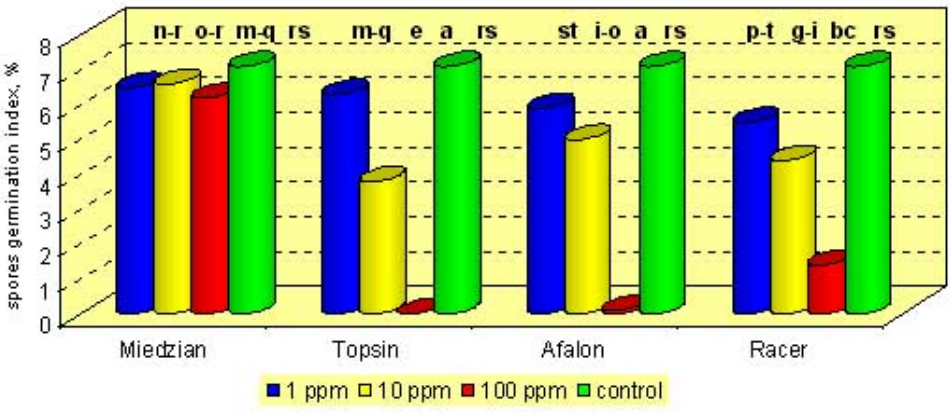
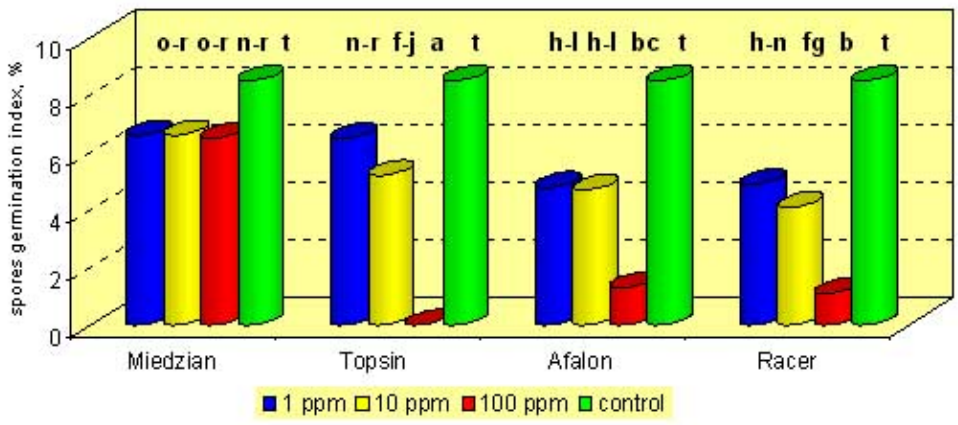


Fig. 9. *Trichoderma viride* Pers ex Gray. spores germination when exposed to fungicides and herbicides (columns marked with different letters differed significantly according to Duncan's test at p = 0.05)



The effect of the abiotic factors researched on the interactions between fungi of *Trichoderma* genus and plant pathogens was defined based on individual biotic effect (IBE). The evaluation was based on the scale [17] in which "0" stood for a steady development of colonies of both fungi. The highest degree of the scale '+8' stood for *Trichoderma* fungus colony totally inhibiting the pathogen development. Negative values were used whenever pathogenic fungi inhibited *Trichoderma* spp. development. The most powerful antagonistic properties of the *Trichoderma* genus fungi tested were noted towards *F. solani* and *R. solani*. However they inhibited *B. cinerea* growth less considerably. All the metal ions and pesticides tested least considerably affected the interaction between *Trichoderma* spp. and *F. solani.* Magnesium ions lowered the antagonistic activity of *T. harzianum* and *T. viride* towards *B. cinerea,* and slightly increased the value of IBE of *T. pseudokoningii* towards this pathogen (Table 1). The highest-applied concentration of zinc made antagonistic fungi lose completely their capacity to inhibit pathogens development (Table 2). *T. harzianum* isolate was the only one which, despite being treated with a high dose of zinc, still inhibited the growth of *F. solani* and *R. solani*. Zinc ions most considerably affected the activity of mycoparasites towards *B. cinerea*. Zinc weakened the effect of *T. harzianum* and *T. pseudokoningii* towards this pathogen, however, it strengthened the antagonism of *T. viride*.

