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Original Research

The Effect of Foliar Fertilizers on the Development and Activity of *Trichoderma* spp.

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Abstract

Research was conducted on the effect of foliar fertilizers containing various macro and microelements (N, Ca, K, B, Cu, Fe, Mn, Mo and Zn) on mycelial growth, spore germination and antagonism of *Trichoderma* isolates. It was found that foliar fertilizers cause changes in the development and antagonism of *Trichoderma* spp. The kind of changes depends on the fungal isolate and fertilizer composition. *T. harzianum* isolate, in which a decrease in spore germination index and considerable changes of mycelial growth and antagonism rate were observed, proved the most sensitive to the chosen fertilizers. Among the selected fertilizers Mikrovit Cu acted most unfavourably, since it most strongly inhibited mycelial growth and *T. harzianum* spore germination, and diminished the antagonism of *Trichoderma* isolates towards *B. cinerea* and *R. solani*. It was found that Mikrovit 1, potassium Alkalin and Wapnovit fertilizers significantly increased the growth rate of *T. harzianum* mycelium. Germination of *T. pseudokonigii* and *T. viride* spores were most stimulated by Mikrovit Mn, whereas Molibdenit inhibited this process in *T. viride*. Potassium Alkalin is a fertilizer which favourably affects the antagonism of *Trichoderma* isolates towards *B. cinerea* and *R. solani*. On the other hand, Mikrovit Mn and Mikrovit Cu preparations diminish biocontrol abilities in the analyzed *Trichoderma* isolates toward pathogens.

Keywords: foliar fertilizers, *Trichoderma* spp., mycelial growth, spore germination, antagonism

Introduction

Trichoderma species reveal antagonistic properties towards many plant pathogen fungi from the genera *Pythium, Verticillum, Sclerotinia, Rhizoctonia, Fusarium* and *Botrytis* [1-5]. Therefore *Trichoderma* spp. are used for biological plant protection. These fungi may be used for seed, bulb and tuber dressing, for spraying plant aboveground parts or they may be supplied to the soil to induce plant resistance [6, 7]. When planning the application of antagonistic *Trichoderma* strains for the purposes of biological control, it is very important to consider the environmental parameters affecting the biocontrol agents. A series of abiotic and biotic environmental parameters influences the biocontrol efficacy of *Trichoderma* [8, 9].

Foliar nutrition is a crucial element of plant breeding technology applied in intensive agricultural production. This form of fertilization makes possible to quickly supply the plants in deficit nutrients. The main advantage of foliar nutrition is its fast activity and high degree of supplied macro- and microelement utilization [10]. It was also indicated that, apart from nutritional properties, these fertilizers positively affect plant healthiness, inhibiting the development of pathogens [11-15].

As foliar fertilizers are applied in agriculture with increasing frequency, they also affect fungi. Research was

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Table 1. Analyzed foliar fertilizers manufactured by Intermag, Olkusz, Poland.

Foliar fertilizers	Composition
Mikrovit 1	0.2% B, 0.4% Cu, 0.6% Fe, 0.6 Mn, 0.02% Mo, 0.4% Zn Cu, Fe, Mn, Zn chelated by EDTA
Alkalin potassium	25% K_2O, 360 g K_2O per 1 l of fertilizer 3% N (N-NH_2), 43 g N-NH_2 per 1 l of fertilizer 1.1% Si
Wapnovit	10% N (N-NO_3), 150 g N-NO_3 per 1 l of fertilizer 17% CaO, 260 g CaO per 1 l of fertilizer 0.050% B, 0.020% Cu, 0.020% Zn
Borvit	8% B (boron ethanolamine), 105 g B per 1 l of fertilizer
Molibdenit	3% Mo (ammonium molybdenate), 33 g Mo per 1 l of fertilizer
Mikrovit Mn	6% Mn (sulphate anion), 75 g Mn per 1 l of fertilizer, Mn complexed by sodium salt of aspartic acid stabile in solution with pH 3-7.5
Mikrovit Fe	3% Fe (sulphate), 34 g Fe per 1 l of fertilizer, Fe chelated by EDTA 1.5 stabile in solutions with pH 3-7.5
Mikrovit Cu	6% Cu (sulphate anion), 74 g Cu per 1 l of fertilizer, Cu complexed by IDHA 2.5 stabile in solutions with pH 3-9

undertaken on the impact of selected fertilizers on mycelial growth, spore germination and antagonism of *Trichoderma* spp.

