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

Danuta Pięta, Alina Pastucha
University of Life Sciences in Lublin

Abstract. The purpose of the paper was to establish the protective effect of the microbiological material prepared from the cells of *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 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.* Among the applied microbiological material, the best protective effect against plant pathogens was observed for bacterium *Bacillus* sp. Bk 30 and its post-culture liquid.

Key words: pea, *Bacillus* sp, *Pseudomonas* sp., post-culture liquids

INTRODUCTION

Losses in plant cultivation caused by pathogenic fungi are always remarkable. At present fungicides are mainly used to protect plants from diseases. According to Borecki [1984], chemical preparations cause the appearance of new fungi genotypes which are more pathogenic towards plants. Besides, fungicides infect the agricultural environment, and the remains of chemical compounds are accumulated in the yield. When no chemical preparations are used, strong plant infection takes place. The presence of fungi in plant tissues causes their infection by mitotoxins [Chełkowski 1985]. More and more frequently non-chemical methods are sought for plant protection. For this purpose, attempts to use antagonistic bacteria of *Bacillus* spp. and *Pseudomonas* spp. are made to control plant diseases [Saniewska et al. 1995, Goel et al. 2000, Manwar et al. 2000, Yeole and Dube 2000, Lewosz 2002, Pięta et al. 2002].

Corresponding author – Adres do korespondencji: Danuta Pięta, Department of Phytopathology, University of Life Science in Lublin, 7 Leszczyńskiego Str., 20-069 Lublin, e-mail: danuta.pieta@up.lublin.pl

Antagonistic *Pseudomonas* spp. and *Bacillus megaterium* exudate hexanal aldehydes against *Botrytis cinerea* [Abanda-Nipwatt et al. 2006]. Domenech et al. [2006] reported that *Bacillus subtilis, Pseudomonas fluorescens and Chryseobacterium balustrinum* as well as a product LS 213 (made from a mixture of *Bacillus subtilis, B. amyloliquefaciens* and chitosan), were effective in protecting the plants of tomato and pepper from soil-borne pathogenic fungi. Those bacteria formed different metabolites of fungicidal or fungistatic properties. Antagonistic bacteria – through the exudation of siderophores [Hamdan et al. 1991, Henry et al. 1991, Manwar et al. 2000, Yeole and Dube 2000] or antibiotics [Howie and Suslov 1991, Cartwright et al. 1995, Rasales et al. 1995, Goel et al., 2000, Srivastava and Shalini 2008] – protected different species of plants from infection by pathogenic fungi. That is the reason why the application of both the microbiological material made from the cells of antagonistic bacteria and their post-culture liquids containing different metabolites of those bacteria may prove effective in the protection from plant pathogens.

The purpose of the present studies was to examine the effectiveness of the protective effect of bacteria *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 and their post-culture liquids from pathogenic fungi infecting pea (*Pisum sativum* L.)

