

THE EFFECT OF BIOPREPARATIONS ON THE FORMATION OF RHIZOSPHERE MICROORGANISM POPULATIONS OF SOYBEAN (*Glycine max* (L.) Merrill.)

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Abstract. The object of a field experiment conducted in the years 2003–2004 was the rhizosphere soil of soybean ‘Mazowia’ cv. Before the sowing, the seeds were dressed with biopreparations (Biochikol 020 PC, Biosept 33 SL, Polyversum) or fungicide (Oxafun T). The non-dressed seeds constituted the control.

The microbiological analysis of the rhizosphere soil of soybean, carried out at anthesis, showed that the greatest number of bacteria in total and bacteria *Bacillus* spp. and *Pseudomonas* spp. occurred after the application of Biochikol 020 PC, while the lowest occurred in the control combination. The smallest number of fungi was observed after the application of Biochikol 020 PC or Oxafun T, slightly bigger after the introduction of Biosept 33 SL or Polyversum into the soil, while the biggest in the control combination. Fungi from the genera of *Alternaria*, *Fusarium*, *Pythium* and *Gliocladium*, *Penicillium*, and *Trichoderma* were most frequently isolated from the rhizosphere of soybean. After the application of biopreparations the proportion of pathogenic fungi in the rhizosphere of soybean was much smaller than in the control. The highest proportion of *Gliocladium* spp. occurred after the application of Polyversum or Biochikol 020 PC, while that of *Trichoderma* spp. after the introduction of Biochikol 020 PC or Biosept 33 SL. Besides, tests on dishes showed that the biopreparations and the fungicide used in the experiment contributed to increased numbers of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp. and *Trichoderma* spp.).

Key words: soybean, rhizosphere, biopreparations

INTRODUCTION

Contemporary agriculture aims at increasing the yields of cultivated plants through the application of proper agricultural treatments, based on thorough knowledge of the soil environment. The soil, as the natural environment of life of different microorga-

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nisms, constitutes a proper ecological niche for them, where a number of biotic and abiotic factors interact [Barabasz 2004]. Thanks to microorganisms the soil can be resistant towards certain plant pathogens [Badura 2004]. Besides, soil microorganisms are closely connected with the life of plants, stimulating or inhibiting their growth and development [Barabasz 2004].

Rhizosphere soil is characterized by the highest biological activity. The composition of microorganism communities in the root zone is formed both under the effect of secondary metabolites secreted by these microorganisms and root exudates as well as compounds formed from the decomposition of husking root cells [Badura 2004, Lewosz 2002, Lilierothe et al. 1990, Manwar et al. 2000, Parke 1990]. Soil microorganisms are sensitive indicators of soil contamination with different chemical substances, including chemical preparations of plant protection [Barabasz 2004].

Lately much interest has been aroused by biological methods of plant protection based on the utilization of antagonistic bacteria and fungi (components of numerous biopreparations), organic compounds or plant preparations inducing the plants' resistance to pathogen, and the methods of transforming plants by means of resistance genes [Biesiada et al. 2002, Borkowski et al. 2004, Mazur et al. 2003, Orlikowski and Skrzypczak 2003, Patkowska 2005, Patkowska and Pięta 2004, Tvaruzkova 2004, Wojdyła 2004]. That is the reason why in practice it is recommended to replace pesticides with biopreparations used for the dressing of bulbs, onions and seeds as well as spraying the plants [Mazur et al. 2003, Orlikowski and Skrzypczak 2003, Patkowska 2005]. Biopreparations that have been given much attention recently include Biochikol 020 PC, Biosept 33 SL and Polyversum. Chitosan, contained in Biochikol 020 PC, induces plants' resistance and protects them from infection by viruses, bacteria and fungi [Borkowski et al. 2004, Patkowska and Pięta 2004, Pospieszny 1997, Romanazzi et al. 2003, Sobiczewski et al. 2004]. Biosept 33 SL, containing 33% grapefruit extract, acts directly on pathogenic factors and it induces plants' resistance to certain pathogens [Janas et al. 2002, Orlikowski and Skrzypczak 2003, Saniewska 2002, Wojdyła 2002]. On the other hand, Polyversum, based on the oospores of mycoparasite *Pythium oligandrum*, as a stimulant of resistance can effectively protect plants from infection by soil and phyllospheric pathogens [Benhamou et al. 1999, Le Floch et al. 2003, Orlikowski and Jaworska-Marosz 2002, Orlikowski et al. 2002, Patkowska 2005, Pharand et al. 2002, Picard et al. 2000].