Material and Methods

The research material was made up of three antagonistic fungal isolates obtained from the collection of Department of Agricultural Environment Protection, Agricultural University of Kraków: Trichoderma harzianum Rifai, Trichoderma pseudokoningii Rifai and Trichoderma viride Pers. ex Gray., as well as of pathogenic fungal species including Botrytis cinerea Pers., 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 foliar fertilizers (Mikrovit 1, Alkalin potassium, Wapnovit, Borvit, Molibdenit, Mikrovit Mn, Mikrovit Fe, Mikrovit Cu) (Table 1) was studied at the concentrations of 1, 10 and 100 ppm (mg·kg-1).

The *in vitro* effect of foliar fertilizers on fungal linear growth was examined with the poisoned medium method [16]. A solid glucose-potato medium (PDA) was prepared with the addition of foliar fertilizers. 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 foliar fertilizers. The results obtained were expressed as the growth rate [17].

Germination capacity of *Trichoderma* conidia in the presence of foliar fertilizers was evaluated with the method described by Burgieł [17]. In solutions of foliar fertilizers 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 [17].

The results of the experiments were verified statistically with variance analysis assumed for three-factor experiments (factor A – foliar fertilizers studied, factor B – concentration of the foliar fertilizers, factor C – *Trichoderma* species). Significance of differences was verified with Duncan's test. Statistical computations were carried out using the STAT computer programme.

The correlations between antagonistic fungi and *Botrytis cinerea* or *Rhizoctonia solani* were defined with the biotic series method following Mańka [18]. The analyzed fungi were inoculated at a distance of 2 cm from one another to the central part of Petri plate with PDA medium supplemented with the analyzed fertilizers at concentrations of 10 or 100 ppm. 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 the 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

On the basis of the conducted experiments it was found that the effect of selected fertilizers on mycelial growth, spore germination and antagonism depended on the *Trichoderma* isolate and the kind of preparation.

In studies examining the effect of fertilizers on mycelial growth in control experiment without foliar fertilizers the *T. harzianum* isolate revealed significantly the fastest growth rate (Table 2). On the other hand, notably the lowest mycelial growth rate was observed in the case of the *T. viride* isolate. The greatest change of growth rate under the influence of foliar fertilizers was detected in the *T. harzianum* isolate. Mikrovit 1, potassium Alkalin and

Table 2. Growth rate of *Trichoderma* spp. exposed to foliar fertilizers.

Foliar fertilizers and concentration [ppm]		Growth rate [mm/day]*		
		Trichoderma harzianum	*Trichoderma pseudokoningii*	*Trichoderma viride*
Mikrovit 1	1	106.58 m-o	101.24 h-j	96.45 bc
	10	106.25 m-o	101.44 h-j	96.81 b-d
	100	107.14 no	101.19 g-j	96.70 b-d
Alkalin potassium	1	106.39 m-o	104.47 k-m	94.14 a
	10	105.70 l-n	102.06 ij	96.54 bc
	100	106.83 no	100.85 g-j	96.24 a-c
Wapnovit	1	106.92 no	101.28 h-j	96.47 bc
	10	107.14 no	101.39 h-j	96.66 b-d
	100	107.95 o	101.42 h-j	96.37 bc
Borvit	1	106.06 l-o	103.00 jk	96.22 a-c
	10	106.78 no	100.91 g-j	97.27 cd
	100	106.11 l-o	100.99 g-j	96.48 bc
Molibdenit	1	100.35 g-i	100.15 g-i	96.30 a-c
	10	99.65 e-i	99.63 e-h	97.55 c-e
	100	98.80 d-g	99.38 e-h	96.43 bc
Mikrovit Mn	1	99.43 e-h	100.38 g-i	95.55 a-c
	10	99.93 f-i	100.00 g-i	96.38 bc
	100	100.28 g-i	100.08 g-i	97.08 b-d
Mikrovit Fe	1	100.78 g-j	99.55 e-h	96.55 bc
	10	97.13 b-d	100.05 g-i	96.48 bc
	100	94.13 a	100.63 g-i	96.55 bc
Mikrovit Cu	1	97.80 c-f	101.38 h-j	96.93 b-d
	10	96.28 a-c	100.08 g-i	96.58 b-d
	100	94.78 ab	100.18 g-i	96.05 a-c
Control (without foliar fertilizers)		104.15 kl	100.69 g-i	96.31 a-c

