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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 *Altenaria alternata, Ascochyta pisi, Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum* f. sp. *pisi, 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. *pisi* 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 one 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

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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, Dubay 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 carboxymethylocelulosis, chitinasis, β-1,3 glucanasis and volatile lacton as well as 6-penthyl 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 glucanasis, chitinasis, proteasis 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 m^2, 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 $t \cdot ha^{-1}$) to 542.3 g (converted into 1.81 $t \cdot ha^{-1}$) (tab. 1). The smallest yield was gathered from control plants. A significantly higher yield as compared to the control was harvestedd 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.

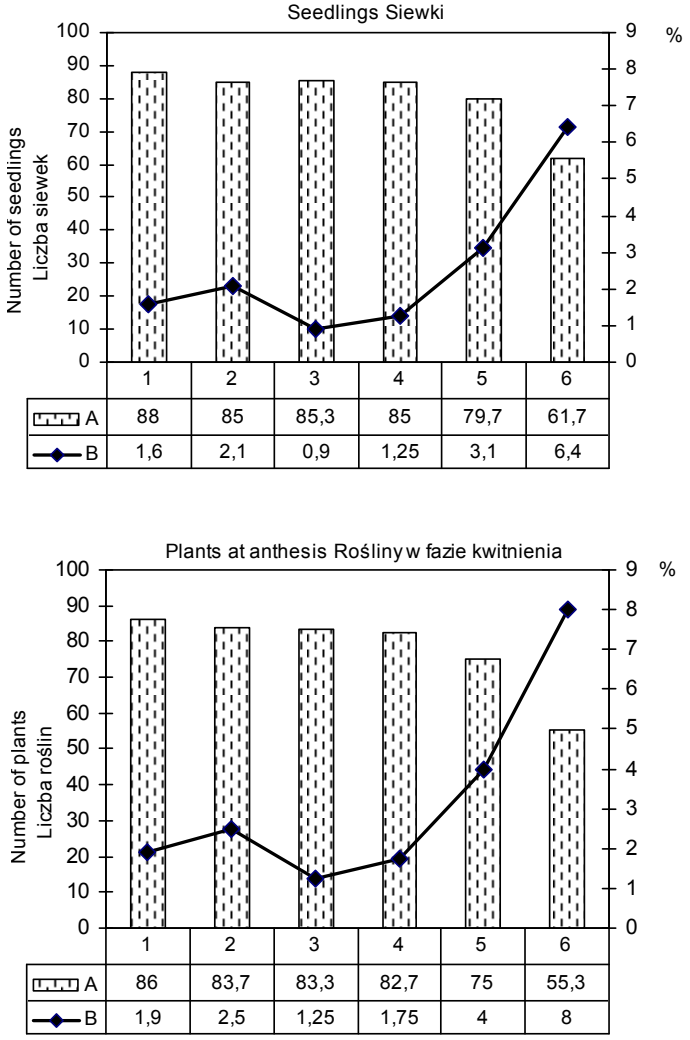

Fig. 1. Number and healthiness of pea plants in individual experimental (mean from the years 2005–2007): 1 – conidia of *T. harzianum*, 2 – conidia of *G. fimbriatum*, 3 – post culture liquids *T. harzianum*, 4 – post culture liquids *G. fimbriatum*, 5 – Zaprawa Oxafun T, 6 – control, A – number of plants, 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 pohodowlany *T. harzianum*, 4 – płyn pohodowlany *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 $t \cdot ha^{-1}$	Mean the percentage of infected seeds Średni udział porażonych nasion
The seeds dressed with *Trichoderma harzianum* G 220 Nasiona zaprawiane *Trichoderma harzianum* G 220	1.51*[c]	8.1*[b]
Seeds dressed with *Gliocladium fimbriatum* S 151 Nasiona zaprawiane *Gliocladium fimbriatum* S 151	1.81[c]	7.25[b]
Seeds soaked in post-culture liquids of *Trichoderma harzianum* G 220 Nasiona moczone w płynie pohodowlanym *Trichoderma harzianum* G 220	1.64[c]	5.8[a]
Seeds soaked in post-culture liquids of *Gliocladium fimbriatum* S 151 Nasiona moczone w płynie pohodowlanym *Gliocladium fimbriatum* 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 vere 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 *Altenaria 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 wyosobnione z porażonych roślin grochu w fazie kwitnienia w latach 2005–2007