The purpose of the present studies was to determine the effect of biopreparations: Biochikol 020 PC, Biosept 33 SL and Polyversum, on the formation of microorganism communities in soybean rhizosphere.

MATERIAL AND METHODS

Field experiments were conducted in the years 2003-2004 at the Experimental Station of Czesławice near Lublin, on grey brown podsolic soil, constituting the second complex of agricultural suitability (good wheat complex). The object of the studies was rhizosphere soil of soybean 'Mazowia' cv. Before the sowing, the seeds were dressed with 2.5% Biochikol 020 PC, 0.2% Biosept 33 SL, Polyversum (in the quantity of 1 g of

preparation $\times 100 \text{ g}^{-1}$ seeds) or Oxafun T (active substance: carboxin 37.5% + tiuram 37.5%) in the quantity of $2 \text{ g} \times \text{kg}^{-1}$ seeds). A control combination was also considered, in which the seeds were not dressed. Each combination included 4 plots (4 replications), where 100 seeds were sown on each. The second protective treatment, consisting of spraying the plants with biopreparation or Bravo Plus 500 SC (active substance: chlorotalonil 50%) was performed at the beginning of anthesis.

In each year of the studies at full anthesis the rhizosphere soil was sampled for a laboratory microbiological analysis. This analysis was conducted according to the method described by Martyniuk et al. [1991]. The isolates of fungi from genera *Gliocladium* and *Trichoderma* (all isolates) and bacteria from genera *Bacillus* and *Pseudomonas* (500 isolates each) obtained in such a way were used to establish their antagonistic effect towards the following fungi pathogenic towards soybean: *B. cinerea*, *F. culmorum*, *F. oxysporum*, f. sp. *glycines*, *F. solani*, *P. irregularare*, *R. solani* and *S. sclerotiorum*. The interaction of these microorganisms was determined in accordance to the method described by Martyniuk et al. [1991] and Mańska and Mańska [1992].

The results referring to the number of microorganisms were statistically analyzed and the significance of differences was established on the basis of Tukey's confidence intervals [Oktaba 1987].

RESULTS AND DISCUSSION

The number of bacteria and fungi obtained as a result of a laboratory microbiological analysis of the rhizosphere soil of soybean growing in particular experimental combinations varied (tab. 1). The total number of bacteria in 1 g d.w. of soil ranged from $1.04 \cdot 10^6$ to $8.87 \cdot 10^6$ cfu. The most bacteria totally (on average, $8.67 \cdot 10^6$ cfu) were obtained from the rhizosphere of soybean grown from the seeds dressed with Biochikol 020 PC. A slightly smaller number was obtained in the case of the combination with seed dressing with Biosept 33 SL or Oxafun T (respectively, $5.35 \cdot 10^6$ and $5.14 \cdot 10^6$ cfu, on average), while the smallest in the control combination. The number of bacteria from genus *Pseudomonas* was smaller than the number of bacteria from genus *Bacillus*. The highest number of *Bacillus* spp. and *Pseudomonas* spp. occurred in the rhizosphere of soybean after the application of Biochikol 020 PC (on average, $4.43 \cdot 10^6$ and $0.90 \cdot 10^6$ cfu). In each year of studies the total number of fungi in 1 g of d.w. of soybean rhizosphere soil in the combinations with biopreparation or fungicide was almost three times as small as in the control combination. The mean number of fungi in the rhizosphere of soybean growing in the combination with Biochikol 020 PC or Zaprawa Oxafun T was the smallest (respectively, $26.37 \cdot 10^3$ and $29.16 \cdot 10^3$ cfu). A slightly bigger number of fungi occurred in the rhizosphere of soybean after the application of Biosept 33 SL or Polyversum (respectively, $33.28 \cdot 10^3$ and $35.88 \cdot 10^3$ cfu), while the biggest in the control combination, i.e. without seed dressing (tab. 1). The biopreparations introduced into the soil probably had a positive effect on the composition of microorganism communities in the rhizosphere of soybean. As observed by Myśkow [1989], proper proportions exist between the populations of microorganisms in the soil. Intensive growth of fungi is accompanied by poor development of bacteria and vice versa.