Table 1. Individual biotic effect (IBE) for *Trichoderma* spp. growing when exposed to magnesium ions against phytopathogens

Mg ion concentration ppm	*Trichoderma. harzianum* Rifai			*Trichoderma pseudokoningii* Rifai			*Trichoderma viride* Pers. ex Gray.		
	B.c.	*F.s.*	*R.s.*	*B.c.*	*F.s.*	*R.s.*	*B.c.*	*F.s.*	*R.s.*
10	+1	+7	+6	+5	+7	+7	+5	+6	+7
50	+2	+7	+6	+5	+7	+7	+6	+7	+7
100	+1	+7	+6	+4	+7	+7	+4	+7	+7
250	+2	+7	+6	+5	+7	+7	+4	+7	+7
500	+3	+7	+6	+5	+7	+7	+3	+7	+6
Control - without Mg ions	+5	+7	+6	+4	+7	+6	+5	+7	+7

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

Table 2. Individual biotic effect (IBE) for *Trichoderma* spp. growing when exposed to zinc ions against phytopathogens

Zn ion concentration ppm	*Trichoderma harzianum* Rifai			*Trichoderma pseudokoningii* Rifai			*Trichoderma viride* Pers. ex Gray.		
	B.c.	*F.s.*	*R.s.*	*B.c.*	*F.s.*	*R.s.*	*B.c.*	*F.s.*	*R.s.*
50	+2	+7	+7	+5	+7	+6	+8	+7	+8
100	+3	+7	+7	+4	+7	+7	+8	+7	+8
200	+2	+7	+5	+2	+7	+5	+6	+7	+8
300	+4	+7	+5	+4	+7	+5	+6	+7	+7
1000	+6	+7	+7	-7	+7	+4	+6	+7	+7
3000	-2	+7	+5	-8	-8	-8	-5	-5	-8
Control – without Zn ions	+5	+7	+6	+4	+7	+6	+5	+7	+7

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

Out of all the pesticides researched, Topsin M showed most unfavourable to the antagonistic activity of selected mycoparasites. Out of all the fungi tested, *T. harzianum* isolate was least susceptible to the preparations researched and maintained its antagonistic properties (Table 3). The herbicides applied decreased the activity of *T. viride* towards *B. cinerea.*

Table 3. Individual biotic effect for *Trichoderma* spp. growing when exposed to fungicides or herbicides against phytopathogens

Fungicide, 10 ppm Herbicide, 100 ppm	*Trichoderma harzianum* Rifai			*Trichoderma pseudokoningii* Rifai			*Trichoderma viride* Pers. ex Gray.		
	B.c.	*F.s.*	*R.s.*	*B.c.*	*F.s.*	*R.s.*	*B.c.*	*F.s.*	*R.s.*
Miedzian 50 WP	+6	+7	+6	+6	+7	+3	+6	+7	+5
Topsin M 70 WP	+3	+6	+4	-2	+6	-5	+2	+6	-4
Afalon 450 SC	+5	+7	+6	+5	+7	+6	+3	+7	+6
Racer 250 EC	+4	+7	+7	+3	+7	+5	+3	+7	+6
Control – without preparation	+5	+7	+6	+4	+7	+6	+5	+7	+7

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

The results of the experiments into the effect of selected abiotic factors on effectiveness of *Trichoderma* genus fungi when pea seed-dressing was applied are given in Tables 4-6. Biological protection from all phytopathogens with antagonistic fungi was equally effective as the standard chemical dressing Oxafun T. The factors applied in the experiment modified the antagonistic effect of *Trichoderma* genus fungi applied in a form of seed dressing. The effect of the factors studied was considerably related to the saprophyte – pathogen relationship and the direction of changes depended on the fungus species as well as the factor kind. It was observed that in most objects the factors studied had a negative effect on the applicability of *Trichoderma* genus fungi to seed dressing. Magnesium ions treatment of selected saprophytic fungi showed an especially negative effect of pea seed dressing with *T. harzianum* and *T. pseudokoningii* fungi against *R. solani* (Table 4). There was noted an unfavourable effect of zinc ions on the applicability of *T. harzianum* and *T. pseudokoningii* to pea seed dressing against *B. cinerea*. Zinc ions, on the other hand, showed a positive effect on the antagonistic activity of the saprophytes researched towards *F. solani* and *R. solani* (Table 5). The fungicides and herbicides researched significantly inhibited antagonistic properties of saprophytes towards the plant pathogens tested (Table 6).