MATERIAL AND METHODS

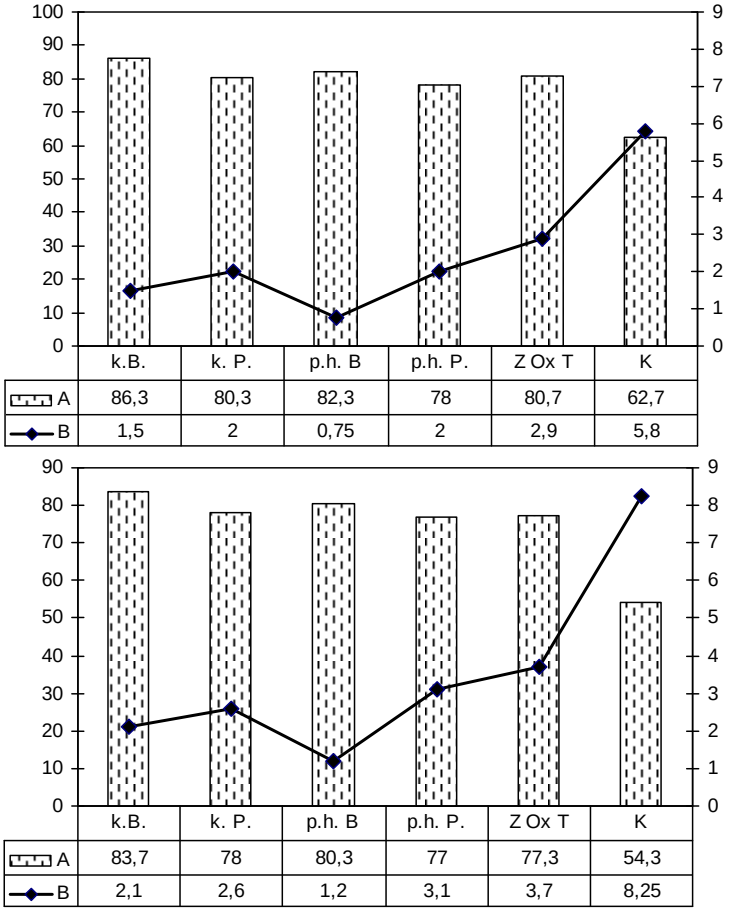
The studies were conducted in the years 2005–2007 on a field of the Experimental Station at Czesławice near Nałęczów. The object of studies were the plants of pea cv. 'Sześciotygodniowy Tor', which grew from the seeds dressed with the cells of antagonistic bacteria *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 as well as with the post-culture liquids of those bacteria. Pea seeds dressing was carried out directly before the sowing [Patkowska and Pastucha 2005]. In order to compare the protective effect of the cells of antagonistic bacteria and their post-culture liquids, the experiment also considered a combination with chemical seed dressing with Zaprawa Oxafun T and the control combination without dressing. The post-culture liquids of the bacteria used in the experiment were obtained from their culture on a liquid medium PDB (Difco) [Saniewska et al. 1995]. Each experimental combination included 4 plots (replications) with the area of $3m^2$, where 100 seeds were sown on each. The bacteria used in thee experiment were from the soil cultivation environment of pea, and their antagonistic effect towards pathogenic fungi was established using the method by Martyniuk et al. [1991].

During the vegetation, two field observations were performed on the plots of particular experimental combinations, i.e. in the phase of seedlings and at anthesis of pea plants, thus determining their number and healthiness. The seedlings as well as the plants at anthesis with inhibited growth and with disease symptoms in the form of necrotic spots were taken in the quantity of 5 for a laboratory mycological analysis conducted with the method described by Pięta et al. [1998]. After the harvest, the size and quality of the pea yield were established.

The obtained results concerning the number and healthiness of the seedlings and plants at anthesis as well as the yielding were statistically analyzed. The significance of differences was determined on the basis of Tukey's confidence semi-intervals [Oktaba 1987].

RESULTS

The first observation found out different numbers of seedlings on the plots of particular experimental combinations (fig. 1). The greatest number of seedlings grew on the plots sown with the seeds dressed with the cells of *Bacillus* sp. Bk 30 and the post-



k. B. – cells *Bacillus* sp. Bk 30, k. P. – cells *Pseudomonas* sp. Pk 28, p.h.B. – post-culture liquids *Bacillus* sp. Bk 30, p.h. P. – post-culture liquids *Pseudomonas* sp. Pk 28, Z Ox. T – Zaprawa Oxafun T, K – Control

B. – komórki *Bacillus* sp. Bk 30, k. P. – komórki *Pseudomonas* sp. Pk 28, p.h.B – płyn pohodowlany *Bacillus* sp. Bk 30, p.h.P – płyn pohodowlany *Pseudomonas* sp. Pk 28, Z Ox. T – Zaprawa Oxafun T, K – Kontrola

A – number plants – liczba roślin; B – % infected plants, B – % porażonych roślin

Fig. 1. Number and healthiness of pea plants in individual experimental (mean from the yars 2005–2007)

Rys. 1. Liczebność i zdrowotność roślin grochu w poszczególnych kombinacjach doświadczenia (średnie z lat 2005–2007)

culture liquid of this bacterium. On the other hand, the smallest number of seedlings grew on control plots, which were sown with non-dressed seeds. At that time the seedlings with inhibited growth and yellowing lower leaves occurred on all the plots. After the seedlings were taken out from the soil, necrotic spots were visible on their roots and the stem base (photo 1). The proportion of such seedlings on the plots of all experimen-

Photo 1. Necrosis of the roots and stem base of pea seedlings (phot. D. Pięta)
Fot. 1. Nekroza korzeni i podstawy łodygi siewek grochu (fot. D. Pięta)

Photo 2. Plants at anthesis of pea infected from soil-borne of fungi (phot. D. Pięta)
Fot. 2. Rośliny grochu w fazie kwitnienia porażone przez grzyby przeżywające w glebie (fot. D. Pięta)

tal combinations ranged from 0.75% to 5.8%. The smallest number of seedlings was observed on the plots sown with the seeds wetted in the post-culture liquid of *Bacillus* sp. Bk 30, while the greatest number was found on control plots (fig. 1). Few infected seedlings were found on the plots in the combinations with dressing the seeds with the cells of *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 as well as the post-culture liquid of *Pseudomonas* sp. Pk 28 (fig. 1).

