

MAY THE POST-CULTURE LIQUIDS OF BACTERIA INFLUENCE ON SOYBEAN [*Glycine max* (L.) Merrill] HEALTHINESS?

Elżbieta Patkowska, Marzena Błazewicz-Woźniak

University of Life Science in Lublin

Abstract. Soybean is one of the most valuable plants cultivated in the world. Soybean seeds and its products contain fibre, lecithin, vitamins, especially from the B group, mineral elements, isoflavones (genistein and daidzein), phytic acid, proteanase and saponin inhibitors. The object of the studies were the plants of soybean 'Aldana' cv. grown from the seeds dressed directly before the sowing with post-culture liquids *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73. The studies also considered a combination with chemical seed dressing with Zaprawa Oxafun T and a combination without any dressing. The effectiveness of post-culture liquids of antagonistic bacteria was estimated in protecting soybean from soil fungi. Post-culture liquids had a positive effect on the number, health status and yielding of the studied plants. Soybean was mainly infected by *Fusarium culmorum*, *F. oxysporum*, *Phoma exigua*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Species *Phomopsis sojae* was additionally isolated from the infected plants. Those fungi were isolated much less frequently from the plants in combinations with post-culture liquids of antagonistic bacteria than from reference plants. A reverse relation was observed in the case of the occurrence of saprobic fungi from genera *Gliocladium*, *Penicillium* and *Trichoderma*.

Key words: biological control, *Bacillus* sp., *Pseudomonas* sp., phytopathogens

INTRODUCTION

In the protection of cultivated plants, including the Fabaceae, which soybean [*Glycine max* (L.) Merrill] belongs to, chemical methods are more and more frequently replaced by the biological methods based on the use of biotechnical preparations [Orlikowski and Skrzypczak 2003, Shovan et al. 2008, Kurzawińska and Mazur 2009, Patkowska 2009a, 2009b, Ye Li-min et al. 2009, Kućmierz et al. 2010] or by the microbiological material of antagonistic microorganisms [Abanda-Nipwatt et al. 2006,

Domenech et al. 2006, Dipali et al. 2007, Khilari et al. 2008, Akhukorala et al. 2009, Dehestani et al. 2010, Patkowska 2010, Patkowska and Pięta 2010]. Biological methods are mainly used in ecological cultivations. The efficiency of such methods in controlling pathogenic fungi towards soybean was shown, for example, by Rajeswardi and Kumari [2009] and Zhang and Xue [2010].

The literature of the subject provides information on controlling phytopathogens species by antagonistic fungi (*Gliocladium* spp., *Trichoderma* spp.) and bacteria (*Bacillus* spp., *Pseudomonas* spp.), whose effect is based on antibiosis, competition or parasitism [Abanda-Nipwatt et al. 2006, Domenech et al. 2006, Srivastava and Shalini 2008, Dehestani et al. 2010, Farhan et al. 2010, Pedersen et al. 2010]. The commonly known antagonists of genera *Pseudomonas* and *Bacillus* include such species as *P. fluorescens* (Flügge) Migula, *P. putida* Trevisan, *P. cepacia* Kluver, *P. aureofaciens* Kluver, *P. chlororaphis* Guign. et Sauvag., *B. subtilis* Ehrenberg, *B. mycoides* Cohn, *B. polymyxa* (Prazmowski) Mace [Saniewska et al. 1995, Abanda-Nipwatt et al. 2006, Domenech et al. 2006, Chincholkar et al. 2007, Srivastava and Shalini 2008, Akhukorala et al. 2009, Sandikar and Awasthi 2009/2010, Dehestani et al. 2010, Farhan et al. 2010, Pedersen et al. 2010, Selin et al. 2010, Thasana et al. 2010].

The microbiological material prepared from antagonistic microorganisms and their post-culture liquids containing secondary metabolites can be used in dressing the propagative material [Roberti et al. 2002, Khilari et al. 2008, Patkowska 2010]. These methods were used, for example, in limiting the occurrence of fungi pathogenic towards runner bean [Patkowska 2010, Patkowska and Pięta 2010]. High effectiveness of the post-culture liquid of bacteria *Bacillus* spp. and *Pseudomonas* spp. in limiting the growth and development of phytopathogens species results from the effect of secondary metabolites such as iron chelating compounds (siderophores), antibiotics, substances inducing plant resistance (salicylic and antranilic acids), enzymes degrading the elements of cell walls of fungi (glucanases, endochitinases) and hormonal substances [Roberti et al. 2002, Dipali et al. 2007, Dehestani et al. 2010, Patkowska 2010, Pedersen et al. 2010].

The purpose of the paper was to establish the effectiveness of post-culture liquids *Bacillus* sp. B 73 and *Pseudomonas* sp. Ps 255 in protecting soybean from soil-borne pathogenic fungi.

