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THE OCCURRENCE OF ANTAGONISTIC MICRO--ORGANISMS IN THE SOIL AFTER THE CULTIVATION OF SPRING RYE AND COMMON VETCH

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Abstract. The object of the studies were saprophytic bacteria and fungi isolated from the soil after the cultivation of spring rye and common vetch. The studies were conducted in laboratory conditions. The antagonistic effect of the isolates of *Bacillus* spp., *Pseudomonas* spp., *Trichoderma hamatum* and *Trichoderma koningii* was determined towards such pathogenic fungi as *Alternaria alternata., Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum, Fusarium solani, Phoma exigua* var *exigua, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* It turned out on the basis of the obtained results that the highest antagonistic effect was characteristic of the isolates of *Trichoderma koningii*, while the smallest of *Trichoderma hamatum.* Among the examined bacteria isolates *Pseudomonas* spp. appeared to be a poorer antagonist than *Bacillus* spp.

Key words: antagonistic bacteria, antagonistic fungi, *Bacillus* spp., *Pseudomonas* spp., *Trichoderma koningii, Trichoderma hamatum*

INTRODUCTION

Cultivated plants in the period of vegetation can stimulate or inhibit the development of particular populations of microorganisms in the soil. Such an effect can be influenced by the compounds secreted by the roots or the husking root cells [Schroth and Hildebrand 1964, Rovira 1969, Sytnik et al. 1977, Milczak and Piotrowski 1980, Piotrowski and Milczak 1982, Funck-Jensen and Hockenhull 1984, Pięta 1988] or by the chemical composition of decomposing plant residues [Huber and Watson 1970, Bojarczuk 1974, Pięta 1999, Pięta et al. 1999, Pięta and Bełkot 2002]. Root exudates are a rich source of aminoacids, organic acid sugars, metal ion vitamins, phenolic acids and their derivatives [Funck-Jensen and Hockenhull 1984, Pięta 1988]. Among the mentioned compounds, sugars and acidic aminoacids have a stimulating effect, while the phenolic compounds, aromatic and alkaline aminoacids as well as organic acids inhibit the development

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of pathogenic fungi [Milczak and Piotrowski 1980, Piotrowski and Milczak 1982, Pięta 1988].

Special attention should be paid to the stimulating effect of root exudates and plant residues on the development of antagonistic bacteria and fungi towards pathogenic fungi living in the soil. Among bacteria, the greatest antagonistic effect towards phytopathogens is shown by *Bacillus* spp. and *Pseudomonas* spp. [Defago and Haas 1990, Keel 1992]. In the case of fungi, such properties are characteristic of *Trichoderma* spp. and *Gliocladium* spp. [Papavizas 1985, Łacicowa and Pięta 1989]. These antagonistic microorganisms not only inhibit the growth and development of phytopathogens but they also shorten their life in the soil. This effect of antagonists takes place through competition, antibiosis and parasitism [Huang 1978, Howell and Stipanovic 1980, 1983, Dos Santos and Dhingra 1982, Łacicowa and Pięta 1985a, 1985b, Ahl et al. 1986, Weller 1988, Defago and Haas 1990, Keel 1992, Pietr and Sobiczewski 1993, Dowling and O'Gara 1994, Lin at al. 1994, Mukherjee et al. 1995]. The soil containing a big number of propagation units of antagonists is defined as "resistant". One of the ways of increasing the communities of antagonists in the soil is their introduction into this environment or the creation of optimum conditions for their growth and development.

The purpose of the studies was to determine the effect of spring rye and common vetch on the populations of antagonistic bacteria and fungi in the soil.

MATERIAL AND METHODS

The studies on antagonists in the soil after spring rye cultivation considered 300 isolates of *Bacillus* spp. 300 of *Pseudomonas* spp. and 25 isolates of *Trichoderma hamatum* (Bonord.) Bain. as well as 31 isolates of *Trichoderma koningii* Oud. isolated from this soil. In the case of the soil after the cultivation of common vetch, the same number of isolates of *Bacillus* spp. and *Pseudomonas* ssp. as used as well as 9 isolates of *T. hamatum* and 13 isolates of *T. koningii.*

