

THE EFFECTIVENESS OF ANTAGONISTIC FUNGI AND THEIR POST-CULTURE LIQUIDS IN PEA (*Pisum sativum* L.) PROTECTION AGAINST DISEASES

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Abstract. The aim of research was to determine the protective effect of the microbiological material *Trichoderma harzianum* G 220 and *Gliocladium fimbriatum* S 151 and their post-culture liquids against soil-borne pathogenic fungi. The use of biological control improved the emergences, healthiness of pea plants and their yielding. Despite using the microbiological material for seed dressing, pea plants were infected by *Alternaria alternata*, *Ascochyta pisi*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *pisii*, *Fusarium solani*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

Key words: pea, *Trichoderma harzianum*, *Gliocladium fimbriatum*, post-culture liquids

INTRODUCTION

Among papilionaceous plants, pea is a plant of considerable importance due to the nutritional value of its protein. Hence, this plant is the object of studies, concerning of pea diseases and their control. The infecting fungi include *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Ascochyta* spp., *Fusarium* spp. and *Pythium* spp. These pathogens cause pre- and post-emergence infection, root rot and premature decay of plants. Besides, *Fusarium oxysporum* f. sp. *pisii* causes fusarious wilt of plants [Hagedorn 1989, Filipowicz 1983, 1993, Shalini and Dahroo 2003]. The enumerated pathogens considerably reduce the size and quality of the seed yield. The harmfulness of the pathogens under discussion results from the fact that they are seed-borne pathogens. Besides, they can develop as saprophytes in the soil or parasitize on other cultivated plants.

The use of chemical protection against soil-borne plant pathogens creates a danger of fungicides contaminating the agricultural environment. Alternative methods of plant

protection became increasingly popular. Within these methods, antagonistic microorganisms are of high importance [Liu 1988, Gurha 2001, El-Kafrawy 2002, Pięta et al. 2002]. Such microorganisms include *Trichoderma* spp. and *Gliocladium* spp. [Łacicowa and Pięta 1990, 1994b, Lin et al. 1994, Elad 2000]. Abundant information in the literature point to the protective effect of these microorganisms against plant pathogens of different plants [Papavizas 1985, Pastucha 1999, Rasami et al. 2001, Dubai 2002, Pięta et al. 2002, 2003].

At present, post-culture liquids of antagonistic fungi are used to control the diseases of cultivated plants [Indra and Thribuvanamala 2002, Roberti et al. 2001, 2002]. According to Stefanowa et al. [1999], post-culture filtrates of the isolates of *Trichoderma harzianum* A-34 and *Trichoderma viride* A-86, containing carboxymethylcellulosis, chitinase, β -1,3 glucanase and volatile lactone as well as 6-pentyl α -pyrone effectively inhibited the growth of *Phytophthora nicotiana*, *Rhizoctonia solani* and *Pythium* spp. A similar effect occurred in the case of post-culture liquids of *T. harzianum*, *T. viride* and *T. virens*, which inhibited the growth of the mycelium and the germination of sclerotia of *Rhizoctonia solani* [Hazarika and Das 1998]. An important role in the antagonistic action of these microorganisms is played by glucanase, chitinase, protease causing degradation of the cell walls of pathogenic fungi [Roberti et al. 2002].

The purpose of the present study was to determine the protective effect of the microbiological material of *Trichoderma harzianum* and *Gliocladium fimbriatum* and their post-culture liquids towards the fungi pathogenic to pea.

MATERIAL AND METHODS

The studies were conducted in the years 2005–2007 at the Experimental Station at Czesławice near Nałęczów on a field of a six-year-old pea monoculture with naturally accumulated infection material. The object of the studies were pea plants cv. ‘Sześciotygodniowy Tor’ grown out of the seeds dressed with the microbiological material consisting of the conidia of *Trichoderma harzianum* G 220 and *Gliocladium fimbriatum* S 151 or the post-culture liquid of those fungi species. The dressing was performed directly before the seed sowing. During dressing the seeds were put on the surface of the 14-days-old cultures of examined fungi species following shaking them for two minutes or they were dipped in the post-culture liquid for five minutes. The post-culture liquid was obtained after 8-days’ growth of *Trichoderma harzianum* G 220 and *Gliocladium fimbriatum* S 151 in a liquid medium PDB (Potato Dextrose Broth) (Difco) at the temperature of 22°C [Mishra and Behr 1976]. The study considered a combination with chemical seed dressing with Zaprawa Oxafun T and the control combination, i.e. without any dressing. Each combination included 4 replicates (4 plots), each with the area of 3.0 m², where 100 seeds were sown on each. During the vegetation two observations were performed in each year of the study – in the phase of seedlings and at anthesis. These observations determined the number of plants and their healthiness on particular plots of the experiment. The seedlings and the plants at anthesis that were considered to be infected were subjected to laboratory mycological analysis. Five seedlings and five

plants at anthesis that showed disease symptoms were taken from each experimental combination.

After the plants were picked up and dried, the seed yield and the proportion of seeds with spots were established. The mycological analysis of the plant material and the seeds were carried out according to the method described by Łacicowa and Pięta [1994a].

Results concerning the number, healthiness and yielding of pea plants were statistically analyzed, and the significance of differences was determined on the basis of Tukey's confidence intervals [Oktaba 1987].

