A POSSIBILITY OF USING GRAPEFRUIT EXTRACT, CHITOSAN AND *PYTHIUM OLIGANDRUM* TO PROTECT SOYBEAN (*GLYCINE MAX* (L.) MERRILL) FROM PATHOGENS

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1. Introduction

For twenty years intensive studies on searching for new methods of biological control of plant diseases have been conducted. One of such methods is the use of biopreparations. Nowadays in Poland Biosept 33 SL (grapefruit extract), Biochikol 020 PC (chitosan) and Polyversum (oospores of *Pythium oligandrum*) are registered. Abundant information from literature report that biopreparations on the basis of both organic compounds and antagonistic microorganisms inhibit the occurrence of different plant diseases [1 - 8].

2. Materials and methods

The object of studies in the years 2005 - 2006 were the seedlings and plants of soybean at anthesis which grew from the seeds dressed with 0.2% Biosept 33 SL, 2.5% Biochikol 020 PC and Polyversum in a dose of 1 g of the preparation × 1 kg^{-1} seeds. At the beginning of anthesis the plants were sprayed with the same biopreparations that had been used for seed dressing. The experiment considered the combination with a chemical preparation Zaprawa Oxafun T for seed dressing, and Bravo Plus 500 SC for spraying plants at anthesis. Plants without any protective treatment constituted the control. During the vegetation period the studies established the population and healthiness of plants in particular phases of development, i.e. in the phase of 6-week-old seedlings and plant at full anthesis – 7 days after the spraying. The diseased plants were taken for laboratory mycological analysis, which was carried out according to the method described by Pięta [9]. After the harvest the yield weight and the proportion of spotted seeds were determined in particular experimental combinations.

The rhizosphere soil was also the object of studies. Sampling the rhizosphere soil and its microbiological analysis were according to the method described by Patkowska et al. [10] and Martyniuk [11]. In order to obtain 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}. Tryptic 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 [12] 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.

The results were statistically analyzed and the significance of differences was determined on the basis of Duncan's [13] confidence semi-intervals.

3. Results and discussion

It was found out on the basis of the obtained results of the observations both in the phase of seedlings and at full anthesis that the number and healthiness of plants were the best in the combination with Biosept 33 SL (Table 1). A lot of plants were in the plots with the combinations with biopreparations (Polyversum, Biochikol 020 PC) and with chemical preparations (Zaprawa Oxafun T + Bravo Plus 500 SC) (Figure 1). The smallest number of plants with the highest share of the diseased ones was found on control plots (Figure 1). The results of studies confirmed the information on the effectiveness of the tested biopreparations in controlling diseases in different plants [1 - 8].

The highest yield with the best quality seeds was obtained from the plants treated with Biosept 33 SL (Figure 2). A high yield was also obtained in the combination with Polyversum and Biochikol 020 PC and with chemical preparations. The lowest seed yield with a big proportion of seeds with spots was obtained from control plants (Figure 2).

The greatest amount of *Fusarium* spp. (*F. culmorum, F. oxysporum* and *F. solani*) was obtained as a result of the mycological analysis (Table 1). The period of vegetation in the years

Table 1. *Fungi frequently isolated from soybean plants in individual experimental (sum from the years 2005-2006).*

Fungus species	Number of isolates					Total
	Polyversum	Biochikol 020 PC	Biosept 33 SL	Zaprawa Oxafun T + Bravo Plus 500 SC	Control	
Alternaria alternata	20	19	8	26	36	109
Botrytis cinerea	2			5	7	14
Fusarium spp.	76	81	70	136	189	552
Fusarium culmorum	13	11	15	23	27	89
Fusarium oxysporum	51	45	44	70	103	313
Fusarium solani	11	22	9	39	53	134
Gliocladium spp.	26	54	34	18	19	151
Phoma exigua	6	17	7	22	31	83
Pythium irregulare	4	6	4	15	22	51
Rhizoctonia solani	9	7		21	29	66
Sclerotinia sclerotiorum	2	1		6	9	18
Trichoderma spp.	29	70	31	14	16	160
Total	**249**	**333**	**222**	**395**	**541**	**1740**