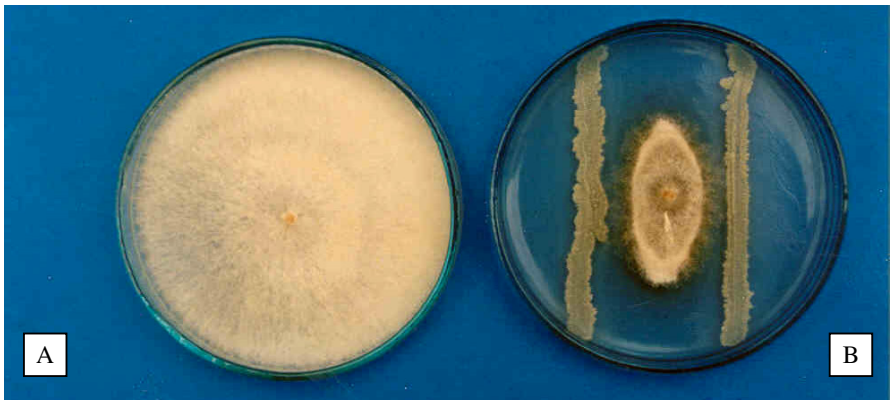
The above-mentioned isolates of bacteria and fungi were tested towards such pathogenic fungi as *Alternaria alternata*, *Botrytis cinerea*, *Fusarium culmorm*, *Fusarium oxysporum*, *Fusarium solani*, *Phoma exigua* var. *exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* Isolates of these fungi came from the collection of the Department of Phytopathology of Agricultural University in Lublin. Their pathogenicity were determined in laboratory and phytotrone tests.

In order to determine the antagonistic effect of the examined bacteria towards pathogenic fungi laboratory tests were conducted according to the method and scale described by Martyniuk et al. [1991]. They took into consideration five degrees, i.e. 0° – lack of inhibition zone, 1–0 – inhibition zone 1–2 mm, 2° – inhibition zone 3–5 mm, 3° – inhibition zone 6–10 mm, 4° – inhibition zone of over 10 mm. With a view of determining the effect of bacteria on pathogenic fungus a scale considering growth inhibition of the colony of the studied phytopathogen was used, which comprises: 0° – lack of inhibited growth of the fungus, 1° – colony growth inhibited up to 20%, 2° – colony growth inhibited up to 50%, 3° – colony growth inhibited up to 80%, 4° – colony growth inhibited up to 100% [Pięta 1999].

The effect of saprophytic fungi on pathogenic fungi was determined by the method of biotic rows [Mańka 1974, Mańka and Mańka 1992], and the individual effect of the antagonistic effect was estimated on the basis of a scale given by Mańka and Kowalski [1968].