MATERIALS AND METHODS

The object of the field studies conducted in the years 2009–2011 in the field of the Experimental Station in Felin belonging to the University of Life Sciences in Lublin were the plants of soybean ‘Aldana’ cv. grown out of the seeds dressed directly before the sowing with the post-culture liquids of *Bacillus* sp. B 73 and *Pseudomonas* sp. Ps 255 [Patkowska 2010]. The bacteria used in the experiment came from the soil environment of soybean cultivation, and their antagonistic effect towards fungi pathogenic for this plant was established using the method described by Pięta and Patkowska [2003]. The post-culture liquid of the bacteria was obtained as a result of their culture on a liquid medium PDB (Difco) at the temperature of 24°C for 4 days [Saniewska et al.

1995]. The studies also considered a combination with chemical seed dressing with Zaprawa Oxafun T (active ingredient: carboxin 37.5% + thiuram 37.5%) and a control combination, i.e. one with no dressing. Each experimental combination included 4 plots (4 replicates) with the area of 1.25 m². 100 seeds were sown in each plot.

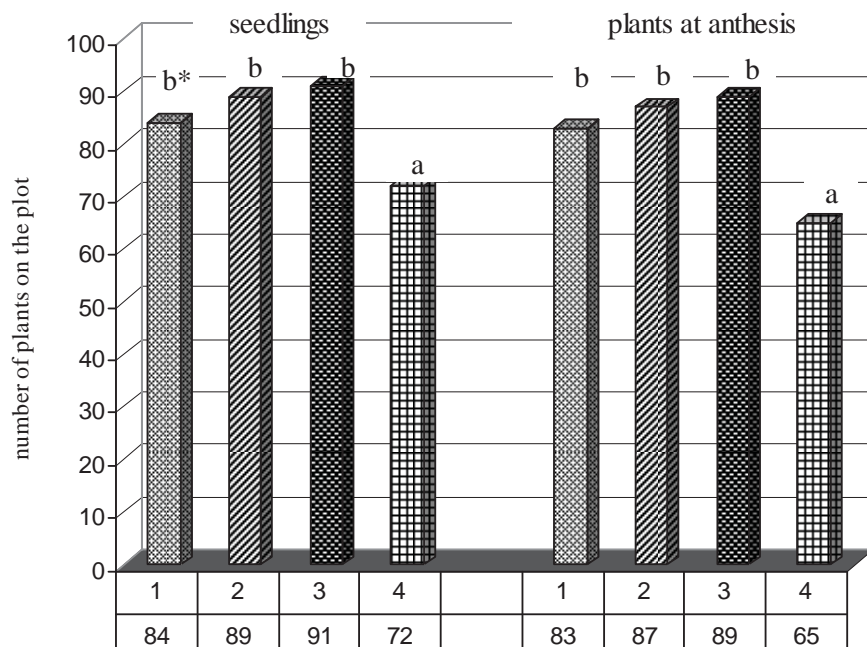
In each year of the studies, observations were carried out twice – at the phase of the seedlings and at anthesis – determining the number of plants in the plots and evaluating their health status. Five plants with apparent signs of necrosis on the roots and the stem base were taken for a laboratory mycological analysis. Fragments of infected plants were laid on mineral medium with the aim of isolating the fungi [Patkowska and Konopiński 2011]. After collecting the plants and drying up the seeds, the yield size and was established and the proportion of infected seeds in the total yield determined. Both the seeds with spots obtained after collecting of plants and those without any spots on the seed cover were subject to mycological analysis (100 seeds in 4 replicates for each combination). The mycological analysis of the plant material and the seeds was conducted according to the method described by Patkowska and Konopiński [2011]. The obtained fungi were taxonomically determined to the species level using the accessible keys and monographs of different taxons listed in the paper by Patkowska and Konopiński [2011]. In the case of fungus *Phomopsis sojae*, the species identification was on a PDA medium with an addition of carnation leaves at the temperature of 24°C [Castillo-Pando et al. 1997].

The obtained results on the number, health status and yielding of plants were statistically analyzed and the significance of differences was determined on the basis of Tukey's confidence intervals [Oktaba 1987].

RESULTS AND DISCUSSION

Field observations conducted at the seedling stage and at anthesis found out different numbers and health status of soybean (fig. 1, 2). The best density of seedlings was found after sowing the seeds dressed with a chemical preparation Zaprawa Oxafun T (the mean number of 91 per plot from three years of studies) (fig. 1). Slightly worse emergence were only obtained after using the post-culture liquids of *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73 (on average, 89 and 84, respectively). The fewest seedlings grew from the seeds without any dressing (on average, 72). In each studied year, seedlings with inhibited growth and with yellowing leaves were observed in particular plots (photo 1). Their proportion in a plot ranged, on average from 6.5% do 18.4% (fig. 2). The greatest number of infected seedlings was observed in the control combination. After dressing the seeds with the chemical preparation and with the post-culture liquids *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73, the number of infected seedlings was small, and their proportion was, respectively, 6.5%, 6.9% and 7.2% (fig. 2).