RESULTS

The first observation indicated different numbers of seedlings growing on the plots of particular experimental combinations (fig. 1). Results concerning the numbers of plants and their healthiness, in the years 2005–2007 were very similar. Therefore in the tables only the mean values were indicated. The greatest number of seedlings, which was 88 on average, grew on the plots sown with the seeds dressed with the conidia of *Trichoderma harzianum* G 220. A lot of seedlings also grew on the plots in the combinations using the conidia of *Gliocladium fimbriatum* S 151 and the post-culture liquids of both antagonists. Much fewer seedlings were found on the plots sown with the seeds dressed with the chemical preparation Zaprawa Oxafun T, while the fewest, 61.7 seedlings on average, were observed on control plots.

Seedlings of inhibited growth and with necrotic spots on the roots and the stem base occurred on all plots. The mean proportion of such seedlings on the plots of particular combinations ranged from 0.9% to 6.4% (fig. 1). The smallest number of seedlings with disease symptoms was observed on the plots sown with the seeds wetted in the post-culture liquid of *T. harzianum* G 220, while the biggest one – on control plots.

The second observation, which fell on the anthesis of pea plants, found out a similar number of plants on the plots of particular combinations and a slightly higher proportion of infected plants. The highest losses of plants and a considerable increase of infected ones occurred on control plots (fig. 1).

The mean yield gathered from plants growing on the plots of particular experimental combinations ranged from 143 g (converted into 0.48 t·ha⁻¹) to 542.3 g (converted into 1.81 t·ha⁻¹) (tab. 1). The smallest yield was gathered from control plants. A significantly higher yield as compared to the control was harvested from plants grown on the plots sown with the seeds dressed with Zaprawa Oxafun T. In the case of the other experimental combinations, i.e. using the conidia of *T. harzianum* G 220 and *G. fimbriatum* S 151 and their post-culture liquids for seed dressing, a high and good quality yield of seeds was obtained. Within the applied microbiological material, *Gliocladium fimbriatum* S 151 proved most effective in protecting pea plants from pathogenic fungi since this combination gave the highest seed yield. On the other hand, the smallest proportion of infected seeds occurred in the yield gathered from the plots of the experimental combination using the post-culture liquid of *T. harzianum* G 220.

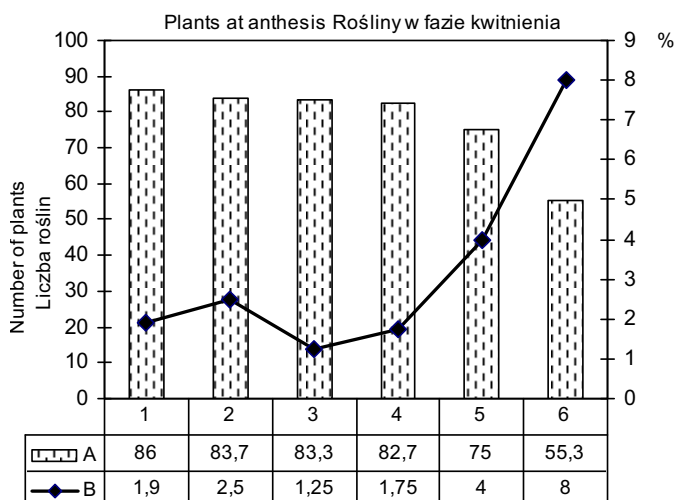
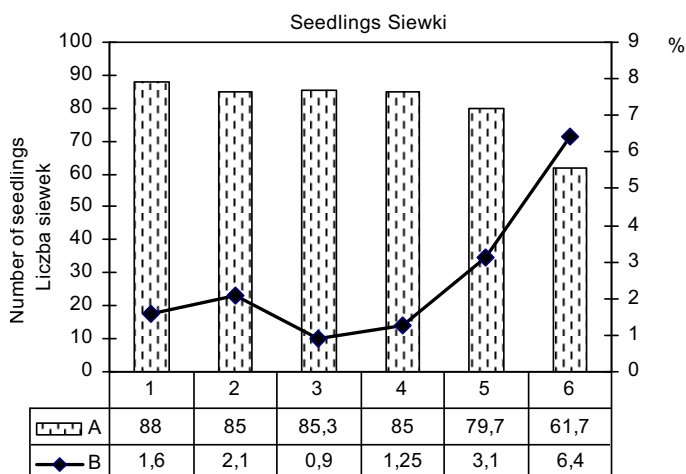


Fig. 1. Number and healthiness of pea plants in individual experimental (mean from the years 2005–2007): 1 – conidia of *T. harzianum*, 2 – conidia of *G. fimbriatum*, 3 – post culture liquids *T. harzianum*, 4 – post culture liquids *G. fimbriatum*, 5 – Zaprawa Oxafun T, 6 – control, A – number of plants, B – % infected plants

Rys. 1. Liczebność i zdrowotność roślin grochu w poszczególnych kombinacjach doświadczenia (średnie z lat 2005–2007): 1 – konidia *T. harzianum*, 2 – konidia *G. fimbriatum*, 3 – płyn pochodzący z *T. harzianum*, 4 – płyn pochodzący z *G. fimbriatum*, 5 – Zaprawa Oxafun T, 6 – kontrola, A – liczba roślin, B – % porażonych roślin

Table 1. Field and healthiness of pea seeds (mean from the years 2005–2007)

Tabela 1. Plon i zdrowotność nasion grochu (średnia z lat 2005–2007)