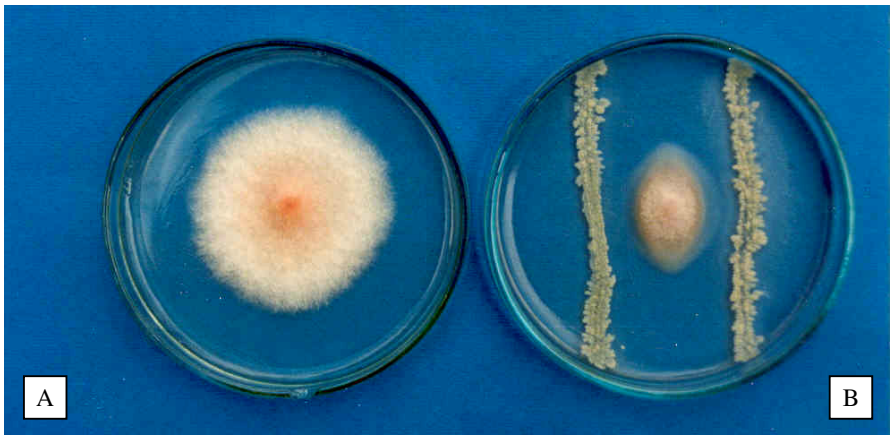
RESULTS AND DISCUSSION

Results of the microbiological analysis of the soil showed that much more isolates of *Trichoderma* spp. were isolated from under the cultivation of spring rye as compared to the soil after the cultivation of spring vetch. The genus *Trichoderma* was represented by *Trichoderma hamatum* and *Trichoderma koningii*. It should be supposed that varying chemical composition of root exudates and plant residues has a significant effect on the composition of microorganism populations. A smaller number of *Trichoderma* spp. isolates in the soil after the cultivation of common vetch is the result of the inhibitory effect of root exudates and the products of decomposition of this plant's tissues. Earlier studies by Darcy [1982] and Papavizas [1985] showed that papilionaceous crops inhibit the growth of *Trichoderma* spp. On the other hand, the studies by Patkowska et al. [2002] and Pięta [1999] showed a stimulating effect of cereals on the growth and development of *Trichoderma* spp. Big populations of *Trichoderma* spp. and their antagonistic abilities suggest that they can play an important role in reducing the propagation units of pathogenic fungi living in the soil. As claimed by Pięta and Bełkot [2002], with a small number of these fungi in the soil, the direct contact with the pathogen is small and hence they have only a slight effect on the reduction of phytopathogens.

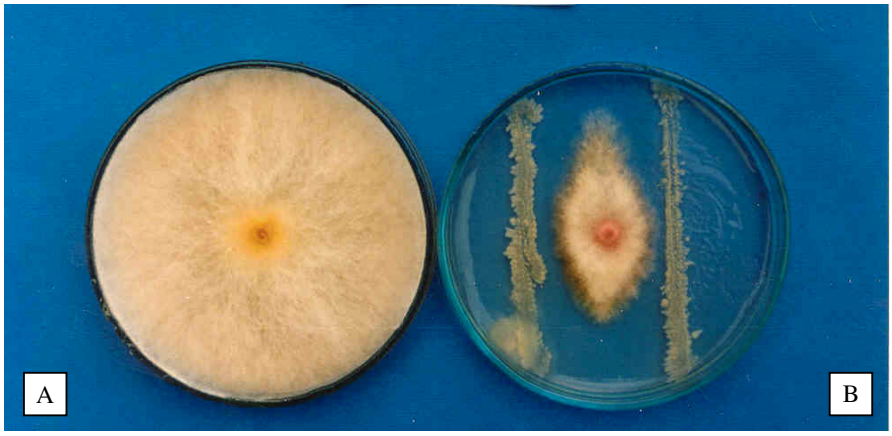


Phot. 1. Eight-day-old cultures of *Rhizoctonia solani* (A – control) and *R. solani* growing with *Bacillus* sp. 17 (B)

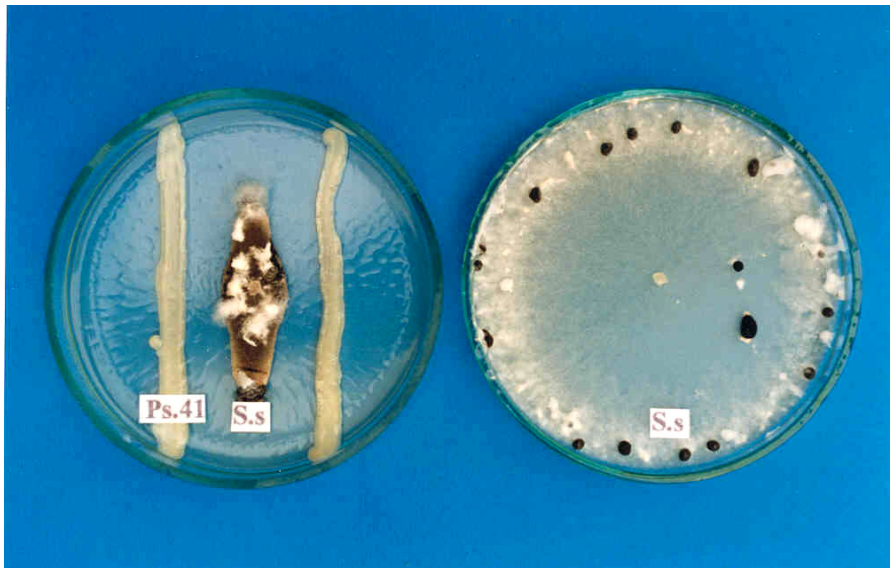
Fot. 1. Dziewięciodniowe kolonie *Rhizoctonia solani* (A – kontrola) i *R. solani* wzrastająca wspólnie z *Bacillus* sp. 17 (B)