Table 1. The number of bacteria and fungi in the rhizosphere soil of soybean plants (mean from the years 2003–2004)
 Tabela 1. Liczebność bakterii i grzybów w ryzoferze soi (średnia z lat 2003–2004)

Experimental combination Kombinacja doświadczenia	Total number of bacteria (mln · g ⁻¹ d. w. of soil)			Number of <i>Bacillus</i> spp. (mln · g ⁻¹ d. w. of soil)			Number of <i>Pseudomonas</i> spp. (mln · g ⁻¹ d. w. of soil)			Total number of fungi (thous. · g ⁻¹ d. w. of soil)		
	2003	2004	mean średnia	2003	2004	mean średnia	2003	2004	mean średnia	2003	2004	mean średnia
Biochikol 020 PC	8.48 ^e	8.87 ^d	8.67 ^d	5.20 ^d	3.67 ^e	4.43 ^e	1.12 ^c	0.69 ^{bc}	0.90 ^c	29.70 ^b	23.05 ^a	26.37 ^a
Biosept 33 SL	6.50 ^c	4.21 ^c	5.35 ^c	3.22 ^b	0.63 ^d	1.92 ^c	0.81 ^{bc}	0.51 ^{ab}	0.66 ^b	37.78 ^c	28.78 ^b	33.28 ^b
Polyversum	4.33 ^b	3.88 ^c	4.10 ^b	1.84 ^a	0.47 ^c	1.15 ^b	1.08 ^c	0.83 ^c	0.95 ^c	42.19 ^d	29.58 ^b	35.88 ^b
Oxafun T	7.54 ^d	2.75 ^b	5.14 ^c	3.97 ^c	0.27 ^b	2.12 ^d	0.51 ^a	0.55 ^{ab}	0.53 ^a	19.53 ^a	38.80 ^c	29.16 ^a
Control (Kontrola)	2.98 ^a	1.04 ^a	2.01 ^a	1.53 ^a	0.16 ^a	0.84 ^a	0.49 ^a	0.43 ^a	0.46 ^a	115.47 ^e	66.46 ^d	90.96 ^c

* mean in columns followed by the same letter do not differ significantly at P ≤ 0.05

* średnie wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy P ≤ 0,05

Table 2. Fungi often isolated from the rhizosphere of soybean plants (total from the years 2003–2004)
 Tabela 2. Grzyby często izolowane z ryzosfery soi (suma z lat 2003–2004)