Experimental combination Kombinacja doświadczenia	Mean the yield of pea seeds Średni plon nasion t·ha ⁻¹	Mean the percentage of infected seeds Średni udział porażonych nasion
The seeds dressed with <i>Trichoderma harzianum</i> G 220 Nasiona zaprawiane <i>Trichoderma harzianum</i> G 220	1.51* ^c	8.1* ^b
Seeds dressed with <i>Gliocladium fimbriatum</i> S 151 Nasiona zaprawiane <i>Gliocladium fimbriatum</i> S 151	1.81 ^c	7.25 ^b
Seeds soaked in post-culture liquids of <i>Trichoderma harzianum</i> G 220 Nasiona moczone w płynie pochodzącym <i>Trichoderma harzianum</i> G 220	1.64 ^c	5.8 ^a
Seeds soaked in post-culture liquids of <i>Gliocladium fimbriatum</i> S 151 Nasiona moczone w płynie pochodzącym <i>Gliocladium fimbriatum</i> S 151	1.78 ^c	6.2 ^a
The seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	1.18 ^b	11.25 ^c
Control Kontrola	0.48 ^a	17.2 ^d

*means values in the columns do not differ significantly at ($P \leq 0.05$), if marked with the same letter

* średnie wartości w kolumnach nie różnią się istotnie przy ($P \leq 0.05$), jeśli są oznaczone tą samą literą

There were obtained 1303 fungi isolates were being a result of the laboratory mycological analysis of the roots and the stem base of the infected pea seedlings. The colonies of *Fusarium* spp. occurred most frequently in the group of the obtained fungi (tab. 2). This genus was represented by *F. culmorum*, *F. oxysporum* f. sp. *pisi*, *F. equiseti* and *F. solani*. Among the enumerated species, *F. oxysporum* f. sp. *pisi* was isolated in the greatest numbers, both from the examined roots and the stem base of pea seedlings. Besides, such pathogenic fungi as *Alternaria alternata*, *Ascochyta pisi*, *Pythium irregulare* and *Rhizoctonia solani* were isolated from the infected organs of seedlings. The enumerated plant pathogens were isolated more numerously from the roots and the stem base of the seedlings taken from the plots of the combination with Zaprawa Oxafun T and the control as compared to the other plots of the experimental combination.

The 1880 fungi colonies were obtained as a result of the mycological analysis of infected plants at anthesis (tab. 3). The most fungi colonies were isolated from control plants, i.e. without any protective treatments on plants. A lot of fungi isolates were obtained from the plants taken from the plots of the combination with Zaprawa Oxafun T. In the case of using the conidia of *T. harzianum* G 220 and *G. fimbriatum* S 151 and the post-culture liquids of these fungi for the dressing of pea seeds, on average twice as few fungi colonies were obtained as compared to the use of a chemical preparation.

The most numerous colonies were *Fusarium* spp., and its isolates constituted 26.9% of all the isolations. Genus *Fusarium* was represented by *F. culmorum*, *F. oxysporum* f. sp. *pisi*, and *F. solani* (tab. 3). Within the group of the enumerated species, *F. oxysporum* f. sp.

Table 3. Fungi isolated from infected plants at anthesis of pea in the years 2005–2007
 Tabela 3. Grzyby wyisobnione z porażonych roślin grochu w fazie kwitnienia w latach 2005–2007

Fungus species Gatunek grzyba	Experimental combination / Number of isolates – Kombinacja doświadczenia / Liczba izolatów														
	Seeds dressed with <i>Trichoderma</i> <i>harzianum</i> G 220		Seeds dressed with <i>Gliocladium fimbriatum</i> S 151		Seeds soaked in post-culture <i>Trichoderma</i> <i>harzianum</i> G 220		Seeds soaked in post-culture <i>Gliocladium fimbriatum</i> liquids of <i>Gliocladium fimbriatum</i> S 151		Seeds dressed with Zaprawa Oxafun T		Total Razem				
	r/k	sb/pl	r/k	sb/pl	r/k	sb/pl	r/k	sb/pl	r/k	sb/pl	r/k	sb/pl			
<i>Acremonium roseum</i> (Oud.) W. Gams	1	2	-	-	2	3	1	1	3	4	6	4	13	14	27
<i>Alternaria alternata</i> (Fr.) Keissler	5	4	3	1	3	6	6	3	12	11	14	20	43	45	88
<i>Ascochyta pisii</i> Libert	1	5	-	2	1	1	3	2	11	8	18	22	34	40	74
<i>Aspergillus niger</i> Van Tiegh	3	-	-	-	2	1	-	-	2	4	8	10	15	15	30
<i>Aureobasidium pullulans</i> (de Bary) Arnaud	-	2	1	1	-	-	1	-	1	2	3	2	6	7	13
<i>Botrytis cinerea</i> Pers.	1	2	2	3	1	1	5	3	10	13	11	11	30	33	63
<i>Cladosporium cladosporioides</i> (Fres) de Vries	6	9	3	13	7	7	5	4	6	7	7	13	34	53	87
<i>Epicoccum purpurascens</i> Ehr. ex. Schl.	2	1	-	2	1	2	3	2	6	8	3	2	15	17	32
<i>Fusarium culmorum</i> (W. G. Sm.) Gams	5	1	4	6	4	3	6	5	12	13	17	18	48	46	94
<i>Fusarium oxysporum</i> Schl. f. sp. <i>pisii</i> (van Hall)	10	17	11	17	13	20	22	31	30	42	35	51	121	178	299
<i>Fusarium solani</i> (Mart.) Sacc.	7	6	4	6	5	8	6	9	12	17	16	17	50	63	113
<i>Gliocladium catenulatum</i> Gilman Abbott	3	6	7	3	4	3	7	3	2	2	-	25	17	42	42
<i>Gliocladium fimbriatum</i> Gilman Abbott	16	6	14	13	8	6	6	4	1	-	2	2	47	31	78
<i>Gliocladium roseum</i> Bänier	8	1	4	4	8	4	4	5	3	2	1	2	28	18	46
<i>Hamicola grisea</i> Domsch	-	-	2	1	2	3	3	1	4	6	7	7	18	18	36
<i>Macrorhynchium</i> Wehmer	3	4	10	-	3	7	3	4	1	5	3	4	23	24	47
<i>Macrorhynchium</i> Fresenius	1	1	-	-	-	-	-	2	2	1	3	6	6	10	16
<i>Papulaspora irregularis</i> Hosson	-	-	-	-	-	2	3	5	-	-	4	2	7	9	16
<i>Penicillium expansum</i> Link ex. S. F. Gray	15	10	-	2	4	3	4	2	1	4	1	2	21	23	44
<i>Penicillium purpurogenum</i> Stoll.	-	1	1	2	6	3	6	4	5	1	7	5	25	16	41
<i>Penicillium verrucosum</i> Dierckx	5	-	4	1	3	5	1	1	6	7	9	12	28	26	54
<i>Penicillium westii</i> Samson et al.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium verrucosum</i> Dierckx	-	2	-	-	-	-	2	3	2	2	4	5	8	12	20
<i>Phoma eupyrena</i> Sacc.	2	1	3	2	1	-	-	4	4	5	6	15	18	33	33
<i>Phoma exigua</i> Desm.	2	1	-	3	1	-	2	1	4	6	7	8	16	19	35
<i>Rhizoctonia solani</i> Kühn	1	5	-	3	2	5	4	6	17	22	22	27	46	68	114
<i>Rhizopus nigricans</i> Ehrenberg	2	3	6	3	2	3	3	3	7	15	21	21	41	48	89
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	-	3	3	-	-	1	3	-	12	11	13	19	31	34	65
<i>Trichoderma harzianum</i> Rifai	11	8	8	6	8	7	6	4	6	1	3	40	29	69	69
<i>Trichoderma koningii</i> Oud.	6	7	9	7	10	5	8	4	2	3	2	37	26	63	63
<i>Trichoderma viride</i> Pers. ex S. F. Gray	6	7	5	4	10	5	2	5	-	4	3	1	26	26	52
Total – Razem	122	115	105	105	110	114	121	121	184	226	255	302	897	983	1880