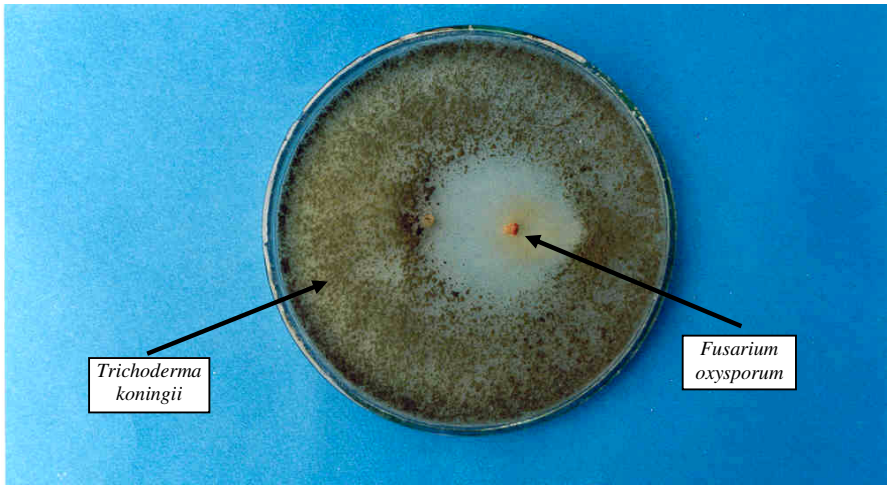
Phot. 2. Eight-day-old cultures of *Fusarium oxysporum* (A – control) and *F. oxysporum* growing with *Bacillus* sp. 41 (B)
Fot. 2. Dziewieciodniowe kolonie Fusarium oxysporum (A – kontrola) i *F. oxysporum* wzrastająca wspólnie z *Bacillus* sp. 41 (B)



Phot. 3. Eight-day-old cultures of *Fusarium culmorum* (A – control) and *F. culmorum* growing with *Bacillus* sp. 100 (B)
Fot. 3. Dziewięciodniowe kolonie *Fusarium culmorum* (A – kontrola) i *F. culmorum* wzrastająca wspólnie z *Bacillus* sp. 100 (B)



Phot. 4. Eight-day-old cultures of *Sclerotinia sclerotiorum* (S.s. – control) and *S. sclerotiorum* growing with *Pseudomonas* sp. 41 (Ps. 41 and S.s.)
Fot. 4. Dziewięciodniowe kolonie *Sclerotinia sclerotiorum* (S.s. – kontrola) i *S. sclerotiorum* wzrastająca wspólnie z *Pseudomonas* sp. 41 (Ps. 41 i S.s.)



Phot. 5. Eight-day-old cultures of *Trichoderma koningii* and *Fusarium oxysporum* growing together
Fot. 5. Dziewięciodniowa kolonie *Trichoderma koningii* wzrastająca wspólnie z *Fusarium oxysporum*

It can be stated on the basis of the studies that the isolates of *Trichoderma koningii* were distinguished by the greatest antagonistic effect (tab. 1), while *Trichoderma hamatum* proved to be a little weaker antagonist towards the studied pathogenic fungi. Among the tested bacteria isolates, *Bacillus* spp. proved to be better antagonists than *Pseudomonas* spp. (tab. 1). The isolates of the studied *Bacillus* spp., *Pseudomonas* spp., *Trichoderma koningii* and *Trichoderma hamatum* were characterized by a negative effect on phytopathogens. Antagonistic isolates, especially certain isolates of *Bacillus* spp., *Pseudomonas* spp. and *Trichoderma koningii* inhibited the growth and development of the colonies of pathogenic fungi, frequently forming an inhibition zone (phot. 1-5). The results concerning the antagonistic effect of particular populations of microorganisms confirmed the studies carried out by Łacicowa and Pięta [1985a, 1985b], Papavizas [1985], Pięta [1993, 1999] and Pięta et al. [1999]. According to Łacicowa and Pięta [1985b], *Trichoderma koningii* turned out to be the most active parasite. As a result of contact, it caused the decay of *F. culmorum, F. oxysporum, F. solani, F. avenaceum* and *F. graminearum* as early as after 10 days. The results discussed here confirmed earlier findings about considerable competitive abilities of *Trichoderma* spp, especially *T. koningii* [Papavizas 1985]. Considerable activity of this species is also determined by the formation of antibiotic substances that have a toxic effect on pathogenic fungi [Papavizas 1985, Simon at al. 1988].