In the anthesis stage, a slight loss of plants in particular plots was observed and a small increase of the proportion of infected plants, with distinct necrotic spots on the stem base and on the roots (fig. 1, 2). The greatest number of plants in the plot was observed after the application of the post-culture liquid *Pseudomonas* sp. Ps 255 and Zaprawa Oxafun T (on average, 87 and 89, respectively), while the lowest in the control



* means for individual growth stage of plants not differ significantly if they marked the same letter ($P < 0.05$)

Fig. 1. Number of soybean plants (mean from the years 2009–2011): 1 – post-culture liquids of *Bacillus* sp. B 73, 2 – post-culture liquids of *Pseudomonas* sp. Ps 255, 3 – Zaprawa Oxafun T, 4 – control

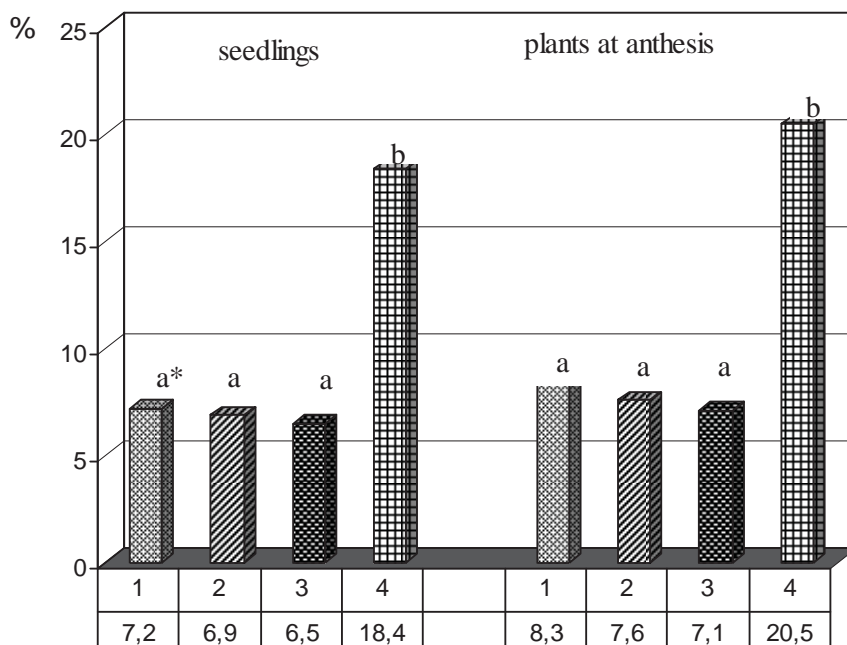
combination (65, on average) (fig. 1). The proportion of infected flowering plants on the plot ranged, on average, between 7.1% and 20.5% (fig. 2). The greatest number of infected plants were found in the control combination.

The size and quality of the obtained seed yield was directly proportional to the number and healthiness of plants (tab. 1). In particular years of studies, the yield of seeds ranged between 145 g from a plot (in the control) and 512 g from a plot (after dressing the seeds with the post-culture liquid of *Pseudomonas* sp. Ps 255) (tab. 1). The highest mean yield of seeds was collected from plants after using the post-culture liquid of *Pseudomonas* sp. Ps 255 (the mean value from three years of studies, 429 g from a plot). A good yield was also found for the plants grown from the seeds dressed with a post-culture liquid of *Bacillus* sp. B 73 (the mean value 370 g) and Zaprawa Oxafun T (on average, 393 g). Significantly, the lowest yield of seeds was harvested from the plants in the reference combination (on average 205 g from a plot) (tab. 1).

The obtained yield contained small seeds, with necrotic spots on the seed cover (photo 2). In particular years of studies, the proportion of such seeds in the total yield ranged between 3% and 10% (tab. 1). The greatest number of infected seeds was collected from the plants in the reference combination, and their proportion was 9.2%, on

average, from three years of studies. A slightly smaller proportion of infected seeds was found after the application of Zaprawa Oxafun T (on average, 5.8%). Significantly, the smallest number of infected seeds was observed for the combinations with the post-culture liquids of *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73 as their proportion was, on average, 4.3% and 4.5%, respectively (tab. 1).

Post-culture liquids of the studied antagonistic bacteria used in the pre-sowing dressing of soybean seeds proved effective in protecting this plant from soil-borne pathogenic fungi, which is confirmed by good emergence, the healthiness of plants and their yielding. A similar effect of post-culture liquids of other bacteria as well as antagonistic fungi of *Trichoderma* spp. and *Gliocladium* spp. was observed in earlier studies on the protection of runner bean from soil-borne fungi [Patkowska 2010, Patkowska and Pięta 2010]. The protective effect of *Pseudomonas* spp. Ps 255 and *Bacillus* spp. results from their antagonistic effect on plant pathogens through antibioses and competition [Abanda-Nipwatt et al. 2006, Domenech et al. 2006, Srivastava and Shalini 2008, Dehestani et al. 2010, Farhan et al. 2010]. Such properties probably characterized the bacteria whose isolates were used in the present studies.