A slightly smaller number of plants were found during the second observation but the proportion of infected plants increased (fig. 1). Like in the case of seedlings, the greatest number of plants was found on the plots in the combination with *Bacillus* sp. Bk 30 cells and the post-culture liquid of this bacterium used for seed dressing, while the smallest number was observed in the control combination. Infected plants at anthesis had distinct disease symptoms in the form of vast necrotic spots on the roots and the stem base (photo 2). Such plants bloomed poorly or they did not set any pods at all, and they sometimes died out. The proportion of diseased plants on the plots of particular combinations ranged from 1.2% to 8.25% (fig. 1).

After the plants were picked up and dried up, the size and quality of the yield were established (tab. 1). The size of the seed yield was proportional to the number and healthiness of plants on the plots. The highest yield was gathered from the plants in the combination with *Bacillus* sp. Bk 30 cells used for seed dressing. On the other hand, the smallest number of seeds was gathered from the plants growing in the control combination. The seeds which were small and had brown spots on the cover occurred in all samples. The proportion of such seeds ranged from 1.92% (in the yield of the combination with post-culture liquid of *Bacillus* sp. Bk 30) to 11.92% (in the yield of the control combination), on average (tab. 1).

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 in t/ha Średni plon nasion w t z ha	Mean the percentage of infected seeds Średni udział porażonych nasion
The seeds dressed with *Bacillus* sp. Bk 30 Nasiona zaprawiane *Bacillus* sp. Bk 30	1.44^{c}*	5.75^{b}
The seeds dressed with *Pseudomonas* sp. Pk 28 Nasiona zaprawiane *Pseudomonas* sp. Pk 28	1.10^{b}	4.92^{b}
Seeds soaked in post-culture liquids *Bacillus* sp. Bk 30 Nasiona moczone w płynie pohodowlanym *Bacillus* sp. Bk 30	1.17^{b}	1.92^{a}
Seeds soaked in post-culture liquids *Pseudomonas* sp. Pk 28 Nasiona moczone w płynie pohodowlanym *Pseudomonas* sp. Pk 28	0.99^{b}	4.58^{b}
The seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	1.14^{b}	6.58^{b}
Control – Kontrola	0.64^{a}	11.92^{c}

*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 nie są oznaczone tą samą literą

Table 2. Fungi isolated from infected seedlings of pea in the years 2005–2007
Tabela 2. Grzyby wyosobnione z porażonych siewek grochu w latach 2005–2007