Table 4. Effectiveness of *Trichoderma* spp. treated with magnesium ions applied as pea seed dressing against pathogenic fungi

Seed dressing		Index of pea seedling infection with pathogenic fungi %		
Antagonistic fungus	Treating the antagonist with Mg ions, ppm	*Botrytis cinerea* Pres.	*Fusarium solani* Sacc.	*Rhizoctonia solani* Kühn
Trichoderma harzianum Rifai	50	56.5 op	28.0 c-i	47.3 l-o
	250	54.3 n-p	30.8 d-j	46.5 l-o
	500	51.3 m-p	28.0 c-i	45.5 l-o
	with no ions	31.0 d-j	26.0 c-g	13.0 b
Trichoderma pseudokoningii Rifai	50	47.3 l-o	31.5 d-j	47.0 l-o
	250	46.5 l-o	43.3 k-n	52.5 n-p
	500	54.5 n-p	59.5 p	36.0 g-l
	with no ions	31.0 d-j	20.0 b-d	17.0 bc
Trichoderma viride Pers. ex Gray.	50	46.3 l-o	39.5 i-m	40.5 j-m
	250	45.5 l-o	35.5 f-l	36.0 g-l
	500	53.3 n-p	39.5 i-m	38.0 h-l
	with no ions	32.0 e-k	27.0 c-h	20.0 b-d
Oxafun T seed dressing		24.0 b-f	22.0 b-e	13.0 b
Non-seed dressed		77.0 q	53.0 n-p	40.0 j-m
Non-seed dressed on the pathogen-free medium		0.0 a		

Means in columns marked with different letters differed significantly according to Duncan's test at p = 0.05

Table 5. Effectiveness of *Trichoderma* spp. treated with zinc ions applied as pea seed dressing against pathogenic fungi

Seed dressing		Index of pea seedling infection with pathogenic fungi %		
Antagonistic fungus	Treating the antagonist with Zn ions, ppm	*Botrytis cinerea* Pres.	*Fusarium solani* Sacc.	*Rhizoctonia solani* Kühn
Trichoderma harzianum Rifai	50	57.3 gh	18.5 a-d	7.8 ab
	200	45.0 e-g	15.5 a-c	9.8 ab
	1000	62.5 g-i	24.0 a-e	6.8 a-b
	with no ions	31.0 b-f	26.0 b-e	13.0 ab
Trichoderma pseudokoningii Rifai	50	42.5 d-g	24.0 a-e	26.5 b-e
	200	28.8 b-f	8.3 ab	29.3 b-f
	1000	82.0 i	11.8 ab	6.5 ab
	with no ions	31.0 b-f	20.0 a-e	17.0 a-d
Trichoderma viride Pers. ex Gray.	50	59.5 gh	12.3 ab	24.8 a-e
	200	27.5 b-e	30.0 b-f	24.0 a-e
	1000	33.3 b-f	24.3 a-e	13.8 ab
	with no ions	32.0 b-f	27.0 b-e	20.0 a-e
Oxafun T seed dressing		24.0 a-e	22.0 a-e	13.0 ab
Non-seed dressed		77.0 hi	53.0 fg	40.0 c-g
Non-seed dressed on the pathogen-free medium		0.0 a		

Means in columns marked with different letters differed significantly according to Duncan's test at p = 0.05

Table 6. Effectiveness of *Trichoderma* spp. treated with fungicides or herbicides applied as pea seed dressing against pathogenic fungi

Seed dressing		Index of pea seedling infection with pathogenic fungi %		
Antagonistic fungus	Treating the antagonist with 10 ppm of fungicide or 100 ppm of herbicide	*Botrytis cinerea* Pres.	*Fusarium solani* Sacc.	*Rhizoctonia solani* Kühn
Trichoderma harzianum Rifai	Miedzian 50 WP	68.0 s-y	59.0 n-w	71.0 t-z
	Topsin M 70 WP	44.0 g-o	100.0 B	54.3 l-u
	Afalon 450 SC	87.3 x	63.3 q-w	35.3 d-l
	Racer 250 EC	52.7 l-s	49.3 j-r	37.7 e-m
	with no preparation	31.0 c-i	26.0 b-g	13.0 a-b
Trichoderma pseudokoningii Rifai	Miedzian 50 WP	73.7 v-A	44.7 h-p	39.7 e-l
	Topsin M 70 WP	67.3 r-y	88.7 z-B	34.0 c-k
	Afalon 450 SC	72.0 t-x	70.7 t-w	38.0 e-n
	Racer 250 EC	71.0 t-x	69.7 s-w	29.0 b-h
	with no preparation	31.0 c-i	20.0 b-c	17.0 b-c
Trichoderma viride Pers. ex Gray.	Miedzian 50 WP	83.3 y-B	72.3 u-A	46.0 i-q
	Topsin M 70 WP	81.0 x-A	52.7 l-t	58.3 m-w
	Afalon 450 SC	59.0 p-t	79.3 w-x	46.3 h-q
	Racer 250 EC	72.3 t-x	64.3 r-w	40.7 f-o
	with no preparation	32.0 c-i	27.0 b-h	20.0 b-c
Oxafun T seed dresing		24.0 b-f	22.0 b-e	13.0 a-b
Non-seed dressed		77.0 w-A	53.0 l-t	40.0 e-m
Non-seed dressed on the pathogen-free medium		0.0 a		