*means marked with different letters differed significantly according to Duncan's test at p = 0.05

Wapnovit apparently increased the mycelial growth rate of *T. harzianum*. On the other hand, Molibdenit, Mikrovit Mn, Mikrovit Fe and Mikrovit Cu preparations resulted in a markedly declined growth rate of *T. harzianum*. All fertilizers affected the mycelial growth of this isolate already when applied at a concentration of 1 ppm. However, in *T. pseudokonigii* a marked increase in mycelial growth rate was observed after the application of 1 ppm potassium Alkalin and Borvit. The studied fertilizers did not cause any changes of mycelial growth in *T. viride*.

The applied foliar fertilizers also affected the spore germination of the studied saprophytes (Table 3). In the control combination, the significantly highest spore germination index was noted for the *T. harzianum* isolate, whereas the *T. pseudokoningii* isolate produced the markedly smallest number of hyphae. Foliar preparations caused a significant decline in the spore germination index value of *T. harzianum*. Mikrovit Cu most strongly inhibited the germination process. A notable stimulation of *T. harzianum* spore germination was observed only in combination with Molibdenit and Borvit fertilizers in 1 and 10 ppm concentrations, respectively, whereas in *T. pseudokoningii* and *T. viride* isolates, the analyzed foliar fertilizers, except potassium Alkalin, caused an increase in the number of produced hyphae. Significantly, the largest number of germinating spores in these fungi was registered in combination with Mikrovit Mn. However, an increase in the fertilizer concentration markedly raised the germination index. In *T. viride*, the spore germination index declined apparently under the influence of 100 ppm concentrations of Mikrovit Fe and Mikrovit Cu. Molibdenit affected the hyphae formation by *T. viride* spores, the most negatively since it significantly decreased germination index.

The analyzed foliar fertilizers caused changes in the biological activity of *Trichoderma* spp. towards *B. cinerea* and *R. solani* (Table 4). The greatest changes in the antagonistic effect on the pathogens were observed in the case of the *T. harzianum* isolate. Mikrovit 1, potassium Alkalin, Wapnovit and Borvit fertilizers used in the analyzed concentrations stimulated the antagonism of *T. harzianum*. The *T. harzianum* isolate totally blocked pathogen development in combinations with these fertilizers. On the other hand, Molibdenit and Mikrovit Cu diminished *T. harzianum* antagonism towards *B. cinerea* and *R. solani*, whereas Mikrovit Mn negatively influenced *T. harzianum* activity towards *B. cinerea*. A decline in IEB was also noted in *T. viride* and *B. cinerea* relationship under the influence of Molibdenit in 10 ppm concentration, Mikrovit Mn and Mikrovit Cu fertilizers. However, *T. viride* activity towards *R. solani* was most strongly diminished by Mikrovit 1. Foliar fertilizers did not cause any greater changes in the effect of *T. pseudokoningii* on the pathogens except for potassium Alkalin, which stimulated *T. pseudokoningii* activity.

Discussion of Results

Conducted research revealed that foliar fertilizers cause changes in the development and antagonism of *Trichoderma* spp. The types of these changes depend on the fungal isolate and the fertilizer composition. The examined *T. harzianum* isolate proved the most sensitive to the selected fertilizers: a decline in spore germination rate and significant changes of mycelial growth rate and antagonism were recorded. Among the selected fertilizers, Mikrovit Cu proved the most unfavourable for *Trichoderma* spp., since it most strongly inhibited mycelial growth and declined *Trichoderma* antagonism towards *B. cinerea* and *R. solani*. It was found that Mikrovit 1, potassium Alkalin and

Wapnovit fertilizers markedly increased the mycelial growth rate of *T. harzianum*. Spore germination of *T. pseudokonigii* and *T. viride* was mostly stimulated by Mikrovit Mn. On the other hand, Molibdenit most inhibited this process in *T. viride*. Potassium Alkalin is a fertilizer which positively affects the antagonism of *Trichoderma* isolates towards *B. cinerea* and *R. solani*, whereas Mikrovit Mn and Mikrovit Cu preparations decrease biocontrol abilities of the analyzed *Trichoderma* isolates towards the tested pathogens.