r/k – root/ korzeń; sb/ pl – stem base/ podstawa łodygi

Table 4. Fungi isolated from pea seeds in the years 2005–2007
Tabela 4. Grzyby wyisobnione z nasion grochu w latach 2005–2007

Fungus species Gatunek grzyba	Experimental combination / Number of isolates – Kombinacja doświadczenia / Liczba izolatów												Total Ogółem			
	Seeds dressed with <i>Trichoderma</i> <i>harzianum</i> G.220		Seeds dressed with <i>Gliocladium</i> <i>finbricitatum</i> S.151		Seeds soaked in post-culture liquids of <i>Trichoderma</i> <i>harzianum</i> G.220		Seeds soaked in post-culture liquids of <i>Gliocladium</i> <i>finbricitatum</i> S.151		Seeds dressed with Zaprawa Oxafun T Nasiona		Control Kontrola					
	1	2	1	2	1	2	1	2	1	2	1	2		1	2	
<i>Acremonium roseum</i> (Oud.) W. Gams	-	-	6	-	-	9	2	4	1	10	5	4	2	33	10	43
<i>Alternaria alternata</i> (Fr.) Keissler	15	7	19	5	9	6	1	10	3	15	6	17	7	85	30	115
<i>Ascochyta pisi</i> Libert	1	-	5	2	2	-	-	2	1	4	2	6	2	20	7	27
<i>Botrytis cinerea</i> Pers.	-	-	2	-	-	-	-	3	1	15	6	21	9	41	16	57
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	9	5	4	2	5	2	7	4	4	8	2	11	3	44	18	62
<i>Epicoccum purpurascens</i> Ehr. ex. Schl.	2	1	1	3	6	1	8	3	3	8	2	4	2	29	12	41
<i>Fusarium equiseti</i> (Corda) Sacc.	2	1	2	1	3	1	2	2	1	-	-	1	1	10	3	13
<i>Fusarium graminearum</i> Schwabe	3	1	1	-	2	-	1	-	1	10	4	10	5	24	9	33
<i>Fusarium oxysporum</i> Schl.	3	1	4	1	4	1	3	1	1	21	9	31	14	66	26	92
<i>Fusarium poae</i> (Peck.) Wollenw.	4	1	-	-	1	-	-	-	-	9	4	12	6	26	11	37
<i>Fusarium solani</i> (Mart.) Sacc.	-	-	-	-	-	-	-	-	-	3	1	5	3	8	4	12
<i>Fusarium sporotrichioides</i> Sherb.	1	-	2	1	-	-	2	2	1	4	2	6	3	15	6	21
<i>Gliocladium catenulatum</i> Gilman Abbott	7	2	6	2	6	2	8	3	3	6	1	1	1	34	11	45
<i>Gliocladium finbricitatum</i> Gilman Abbott	4	1	6	1	5	2	7	1	1	1	2	3	-	26	7	33
<i>Gliocladium roseum</i> Bänier	4	1	2	-	-	-	2	2	2	4	2	2	1	14	4	18
<i>Humicola grisea</i> Domsch	6	1	1	1	-	-	3	1	1	2	1	1	-	13	4	17
<i>Mucor hiemalis</i> Wehmer	1	-	3	1	3	1	3	1	1	1	-	3	2	14	5	19
<i>Papulaspora irregularis</i> Hotson	2	-	1	-	1	-	1	-	-	-	-	5	2	9	4	13
<i>Penicillium canescens</i> Scopp.	3	2	2	-	2	1	2	2	1	3	1	1	-	13	4	17
<i>Penicillium frequentans</i> Westling	7	1	3	-	3	-	-	-	1	3	1	3	2	19	5	24
<i>Penicillium verrucosum</i> Dierckx var. <i>cycloclum</i> (West.) Samson et al.	2	-	1	1	-	-	3	1	1	2	1	1	1	9	4	13
<i>Penicillium verrucosum</i> var. <i>verrucosum</i> (West.) Samson et al.	-	-	3	1	8	3	4	-	-	8	2	11	5	34	11	45
<i>Rhizoctonia solani</i> Kühn	3	-	4	-	2	-	1	1	1	13	5	17	8	40	14	54
<i>Rhizopus nigricans</i> Ehrenberg	5	1	10	4	-	2	7	2	5	1	4	2	31	12	43	
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	1	-	-	-	1	-	1	-	16	6	18	6	37	12	49	
<i>Trichoderma hamatum</i> (Bonard) Batm	4	1	1	-	4	3	3	2	1	-	1	4	1	16	8	24
<i>Trichoderma harzianum</i> Rifai	5	2	3	2	7	3	8	2	2	6	1	6	2	35	12	47
<i>Trichoderma koningi</i> Oud.	-	-	2	1	2	2	1	-	2	2	-	1	-	11	5	16
<i>Trichoderma viride</i> Pers. ex S.F. Gray	10	3	2	1	5	3	9	4	4	-	-	8	2	38	13	51
Total – Razem	101	31	96	30	90	31	107	35	183	68	217	92	794	287	1081	