Means in columns marked with different letters differed significantly according to Duncan's test at p = 0.05

DISCUSSION

The experiments presented showed that higher concentrations of the metal ions studied inhibited mycelium growth, weakened sporulation and spore germination of *Trichoderma* spp. isolates. All that is confirmed by the fact that metal ions of biogenic properties, including magnesium, manganese, zinc or copper show their toxicity in higher concentrations only [2]. Kowalski [10] observed that *T. viride* is a fungus especially susceptible to industrial emissions of zinc and lead. The author and his co-researchers [11] consider also *Trichoderma polysporum* and *T. pseudokoningii* fungi to be very susceptible to industrial pollution. A varied effect of mineral nutrition on *T. viride* was also noted by Sierota [27], observing a clearly inhibiting effect of zinc on the development of mycelium of that saprophyte and stimulation of sporulation by magnesium and manganese ions. Fungi pathogenic towards plants reacted to an increased concentration of zinc ions in the medium with a lowered growth rate, and zinc doses of 1000 and 3000 ppm showed a strong inhibition of pathogen development [6]. The highest susceptibility to zinc ions was observed in *R. solani* species.

Literature offers data which seem to confirm the present results showing a powerful fungistatic effect of Topsin M preparation on many fungi. Machowicz-Stefaniak [15] classified this fungicide as very fungicidal, falling into group one of the fungicidal activity as its ED_{50} towards *Botrytis cinerea* was below 1 ppm of a.i. Higher concentrations of Topsin M showed fungicidal towards *B. cinerea*, while other reports inform of a low activity of Topsin M towards agaric pathogens and *Phoma* spp. and *Pseudocercosporella herpotrichoides* fungi [8,25,30]. Reports on the effect of fungicides on antagonistic fungi state that these microorganisms, similarly to pathogens, are susceptible to these substances. Powerful growth inhibitors for *T. viride,* following Klimach and Wieczorek [7], included the following fungicides: Seed Dressing T, Rizolex 50 WP, Dithane M-45 and Rovral 50 WP. Fungal development was not negatively affected only by Previcur 607 SL and Dressing Marshal 25 ST. Machowicz-Stefaniak et al. [16], investigating 11 fungicides containing various active substances, observed that fungicides in the concentration of 100 ppm inhibited growth of *T. harzianum* colonies. Susceptibility of this fungus to the preparations researched differed, depending on the kind of fungicide. However for each preparation a decrease in the concentration of active ingredient was related to a loss of fungistatic activity. Reports by

Appaiah et al. [1] showed, in turn, that inhibiting *T. harzianum* growth as a result of thiuram application was not proportional to an increase in substance concentration. The experiments reported by Sas-Piotrowska and Piotrowski [26] observed an increase in toxicity towards *Trichoderma* spp. fungi due to a combination of fungicides with Apron or Previcur. Out of all the species researched, the lowest susceptibility to the fungicides tested was observed in *T. viride* species. The authors showed also that *T. viride* and *T. koningii* species showed a higher resistance to fungicides than phytopathogenic fungi.

Fungistatic properties can be also attributed to herbicides [4]. The present paper reports on *T. harzianum* isolate being most resistant to herbicides. *In vitro* the highest fungicidal activity was recorded for Afalon 450 and Racer 250.