* means for individual growth stage of plants not differ significantly if they marked the same letter (P < 0.05)

Fig. 2. Proportion of infected plants on the plot (mean from the years 2009–2011): 1 – post-culture liquids of *Bacillus* sp. B 73, 2 – post-culture liquids of *Pseudomonas* sp. Ps 255, 3 – Zaprawa Oxafun T, 4 – control

Table 1. Weight and quality of soybean seeds yield

Experimental combination	Yield of soybean seeds in g from the plot				Percentage of infected seeds in the total yield			
	2009	2010	2011	mean	2009	2010	2011	mean
Seeds soaked in post-culture liquids of <i>Bacillus</i> sp. B 73	342 ^b	279 ^b	490 ^c	370 ^b	4.0 ^a	3.5 ^a	6.0 ^a	4.5 ^a
Seeds soaked in post-culture liquids of <i>Pseudomonas</i> sp. Ps 255	427 ^c	348 ^c	512 ^c	429 ^c	4.5 ^a	3.0 ^a	5.5 ^a	4.3 ^a
Seeds dressed with Zaprawa Oxafun T	450 ^c	361 ^c	368 ^b	393 ^b	5.5 ^b	4.5 ^b	7.5 ^b	5.8 ^b
Control	264 ^a	145 ^a	205 ^a	205 ^a	9.0 ^c	8.5 ^c	10.0 ^c	9.2 ^c

* Means in columns followed by the same letters are not significantly different at $P \leq 0.05$

The species composition of fungi isolated during the mycological analysis from the infected roots, the stem base and the seeds obtained after the harvest is presented in tables 2 and 3. This composition was similar in particular experimental combinations, whereas differences were observed in the quantitative composition of the isolated fungi. Totally, 405 isolates were obtained from the infected seedlings and 503 from the plants at anthesis (tab. 2), while 537 fungi isolates were obtained from the studied seeds (tab. 3). Despite the pre-sowing dressing of the seeds, the following species were often or very often isolated from the seedlings and plants at anthesis: *Fusarium oxysporum* f. sp. *glycines* (12.5% in the total association of the isolated fungi), *Rhizoctonia solani* (10.3%), *Alternaria alternata* (8.1%), *Fusarium culmorum* (7%) and *Phoma exigua* (6.3%) (tab. 2). Besides, soybean seedlings were frequently infected by *Pythium irregulare* (5.4% in the total fungi population), while older plants were often infected by *Sclerotinia sclerotiorum* (4.7%). The fungi enumerated above had been more frequently infected soybean in the combination with Zaprawa Oxafun T and in the reference than after using the post-culture liquids of antagonistic bacteria. A converse relation was observed in the case of saprobic fungi from genera *Gliocladium*, *Trichoderma* and *Penicillium*. Genus *Gliocladium* was represented by the species of *G. catenulatum* and *G. roseum*, whereas genus *Trichoderma* was represented by *T. hamatum*, *T. harzianum*, *T. koningii* and *T. viride*. *Penicillium* spp. included *P. meleagrimum*, *P. verrucosum* var. *cyclopium* and *P. verrucosum* var. *verrucosum*.

A similar relation was observed during the mycological analysis of the studied soybean seeds. The greatest proportion in the total population of fungi isolated from the seeds was made up of the species of *Phomopsis sojae* (14.4%), *Fusarium oxysporum* (8%), *F. culmorum* and *F. equiseti* (po 6.7%), *A. alternata* (6.4%), *S. sclerotiorum* (6.4%) and *R. solani* (6.2%) (tab. 3). The smallest number of those fungi was obtained from the seeds after protecting soybean with post-culture liquids of *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73.

Post-culture liquids of *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73 proved effective in protecting the germinating seeds, and then the roots and the stem base of the

Table 2. Fungi isolated from infected of soybean plants (total from the years 2009–2011)