Fungus species Gatunek grzyba	Experimental combination / Number of isolates Kombinacja doświadczenia / Liczba izolatów														H
	A		B		C		D		E		F		G		
	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	
Acremonium roseum (Oud.) W. Gams	4	4	6	6	4	2	11	7	8	6	4	2	37	27	64
Alternaria alternata (Fr.) Keissler	6	7	3	8	6	6	5	7	11	11	10	8	41	47	88
Ascochyta pisi Libert	4	8	4	7	5	1	2	4	13	7	9	10	37	37	74
Botrytis cinerea Pers.	-	-	-	-	-	-	1	-	3	2	5	4	9	6	15
Cladosporium cladosporioides (Fres) de Vries	3	1	-	7	3	5	-	-	4	2	5	4	15	19	34
Epicoccum purpurascens Ehr. ex. Schl.	3	1	3	5	2	4	5	3	1	1	10	5	24	19	43
Fusarium culmorum (W. G. Sm.) Gams	4	4	4	7	5	6	3	6	7	6	4	6	27	35	62
Fusarium equiseti (Corda) Sacc.	5	1	3	3	3	4	6	5	5	4	8	7	30	24	54
Fusarium oxysporum Schl. f. sp. *pisi* (van Hall.) Snyd. et Hans	10	20	31	29	13	14	13	21	18	23	15	23	100	130	230
Fusarium solani (Mart.) Sacc.	2	1	3	4	3	5	4	2	4	6	5	7	21	25	46
Gliocladium catenulatum Gilman Abbott	6	6	4	5	5	6	10	2	1	3	3	2	29	24	53
Gliocladium roseum Bainier	1	6	3	6	4	5	12	10	2	4	-	1	22	32	54
Humicola grisea Domsch	3	-	1	2	2	1	-	-	4	2	3	5	13	10	23
Mucor hiemalis Wehmer	3	1	-	-	-	3	3	3	1	-	-	-	7	7	14
Mucor mucedo Fresenius	-	-	-	-	-	-	-	-	3	-	-	4	3	4	7
Penicillium canescens Scopp.	1	-	1	4	2	-	-	-	1	-	-	-	5	4	9
Penicillium expansum Link ex S. F. Gray	-	8	5	6	-	3	4	-	5	6	4	3	18	26	44
Penicillium frequentans Westling	1	3	-	1	2	-	-	-	3	1	-	-	6	5	11
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson et al.	3	-	3	4	-	-	1	1	2	1	5	-	14	6	20
Phoma exigua Desm.	-	-	-	-	-	-	2	1	2	4	3	6	7	11	18
Pythium irregulare Buisman	6	4	8	8	6	4	4	7	9	9	8	12	41	44	85
Rhizoctonia solani Kűhn	7	8	6	8	5	4	4	3	9	7	9	17	40	47	87
Rhizopus nigricans Ehrenberg	-	-	1	2	4	4	1	2	2	1	3	-	11	9	20
Talaromyces flavus Stolk et Samson	1	1	10	6	-	-	-	-	1	4	-	-	12	11	23
Trichoderma harzianum Rifai	8	7	7	5	4	18	8	11	7	4	1	2	35	47	82
Trichoderma koningii Oud.	1	3	1	3	13	6	13	7	2	3	-	3	30	25	55
Trichoderma polysporum (Link ex Pers.) Rifai	1	1	3	-	2	-	3	2	-	2	-	-	9	5	14
Total – Razem	83	95	110	136	93	101	115	104	128	119	114	131	643	686	1329

A – The seeds dressed with *Bacillus* sp. Bk 30 – Nasiona zaprawiane *Bacillus* sp. Bk 30
B – The seeds dressed with *Pseudomonas* sp. Pk 28 – Nasiona zaprawiane *Pseudomonas* sp. Pk 28
C – Seeds soaked in post-culture liquids *Bacillus* sp. Bk 30 – Nasiona moczone w płynie poh. *Bacillus* sp. Bk 30
D – Seeds soaked in post-culture liquids *Pseudomonas* sp. Pk 28 – Nasiona moczone w płynie poh. *Pseudomonas* sp. Pk 28
E – The seeds dressed with Zaprawa Oxafun T – Nasiona zaprawiane Zaprawą Oxafun T
F – Control – Kontrola
G – Total – Razem
H – Total – Ogółem
k – root, korzeń; pł – stem base, podstawa łodygi