1 – seeds with spots, nasiona z plamami; 2 – seeds without spots, nasiona bez plam

Table 2. The effect of soil type and fertilization on the length of inflorescence and number of stems of goldenrod plant
 Tabela 2. Wpływ typu gleby i nawożenia na długość kwiatostanu i liczbę łodyg nawłoci

Soil Gleba	Fertilization Nawożenie	Length of inflorescence – Długość kwiatostanu, cm						Number of stems per plant – Liczba pędów na roślinie					
		I year (I rok)		II year (II rok)		III year (III rok)		I year (I rok)		II year (II rok)		III year (III rok)	
		1 st crop 1 zbiór	2 nd crop 2 zbiór	1 st crop 1 zbiór	2 nd crop 2 zbiór	1 st crop 1 zbiór	2 nd crop 2 zbiór	1 st crop 1 zbiór	2 nd crop 2 zbiór	1 st crop 1 zbiór	2 nd crop 2 zbiór	1 st crop 1 zbiór	2 nd crop 2 zbiór
PS	N ₀ P ₀ K ₀	18	23	29	15	1	6	4	6	3			
	N ₁ P ₁ K ₁	20	29	34	19	3	7	6	5	3			
	N ₂ P ₂ K ₂	21	33	38	20	3	7	5	8	4			
	N ₃ P ₃ K ₃	26	32	48	20	4	8	6	6	5			
Mean – Średnio	N ₁ P ₁ K ₁	29	35	45	23	5	8	8	5	5			
	N ₂ P ₂ K ₂	54	30	39	19	3	7	6	6	4			
	N ₃ P ₃ K ₃	29	30	37	21	2	8	5	6	4			
	N ₁ P ₁ K ₁	34	33	38	22	4	10	5	11	5			
PG	N ₂ P ₂ K ₂	36	38	46	22	4	9	6	8	5			
	N ₃ P ₃ K ₃	41	41	52	28	5	11	5	7	6			
	N ₁ P ₁ K ₁	44	45	50	27	5	12	4	7	6			
	Mean – Średnio	37	37	45	24	4	10	5	8	5			
Source of variation Źródło zmienności													
Soil – Gleba	0.87	1.09	1.73	0.84	0.38	0.27	0.38	0.36	0.45	0.81	0.81	0.81	
Fertilization level	1.95	2.46	3.89	1.89	0.86	0.61	0.86	0.86	1.03	1.68	1.68	1.68	
Poziom nawożenia	3.24	4.09	NS	3.13	1.44	1.01	1.44	1.44	NS	NS	NS	NS	
Interaction – Interakcja													

NS, **, * – nonsignificant or significant at $P \leq 0.05$ or 0.1, respectively by Tukey's test; PS – slightly loamy sand, PG – heavy loamy sand; N₀P₀K₀ – control object without mineral fertilization, N₁P₁K₁ – with 20 kg N, 30.3 kg P, 24.1 kg K, N₂P₂K₂ – with 40 kg N, 61.1 kg P, 48.2 kg K, N₃P₃K₃ – with 60 kg N, 91.6 kg P, 72.3 kg K and N₁P₁K₁ – with 80 kg N, 122.2 kg P and 96.4 kg K per hectare
 NS, **, * – nieistotna lub istotna przy $P \leq 0.05$ lub 0.1, odpowiednio wg testu Tukeya; PS – piasek słabo gliniasty, PG – piasek gliniasty mocno pylasty; N₀P₀K₀ – obiekt kontroly bez nawożenia mineralnego, N₁P₁K₁ – z zastosowaniem 20 kg N, 30,3 kg P, 24,1 kg K, N₂P₂K₂ – z zastosowaniem 40 kg N, 61,1 kg P, 48,2 kg K, N₃P₃K₃ – z zastosowaniem 60 kg N, 91,6 kg P, 72,3 kg K i N₁P₁K₁ – z zastosowaniem 80 kg N, 122,2 kg P i 96,4 kg K na 1 hektar

pisi proved to dominate as its isolates constituted 59% of all *Fusarium* spp. colonies. The isolates of *Alternaria alternata*, *Ascochyta pisi*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *pisi*, *Fusarium solani*, *Phoma exigua*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were obtained from the infected plants at anthesis.