Fungus species Gatunek grzyba	Experimental combination / Number of isolates – Kombinacja doświadczenia / Liczba izolatów														**Total** **Ogólem**
	Seeds dressed with *Trichoderma harzianum* G 220 Nasiona zaprawiane *Trichoderma harzianum* G 220		Seeds dressed with *Gliocladium fimbriatum* S 151 Nasiona zaprawiane *Gliocladium fimbriatum* S 151		Seeds soaked in post-culture liquids of *Trichoderma harzianum* G 220 Nasiona moczone w płynie pohodowlanym *Trichoderma harzianum* G 220		Seeds soaked in post-culture liquids of *Gliocla–dium fimbriatum* S 151 Nasiona moczone w płynie pohodowlanym *Glio–cladium fimbriatum* S 151		Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T		Control Kontrola		Total Razem		
	r/k	sb/pł	r/k	sb/pł	r/k	sb/pł	r/k	sb/pł	r/k	sb/pł	r/k	sb/pł	r/k	sb/pł	
Acremonium roseum (Oud.) W. Gams	1	2	-	-	2	3	1	1	3	4	6	4	13	14	**27**
Alternaria alternata (Fr.) Keissler	5	4	3	1	3	6	6	3	12	11	14	20	43	45	**88**
Ascochyta pisi Libert	1	5	1	2	-	1	3	2	11	8	18	22	34	40	**74**
Aspergillus niger van Tiegh	3	-	-	-	2	1	-	-	2	4	8	10	15	15	**30**
Aureobasidium pullulans *(de Bary) Arnaud.*	-	2	1	1	-	-	1	-	1	2	3	2	6	7	**13**
Botrytis cinerea *Pers.*	1	2	2	3	1	1	5	3	10	13	11	11	30	33	**63**
Cladosporium cladosporioides *(Fres) de Vries*	6	9	3	13	7	7	5	4	6	7	7	13	34	53	**87**
Epicoccum purpurascens Ehr. ex. Schl.	2	1	-	2	1	2	3	2	6	8	3	2	15	17	**32**
Fusarium culmorum (W. G. Sm.) Gams	5	1	4	6	4	3	6	5	12	13	17	18	48	46	**94**
Fusarium oxysporum Schl. f. sp. *pisi* (van Hall.) Snyd. et Hans	10	17	11	17	13	20	22	31	30	42	35	51	121	178	**299**
Fusarium solani (Mart.) Sacc.	7	6	4	6	5	8	6	9	12	17	16	17	50	63	**113**
Gliocladium catenulatum Gilman Abbott	3	6	7	3	4	3	7	3	2	2	2	-	25	17	**42**
Gliocladium fimbriatum *Gilman Abbott*	16	6	14	13	8	6	6	4	1	-	2	2	47	31	**78**
Gliocladium roseum Bainier	8	1	4	4	8	4	4	5	3	2	1	2	28	18	**46**
Humicola grisea Domsch	-	-	2	1	2	3	3	1	4	6	7	7	18	18	**36**
Mucor hiemalis Wehmer	3	4	10	-	3	7	3	4	1	5	3	4	23	24	**47**
Mucor mucedo Fresenius	1	1	-	-	-	-	-	2	2	1	3	6	6	10	**16**
Papulaspora irregularis Hotson	-	-	-	-	-	2	3	5	-	-	4	2	7	9	**16**
Penicillium expansum Link ex. S. F. Gray	15	10	-	2	4	3	-	2	1	4	1	2	21	23	**44**
Penicillium purpurogenum Stoll.	-	1-	1	2	6	3	6	4	5	1	7	5	25	16	**41**
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson et al.	5	-	4	1	3	5	1	1	6	7	9	12	28	26	**54**
Penicillium verrucosum Dierckx var. v*errucosum* Samson et al.	-	2	-	-	-	-	2	3	2	2	4	5	8	12	**20**
Phoma eupyrena Sacc.	2	1	3	2	1	-	-	4	4	5	5	6	15	18	**33**
Phoma exigua Desm.	2	1	-	3	1	-	2	1	4	6	7	8	16	19	**35**
Rhizoctonia solani Kühn	1	5	-	3	2	5	4	6	17	22	22	27	46	68	**114**
Rhizopus nigricans Ehrenberg	2	3	6	3	2	3	3	3	7	15	21	21	41	48	**89**
Sclerotinia sclerotiorum (Lib.) de Bary	-	3	3	-	-	1	3	-	12	11	13	19	31	34	**65**
Trichoderma harzianum Rifai	11	8	8	6	8	7	6	4	6	1	1	3	40	29	**69**
Trichoderma koningii Oud.	6	7	9	7	10	5	8	4	2	3	2	-	37	26	**63**
Trichoderma viride 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/ pł – stem base/ podstawa łodygi