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														H
	A		B		C		D		E		F		G		
	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	
Acremonium roseum (Oud.) W. Gams	2	2	3	3	-	-	2	-	4	5	6	4	17	14	31
Alternaria alternata (Fr.) Keissler	6	7	6	9	8	5	8	9	8	12	14	16	50	58	108
Ascochyta pisi Libert	5	7	7	2	7	6	5	1	10	9	15	18	49	43	92
Aspergillus niger van Tiegh	1	-	-	-	4	1	-	14	3	5	6	15	14	35	49
Aureobasidium pullulans (de Bary) Arnaud.	-	-	2	-	2	2	4	3	2	2	3	2	13	9	22
Botrytis cinerea Pers.	7	3	6	5	7	2	9	1	11	9	12	9	52	29	81
Cladosporium cladosporioides (Fres) de Vries	2	4	3	5	4	3	2	3	4	6	9	13	24	34	58
Epicoccum purpurascens Ehr. ex. Schl.	3	2	2	5	2	3	4	3	5	10	5	2	21	25	46
Fusarium culmorum (W. G. Sm.) Gams	9	5	7	11	11	8	5	8	15	13	15	18	62	63	125
Fusarium oxysporum Schl. f. sp. *pisi* (van Hall.) Sny et Hans	20	24	32	33	20	25	25	30	30	41	36	50	163	203	366
Fusarium solani (Mart.) Sacc.	9	11	14	11	9	11	11	19	18	21	19	28	80	101	181
Gliocladium catenulatum Gilman Abbott	4	3	1	3	4	3	2	2	3	1	1	1	15	13	28
Gliocladium fimbriatum Gilman Abbott	3	2	1	2	3	2	4	4	-	1	-	1	11	12	23
Gliocladium roseum Bainier	4	4	5	5	5	2	7	4	4	2	4	4	29	21	50
Humicola grisea Domsch	-	-	1	1	2	2	-	-	3	4	5	6	11	13	24
Mucor hiemalis Wehmer	-	4	8	-	2	-	-	2	-	3	-	2	10	11	21
Mucor mucedo Fresenius	2	6	-	7	-	7	6	5	6	4	7	7	21	36	57
Papulaspora irregularis Hotson	4	3	-	3	6	3	7	-	-	-	3	3	20	12	32
Penicillium expansum Link ex. S. F. Gray	4	-	-	1	-	-	3	4	2	4	1	2	10	11	21
Penicillium purpurogenum Stoll.	5	2	-	7	4	4	1	3	3	2	8	4	21	22	43
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson et al.	6	3	2	-	4	6	3	5	9	6	9	5	33	25	58
Penicillium verrucosum Dierckx var. v*errucosum* Samson et al.	4	-	2	3	4	2	-	-	3	2	4	5	17	12	29
Phoma eupyrena Sacc.	3	4	1	2	2	2	1	3	4	5	6	7	17	23	40
Phoma exigua Desm.	2	3	3	3	4	5	5	3	9	13	14	11	37	38	75
Rhizoctonia solani Kűhn	6	8	10	10	13	8	6	6	18	14	21	27	74	73	147
Rhizopus nigricans Ehrenberg	2	1	2	-	5	4	8	5	6	6	9	16	32	32	64
Sclerotinia sclerotiorum (Lib.) de Bary	5	3	5	4	5	4	7	1	7	7	11	12	40	31	71
Trichoderma harzianum Rifai	3	2	1	3	-	2	1	6	3	1	2	-	10	14	24
Trichoderma koningii Oud.	10	5	9	8	2	6	11	3	1	2	-	3	33	27	60
Trichoderma viride Pers. ex S. F. Gray	4	5	4	2	-	3	6	-	1	2	2	2	17	14	31
Total – Razem	135	123	137	148	139	131	153	147	192	212	247	293	1003	1054	2057

A – The seeds dressed with *Bacillus* sp. Bk 30 – Nasiona zaprawiane *Bacillus* sp. Bk 30
B – The seeds dressed with *Pseudomonas* sp. Pk 28 – Nasiona zaprawiane *Pseudomonas* sp. Pk 28
C – Seeds soaked in post-culture liquids *Bacillus* sp. Bk 30 – Nasiona moczone w płynie poh. *Bacillus* sp. Bk 30
D – Seeds soaked in post-culture liquids *Pseudomonas* sp. Pk 28 – Nasiona moczone w płynie poh. *Pseudomonas* sp. Pk 28
E – The seeds dressed with Zaprawa Oxafun T – Nasiona zaprawiane Zaprawą Oxafun T
F – Control – Kontrola
G – Total – Razem
H – Total – Ogółem
k – root, korzeń; pł – stem base, podstawa łodygi