Fungus species Gatunek grzyba	Experimental combination/Number of isolates – Kombinacja doświadczenia/Liczba izolatów															
	Biochikol 020 PC			Biosept 33 SL			Polyversum			Oxafun T			control – kontrola			total razem
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c	
<i>Alternaria alternata</i> (Fr.) Keissler	5	4	9	1	2	3	4	6	10	5	6	11	7	5	12	45
<i>Aspergillus flavus</i> Link	-	-	-	-	-	-	-	-	-	2	-	2	5	2	7	9
<i>Cladosporium cladosporioides</i> Fres de Vries	4	3	7	2	1	3	2	-	2	4	5	9	4	9	13	34
<i>Fusarium oxysporum</i> Schl.	10	8	18	12	9	21	14	11	25	17	13	30	25	18	43	137
<i>Fusarium solani</i> (Mart.) Sacc	3	4	7	4	1	5	5	7	12	8	7	15	10	6	16	55
<i>Gliocladium catenulatum</i> Gilman Abbott	12	15	27	6	8	14	14	16	30	2	3	5	2	-	2	78
<i>Gliocladium roseum</i> Bainier	7	5	12	3	5	8	4	5	9	1	2	3	1	2	3	35
<i>Humicola grisea</i> Domsch	2	-	2	-	-	-	1	4	5	2	2	4	3	6	9	20
<i>Mucor hiemalis</i> Bainier	2	3	5	1	1	2	-	1	1	2	6	8	2	3	5	21
<i>Mucor mucedo</i> Fresenius	-	-	-	-	-	-	2	2	4	3	4	7	5	7	12	23
<i>Papulaspora irregularis</i> Hotson	-	3	3	-	-	-	-	-	-	-	-	-	1	-	1	4
<i>Penicillium canescens</i> Scopp.	1	-	1	-	-	-	-	-	-	-	-	-	3	-	3	4
<i>Penicillium fellutanum</i> Biourge	-	-	-	1	6	7	2	3	5	10	8	18	36	37	73	103
<i>Penicillium nigricans</i> (Bain.) Than	2	2	4	2	-	2	4	2	6	5	11	16	10	11	21	49
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclosporum</i> (West.)	4	3	7	4	6	10	8	6	14	7	5	12	7	9	16	59
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i>	-	-	-	1	2	3	-	1	1	2	6	8	-	-	-	12
Samson, Stolk et Hadlok	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Pythium irregularare</i> Buisman	4	7	11	6	13	19	10	12	22	16	14	30	57	64	121	203
<i>Rhizopus nigricans</i> Ehrenberg	3	5	8	1	-	1	-	-	-	6	8	14	12	9	21	44
<i>Rhizoctonia solani</i> Kühn	2	-	2	-	-	-	2	4	6	3	2	5	5	7	12	25
<i>Talaromyces flavus</i> (Ben.) Emend. Stolk et Samson	-	-	-	-	-	-	1	-	1	2	4	6	3	-	3	10
<i>Trichoderma aureoviride</i> Rifai	25	17	42	15	13	28	13	8	21	1	3	4	1	2	3	98
<i>Trichoderma harzianum</i> Rifai	3	6	9	2	3	5	1	2	3	-	-	-	1	-	1	18
<i>Trichoderma koningii</i> Oud.	19	20	39	11	9	20	7	5	12	5	5	10	3	2	5	86
<i>Trichoderma polysporum</i> (Link ex Pers.) Rifai	4	8	12	3	6	9	4	6	10	2	4	6	2	2	4	41
<i>Trichoderma viride</i> Pers. ex S. F. Gray	18	12	30	5	8	13	8	12	20	3	4	7	2	-	2	72
Total – Razem	130	125	255	80	93	173	106	113	219	108	122	230	207	201	408	1285

a – 2003 year, b – 2004 year, c – total,
 a – 2003 r., b – 2004 r., c – suma

Totally, 1285 isolates of the most numerously occurring fungi were obtained from the rhizosphere of soybean in particular experimental combinations (tab. 2). The most frequently isolated fungi belonged to the genera of *Alternaria*, *Fusarium*, *Gliocladium*, *Pythium* and *Trichoderma*. Genus *Fusarium* was represented by *F. oxysporum* and *F. solani*, while genus *Gliocladium* was represented by *G. catenulatum* and *G. roseum*. The following species occurred within *Trichoderma* spp.: *T. aureoviride*, *T. harzianum*, *T. koningii*, *T. polysporum* and *T. viride* (tab. 2). Introducing the tested biopreparations or the fungicide into the soil together with soybean seeds contributed to the reduction of the number of pathogenic fungi and increase of the number of fungi with antagonistic effect (*Gliocladium* spp., *Trichoderma* spp.) towards plant pathogens (tab. 2).