Saprobionts were obtained during the mycological analysis of the infected roots and the stem base of pea plants. An especially big number of colonies of *Gliocladium* spp. and *Trichoderma* spp. were obtained from the plants taken from the plots where the microbiological material or the post-culture liquid was used for pre-sowing dressing. A much smaller quantity of these fungi was obtained from the plants grown out of the seeds dressed with a chemical preparation (tab. 3).

The mycological analysis of the seeds showed that the seeds with and without spots were infected by fungi. Totally, 1,081 fungi colonies during three years of studies were obtained (tab. 4). Majority of fungi isolates were originated from spotted seeds as compared to those without any spots. Fungi species occurring on plants during the vegetation and on the seeds were very similar. The most fungi colonies were isolated from the seeds gathered from control plants. Regardless of the applied microbiological material (conidia *T. harzianum* G 220 and *G. fimbriatum* S 151 or their post-culture liquids), a similar number of fungi colonies was obtained.

DISCUSSION

The present studies confirmed the information on the protective effect of *Trichoderma* spp. and *Gliocladium* spp. towards plant infection by pathogenic fungi [Pięta et al. 1998, Pastucha 1999, Pięta and Pastucha 2004, Pastucha and Patkowska 2005]. The obtained results also pointed to the positive effect of post-culture liquids of *T. harzianum* G 220 and *G. fimbriatum* S 151 on reducing the infection of pea by soil-borne pathogenic fungi.

The protective effect of the microbiological material made of the conidia of such antagonistic fungi as *Trichoderma* spp. and *Gliocladium* spp. against plant pathogens is known for a number of cultivated plants [Roznay et al. 1991, Elad 2000, Mesta and Amaresh 2000, Prasad and Rangeswaran 2000, Matcalf and Wilson 2001, Sharma and Sharma 2001, Gherbawy and Yaser 2003].

Introducing the conidia of antagonistic fungi through seed dressing to the soil effectively protects the germinating seeds, and then the roots and the stem base of the seedlings and older plants from infection by plant pathogens. The presence of antagonists in the soil inhibits the growth and development of pathogenic fungi and destroys their endospores, causing the decrease of the populations of plant pathogens [Papavizas 1985, Benhamou and Chet 1996]. On the other hand, antagonistic fungi developing in the rhizosphere of plants creates a barrier protecting them from infection by pathogenic fungi [Mukherjee et al. 1995, Pięta 1997, 1998, Weller 1998, Berg et al. 2002, Weller et al. 2002].

Good emergences, healthiness and yielding of plants point to positive effect of *T. harzianum* G 220 and *G. fimbriatum* S 151.

The use of post-culture liquids for dressing pea seeds as well as the microbiological material of antagonistic fungi proved effective in protecting from infection by soil-borne fungi. It should be supposed that enzymes present in the post-culture liquid, specific toxic compounds, antibiotics produced by *Trichoderma* spp. and *Gliocladium* spp. can effectively protect pea plants from pathogenic fungi [Liu 1988, Avent et al. 1993, Harman et al. 1993, Haran et al. 1995]. The occurrence of such enzymes as chitobiasis, chitinasis, glucanasis, proteasis in the post-culture liquid of the discussed antagonists causes degradation of the cell walls of pathogenic fungi [Roberti et al. 2002]. On the other hand, metabolites produced by *Trichoderma* spp. and *Gliocladium* spp. inhibit the germination of fungi endospores and their growth and development [Singh 1998, Stefanova et al. 1999, Rasami et al. 2001, Küçük and Kivanc 2003, Kumar et al. 2005].

Results obtained in the present studies point to the possibility of using both the microbiological material of *T. harzianum* G 220 and *G. fimbriatum* S 151 as well as their post-culture liquids to protect plants from soil-borne pathogenic fungi.

CONCLUSIONS

1. Dressing the pea seeds with conidia *Trichoderma harzianum* and *Gliocladium fimbriatum* and their post-culture liquids improve of the emergences, healthiness and yielding of the plants.

2. The application of the microbiological material made from conidia of *Trichoderma harzianum* and *Gliocladium fimbriatum* and their post-culture liquids to seeds dressing effectively protected the germinating seeds, seedlings and older plants from infection soil-borne fungi.

3. Pathogenic fungi from genus *Alternaria alternata*, *Ascochyta pisi*, *Botrytis cinerea*, *Phoma exigua*, *Pythium irregulare*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Fusarium* spp. threaten of the pea cultivations.

REFERENCES

- Avent A. G., Hanson J., Trunch A., 1993. Metabolites of *Gliocladium flavofuscum*. Phytochemistry, 32, 1, 197–198.
- Benhamou N., Chet I., 1996. Parasitism of sclerotia of *Sclerotium rolfsii* by *Trichoderma harzianum*: Ultrastructural and cytochemical aspects of the interaction. Phytopathol., 86, 405–416.
- Berg G., Roskot N., Steidle A., Eberl L., Zock A., Smalla K., 2002. Plant-diseases phenotypic diversity of antagonistic rhizobacteria isolated from different *Verticillium dahliae* Kleb. Appl. Environ. Microbiol., 68, 3328–3338.
- Dubay S. C., 2002. Bio-agent based integrated management of collar rot of french bean. Indian Phytopathol., 55, 2, 230–231.
- Elad J., 2000. *Trichoderma harzianum* T 39 preparation for biocontrol of plant diseases – control of *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Cladosporium fulvum*. Biocontrol Sci. and Technology, 10, 4, 499–507.
- El-Kafrawy A. A., 2002. Biological control of bean damping of caused by *Rhizoctonia solani* Egyptian J. Agric. Res., 80, 1, 57–70.

