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THE USE OF BIOSEPT 33 SL, BIOCHIKOL 020 PC AND POLYVERSUM TO CONTROL SOYBEAN (*Glycine max* (L.) Merrill) DISEASES AGAINST PATHOGENS. PART II. MICROORGANISM COMMUNITIES IN THE RHIZOSPHERE SOIL OF SOYBEAN

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Abstract. The purpose of the studies was to determine the effect of such preparations as Biochikol 020 PC, Polyversum and Biosept 33 SL on the formation of microorganisms communities in the rhizosphere soil of soybean. Results of the studies pointed to a positive effect of biopreparations on the increase number of cfu total bacteria, *Bacillus* spp. and *Pseudomonas* spp. After the application of biopreparations balance between pathogens and saprobionts was maintained in the rhizosphere soil of soybean. Such relation did not exist after using chemical preparations. Biopreparations increased the number of antagonistic bacteria – *Bacillus* spp., *Pseudomonas* spp. and fungi – *Gliocladium* spp., *Trichoderma* spp.

Key words: microorganism communities, rhizosphere soil, antagonistic bacteria, antagonistic fungi

INTRODUCTION

The soil contains communities of populations of both bacteria and fungi that have a varying effect on each other and on plants. A number of researchers divide microorganisms into those positively, negatively and neutrally affecting a plant. On the other hand, microorganisms can be neutral towards each other or they can have an antagonistic effect though antibiosis, competition or parasitism. Root exudates affect the quantitative and qualitative composition in the period of vegetation [Rovira 1965, 1969, Vancura and Stanek 1975, Pięta 1988, Darcy 1982, Funck-Jensen and Hockenhull 1984, Odham et al. 1986]. Besides, the composition of microorganism populations is also affected by biotic and abiotic factors. The abiotic ones include the conditions of the

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environment and the chemical preparations or biopreparations introduced as seed dressing or used to spray the plants.

The purpose of the studies was to determine the effect of such preparations as Biochikol 020 PC (a.s. chitosan), Polyversum (a.s. *Pythium oligandrum*) and Biosept 33 SL (grapefruit extract) on the formation of microorganisms communities in the rhizosphere soil of soybean.

MATERIALS AND METHODS

The objects of the studies were microorganism communities (bacteria and fungi) occurring in the rhizosphere soil of soybean 'Mazowia' cv., whose seeds were dressed with biopreparations and the plants in the phase 60 600 (open first flowers), according to "The key to determine developmental phases of mono- and dicotyledonous plants in BBCH scale" [IOR 2005], sprayed with the same preparations. In order to compare the results obtained after applying biopreparations a combination with a chemical preparation was considered, which means that the seeds were dressed with Zaprawa Oxafun T and the plants in phase 60 600 were sprayed with Bravo Plus SC. Besides, a control combination was considered, i.e. without any protective treatment. The manner of setting the experiment, sampling the rhizosphere soil and the microbiological analysis were according to the method described by Patkowska [2006] and Martyniuk et al. [1991]. In order to obtained the total number cfu of bacteria in the rhizosphere soil of soybean, PDA medium was used with an addition of yeast extract and dilutions of soil solution 10^{-5}, 10^{-6}, 10^{-7}. Triptic Soy Agar and dilutions 10^{-4}, 10^{-5}, 10^{-6} were used to isolate *Bacillus* spp., whereas medium Pseudomonas Agar F and water dilutions of the soil solution 10^{-2}. 10^{-3} and 10^{-4} were used for *Pseudomonas* spp. Martin medium and the dilutions 10^{-2}, 10^{-3} and 10^{-4} were used to calculate the total number cfu of fungi occurring in the rhizosphere soil [Martin 1950].

Among the isolated bacteria colonies 500 isolates of *Bacillus* spp. and P*seudomonas* spp. were used to study the their antagonistic effect towards *Alternaria alternata, Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Pythium irregulare, Phoma exigua, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* In the case of the isolated fungi, *Gliocladium* spp. and *Trichoderma* spp. were subjected to laboratory tests. The antagonistic effect of the studied microorganisms was determined using the method by Martyniuk et al. [1991], whereas biotic tests described by Mańka and Mańka [1992] were used for the fungi.