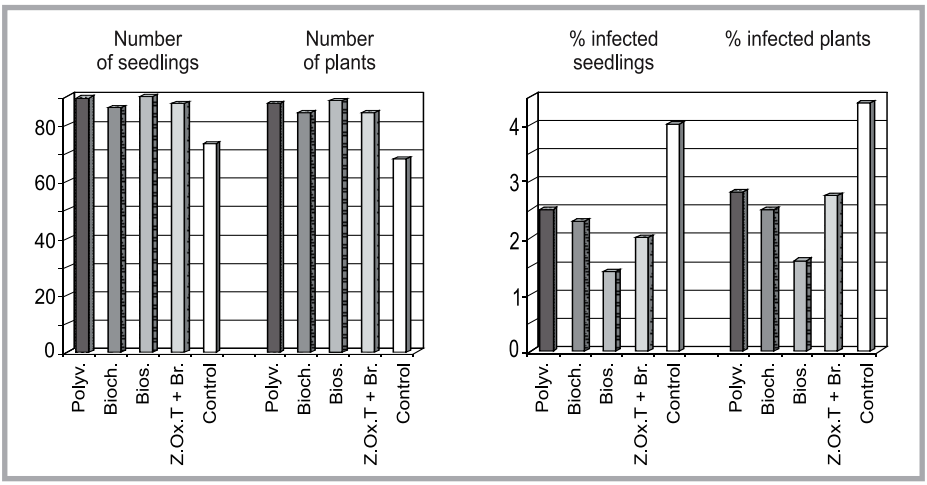

Figure 1. *Number and healthiness of soybean plants on plots (mean from the years 2005-2006); Polyv. - Polyversum, Bioch. - Biochikol 020 PC, Bios. - Biosept 33 SL, Z.Ox.T - Zaprawa Oxafun T, Br. - Bravo Plus 500 SC.*

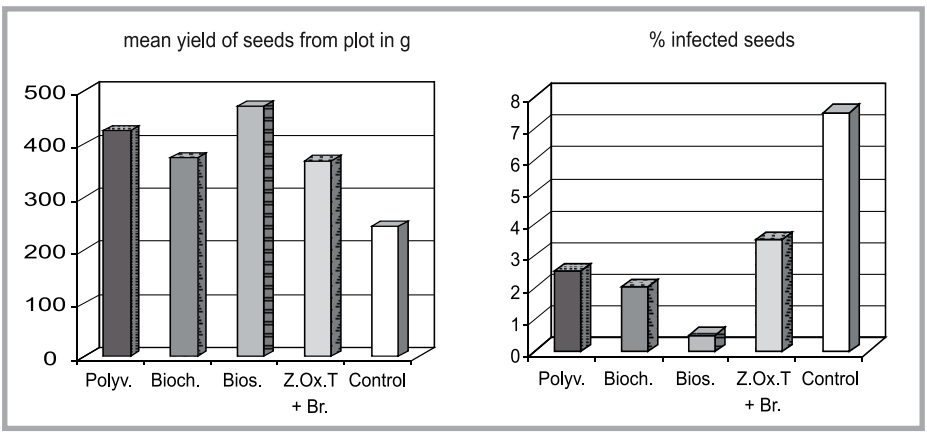

Figure 2. *Yield and participation of infected soybean seeds (mean from the years 2005-2006); Polyv. - Polyversum, Bioch. - Biochikol 020 PC, Bios. - Biosept 33 SL, Z.Ox.T - Zaprawa Oxafun T, Br. - Bravo Plus 500 SC.*