Similar results, to the present ones, showing an unfavourable effect of Afalon on pathogenic fungi were reported by Mazurkiewicz-Zapałowicz and Janowicz [18]. A negative reaction to Afalon was also noted in *Trichoderma* genus fungi. Nowak [21] observed an increased *Trichoderma* spp. colony growth inhibition intensity with increasing concentration of Afalon in the medium. As a result, with Afalon in the amount of 1000 $mg \cdot dm^{-3}$ of the medium, there was recorded a complete *Trichoderma* spp. mycelium growth inhibition. Growth of saprophytic fungi of *Trichoderma* genus can be also limited by other herbicides like Gesard 500 SC, Roundup or preparations containing atrazine, however they usually show a negative effect in higher concentrations only [5,7,31]. The group of preparations which even in higher doses show no inhibiting effect on the development of *Trichoderma* genus fungi included Aminopielik Super 464 SL and herbicides based on sulphorinate [5,7].

Trichoderma genus fungi are among most effective saprophytic antagonistic fungi, widely applied in protection of agricultural and horticultural plants and trees [9,22,23]. The experiments carried out confirmed the applicability of the *Trichoderma* genus isolates tested to plant protection against pathogens. The mycoparasites investigated in *in vitro* tests showed powerful antagonistic properties towards *F. solani* and *R. solani*, while *B. cinerea* growth inhibition was slightly lower.

The infection experiments reported in the present paper showed an effective protective effect of the *Trichoderma* spp. isolates studied on phytopathogens. Microbiological seed dressing with the saprophytic fungi species tested enhanced the pea seedling health status. The effectiveness of *Trichoderma* genus fungi towards all the pathogens used equalled the effectiveness of Oxafun T, a chemical seed dressing.

The application of *Trichoderma* spp. fungi as microbiological preparation components used for seed dressing is reported in numerous papers which cover protecting germinating seeds and then plant roots from infection with phytopathogens [12,14]. Clear protective effects of *Trichoderma* spp. under provoking conditions and exposed to a considerable inoculation potential of pathogenic fungi suggest that a similar effect can be obtained in the field. All that is confirmed by field experiments with pea and bean monocultures which showed that seed dressing with *Trichoderma* spp. enhances the emergence and inhibits plant infection with soil pathogens [13,24]. The application of *Trichoderma* spp. to seed dressing also makes significantly higher pea and bean yields possible.

The present results show that treating *Trichoderma* genus fungi with metal ions, fungicides or herbicides can change antagonisms of saprophytic fungi towards soil pathogens. Changes in the antagonistic or pathogenic activity in fungi as a result of environmental conditions were also recorded by other authors. Sierota [28] reports on magnesium, manganese and zinc in the concentration of 100 ppm enhancing the inhibitory effect of leachates of *T. viride* on brown root rot. All that can suggest that these elements participate in processes of antagonistic substances formation. It was observed that mutations of *Trichoderma harzianum* strains to obtain resistance to benomyl and iprodione fungicides, in general, decrease the mycoparasitic activity of strains towards a series of phytopathogenic fungi of *Fusarium, Phytophthora, Rhizoctonia* and *Verticillium daliae* genera [29].

The present results and the literature reports show that the effect of anthropogenic origin factor can significantly disturb ecosystem balance between pathogenic and antagonistic organisms conditioning plant health status.

CONCLUSIONS

1. The abiotic factors studied affected the *Trichoderma* genus fungi tested and the fungal reactions depended on the kind of factor, its concentration and on the fungal isolate.
2. Zinc ions applied in 1000 and 3000 ppm concentrations inhibited the growth and germination of *Trichoderma* genus fungi spores, while zinc ions in the doses of 50, 100, 200, 300 ppm of a.i. stimulated *T. harzianum* genus growth.

3. Topsin M 70 WP showed a fungicidal effect in the dose of 100 ppm of a.i, blocking mycelium growth and *Trichoderma* genus fungi spores germination.
4. *In vitro* experiments revealed strong fungistatic properties of Dispersive Afalon 450 SC and Racer 250 WP herbicides in the dose of 100 ppm of a.i. towards the *Trichoderma* spp. isolates tested.
5. Microbiological pea seed dressing against phytopathogens with *Trichoderma* genus fungi untreated with the abiotic factors researched enhanced the pea seedling health status.
6. The abiotic factors studied, including metal ions, fungicides and herbicides, deteriorated the antagonistic properties of *Trichoderma* genus fungi used as pea seed dressing against *Botrytis cinerea, Fusarium solani* and *Rhizoctonia solani* pathogenic fungi.

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Joanna Dłużniewska
Department of Agricultural Environment Protection
Agriculture University of Cracow
al. Mickiewicza 21, 31-120 Cracow, Poland
e-mail: rrdluzni@cyf-kr.edu.pl