RESULTS AND DISCUSSION

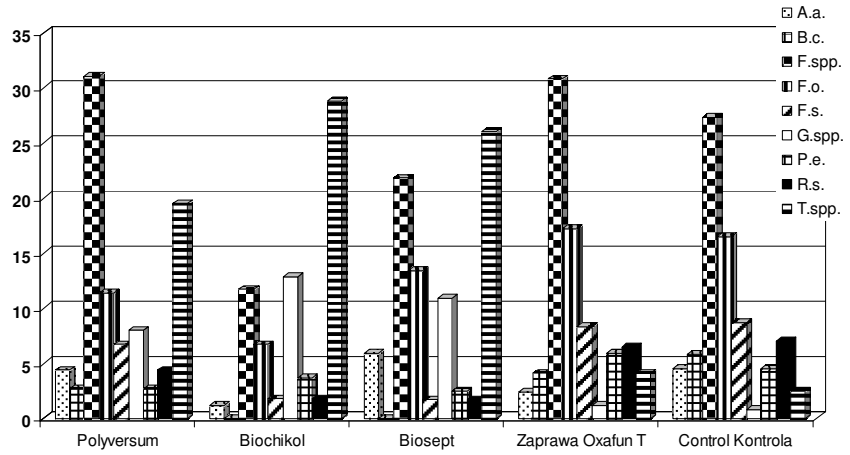
Results of the studies pointed to a positive effect of biopreparations on the increase of cfu total bacteria, *Bacillus* spp. and *Pseudomonas* spp. (tab. 1). In each year of the studies the greatest number of cfu total bacteria occurred in the rhizosphere soil of soybean after using biopreparation Biosept 33 SL, while the smallest amount was found in the control soil (tab. 1). A similar number of cfu was observed in the case of *Pseudo-*

Table 1. Number of bacteria and fungi isolated from ryzosphere of soybean
Tabela 1. Liczba bakterii i grzybów izolowana z ryzosfery soi

Experimental combination Kombinacja doświadczenia	Total number cfu bacteria (mln · g^{-1} d.w. of soil) Ogólna liczba j.t.k. bakterii (mln · g^{-1} s.m. gleby)			Number cfu *Bacillus* spp. (mln · g^{-1} d.w. of soil) Liczba j.t.k. *Bacillus* spp. (mln · g^{-1} s.m. gleby)			Number cfu *Pseudomonas* spp. (mln · g^{-1} d.w. of soil) Liczba j.t.k. *Pseudomonas* spp. (mln · g^{-1} s.m. gleby			Total number cfu fungi (thous. · g^{-1} d.w. of soil) Ogólna liczba j.t.k. grzybów (tys. · g^{-1} s.m. gleby)		
	2005	2006	mean średnia	2005	2006	mean średnia	2005	2006	mean średnia	2005	2006	mean średnia
Polyversum	11.94^{c}*	3.41c	7.68c	2.59b	2.27c	2.43b	0.33c	0.16b	0.25b	28.85ab	15.87b	22.36b
Biochikol 020 PC	9.26b	3.56c	6.41b	3.90d	1.86b	2.88b	0.26b	0.20c	0.23b	29.72b	12.04a	20.88a
Biosept 33 SL	14.98d	4.68d	9.83d	3.43c	1.07a	2.25b	0.48d	0.41d	0.45c	27.57a	14.68b	21.13ab
Zaprawa Oxafun T + Bravo Plus 500 SC	11.62c	2.28b	6.95b	3.35c	1.01a	2.18b	0.31c	0.09a	0.20ab	39.67c	18.25c	28.96c
Control – Kontrola	5.63a	1.51a	3.57a	1.68a	1.07a	1.38a	0.21a	0.13ab	0.17a	41.36d	24.24d	32.80d

*Means in columns differ significantly ($P \leq 0.05$), if they are not marked with the same letter
Średnie w kolumnach różnią się istotnie ($p \leq 0,05$), jeśli nie są oznaczone tymi samymi literami

monas spp. On the other hand, the most cfu *Bacillus* spp. was found in the rhizosphere soil after using Biochikol 020 PC, and the smallest amount – in the rhizosphere soil of the control, i.e. without any protective treatment. The number of cfu fungi in the studied samples of soil was reverse to the number of bacteria. The most fungi colonies were isolated from the control soil, and the fewest – from the soil after using biopreparations (tab. 1). According to the studies conducted by Myśków [1989], definite proportions occur between microorganisms. When the bacteria are numerous, the development of fungi is weakened and vice versa.



A.a. – *A. alternata*, B.c. – *B. cinerea,* F.spp. – *Fusarium* total – ogółem, F.o. – *F. oxysporum*, F.s. – *F. solani*, G. spp. – *Gliocladium* total – ogółem, P.e. – *P. exigua*, R.s. – *R solani*, T. spp. – *Trichoderma* total – ogółem

Fig. 1. Fungi frequently isolated from the rhizosphere soil of soybean in individual experiments (mean % from the years 2005–2006)

Rys. 1. Grzyby często izolowane z gleby ryzosferowej w poszczególnych kombinacjach doświadczenia (średnie z lat 2005–2006)