Fungus species	Experimental combination / Number of isolates											
	Seeds soaked in post-culture liquids of <i>Bacillus</i> sp. B 73		Seeds soaked in post-culture liquids of <i>Pseudomonas</i> sp. Ps 255		Seeds dressed with Zaprawa Oxaftun T		Control		Total		Total (%)	
	a	b	a	b	a	b	a	b	a	b		
<i>Acremonium murorum</i> (Corda) W. Gams	3	-	1	-	4	-	-	-	2	-	8 (0.9)	
<i>Acremonium strictum</i> W. Gams	1	6	-	5	-	6	1	4	8	21	23 (2.6)	
<i>Alternaria alternata</i> (Fr.) Keissler	5	12	5	10	6	12	9	15	25	49	74 (8.1)	
<i>Alternaria tenuissima</i> (Kunze) Wiltshire	-	2	-	2	3	3	3	5	6	12	18 (2.0)	
<i>Botrytis cinerea</i> Pers.	-	2	-	2	3	4	4	4	7	15	22 (2.4)	
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	1	-	-	-	5	1	3	4	9	5	14 (1.5)	
<i>Cladosporium herbarum</i> (Pers.) Link	-	-	1	-	4	1	4	5	9	6	15 (1.7)	
<i>Epicoccum nigrum</i> Link	-	1	-	-	4	1	4	2	8	4	12 (1.3)	
<i>Fusarium avenaceum</i> (Corda ex Fr.) Sacc.	-	-	-	-	3	-	4	-	7	-	7 (0.8)	
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	4	7	4	8	5	11	10	15	23	41	64 (7.0)	
<i>Fusarium equiseti</i> (Corda) Sacc.	1	2	1	-	4	4	6	6	12	12	24 (2.6)	
<i>Fusarium oxysporum</i> Schl. f.sp. <i>glycines</i> Amst. Amst.	10	16	9	14	12	18	20	15	51	63	114 (12.5)	
<i>Fusarium solani</i> (Mart.) Sacc.	3	2	1	3	3	4	6	6	13	15	28 (3.1)	
<i>Gliocladium catenulatum</i> Gilim. et Abbott	2	5	4	6	1	2	-	-	7	13	20 (2.2)	
<i>Gliocladium roseum</i> Bainier	4	6	5	7	3	2	-	-	12	15	27 (3.0)	
<i>Humicola grisea</i> Traeen	-	-	-	-	2	3	3	5	5	8	13 (1.4)	
<i>Penicillium chrysogenum</i> Thom	5	4	6	5	-	2	1	-	12	11	23 (2.5)	
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling) Samson, Stolk et Hadlok	4	2	5	3	1	2	2	-	12	7	19 (2.1)	
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i> Samson, Stolk et Hadlok	6	4	8	5	5	3	2	1	21	13	34 (3.8)	
<i>Phoma exigua</i> Desm. var. <i>exigua</i>	2	8	1	6	7	8	10	15	20	37	57 (6.3)	
<i>Pythium irregulare</i> Buisman	11	-	10	-	12	-	16	-	49	-	49 (5.4)	
<i>Rhizoctonia solani</i> Kühn	8	10	6	7	10	12	18	22	42	51	93 (10.3)	
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	-	9	-	8	-	11	-	15	-	43	43 (4.7)	
<i>Trichoderma hamatum</i> (Bon.) Bainier	3	4	6	5	2	2	1	-	12	11	23 (2.5)	
<i>Trichoderma harzianum</i> Rifai	4	5	4	6	-	1	-	-	8	12	20 (2.2)	
<i>Trichoderma koningii</i> Oud.	1	8	3	11	-	4	-	-	4	23	27 (3.0)	
<i>Trichoderma viride</i> Pers.	8	6	9	8	1	2	3	-	21	16	37 (4.1)	
Total	86	121	89	121	100	119	130	142	405	503	908 (100.0)	

a – seedlings, b – plants at anthesis

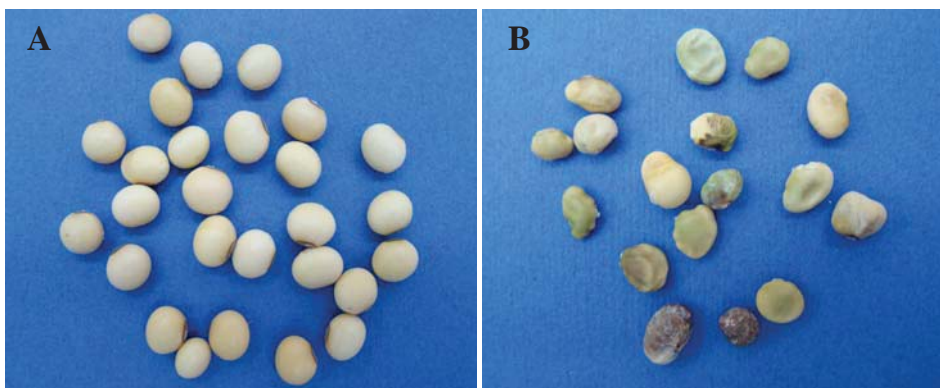
Table 3. Fungi isolated from soybean seeds (total from the years 2009–2011)

