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Original Research

Effect of Bio-Products on Bean Yield and Bacterial and Fungal Communities in the Rhizosphere and Non-Rhizosphere

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Abstract

This study presents the effect of biopreparations (Polyversum, Biochikol 020 PC and Biosept 33 SL) on fungal and bacterial rhizosphere and non-rhizosphere communities after seed dressing and spraying of *Phaseolus vulgaris* plants. The use of biopreparations has a positive effect on the communities of bacteria and fungi in soil under the cultivation of this plant. The number of cfu of the studied microorganisms in the non-rhizosphere soil was slightly lower than in the rhizosphere. Biochikol 020 PC and Biosept 33 SL increased the number of cfu of bacteria *Bacillus* spp. and *Pseudomonas* spp. and decreased the population of soil-borne fungi. Different species were isolated within the fungi and they belonged to the following genera: *Altenaria, Fusarium, Rhizoctonia, Sclerotinia, Gliocladium, Penicillium* and *Trichoderma*. The most antagonistic bacteria and fungi were obtained after introducing biopreparations Biochikol 020 PC or Biosept 33 SL. The smallest number of antagonists were found in the soil after dressing the bean seeds with Zaprawa Oxafun T and spraying the plants with fungicide Bravo Plus 500 SC and in the control combination. Besides, the applied biopreparations and fungicides had a positive effect on *Pheseolus vulgaris* yielding.

Keywords: Biochikol 020 PC, Biosept 33 SL, Polyversum, Zaprawa Oxafun T, Bravo Plus 500 SC, *Phaseolus vulgaris*, yielding, bacteria, fungi

Introduction

Beans belong to the important plants cultivated in southeastern Poland. Because of favorable soil and climatic conditions, the bean cultivation is concentrated mainly in the Lublin region, where infrequent crop rotation is conductive to pathogen accumulation in the soil. Earlier research [1, 2] showed that plants of this crop were infected by *Botrytis cinerea* Pers., *Rhizoctonia solani* Kühn, *Sclerotinia sclerotiorum* (Lib.) de Bary and *Fusarium* spp. These fungi infected the bean plants at each growth stage, causing necrosis of the underground and aboveground parts, as well as damping-off and tracheomycosis that reduces the size and quality of the yield [2]. The chemical method based on the application of fungicides mainly for seed dressing has so far been a commonly used method for protecting the bean plants from soil-borne pathogens. Increasing knowledge about the consequences for the environment and the possibilities of crop contamination resulting from the use of chemicals point to the need of partial or complete introduction of a non-chemical method of plant protection [1, 2].

Soil – being a natural environment for different microorganisms – constitutes their proper ecological niche where a number of biotic and abiotic factors interact. Soil microorganisms, closely connected with the life of plants,

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stimulate or inhibit their growth and development [3]. The greatest biological activity is characteristic of the rhizosphere soil [4-7].

Microorganbial communities in the cultivated environment are very important, since they affect the health and, consequently, yield of plants [6, 8, 9]. The biological control of different plant species from pathogenic factors consists, for example, in replacing pesticides with biopreparations based on antagonistic microorganisms, and plant extracts or organic compounds [10-15]. In recent years much attention has been paid to the protective effect of such biopreparations as Polyversum, Biochikol 020 PC or Biosept 33 SL.

Polyversum, based on *Pythium oligandrum* oospores, Biochikol 020 PC, whose active substance is chitosan, and Biosept 33 SL, containing 33% of grapefruit extract, may affect microorganism communities in the soil; they interact with fungal pathogens and they induce plants resistance to certain plant pathogens [10-16]. That is the reason why in practice it is recommended to replace pesticides with these biopreparations used for the dressing of bulbs, onions and seeds, as well as spraying the plants [14, 17, 18].

The compounds contained in grapefruit extract such as 7-geranoxycumarine, triclosan or benzetonine chloride can inhibit the development of bacteria and fungi [19, 20]. The studies by Orlikowski [12] on the mechanism of the effect of grapefruit extract on *Phytophthora cryptogea* showed that it limited the growth of mycelium, inhibited the formation of zoosporangia and germination of this pathogen's zoospores. Besides, grapefruit extract introduced to peat substrate inhibited the growth of mycelium, the formation of conidial spores and chlamydospores of *Fusarium oxysporum* f. sp. *dianthi*, thereby reducing the number of propagation units of this fungus in the medium [14]. The studies conducted by Orlikowski and Skrzypczak [14] on protection of tulips from *Botrytis tulipae* also confirmed the direct effect of this product on the pathogen, since it inhibited the formation of mycelial filaments and conidial spores of *B. tulipae*.