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

Fungus species Gatunek grzyba	Experimental combination / Number of isolates – Kombinacja doświadczenia / Liczba izolatów														**Total Ogólem**
	Seeds dressed with *Trichoderma harzianum* G 220 Nasiona zaprawiane *Trichoderma harzianum* G 220		Seeds dressed with *Gliocladium fimbriatum* S 151 Nasiona zaprawiane *Gliocladium fimbriatum* S 151		Seeds soaked in post-culture liquids of *Trichoderma harzianum* G 220 Nasiona moczone w płynie pohodowlanym *Trichoderma harzianum* G 220		Seeds soaked in post-culture liquids of *Gliocladium fimbriatum* S 151 Nasiona moczone w płynie pohodowlanym *Gliocladium fimbriatum* S 151		Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T		Control Kontrola		Total Razem		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Acremonium roseum (Oud.) W. Gams	-	-	6	-	9	2	4	1	10	5	4	2	33	10	**43**
Alternaria alternata (Fr.) Keissler	15	7	19	5	9	2	10	3	15	6	17	7	85	30	**115**
Ascochyta pisi Libert	1	-	5	2	2	-	2	1	4	2	6	2	20	7	**27**
Botrytis cinerea Pers.	-	-	2	-	-	-	3	1	15	6	21	9	41	16	**57**
Cladosporium cladosporioides *(Fres) de Vries*	9	5	4	2	5	2	7	4	8	2	11	3	44	18	**62**
Epicoccum purpurascens Ehr. ex. Schl.	2	1	1	3	6	1	8	3	8	2	4	2	29	12	**41**
Fusarium equiseti (Corda) Sacc.	2	1	2	1	3	-	2	-	-	-	1	1	10	3	**13**
Fusarium graminearum Schwabe	-	-	1	-	2	-	1	-	10	4	10	5	24	9	**33**
Fusarium oxysporum Schl.	3	1	4	1	4	-	3	1	21	9	31	14	66	26	**92**
Fusarium poae (Peck.) Wollenw.	4	1	-	-	1	-	-	-	9	4	12	6	26	11	**37**
Fusarium solani (Mart.) Sacc.	-	-	-	-	-	-	-	-	3	1	5	3	8	4	**12**
Fusarium sporotrichioides Sherb.	1	-	2	1	-	-	2	-	4	2	6	3	15	6	**21**
Gliocladium catenulatum Gilman Abbott	7	2	6	2	6	2	8	3	6	1	1	1	34	11	**45**
Gliocladium fimbriatum Gilman Abbott	4	1	6	1	5	2	7	1	1	2	3	-	26	7	**33**
Gliocladium roseum Bainier	4	1	2	-	-	-	2	-	4	2	2	1	14	4	**18**
Humicola grisea Domsch	6	1	1	1	-	-	3	1	2	1	1	-	13	4	**17**
Mucor hiemalis Wehmer	1	-	3	1	3	1	3	1	1	-	3	2	14	5	**19**
Papulaspora irregularis Hotson	2	-	1	-	1	2	-	-	-	-	5	2	9	4	**13**
Penicillium canescens Scopp.	3	2	2	-	2	1	2	-	3	1	1	-	13	4	**17**
Penicillium frequentans Westling	7	1	3	-	3	-	-	1	3	1	3	2	19	5	**24**
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson et al.	2	-	1	1	-	-	3	1	2	1	1	1	9	4	**13**
Penicillium verrucosum var. *verrucosum* (West.) Samson et al.	-	-	3	1	8	3	4	-	8	2	11	5	34	11	**45**
Rhizoctonia solani Kühn	3	-	4	-	2	-	1	1	13	5	17	8	40	14	**54**
Rhizopus nigricans Ehrenberg	5	1	10	4	-	2	7	2	5	1	4	2	31	12	**43**