Table 4. Fungi isolated from seeds of pea 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														H
	A		B		C		D		E		F		G		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Acremonium roseum (Oud.) W. Gams	12	-	12	4	11	6	7	3	13	7	5	3	60	23	83
Alternaria alternata (Fr.) Keissler	32	10	10	5	7	1	14	10	14	10	19	16	96	52	148
Ascochyta pisi Libert	2	-	3	-	3	1	4	2	5	1	1	8	18	12	30
Botrytis cinerea Pers.	7	1	10	3	9	4	8	2	14	9	17	13	65	32	97
Cladosporium cladosporioides (Fres) de Vries	6	2	5	1	6	3	8	4	5	1	6	6	36	17	53
Epicoccum purpurascens Ehr. ex. Schl.	4	1	1	-	-	1	1	1	2	1	1	1	9	5	14
Fusarium equiseti (Corda) Sacc.	3	1	3	1	-	-	2	-	6	2	7	4	21	8	29
Fusarium graminearum Schwabe	4	1	3	1	3	1	2	-	9	5	9	8	30	16	46
Fusarium oxysporum Schl.	7	2	9	4	4	2	9	4	15	12	22	17	66	41	107
Fusarium poae (Peck.) Wollenw.	7	3	9	3	5	2	13	4	14	11	24	17	72	40	112
Fusarium solani (Mart.) Sacc.	1	-	-	-	2	1	4	2	3	5	8	12	18	20	38
Fusarium sporotrichioides Sherb.	-	-	2	1	5	2	6	1	4	2	6	3	23	9	32
Gliocladium catenulatum Gilman Abbott	5	2	9	2	5	1	5	5	4	1	1	-	29	11	40
Gliocladium fimbriatum Gilman Abbott	-	-	3	1	3	1	-	-	1	1	3	-	10	3	13
Humicola grisea Domsch	4	2	4	2	4	1	5	2	3	2	3	4	23	13	36
Mucor hiemalis Wehmer	1	-	2	1	5	2	4	1	4	2	3	3	19	9	28
Papulaspora irregularis Hotson	3	1	-	-	-	-	2	1	1	1	2	-	8	3	11
Penicillium canescens Scopp.	1	-	2	-	2	-	4	2	1	-	2	-	12	2	14
Penicillium frequentans Westling	3	2	4	2	-	-	5	1	3	1	3	2	18	8	26
Penicillium verrucosum var. *verrucosum* (West.) Samson et al.	-	-	6	2	2	-	8	3	2	4	8	1	26	10	36
Phoma exigua Desm. var. *exigua*	-	-	1	1	1	1	-	-	3	1	5	2	10	5	15
Rhizoctonia solani Kűhn	9	4	5	2	6	2	10	3	14	6	22	12	66	29	95
Rhizopus nigricans Ehrenberg	4	2	4	2	-	-	2	1	5	1	3	2	18	8	26
Sclerotinia sclerotiorum (Lib.) de Bary	2	-	4	1	4	-	3	-	8	3	8	7	29	11	40
Trichoderma harzianum Rifai	2	-	7	3	2	1	5	1	4	1	5	2	25	8	33
Trichoderma viride Pers. ex S.F. Gray	-	2	3	-	2	-	4	2	-	2	1	1	10	7	17
Total – Razem	119	36	121	42	91	33	135	55	157	92	194	144	817	402	1219

A – The seeds dressed with *Bacillus* sp. Bk 30 – Nasiona zaprawiane *Bacillus* sp. Bk 30
B – The seeds dressed with *Pseudomonas* sp. Pk 28 – Nasiona zaprawiane *Pseudomonas* sp. Pk 28
C – Seeds soaked in post-culture liquids *Bacillus* sp. Bk 30 – Nasiona moczone w płynie poh. *Bacillus* sp. Bk 30
D – Seeds soaked in post-culture liquids *Pseudomonas* sp. Pk 28 – Nasiona moczone w płynie poh. *Pseudomonas* sp. Pk 28
E – The seeds dressed with Zaprawa Oxafun T – Nasiona zaprawiane Zaprawą Oxafun T
F – Control – Kontrola
G – Total – Razem
H – Total – Ogółem
1 – seeds with spots, nasiona z plamami; 2 – seeds without spots, nasiona bez plam

Both the infected seedlings and plants at anthesis were subjected to a laboratory mycological analysis. Totally, 1329 fungi isolates were isolated from the roots and the stem base, while 2,057 isolates were obtained from the same organs of plants at anthesis (tab. 2, 3). The largest populations isolated from the analyzed organs of seedlings and plants at anthesis included *Fusarium* spp. represented by *F. culmorum, F. oxysporum* f. sp. *pisi* and *F. solani* . Among the enumerated species, *F. oxysporum* f. sp. *pisi* was

the pathogen isolated in the greatest quantities. Besides, pea seedlings were infected by *A. alternata, B. cinerea, P. exigua, P. irregulare* and *R. solani* (tab. 2). On the other hand, plants at anthesis were infected by the same species and – additionally – by *A. pisi* and *S. sclerotiorum* (tab. 3). The most fungi isolates – regardless of the developmental phase of plants – were isolated from control plants, i.e. without seed dressing. Much fewer fungi colonies were isolated from plants grown out of the seeds dressed with the microbiological material (tab. 2, 3).