- Filipowicz A., 1983. Grzyby zasiedlające wędnący groch siewny oraz podatność na nie różnych odmian tej rośliny. Zesz. Prob. Post. Nauk Rol. 275, 107–120.
- Filipowicz A., 1993. Fungi colonizing and decaying plants of pea (*Pisum sativum* L.). Phytopath. Pol., (XVIII), 6, 73.
- Gherbawy Y. A. M. H., Yaser M., 2003. Fungicides and some biological controller agents effect on the growth of *Fusarium oxysporum* causing paprika wilt. Rev. Plant Pathol., 83 (5), 585.
- Gurha S. N., 2001. Effect of some *Trichoderma* spp. on the growth of different isolates of *Fusarium oxysporum* f. sp. *ciceris*, in vitro. Ann. Plant Protect. Sci., 9, 2, 332–334.
- Hagedorn D. J., 1989. Compendium of pea diseases. The American Phytopath. Soc., USA.
- Harman G. E., Hayes C. K., Lorito M., Di Pietro A., Brodway R. M., Tronsmo A., 1993. Chitinolytic enzymes produced by *Trichoderma harzianum*, purification of chitinobiosidase. Phytopathology, 83, 313–318.
- Haran S., Schickler H., Oppenheim A., Chet I., 1995. New components of the chitinolytic system of *Trichoderma harzianum*. Mycol. Res., 99, 441–446.
- Hazarika D. K., Das K. K., 1998. Biological management of root rot of french bean (*Phaseolus vulgaris* L.) calised by *Rhizoctonia solani*. Plant Dis. Res., 13, 2, 101–105.
- Indra N., Thribuvanamala G., 2002. Antagonisms of *Trichoderma* spp. against *Macrophomina phaseolina* causing root rot of black gram. Plant Dis. Res., 17, 1, 142–144.
- Kumar S., Sharma S., Sharma B. K., Thakur D. P., 2005. Effect of culture filtrate of antagonists on *Sclerotinia graminicola* sporangial formation and germination, India. Environ. Ecology, 23 (Special 4), 832–837.
- Kücüő C., Kivanc M., 2003. Isolation of *Trichoderma* spp. and Determination of Their Antifungal. Bioch. Physiol. Features. Turk. J. Biol., 27, 247–253.
- Lin A., Lee T. M., Dern J. C., 1994. Tricholin, a new antifungal agent from *Trichoderma viride* and its action in biological control of *Rhizoctonia solani*. Antibiotics, 47, 7, 799–805.
- Liu H. Y., 1988. Hyperparasitism of *Gliocladium deliquescens* and *Penicillium vermiculatum* on *Sclerotinia sclerotiorum*. Plant Protect. Bull., Tajwan, 31, 175–184.
- Łacicowa B., Pięta D., 1990. Skuteczność zaprawiania nasion fasoli *Trichoderma koningii* i *Gliocladium catenulatum* w warunkach zagrożenia chorobowego przez niektóre grzyby przeżywające w glebie. Phytopatologia Polonica, XI, 16–24.
- Łacicowa B., Pięta D., 1994a. Ochronne działanie mikrobiologicznego zaprawiania nasion grochu (*Pisum sativum* L.) przeciwko chorobotwórczym grzybom odglebowym. Annales UMCS, Horticultura, s. EEE, II, 165–171.
- Łacicowa B., Pięta D., 1994b. Skuteczność mikrobiologicznego zaprawiania nasion fasoli wielokwiatowej (*Phaseoli coccineus* L.) w warunkach zagrożenia chorobowego przez grzyby przeżywające w glebie. Annales UMCS, Horticultura, s. EEE, II, 157–164.
- Matcalf D. A., Wilson C. R., 2001. The process of antagonisms of *Sclerotium cepivorum* in white rot affected onion roots by *Trichoderma koningii*. Plant Pathology, 50 (2), 249–257.
- Mesta R. K., Amaresh Y. S., 2000. Biological control of *Sclerotium* wilt of sunflower. Plant Dis. Res., 15 (2), 202–203.
- Mishra C. B. P., Behr L., 1976. Der Einfluss von Kulturfiltraten von *Fusarium culmorum* (W. G. Sm.) Sacc. *Fusarium avenaceum* (Fr.) Sacc. und *Fusarium nivale* (Fr.) Ces. (*Griphosphaeria nivalis* Müller et v. Arx) auf die Keimung des Weizen. Arch. Phytopathol., Pflanzenschutz., 12, 373–377.
- Mukherjee P. K., Mukhopadhyay A. N., Sarmah D. K., Shrestha S. M., 1995. Comparative antagonistic properties of *Gliocladium virens* and *Trichoderma harzianum* on *Sclerotium rolfsii* and *Rhizoctonia solani* – it's relevance to understanding the mechanisms of biocontrol. J. Phytopathol., 143, 275–279.