Sclerotinia sclerotiorum (Lib.) de Bary	1	-	-	-	1	-	1	-	16	6	18	6	37	12	**49**
Trichoderma hamatum (Bonard) Baim	4	1	1	-	4	3	3	2	-	1	4	1	16	8	**24**
Trichoderma harzianum Rifai	5	2	3	2	7	3	8	2	6	1	6	2	35	12	**47**
Trichoderma koningii Oud.	-	-	2	1	2	2	4	2	2	-	1	-	11	5	**16**
Trichoderma viride 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)	
		1st crop 1 zbiór	1st crop 1 zbiór	2nd crop 2 zbiór	1st crop 1 zbiór	2nd crop 2 zbiór	1st crop 1 zbiór	1st crop 1 zbiór	2nd crop 2 zbiór	1st crop 1 zbiór	2nd crop 2 zbiór
	$N_oP_oK_o$	18	37	23	29	15	1	6	4	6	3
	$N_1P_1K_1$	20	46	29	34	19	3	7	6	5	3
PS	$N_2P_2K_2$	21	54	33	38	20	3	7	5	8	4
	$N_3P_3K_3$	26	65	32	48	20	4	8	6	6	5
	$N_4P_4K_4$	29	66	35	45	23	5	8	8	5	5
Mean – Średnio		23	54	30	39	19	3	7	6	6	4
	$N_oP_oK_o$	29	56	30	37	21	2	8	5	6	4
	$N_1P_1K_1$	34	53	33	38	22	4	10	5	11	5
PG	$N_2P_2K_2$	36	57	38	46	22	4	9	6	8	5
	$N_3P_3K_3$	41	57	41	52	28	5	11	5	7	6
	$N_4P_4K_4$	44	56	45	50	27	5	12	4	7	6
Mean – Średnio		37	56	37	45	24	4	10	5	8	5
Source of variation Źródło zmienności											
Soil – Gleba		0.87	1.09	1.73	1.69	0.84	0.38	0.27	0.38	0.45	0.36
Fertilization level Poziom nawożenia		1.95	2.46	3.89	3.78	1.89	0.86	0.61	0.86	1.03	0.81
Interaction – Interakcja		3.24	4.09	NS	NS	3.13	1.44	1.01	1.44	1.68	NS

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; $N_oP_oK_o$ – control object without mineral fertilization, $N_1P_1K_1$ – with 20 kg N, 30.3 kg P, 24.1 kg K, $N_2P_2K_2$ – with 40 kg N, 61.1 kg P, 48.2 kg K, $N_3P_3K_3$ – with 60 kg N, 91.6 kg P, 72.3 kg K and $N_4P_4K_4$ – 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; $N_oP_oK_o$ – obiekt kontrolny bez nawożenia mineralnego, $N_1P_1K_1$ – z zastosowaniem 20 kg N, 30,3 kg P, 24,1 kg K, $N_2P_2K_2$ – z zastosowaniem 40 kg N, 61,1 kg P, 48,2 kg K, $N_3P_3K_3$ – z zastosowaniem 60 kg N, 91,6 kg P, 72,3 kg K i $N_4P_4K_4$ – 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 *Altenaria 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 Rangeshwaran 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, Kücük 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.

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SKUTECZNOŚĆ GRZYBÓW ANTAGONISTYCZNYCH I ICH PŁYNÓW POHODOWLANYCH W ZWALCZANIU CHORÓB 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 irregulare, Rhizoctonia solani* i *Sclerotinia sclerotiorum.*

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

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