Some microelements which are components of foliar fertilizers reveal a fungistatic effect. This concerns particularly sulphur, copper, zinc, tin, phosphorus and manganese. The compounds of these elements are active substances in applied fungicides [10, 19]. Foliar fertilizers containing iron or manganese ions may control linear growth, biomass increment and sporulation of polyphagus fungi: *Fusarium culmorum, Sclerotinia sclerotiorum*, *Botrytis cinerea* and *Rhizoctonia solani*. The investigations revealed a lack of uniform response of the applied fertilizers and their concentrations [20, 21]. On the other hand, fertilizers based on potassium salts (KNO_3, KCl, K_2SO_4 and KH_2PO_4) applied against *Alternaria solani* and *A. macrospore* inhibited the *in vitro* mycelial growth of these pathogens and blocked the spore germination of *A. solani* [11].

Apart from nitrogen, calcium, potassium and phosphorous, foliar fertilizers also contain numerous microelements, including heavy metals. Although several heavy metal ions are trace elements necessary for the growth of fungi, at high concentrations they are toxic. The toxic effect of metals upon the growth and activity of microorganisms may result from the fact that metals can bind to various biomolecules by covalent bonds. Metals may also unspecifically affect many cell structures and influence metabolic processes through a blockage of enzymes [8, 9, 22]. It was noted that the responses of *Trichoderma* isolates to zinc ions were connected with cotrentrations of this metal. Zinc dosed 1000 and 3000 ppm inhibited mycelial growth and *Trichoderma* spore germination, whereas lower concentrations of this metal stimulated *T. harzianum* growth [8]. Similar responses of *T. harzianum* isolate were registered under the influence of manganese ions. An increase in manganese ion concentration caused a decline in the growth rate of this fungus [23], while magnesium did not cause any changes of *Trichoderma* spp. growth [8]. Variable effect of mineral nutrition on *Trichoderma* spp. was also observed in other studies. It was demonstrated that zinc apparently inhibited mycelial growth, whereas manganese ions stimulated *T. viride* spore germination [24].

The effect of ten metals (aluminium, copper, nickel, cobalt, cadmium, zinc, manganese, lead, mercury and iron) on mycelial growth of *Trichoderma* strains was investigated. Mycelial growth was influenced significantly by the metals. The lowest IC50 values were found for copper, while the highest for aluminium [9, 25]. JingHua et al. [26] studied the effects of copper, zinc, iron, boron, molybdenum, calcium, manganese, magnesium and potassium on the efficiency of *Trichoderma* strain T23 in controlling *Fusarium* spp. Ammonium molybdate, ferrous sulphate, calcium sulphate and potassium dihydrogen improved the mycelial growth and sporulation of *Trichoderma* T23.

The results presented in this paper indicate that treatment with foliar fertilizers may lead to changes in the antagonisms of saprophytic fungi toward pathogens. Changes in the antagonistic activity of *Trichoderma* spp. under the influence of macro and microlements were observed also by other authors. The antagonistic activity of *T. harzianum* on *Sclerotium rolfsii* on solid culture media was stimulated in the presence of nitrogen fertilizers [27].

Table 3. Conidial germination of *Trichoderma* spp. exposed to foliar fertilizers.

Foliar fertilizers and concentration [ppm]		Index of conidial germination [%]*		
		Trichoderma harzianum	*Trichoderma pseudokoningii*	*Trichoderma viride*
Mikrovit 1	1	9.1 q-u	3.4 b-f	8.6 p-t
	10	10.0 t-w	4.7 f-i	8.8 p-u
	100	11.0 v-x	7.8 n-q	13.2 zA
Alkalin potassium	1	15.2 B	2.7 b-d	6.3 j-m
	10	10.2 u-w	4.0 d-h	9.7 r-v
	100	8.8 p-u	0.5 a	9.6 r-u
Wapnovit	1	12.4 yz	6.5 k-n	8.2 o-r
	10	7.9 n-q	5.7 i-l	12.3 x-z
	100	8.4 p-s	10.2 u-w	15.5 B
Borvit	1	13.4 zA	6.7 k-n	9.5 r-u
	10	22.2 G	3.2 b-e	11.1 w-y
	100	4.8 f-i	3.5 c-f	12.6 z
Molibdenit	1	28.9 I	14.2 AB	21.2 FG
	10	39.8 K	17.3 C	5.4 h-k
	100	9.8 s-w	8.7 p-u	0.3 a
Mikrovit Mn	1	4.2 e-h	5.1 g-j	24.5 H
	10	13.5 zA	15.1 B	17.6 C
	100	6.9 l-o	55.5 M	47.5 L
Mikrovit Fe	1	9.5 r-u	7.6 m-p	18.3 CD
	10	6.5 k-n	12.6 z	28.1 I
	100	2.3 bc	25.3 H	9.4 r-u
Mikrovit Cu	1	4.5 e-i	31.2 J	12.9 zA
	10	4.8 f-i	20.5 EF	12.9 zA
	100	3.7 c-g	6.3 j-m	5.8 i-l
Control (without foliar fertilizers)		19.3 DE	2.0 b	11.1 w-y