Fungus species	Experimental combination / Number of isolates											
	Seeds soaked in post-culture liquids of <i>Bacillus</i> sp. B 73		Seeds soaked in post-culture liquids of <i>Pseudomonas</i> sp. Ps 255		Seeds dressed with Zaprawa Oxafun T		Control		Total		Total (%)	
	a	b	a	b	a	b	a	b	a	b		
<i>Acremonium murorum</i> (Corda) W. Gams	2	-	-	-	-	-	-	-	2	-	2 (0.4)	
<i>Alternaria alternata</i> (Fr.) Keissler	6	3	4	-	5	1	10	5	25	9	34 (6.4)	
<i>Alternaria tenuissima</i> (Kunze) Wiltshire	-	-	2	1	-	-	9	-	11	1	12 (2.2)	
<i>Aspergillus flavus</i> Link	4	-	5	2	3	1	9	-	21	3	24 (4.5)	
<i>Botrytis cinerea</i> Pers.	2	-	1	-	1	-	4	-	8	-	8 (1.4)	
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	2	1	3	3	1	1	7	2	13	7	20 (3.7)	
<i>Epicoccum nigrum</i> Link	3	-	2	-	1	-	-	-	6	-	6 (1.1)	
<i>Fusarium avenaceum</i> (Corda ex Fr.) Sacc.	4	-	3	-	3	-	11	2	21	2	23 (4.3)	
<i>Fusarium culmorum</i> (W.G.Sm.) Sacc.	7	4	5	1	5	3	8	3	25	11	36 (6.7)	
<i>Fusarium equiseti</i> (Corda) Sacc.	5	2	3	3	6	2	10	5	24	12	36 (6.7)	
<i>Fusarium graminearum</i> Schwabe	-	-	1	-	-	-	6	1	7	1	8 (1.4)	
<i>Fusarium oxysporum</i> Schl.	7	5	7	-	6	2	11	5	31	12	43 (8.0)	
<i>Fusarium solani</i> (Mart.) Sacc.	5	-	-	-	3	-	8	4	16	4	20 (3.7)	
<i>Gliocladium catenulatum</i> Gilman et Abbott	2	5	2	6	1	3	-	-	5	14	19 (3.5)	
<i>Penicillium chrysogenum</i> Thom	2	-	1	-	1	-	4	2	8	2	10 (1.9)	
<i>Penicillium decumbens</i> Thom	2	-	1	-	2	1	3	1	8	2	10 (1.9)	
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling) Samson, Stolk et Hadlok	4	1	1	-	-	-	-	-	5	1	6 (1.1)	
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i> Samson, Stolk et Hadlok	3	1	2	-	4	1	-	-	9	2	11 (2.0)	
<i>Phoma exigua</i> Desm. var. <i>exigua</i>	3	-	3	-	4	-	10	3	20	3	23 (4.3)	
<i>Phomopsis sojae</i> Lehman	12	6	10	6	11	5	18	9	51	26	77 (14.4)	
<i>Rhizoctonia solani</i> Kühn	6	2	2	-	5	-	15	3	28	5	33 (6.2)	
<i>Rhizopus nigricans</i> Ehr.	2	-	-	-	-	-	-	-	2	-	2 (0.4)	
<i>Sclerotinia sclerotiorum</i> (Lib.) de Bary	6	2	3	-	5	-	12	6	26	8	34 (6.4)	
<i>Trichoderma koningii</i> Oud.	4	4	3	5	2	3	-	-	9	12	21 (3.9)	
<i>Trichoderma viride</i> Pers.	1	6	2	8	-	2	-	-	3	16	19 (3.5)	
Total	94	42	68	35	69	23	155	51	386	151	537 (100.0)	

a – seeds with spots, b – seeds without spots



Phot. 1. Necrosis on the roots and stem base of soybean seedlings (photo by E. Patkowska)



Phot. 2. Healthy (A) and infected (B) of soybean seeds (photo by E. Patkowska)

seedlings and older plants from infection by soil-borne pathogenic fungi. The big effectiveness of those liquids was probably based on the activity of secondary metabolites. Such properties were confirmed in many studies [Roberti et al. 2002, Dipali et al. 2007, Dehestani et al. 2010, Patkowska 2010, Pedersen et al. 2010]. An important role in the protective effect is played, for example, by enzymes present in the post-culture liquids [Roberti et al. 2002, Dipali et al. 2007]. The application of post-culture liquids of antagonistic bacteria in seed dressing of runner bean was also effective in protecting the seedlings and the roots of older plants from infection by soil-borne fungi [Patkowska

2010, Patkowska and Pięta 2010]. Besides, secondary metabolites of *B. subtilis* SB 24 effectively protected soybean plants from infection by *S. sclerotiorum* [Zhang and Xue 2010]. On the other hand, soaking the seeds of lentil in post-culture filtrates of *P. fluorescens* inhibited the plants' infection by *F. oxysporum* f. sp. *lentis* [De et al. 2003].

The obtained results point to the possibility of practical application of post-culture liquids of *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73 in protecting soybean from soil-borne fungi as an alternative to chemical protection. Applying the microbiological material on the surface of seeds, grain, bulbs, tubers or roots of soybean is considered to be the most effective method preventing the process of infection. It allows the antagonistic microorganisms to colonize the surface of the propagative material, and then – as a result of their permanent growth – to protect the sprawling root system throughout the vegetation period, thus becoming a specific biological protection against plant pathogens [Yedidia et al. 2000, Patkowska 2010, Patkowska and Pięta 2010, Zhang and Xue 2010].

CONCLUSIONS

1. Post-culture liquids of antagonistic bacteria used in pre-sowing seed dressing of soybean can improve the healthiness and yielding of this plant.

2. The application of post-culture liquids of antagonistic bacteria to the surface of soybean seeds can limit the plant infection by *Fusarium culmorum*, *F. oxysporum*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Phomopsis sojae*.

3. There is a possibility of using the post-culture liquids of *Pseudomonas* sp. Ps 255 and *Bacillus* sp. B 73 in the protection of soybean from soil-borne fungi as a method alternative to chemical protection.