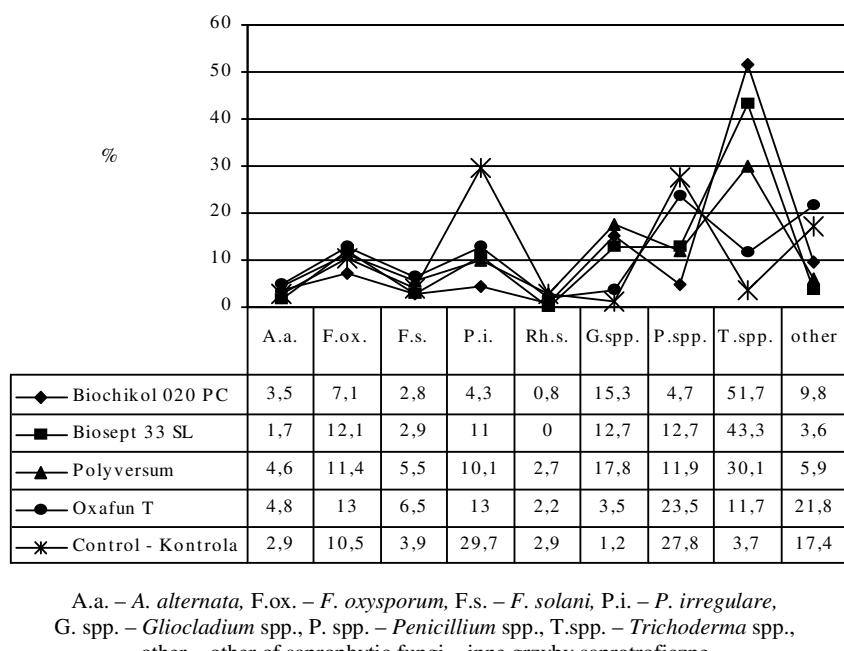


Fig. 1. Participation of fungi isolated from the rhizosphere of soybean (mean from the years 2003–2004)

Rys. 1. Udział grzybów wyizolowanych z ryzosfery soi (suma z lat 2003–2004)

The proportion of fungi recognized as pathogenic (unpublished results of pathogenicity tests) such as *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium solani*, *Pythium irregularare* and *Rhizoctonia solani*, isolated from the rhizosphere of soybean in particular experimental combinations, ranged from 1.7% to 29.7% (fig. 1). In the case of *F. oxysporum*, *F. solani* and *P. irregularare* their smallest proportion occurred in the rhizosphere of soybean after applying Biochikol 020 PC, and it was 7.1%, 2.8% and

4.3%, respectively. The proportion of species from genera *Gliocladium* and *Trichoderma* was higher in the rhizosphere of soybean in the combinations with biopreparations as compared with the combinations with fungicide or the control. The highest proportion of *Gliocladium* spp. was observed after the application of Polyversum or Biochikol 020 PC (17.8% and 15.3%, respectively), while the lowest in the control (1.2%). The proportion of *Trichoderma* spp. was the highest in the rhizosphere of soybean grown from the seeds dressed with Biochikol 020 PC (51.7%) or Biosept 33 SL (43.3%). It was slightly smaller in the combination with Polyversum (30.1%), and the smallest in the control, where the seeds were not dressed (3.7%) (fig. 1).