*means marked with different letters differed significantly according to Duncan's test at p = 0.05

Table 4. Individual biotic effect (IBE) of *Trichoderma* spp. on phytopathogens when exposed to foliar fertilizers.

Foliar fertilizers and concentration [ppm]		*Trichoderma harzianum*		*Trichoderma pseudokoningii*		*Trichoderma viride*	
		B. cinerea	*R. solani*	*B. cinerea*	*R. solani*	*B. cinerea*	*R. solani*
Mikrovit 1	10	+8	+8	+7	+6	+5	+5
	100	+8	+8	+5	+6	+4	+4
Alkalin potassium	10	+8	+8	+8	+8	+6	+7
	100	+8	+8	+6	+8	+6	+6
Wapnovit	10	+8	+8	+7	+7	+5	+6
	100	+8	+8	+7	+7	+6	+8
Borvit	10	+8	+8	+7	+8	+6	+5
	100	+8	+8	+7	+6	+6	+7
Molibdenit	10	+4	+3	+6	+6	+2	+6
	100	+3	+4	+6	+6	+6	+6
Mikrovit Mn	10	+3	+4	+6	+5	+3	+7
	100	+3	+6	+5	+5	+3	+4
Mikrovit Fe	10	+5	+5	+6	+5	+5	+6
	100	+5	+4	+7	+6	+4	+8
Mikrovit Cu	10	+2	+4	+5	+5	+4	+7
	100	+3	+4	+5	+5	+2	+6
Control (without foliar fertilizers)		+6	+6	+6	+6	+5	+7

The confrontation of *T. harzianum* with sclerotia of *S. rolfsii* separately with urea, sulphate ammonium and nitrate potassium showed an increase in antagonistic activity. It was also noted that soil treatment with *Trichoderma* in combination with ammonium molybdate, manganese sulphate and calcium sulphate significantly reduced melon wilt disease index [26]. On the other hand, reported that magnesium, manganese and zinc in 100 ppm concentrations positively affected the inhibitory activity of *T. viride* filtrates on root polypore [28]. It may evidence the share of these elements in the processes of antagonistic substance formation. It was demonstrated that biocontrol abilities of *T. viride* towards pathogens are more connected with intensity and course of metabolic processes and the form of bioavailable alimentary compounds than with their abundance in the medium [28]. The capability of *T. harzianum* T22 to solubilize *in vitro* some insoluble or sparingly soluble minerals was also investigated [29]. *T. harzianum* T22 can solubilize various plant nutrients, such as rock phosphate, Fe^{3+}, Cu^{2+}, Mn^{4+} and Zn^{0}, that can be unavailable to plants. T22 reduces oxidized metallic ions to increase their solubility and also produces siderophores that chelate iron. Solubilization of metal oxides by *Trichoderma* spp. involves both chelation and reduction. Both of these mechanisms also play a role in biocontrol of plant pathogens [29].

Data about the effects of such fertilizers on *Trichoderma* strains are of special importance if *Trichodrma* as a biocontrol agent is planned to be applied in inhibitory effects towards a biocontrol *Trichoderma* strain, their combination could result in diminished biocontrol effect, while on the other hand, synergistic interactions may be beneficial.

The studies on the effects of different environmental factors on mycoparasitic *Trichoderma* strains indicate the necessity to broaden knowledge about the ecophysiology of this genus. The application of *Trichoderma* strains with improved tolerance of unfavorable environmental conditions could increase the efficacy of biological control.

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