REFERENCES

- Abanda-Nipwatt D., Krimm U., Schreiber L., Schwab W., 2006. Dual antagonism of aldehydes and epiphytic bacteria from strawberry leaf surfaces against the pathogenic fungus *Botrytis cinerea* *in vitro*. *BioControl* 51, 3, 279–291.
- Akhukorala S.N.P., Fernando W.G.D., Rashid K.Y., 2009. Identification of antifungal antibiotics of *Bacillus* species isolated from different microhabitats using polymerase chain reaction and MALDI-TOF mass spectrometry. *Can. J. Microbiol.* 55, 9, 1021–1032.
- Castillo-Pando M.S., Nair N.G., Emmett R.W., Wicks T.J., 1997. Inhibition in pycnidial viability of *Phomopsis viticola* on canes *in situ* as an aid to reducing inoculum potential of cane and leaf blight disease of grapevines. *Australas. Plant Pathol.* 26, 21–25.
- Chincholkar S.B., Chaudhari B.L., Rane M.R., Sarode P.D., 2007. Fungal phytopathogen suppression using siderophoregenic bioinoculants. [in:]: *Biological control of plant diseases* (eds. Chincholkar S. B., Mukerji K.G.). Binghampton, USA, Haworth Food & Agricultural Products Press, 401–417.
- De R.K., Dwivedi R.P., Narain U., 2003. Biological control of lentil wilt caused by *Fusarium oxysporum* f. sp. *lentis*. *Ann. Plant Prot. Sci.* 11, 1, 46–52.
- Dehestani A., Kazemitabar K., Ahmadian G., Jelodar N.B., Salmanian A.H., Seyedi M., Rahimian H., Ghasemi S., 2010. Chitinolytic and antifungal activity of a *Bacillus pumilis* chitinase expressed in *Arabidopsis*. *Biotech. Letters* 32, 4, 539–546.

- Dipali W., Ingle R.W., Deshmukh R.W., 2007. Screening of vegetable market wastes fungi for their cellulolytic activities *in vitro*. J. Plant Dis. Sci. 2, 1, 91–93.
- Domenech J., Reddy M.S., Kloepper J.W., Ramos B., Gutierrez-Mañero J., 2006. Combined application of the biological produkt LS213 with *Bacillus*, *Pseudomonas* or *Chryseobacterium* from growth promotion and biological control of soil-borne diseases in pepper and tomato. Biolog. Control 51, 2, 245–258.
- Farhan H.N., Hameed A.T., Aobad H.M., 2010. The biological activity of some *Pseudomonas* sp. isolates on growth of three plant pathogenic fungi under incubator conditions. Advances in Environ. Biol. 4, 1, 53–57.
- Khilari K., Mukhopadhyay A.N., Yadav R. S., 2008. Effect of volatile and non-volatile compounds of *T. koningii* and *G. virens* on growth *S. rolfisii*, *R. solani* and *F. oxysporum* f. sp. *ciceri*. Environ. Ecol. 26, 4B, 1961–1964.
- Kućmierz J., Nawrocki J., Klata E., Stadnik U., 2010. Skuteczność kilku preparatów biotechnicznych w zwalczaniu *Rhizoctonia solani* Kühn i *Sclerotinia sclerotiorum* (Lib.) de Bary. Zesz. Prob. Postęp. Nauk Rol. 554, 71–76.
- Kurzawińska H., Mazur S., 2009. The evaluation of *Pythium oligandrum* and chitosan in control of *Phytophthora infestans* (Mont.) de Bary on potato plants. Folia Hortic. 21/2, 13–23.
- Oktaba W., 1987. Metody statystyki matematycznej w doświadczalnictwie. PWN, Warszawa.
- Orlikowski L.B., Skrzypczak Cz., 2003. Biocides in the control of soil-borne and leaf pathogens. Hortic. Veget. Grow. 22, 426–433.
- Patkowska E., 2009a. Effect of bio-products on bean yield and bacterial and fungal communities in the rhizosphere and non-rhizosphere. Polish J. Environ. Stud. 18, 2, 255–263.
- Patkowska E., 2009b. Effect of chitosan and Zaprawa Oxafun T on the healthiness and communities of rhizosphere microorganisms of runner bean (*Phaseolus coccineus* L.). Ecolog. Chem. Eng. S, 16, (S2): 163–174.
- Patkowska E., 2010. Use of chemical dressing and post-culture liquids of antagonistic bacteria in the protection of runner bean (*Phaseolus coccineus* L.). Ecolog. Chem. Eng. A, 17(9), 1153–1160.
- Patkowska E., Konopiński M., 2011. Cover crops and soil-borne fungi dangerous towards the cultivation of salsify (*Tragopogon porrifolius* var. *sativus* (Gaterau) Br.). Acta Sci. Pol., Hortorum Cultus 10 (2), 167–181.
- Patkowska E., Pięta D., 2010. Use of chemical dressing and post-culture liquids of antagonistic fungi in the protection of runner bean (*Phaseolus coccineus* L.) from soil-borne fungi. Ecolog. Chem. Eng. A, 17(9), 1161–1169.
- Pedersen A.L., Ekelund F., Johansen A., Winding A., 2010. Interaction of bacteria-feeding soil flagellates and *Pseudomonas* spp. Biol. and Fertility of Soils 46, 2, 151–158.
- Pięta D., Patkowska E., 2003. The role of antagonistic fungi and bacteria limiting the occurrence of some phytopathogens inhabiting the soybean soil environment. EJPAU, Horticulture, 6, 2. <http://www.ejpau.media.pl>
- Rajeswardi B., Kumari K.V.S.M., 2009. Bioagents and fungicides for the management of seed and seedling diseases of soybean. Indian J. Plant Protec. 37, ½, 127–131.
- 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.
- Sandikar B.M., Awasthi R.S., 2009/2010. Preparation and shelf-live study of *Pseudomonas* and *Bacillus* bioformulations against phytopathogenic *Pythium* and *Fusarium* species. Int. J. Plant Prot. 2, 2, 251–254.
- Saniewska A., Orlikowski L. B., Sobiczewski P., 1995. Effectiveness of *Bacillus* sp. in the control of *Phytophthora cryptogea* Pethybr. et Laff. M. Mańka (ed.), Environmental biotic factors in integrated plant disease control, Polish Phytopathol. Soc., Poznań, 479–184.