The effectiveness of biopreparations used in the experiment in inhibiting the growth of pathogenic fungi and stimulating the growth of antagonistic fungi in the plants' rhizosphere is first of all connected with the effect of biologically active substances. Chitosan present in Biochikol 020 PC not only induces systemic resistance acquired (SAR) in plants but it also contributes to the increase of biomass and the production of chitinolytic enzymes in fungi from genus *Trichoderma* [Pospieszny 1997]. The effect of Biosept 33 SL is related to the presence of endogenous flavonoids of grapefruit and glycosides, including such as naringenin rutinoside, hesperidine, kempferol, dihydrokempferol, quercitin, apigenin rutinoside and nobiletin [Kędzia 2001]. These compounds inhibit the germination of sporules, the growth of sporogenous hypha and vegetative hyphas through damaging the membrane systems, and they inhibit the activity of respiratory enzymes [Dakora 1995]. On the other hand, *Pythium oligandrum* contained in Polyversum can colonize the root system of plants and stay in the rhizosphere throughout the vegetation, making colonization of this sphere by pathogens difficult [Le Floch et al. 2003]. As observed by Orlikowski et al [2002], *P. oligandrum*, introduced to peat medium in the quantity of 25 oospores per 1 g, caused even a 50% reduction of the number of propagation units of the species belonging to genera *Phytophthora* and *Pythium* as well as the special forms of *F. oxysporum*. Besides, watering ivy with a suspension of *P. oligandrum* oospores directly after planting this plant into an infected medium protected as many as 50% plants from infection by *Pythium ultimum*.

Laboratory studies gave 298 bacteria isolates (*Bacillus* spp. and *Pseudomonas* spp.) and 428 fungi isolates (*Gliocladium* spp. and *Trichoderma* spp.), which were antagonistic towards the tested pathogenic fungi (fig. 2). Isolates from genus *Pseudomonas* dominated within antagonistic bacteria (202 isolates in all experimental combinations), while *Trichoderma* constituted the majority within antagonistic fungi (315 isolates). The most antagonistic bacteria and fungi were obtained from soybean rhizosphere after the introduction of biopreparations Biochikol 020 PC, Biosept 33 SL or Polyversum, while the lowest number was achieved after dressing the seeds with Oxafun T and from the control combination (fig. 2).

It should be supposed that introducing the tested biopreparations into the soil together with soybean seeds had a positive effect on the increase of the number of antagonistic microorganisms that could limit the growth and development of pathogenic fungi. As observed by Diaz de Villegas et al. [2002], Lewosz [2002], Manwar et al. [2000] or Sobiczewski et al. [2004], antagonistic *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp. and *Trichoderma* spp. can inhibit the growth of plant pathogens thanks to the exudation of such metabolites as antibiotics, substances inducing plants' resistance (antrami-

lin, salicylic acids), siderophores, enzymes degrading the compounds of cell walls or hormonal substances. Fungi from genus *Trichoderma* are especially considered to be antagonists of a lot of plant pathogens (*Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Sclerotinia* spp., *Verticillium* spp., *Gaeumannomyces* spp.). They possess a lot of