A laboratory mycological analysis of the seeds gave 1219 fungi isolates (tab. 4). More than twice as many fungi colonies were isolated from the seeds with spots as compared to those without them. The most fungi isolates were obtained from the seeds gathered from control plants. The use of the microbiological material in the form of a suspension of the cells of antagonistic bacteria or post-culture liquids of those bacteria caused less infection of the seeds by fungi (tab. 4). Pea seeds were infected by the same fungi species that occurred on plants during the vegetation. Besides, the quantitative composition of particular fungi species in seeds infection was proportional to the number of fungi isolated from the studied plants.

DISCUSSION

The present studies confirmed the information from the literature on the protective effect of antagonistic bacteria such as *Bacillus* spp. and *Pseudomonas* spp. [Saniewska et al. 1995, Pięta et al. 1998, Jian et al. 1999, Babu et al. 2000, Lewosz 2002, Pięta and Patkowska 2003]. Post-culture liquids were as effective as the cells of *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 used for seed dressing in the protection of pea from soil-borne pathogenic fungi. The cells of the studied bacteria and their post-culture liquids used in the experiment had a positive effect on the emergences, healthiness and yielding of pea. The microbiological material (cells of *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28) and the post-culture liquids of the tested bacteria effectively protected the germinating seeds, and next the roots of seedlings and older plants from infection by soil-borne plant pathogens.

According to De et al. [2003], wetting lentil seeds in the post-culture liquid of *P. fluorescens* limited plant infection by *Fusarium oxysporum* f. sp. *lentis.* Studies conducted by Babu et al. [2000] showed that the use of post-culture liquid of *P. fluorescens* on tomato plants reduced the signs of alternariosis on the leaves by 38%. On the other hand, in *in vitro* conditions the post-culture liquids of *Bacillus subtilis* B-903 caused deformation of the hypha and spores of the cell walls of *F. oxysporum* f. sp. *spinacie* [Jian et al. 1999]. Besides, *Bacillus polymyxa* or the post-culture liquid of this bacterium successfully inhibited the development of the red spot on *Hipeastrum* and *Hymenocalis* caused by *Phoma narcissi* [Saniewska 2000]. *In vitro* studies showed that this bacterium was antagonistic towards other species of pathogenic fungi of ornamental plants [Saniewska 2000].

It should be supposed that high effectiveness of the protective effect of bacteria used in the experiment such as *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 was based on competition, parasitism and antibiosis [according to the literature quoted by Pięta 2004].

The tested antagonistic bacteria exudated specific compounds, antibiotics, enzymes, which were contained in post-culture liquids. On the basis of abundant information from the literature, it can be stated that the enumerated antagonistic microorganisms exudate such metabolites limiting the growth and development of plant pathogens as siderophores, antibiotics, salicylic acid and anthranilic acid (resistance inducing substances, enzymes – glucanasis, chitinasis, amylases, proteasis and hormones) [Manwar et al. 2000, Yeole and Dube 2000, Lewisz 2002, Sobiczewski 2002].

CONCLUSIONS

1. The cells of the studied bacteria and their post-culture liquids used in the experiment had a positive effect on the emergences, healthiness and yielding of pea
2. *Bacillus* sp. Bk 30 and *Pseudomonas* sp. Pk 28 and their post-culture liquids effectively protected the germinating seeds, and next the roots of seedlings and older plants from infection by soil-borne pathogens
3. Roots and stem bases of seedlings and plant at anthesis where infected by *Alternaria alternata, Ascochyta pisi, Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum* f. sp. *pisi, Fusarium solani, Phoma exigua, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum*

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BAKTERIE ANTAGONISTYCZNE I ICH PŁYNY POHODOWLANE W OCHRONIE GROCHU (*Pisum sativum* L.) PRZED CHOROBAMI

Streszczenie. Celem pracy było określenie skuteczności ochronnego działania materiału mikrobiologicznego sporządzonego z komórek *Bacillus* sp. Bk 30 i *Pseudomonas* sp. Pk 28 oraz ich płynów pohodowlanych przeciwko grzybom patogenicznym 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*. Spośród stosowanego materiału mikrobiologicznego najskuteczniejszym ochronnym działaniem przed fitopatogenami wyróżniła się bakteria *Bacillus* sp. Bk 30 oraz jej płyn pohodowlany.

Słowa kluczowe: groch, *Bacillus* sp., *Pseudomonas* sp., płyny pohodowlane

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