- Oktaba W., 1987. Metody statystyki matematycznej w doświadczeniach. PWN, Warszawa, 488 pp.
- Papavizas G. S., 1985. *Trichoderma* and *Gliocladium*. Biology, ecology and potential for biocontrol. Ann. Rev. Phytopath., 23, 23–54.
- Pastucha A., 1999. Ochronne działanie mikroorganizmów antagonistycznych w ograniczaniu występowania chorób soi (*Glycine max* (L.) Merrill). Annales UMCS, Horticultura, s. EEE, VII, 119–135.
- Pastucha A., Patkowska E., 2005. Wpływ płynów pochodzących z grzybów antagonistycznych na zdrowotność i plonowanie soi. Acta Agrobotanica, 58, 2, 111–124.
- Pięta D., 1997. Niektóre aspekty wykorzystania mikroorganizmów antagonistycznych do zwalczania chorób roślin. Annales UMCS, Horticultura, s. EEE, V, 1–4.
- Pięta D., 1998. Rola mikroorganizmów antagonistycznych w zwalczaniu chorób roślin. Roczn. AR w Poznaniu, CCCIV, Ogr., 27, 221–227.
- Pięta D., Pastucha A., 2004. Biological methods of protecting common bean (*Phaseolus vulgaris* L.). Folia Univ. Agric. Stein. Agricultura, 239 (95), 301–306.
- Pięta D., Patkowska E., Pastucha A., 1998. The efficiency of microbiological dressing of pea (*Pisum sativum* L.) against pathogenic soilborne fungi. Ann. Agric. Sci., E, Plant Protect., 27, 1/2, 81–89.
- Pięta D., Patkowska E., Pastucha A., Bełkot M., 2002. Wpływ mikroorganizmów antagonistycznych na ograniczenie porażenia soi przez grzyby chorobotwórcze przeżywające w glebie. Acta Sci. Pol., Hortorum Cultus, 1 (1), 23–30.
- Pięta D., Pastucha A., Patkowska E., 2003. The use of antagonistic microorganisms in biological control of bean diseases. Sodininkystė ir Darzininkystė Horticulture and Vegetable Growing, 22, 3, 401–406.
- Prasad R. D., Rangeshwaran R., 2000. Effect of soil application of a granular formulation of *Trichoderma harzianum* on *Rhizoctonia solani* incited seed rot and damping-off of chickpea. J. Mycol. Plant Pathol., 30, 2, 216–220.
- Rasami, Aggarwal, Srivastawa K. D., Singh D. V., 2001. Biological control of loose smut of wheat: seed treatment with *Trichoderma viride* and its influence on plant growth. Ann. Plant Prot. Sci., 9, 1, 63–67.
- Roberti R., de Vero L., Pisi A., Elad Y. (ed.), Freeman S. (ed), Monte E., 2001. Biological control of wheat root rot by antagonistic fungi and their modes of action. IOBC-WPRS Working Group “Biological Control of Fungal and Bacterial Plant Pathogens”. Proceeding of the sixth other Means of Control Sevilla Spain, November 30, December 3. Biull. OILB-SROP, 2001, 24, 3, 13–16.
- Roberti R., Zakrisson E., Flaming F., de Vero L., 2002. Antagonistic fungi producing hydrolytic enzymes, active in degrading the cell wall of some root rot pathogens (*Fusarium* spp.) of wheat. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz, 109, 101–108.
- Roznay Z. D., Kavesi M., Klement Z., Kajana L., 1991. Biological control and dieback disease of apricots. Acta Horticulture, 293, 525–532.
- Shalini V., Dohroo N. P., 2003. Morphological, cultural and pathogenic variability in *Fusarium oxysporum* isolates from pea. Plant Dis. Res., 18 (1), 5–8.
- Sharma M., Sharma S. K., 2001. Biological control of *Dematophora necatrix* causing white root rot of apple. Plant Dis. Res., 16, 1, 40–45.
- Singh Y., 1998. Biological control of *Sclerotinia sclerotiorum*. Plant Dis. Res., 13, 2, 144–146.
- Stefanowa M., Leiva A., Larrinaga L., Coronado M. F., 1999. Metabolic activity of *Trichoderma* spp. isolates for a control of soilborne phytopathogenic fungi. Revista de la Facultad de Agronomía, Universidad del Zulia, 16, 5, 509–516.

Weller D. M., 1998. Biological control of soilborne plant pathogens in the rhizosphere. *Phytopathology*, 26, 379–407.

Weller D. M., Raaijmakers J. M., Gardener B. B., Thomashow L. S., 2002. Responsible for specific soil suppression to plant pathogens. *Ann. Rev. Phytopathology*, 27, 502–509.

SKUTECZNOŚĆ GRZYBÓW ANTAGONISTYCZNYCH I ICH PŁYNÓW POHODOWLANYCH W ZWALCZANIU CHOROÓB GROCHU (*Pisum sativum* L.)

Streszczenie. Celem pracy było określenie skuteczności ochronnego działania materiału mikrobiologicznego *Trichoderma harzianum* G 220 i *Gliocladium fimbriatum* S 151 oraz ich płynów pohodowlanych przeciwko grzybom przeżywającym w glebie. Stosowanie biologicznego zwalczania poprawiło wschody, zdrowotność roślin grochu oraz ich plonowanie. Mimo użycia materiału mikrobiologicznego do zaprawiania nasion, rośliny grochu były porażane przez *Alternaria alternata*, *Ascochyta pisi*, *Botrytis cinerea*, *Fusarium culmorum*, *Fusarium oxysporum* f. sp. *pisi*, *Fusarium solani*, *Phoma exigua*, *Pythium irregularare*, *Rhizoctonia solani* i *Sclerotinia sclerotiorum*.

Słowa kluczowe: groch, *Trichoderma harzianum*, *Gliocladium fimbriatum*, płyny pohodowlane

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