Regardless of the experimental combination, the studies found the greatest number of *Fusarium* spp. within the isolated fungi. The greatest proportion of those fungi in the rhizosphere soil of soybean could be confirmed by their big role in infecting the seedlings and plants at anthesis [Pięta 2006]. Both the seedlings and plants at anthesis were most frequently infected by *Fusarium* spp. The dominating species in the studied samples of rhizosphere soil was *F. oxysporum*, with an exception of the soil from the combination with Biochikol 020 PC (fig. 1). *Fusarium solani* was also frequently isolated from the rhizosphere soil of soybean. The proportion of other fungi such as *Altenaria alternata, Botrytis cinerea, Phoma exigua* and *Rhizoctonia solani* was much lower and differed in particular experimental combinations (fig. 1). On the other hand, *Gliocladium* spp. and *Trichoderma* spp., as antagonistic fungi, were most abundant in the rhizosphere soil of soybean after the application of Biochikol 020 PC and Biosept 33 SL (fig. 1). Chitosan is a compound which stimulates the growth and development of

Table 2. Number of antagonistic bacteria and of antagonistic fungi towards pathogenic fungi
Tabela 2. Liczba antagonistycznych bakterii i grzybów względem grzybów patogenicznych

Antagonistic bacteria and fungi Antagonistyczne bakterie i grzyby	Experimental combination – Kombinacja doświadczenia number of isolates – liczba izolatów				
	Polyversum	Biochikol 020 PC	Biosept 33 SL	Zaprawa Oxafun T+ Bravo Plus 500 SC	Control Kontrola
Bacillus spp.	11	17	10	3	1
Pseudomonas spp.	15	21	17	5	6
Total – Razem	26	38	27	8	7
Gliocladium spp.	6	14	9	2	1
Trichoderma spp.	20	31	22	4	4
Total – Razem	26	44	31	6	5
Total bacteria and fungi Razem bakerie i grzyby	52	82	58	14	12

antagonistic microorganisms, especially *Trichoderma* spp. [Patkowska et al. 2006]. A lot of such antagonists were found in the rhizosphere soil after the application of Polyversum, and sporadically they were found in the control soil after using chemical preparations. Similarly, antagonistic *Bacillus* spp. and *Pseudomonas* spp. occurred in the studied soil samples (tab. 2). It should be expected that abundant occurrence of antagonists can reduce the growth and development of phytopathogens This fact is confirmed by a lot of information in literature [Marin and Hancock 1987, Orlikowski et al. 1999, Orlikowski et al. 2001a, 2001b, Orlikowski et al. 2002, Gajda and Kurzawińska 2004a, 2004b]. According to Allan and Hadwiger [1979], chitosan and grapefruit extract affect pathogenic fungi like a fungicide. In the case of Polyversum, antagonistic fungus *Pythium oligandrum*, which limits the occurrence of pathogenic fungi through competition, superparasitism and antibiosis, is the active factor [Marin and Hancock 1987, Vesely and Kocova 2001]. Hence, it should be supposed that the use of biopreparations not only reduces the occurrence of phytopathogens in the soil but it also protects the plants from pathogenic fungi. A big proportion of antagonists in the soil keeps the balance between the populations of microorganisms and stops the excessive development of pathogenic fungi, by which the soil becomes suppressive.

CONCLUSION

1. Biopreparations such as Biochikol 020 PC, Biosept 33 SL and Polyversum were inhibitors of growth and development of plant pathogens.

2. After the application of biopreparations a balance between pathogens and saprobionts was maintained in the rhizosphere soil of soybean. Such a relation did not exist after using chemical preparations.

3. Biopreparations increased the number of antagonistic bacteria – *Bacillus* spp., *Pseudomonas* spp. and fungi – *Gliocladium* spp., *Trichoderma* spp.

4. The best conditions for the development of antagonists were created by chitosan – a biologically active substance in Biochikol 020 PC.

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ZASTOSOWANIE BIOSEPTU 33 SL, BIOCHIKOLU 020 PC I POLYVERSUM DO ZWALCZANIA CHORÓB SOI (*GLYCINE MAX* (L.) MERRILL) PRZED PATOGENAMI. CZĘŚĆ II. ZBIOROWISKA MIKROORGANIZMÓW W GLEBIE RYZOSFEROWEJ SOI

Streszczenie: Celem badań było określenie wpływu takich biopreparatów, jak Biosept 33 SL, Biochikol 020 i Polyversum na kształtowanie się zbiorowisk mikroorganizmów w glebie ryzosferowej soi. Uzyskane wyniki z badań wskazały na pozytywny wpływ biopreparatów na zwiększenie jtk bakterii ogółem, *Bacillus* spp. i *Pseudomonas* spp. Po zastosowaniu biopreparatów, w glebie ryzosferowej soi była zachowana równowaga między patogenami i saprobiontami. Takiej zależności nie było po zastosowaniu preparatów chemicznych. Biopreparaty zwiększyły liczbę antagonistycznych bakterii – *Bacillus* spp., *Pseudomonas* spp. i grzybów – *Gliocladium* spp., *Trichoderma* spp.

Słowa kluczowe: zbiorowiska mikroorganizmów, gleba ryzosferowa, antagonistyczne bakterie, antagonistyczne grzyby

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