2005 - 2006 was favorable for the development of *Fusarium* spp. and plant infection (characterized by high air temperature). According to Booth [14], Sinclair and Backman [15], these are the fungi that require temperature between 22 and 28 °C for their growth. Besides, *Alternaria alternata, Botrytis cinerea, Phoma exigua, Rhizoctonia solani*, *Pythium irregulare* were isolated from the studied plants as well as *Sclerotinia sclerotiorum* – from plants at anthesis (Table 1). Saprobionts such as *Gliocladium* spp. and *Trichoderma* spp. were also obtained in big numbers (Table 1). According to Dos Santos and Dhingra [16], the number of *Fusarium* spp. colonies considerably drops with abundant occurrence of *Trichoderma*

spp. Antagonistic *Gliocladium* spp. and *Trichoderma* spp., colonizing the rhizosphere of soybean, can constitute a protective barrier against pathogens [17, 18]. Results of the studies pointed to a positive effect of biopreparations on the increase of cfu total bacteria, *Bacillus* spp. and *Pseudomonas* spp. (Figure 3). 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 (Figure 3). The number cfu of *Pseudomonas* spp. was also the highest in the rhizosphere soil of soybean treated with Biosept 33 SL. 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 (Figure 4). According to the studies conducted by Myśków [19], definite proportions occur between microorganisms. When the bacteria are numerous, the development of fungi is weakened and *vice versa*.

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 (Figure 5). Chitosan is a compound which stimulates the growth and development of antagonistic microorganisms, especially *Trichoderma* spp. [20]. 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*

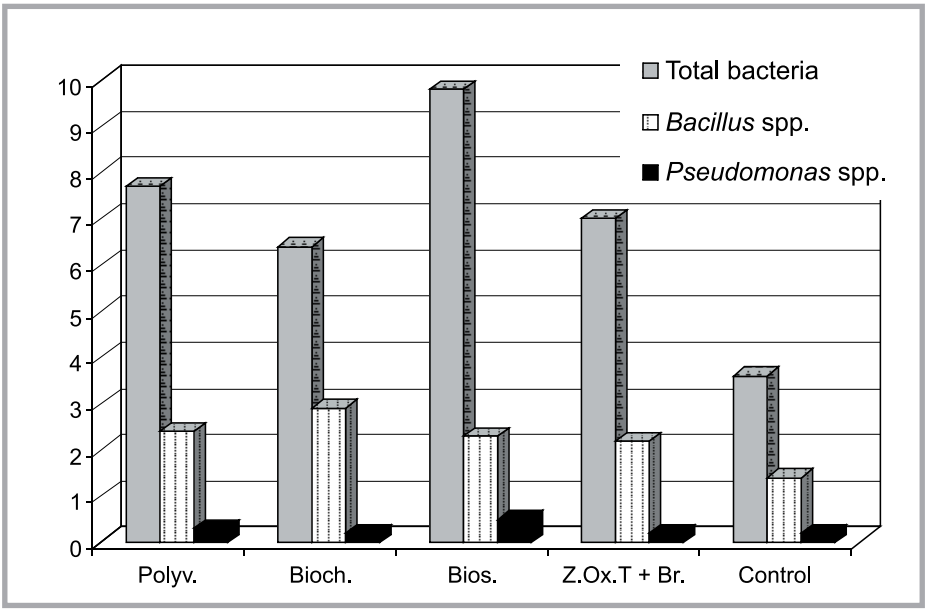

Figure 3. *Number cfu bacteria (in mln • g-1d.w. soil) isolated from rhizosphere of soybean (mean from the years 2005-2006); Polyv. - Polyversum, Bioch. - Biochikol 020 PC, Bios. - Biosept 33 SL, Z.Ox.T - Zaprawa Oxafun T, Br. - Bravo Plus 500 SC.*