Table 1. Antagonistic action of saprotrophic microorganisms to pathogenic fungi
Tabela 1. Antagonistyczne oddziaływanie mikroorganizmów saprotroficznych na grzyby patogeniczne

Antagonistic microorganisms Mikroorganizmy antagonistyczne	Średnia aktywność antagonistyczna wg skali Mean antagonistic activity according to scale								
	A. alternata	*B. cinerea*	*F. culmorum*	*F. oxysporum*	*F. solani*	*P. exigua*	*P. irregulare*	*R. solani*	*S. sclerotiorum*
Trichoderma hamatum	+7	+6	+6	+8	+8	+8	+7	+7	+6
Trichoderma koningii	+8	+8	+8	+8	+8	+8	+8	+8	+8
Bacillus spp.	+3	+4	+4	+5	+5	+5	+4	+4	+5
Pseudomonas spp.	+2	+3	+2	+3	+3	+2	+2	+3	+3

Table 2. Antagonistic bacteria and fungi towards pathogenic fungi
Tabela 2. Bakterie i grzyby o antagonistycznym oddziaływaniu względem grzybów patogenicznych

Bacteria and fungi antagonistic Bakterie i grzyby antagonistyczne	Combination – Kombinacje Number of isolates – Liczba izolatów	
	Soil after spring rye Gleba po uprawie żyta jarego	Soil after common vetch Gleba po uprawie wyki siewnej
Bacillus spp.	18	12
Pseudomonas spp.	40	24
Razem – Total	58	36
Trichoderma hamatum	20	0
Trichoderma koningii	31	8
Razem – Total	51	8
Ogółem – General	109	44

Results of laboratory tests showed that not all studied isolates of *T. hamatum* and *T. koningii* obtained from the soil are antagonists (tab. 2). In the case of the soil after the cultivation of spring rye, isolates occurred that were not antagonists and were neutral towards phytopathogens. There were more of such isolates in the soil after the cultivation of common vetch, especially in *T. hamatum.* Among the examined isolates of *Bacillus* spp. and *Pseudomonas* spp. obtained from the soil after the cultivation of spring rye the studies found out respectively, 6% and 13.3% of those that showed antagonistic activity. On the other hand, the soil after the cultivation of common vetch contained a little smaller number of antagonistic isolates of those bacteria, namely 4% *Bacillus* spp. and 8% *Pseudomonas* spp. (tab. 2). The tested microorganisms showed different antagonistic effects and hence their sole presence cannot determine the phytosanitary condition of the soil [Papavizas 1985, Pietr 1987]. The results point to the necessity of conducting such studies.

CONCLUSIONS

1. Spring rye stimulates the growth and development of *Trichoderma* spp. more than common vetch.
2. The antagonistic effect of *Trichoderma koningii* and *Bacillus* spp. is greater than that of *Trichoderma hamatum* and *Pseudomonas* spp.
3. Not all isolates among *Trichoderma* spp., *Bacillus* spp. and *Pseudomonas* spp. are characterized by the antagonistic effect towards pathogenic fungi.

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WYSTĘPOWANIE MIKROORGANIZMÓW ANTAGONISTYCZNYCH W GLEBIE PO UPRAWIE ŻYTA JAREGO I WYKI SIEWNEJ

Streszczenie. Przedmiotem badań były saprotroficzne bakterie i grzyby wyizolowane z gleby po uprawie żyta jarego i wyki siewnej. Badania przeprowadzono w warunkach laboratoryjnych. Antagonistyczne oddziaływanie izolatów *Bacillus* spp., *Pseudomonas* spp., *Trichoderma hamatum* i *Trichoderma koningii* określano względem takich grzybów patogenicznych jak *Alternaria alternata, Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Phoma exigua* var. *exigua, Pythium irregulare, Rhizoctonia solani* i *Sclerotinia sclerotiorum*. Na podstawie uzyskanych wyników okazało się, że największą aktywnością antagonistycznego działania wyróżniły się izolaty *T. koningii*, a najmniejszą *T. hamatum*. Spośród badanych izolatów bakterii słabszymi antagonistami okazały się *Pseudomonas* spp., niż *Bacillus* spp.

Słowa kluczowe: bakterie antagonistyczne, grzyby antagonistyczne, *Bacillus* spp., *Pseudomonas* spp., *Trichoderma koningii, Trichoderma hamatum*

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