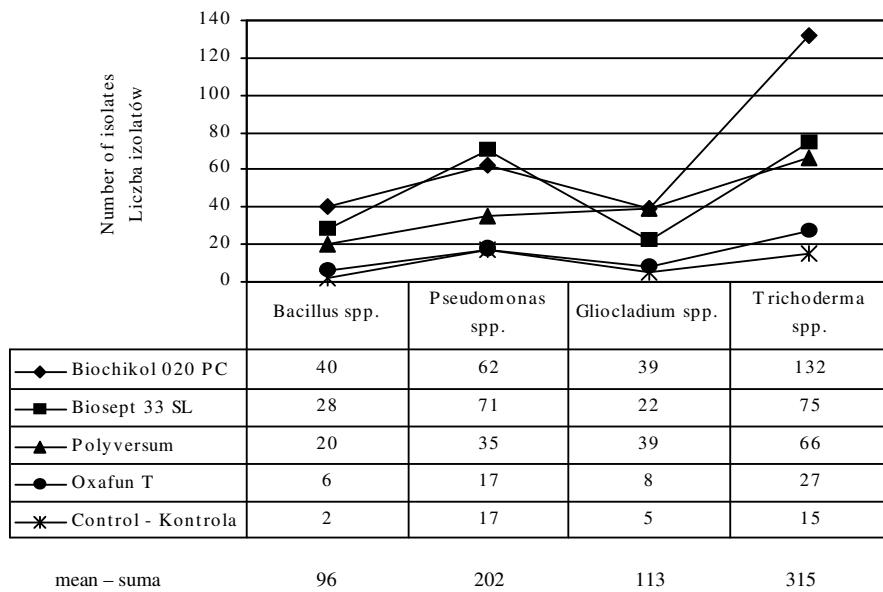


Fig. 2. The number of antagonistic bacteria and fungi isolated from the rhizosphere of soybean (total from the years 2003–2004)

Rys. 2. Liczebność antagonistycznych bakterii i grzybów wyizolowanych z ryzosfery soi (suma z lat 2003–2004)

properties of a good antagonist, namely fast growth, abundant sporulation, common occurrence in the soil environment, exudation of fungistatic substances, production of cellulitic and hydrolytic enzymes, utilization of organic and inorganic compounds or the ability of parasitism [Jaworska et al. 2004]. The studies conducted by Biesiada et al. [2002] showed that species *Trichoderma viride* can inhibit the infection of leek by *Pyrenopeziza terrestris*.

Abundant information from literature as well as the results of the present studies make it possible to include the tested bipreparations into a group of effective biological preparations of plant protection from soil phytopathogens.

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**ODDZIAŁYWANIE BIOPREPARATÓW NA KSZTAŁTOWANIE SIĘ
POPULACJI MIKROORGANIZMÓW RYZOSFEROWYCH SOI
(*Glycine max* (L.) Merrill.)**

Streszczenie. Przedmiotem doświadczenia polowego przeprowadzonego w latach 2003–2004, była gleba ryzosferowa soi odm. ‘Mazowia’. Przed wysiewem nasiona zapisywano biopreparatami (Biochikol 020 PC, Biosept 33 SL, Polyversum) lub fungicydem (Zaprawa Oxafun T). Kontrolę stanowiły nasiona niezaprawiane. Analiza mikrobiologiczna gleby ryzosferowej soi, przeprowadzona w fazie kwitnienia wykazała, że najwięcej bakterii ogółem oraz bakterii *Bacillus* spp. i *Pseudomonas* spp. wystąpiło po zastosowaniu Biochikułu 020 PC, a najmniej w kombinacji kontrolnej. Najmniejszą liczebność grzybów zaobserwowano po zastosowaniu Biochikułu 020 PC lub Zaprawy Oxafun T, nieco większą po wprowadzeniu do gleby Bioseptu 33 SL lub Polyversum, a największą w kombinacji kontrolnej. Z ryzosfery soi najczęściej wyosabniano grzyby z rodzajów *Alternaria*, *Fusarium*, *Pythium* oraz *Gliocladium*, *Penicillium* i *Trichoderma*. Po zastosowaniu biopreparatów udział grzybów chorobotwórczych w ryzosferze soi był znacznie mniejszy aniżeli w kontroli. Największy udział *Gliocladium* spp. wystąpił po zastosowaniu Polyversum lub Biochikułu 020 PC, a *Trichoderma* spp. po wprowadzeniu Biochikułu 020 PC lub Bioseptu 33 SL. Ponadto badania szalkowe wykazały, że użyte w doświadczeniu biopreparaty i fungicyd przyczyniły się do zwiększenia liczebności antagonistycznych bakterii (*Bacillus* spp. i *Pseudomonas* spp.) oraz grzybów (*Gliocladium* spp. i *Trichoderma* spp.).

Słowa kluczowe: soja, ryzosfera, biopreparaty

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