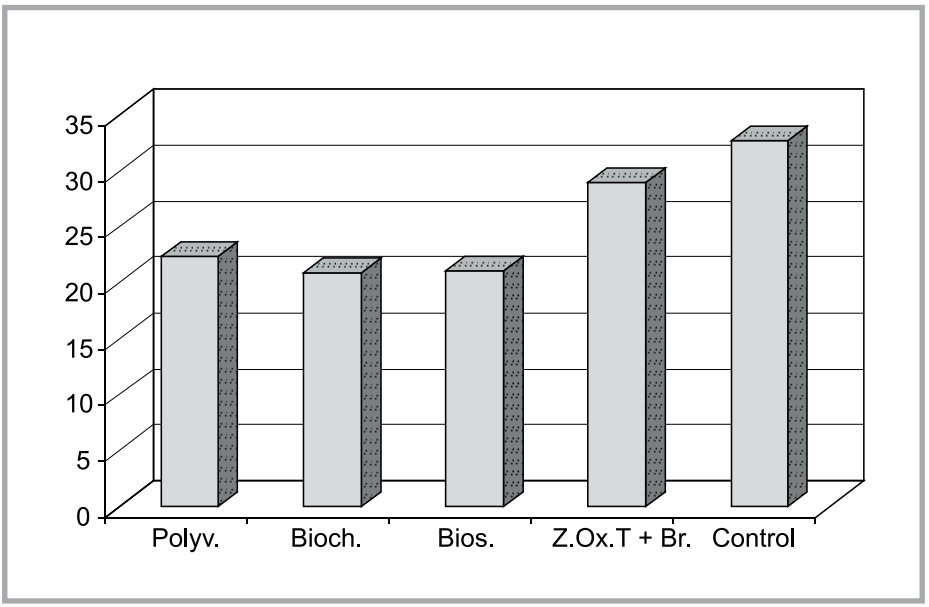

Figure 4. *Number cfu fungi (in thous. • g-1d.w. soil) isolated from rhizosphere of soybean (mean from the years 2005-2006); Polyv. - Polyversum, Bioch. - Biochikol 020 PC, Bios. - Biosept 33 SL, Z.Ox.T - Zaprawa Oxafun T, Br. - Bravo Plus 500 SC.*

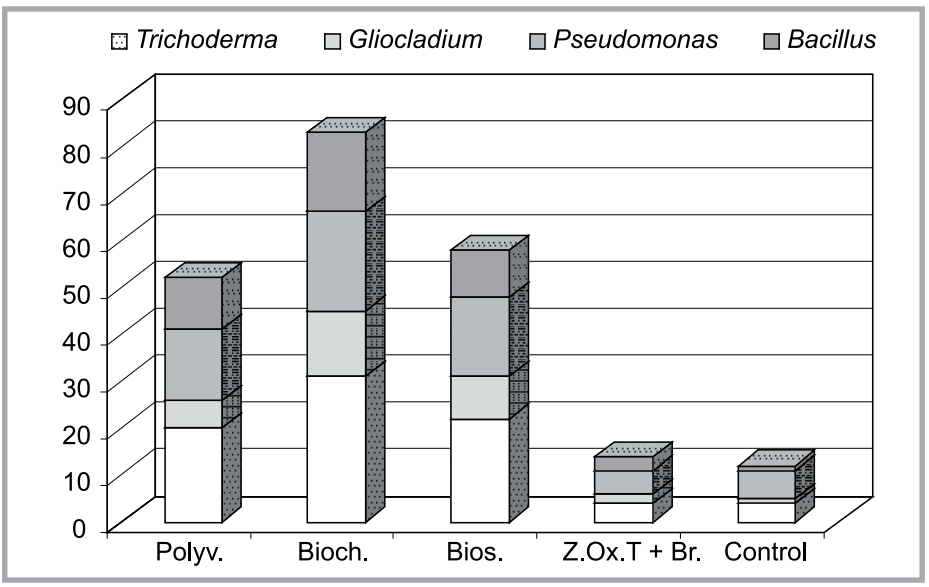

Figure 5. *Number of antagonistic microorganisms (sum from the years 2005 - 2006); Polyv. - Polyversum, Bioch. - Biochikol 020 PC, Bios. - Biosept 33 SL, Z.Ox.T - Zaprawa Oxafun T, Br. - Bravo Plus 500 SC.*

spp. and *Pseudomonas* spp. occurred in the studied soil samples (Figure 5). 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 [3 - 5, 21 - 24]. According to Allan and Hadwiger [25], 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 [21, 26]. Hence, it should be supposed that the, use of bio-preparations 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.

4. References

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