- Selin C., Habibian R., Poritsanos N., Athukorala S.N.P., Fernando D., Kievit T.R.de, 2010. Phenazines are not essential for *Pseudomonas chlororaphis* PA23 biocontrol of *Sclerotinia sclerotiorum*, but do play a role in biofilm formation. *FEMS Microbiol. Ecol.* 71, 1, 73–83.
- Shovan L.R., Bhuiyan M.K.A., Begum J.A., Pervez Z., 2008. In vitro control of *Colletotrichum dematium* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum*. *Int. J. Sustain. Crop Prod.* 3, 3, 10–17.
- Srivastava R., Shalini R., 2008. Antifungal activity of *Pseudomonas fluorescens* against different plant pathogenic fungi. *EJEFChem.* 7, 4, 2789–2796.
- Thasana N., Prapagdee B., Rangkadilok N., Sallabhan R., Aye S.L., Ruchirawat S., Loprasert S., 2010. *Bacillus subtilis* SSE4 produces subtilene A, a new lipopeptide antibiotic possessing an unusual C15 unsaturated β -amino acid. *FEBS Letters* 584, 14, 3209–3214.
- Yedidia I., Benhamou N., Kapulnik Y., Chet I., 2000. Induction and accumulation of PR proteins activity during stages of root colonization by the mycoparasite *Trichoderma harzianum* strain T-203. *Plant Physiol. Biochem.* 38, 863–873.
- Ye Li-min, Xu Fen-fen, Xiong Zhen-huan, Wu Gao-yan, 2009. Effect of chitosan treatment on the yield, quality and diseases of soybean. *Hubei Agricul. Sci.*, China, 07, http://en.cnki.com.cn/Article_en/CJFDTOTAL-HBNY200907020.htm
- Zhang J.X., Xue A.G., 2010. Biocontrol of sclerotinia stem rot (*Sclerotinia sclerotiorum*) of soybean using novel *Bacillus subtilis* strain SB24 under control conditions. *Plant Pathol.* 59, 2, 382–391.

CZY PŁYNY POHODOWLANE BAKTERII MAJĄ WPLYW NA ZDROWOTNOŚĆ SOI [*Glycine max* (L.) Merrill]?

Streszczenie. Soja jest jedną z najbardziej wartościowych roślin uprawnych na świecie. Nasiona i produkty sojowe zawierają błonnik pokarmowy, lecytynę, witaminy, zwłaszcza z grupy B, składniki mineralne, izoflawony (genisteina i daidzeina), kwas fitynowy, inhibitory proteinaz i saponiny. Przedmiotem badań były rośliny soi odm. 'Aldana' wyrosłe z nasion zaprawianych bezpośrednio przed wysiewem płynami pochodzonymi *Pseudomonas* sp. Ps 255 i *Bacillus* sp. B 73. W badaniach uwzględniono również kombinację z chemicznym zaprawianiem nasion Zaprawą Oxafun T oraz kombinację bez żadnego zaprawiania. Określono skuteczność płynów pochodzanych bakterii antagonistycznych w ochronie soi przed grzybami odglebowymi. Płyny pochodzane wpłynęły korzystnie na liczebność, zdrowotność i plonowanie badanej rośliny. Soja porażana była głównie przez *Fusarium culmorum*, *F. oxysporum*, *Phoma exigua*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*. Z porażonych nasion wyosobniono dodatkowo gatunek *Phomopsis sojae*. Grzyby te izolowano znacznie rzadziej z roślin w kombinacjach z zastosowaniem płynów pochodzanych bakterii antagonistycznych, aniżeli z roślin kontrolnych. Odwrotną zależność stwierdzono w przypadku występowania grzybów saprotroficznych z rodzajów *Gladiolium*, *Penicillium* i *Trichoderma*.

Słowa kluczowe: biologiczna ochrona, *Bacillus* sp., *Pseudomonas* sp., fitopatogeny