On the other hand, the effect of *Pythium oligandrum* on pathogens is differentiated. As stated by Benhamou et al. [21], it is mycoparasitism consisting of a direct contact between a pathogenic species and *P. oligandrum*, as a result of which destructive changes occur in the host's filaments. Another kind of effect is antibiosis, which leads to dying out of filaments, despite the lack of a direct contact between the pathogen and the antagonist [21]. Besides, *P. oligandrum* colonizes the root zone of plants, in this way protecting it from infection by pathogenic fungi [22].

Chitosan contained in Biochikol 020 PC induces plant resistance and protects them from infection by viruses, bacteria and fungi [10, 23]. Besides, this bio-product is used as a dressing for papilionaceous plants, or as foliar application inhibited the development of pathogens [15, 18].

In literature there is no information concerning the effect of biopreparations on the composition of microorganisms in the soil environment and plant yield. Hence, the purpose of the present study was to determine the effect of *Pythium oligandrum*, chitosan and grapefruit extract on plant yield and on fungal and bacterial microorganisms community in the non-rhizosphere and rhizosphere soil of common bean growing under threat from soil-borne plant pathogens.

Material and Methods

Field Experiment

Field studies were conducted at the Experimental Farm of Czesławice near Nałęczów in the years 2005-06 on a field of a three-year-long monoculture of common bean.

The experiment was set up in a random blocks scheme with four replications (plot areas – 3.75m^2), on grey-brown podsolic soil belonging to the second soil suitability complex (good wheat complex). 100 bean seeds were sown on each plot in four rows. The spacing between the rows was 30cm, and the seeds were sown 10cm apart.

The object of the studies was non-rhizosphere and rhizosphere soil of common bean of 'Narew' cv. The experiment was established in the first 10 days of May, according to the method described earlier by Patkowska [9]. Before sowing, the seeds were dressed with the following biopreparations: 2.5% Biochikol 020 PC (containing 1.88% of active substance), 0.2% Biosept 33 SL (33% grapefruit extract), Polyversum (containing 10^6 oospores of *Pythium oligandrum* per 1g), applying 1g of the preparation x 100g^{-1} seeds. Besides, Zaprawa Oxafun T was used (active substance: carboxine 37.5% + tiuram 37.5%) in the quantity of 1g x 100g^{-1} seeds. The seeds that were not dressed constituted the control object. Each combination included 4 plots, where 100 seeds were sown on each. The second treatment was carried out at the beginning of anthesis of common bean. It consisted of spraying the aboveground part of the plants with the same preparations that were used for seed dressing, i.e. 2.5% Biochikol 020 PC, 0.2% Biosept 33 SL and 0.1% Polyversum. In the case of the combination with Zaprawa Oxafun T, the plants were sprayed with 0.1% fungicide Bravo Plus 500 SC (a.s. chlorotalonile 50%).

Assessment of Bean Yield

After the plants were picked and dried, the yield of *Phaseolus vulgaris* growing in particular experimental combinations was established and expressed as grams of the dry weight of seeds from a plot.

Analysis of Microbial Community

Eight weeks after sowing, non-rhizosphere and rhizosphere soil samples were taken from particular experimental combinations and laboratory microbiological analysis was conducted, according to the method described by Patkowska [9] and Martyniuk et al. [24]. The manner of soil sampling was in accordance with the method described by Martyniuk et al. [24]. Four plants were dug out as a whole from each plot of particular experimental combinations (i.e.

Table 1. The number of bacteria and fungi in the rhizosphere of common bean.

Treatment	Concentration (%)	Total number of bacteria [cfu·g^{-1} DW of soil] · 10^6			Total number of *Bacillus* spp. [cfu·g^{-1} DW of soil] · 10^6			Total number of *Pseudomonas* spp. [cfu·g^{-1} DW of soil] · 10^6			Total number of fungi [cfu·g^{-1} DW of soil] · 10^3		
		2005	2006	mean	2005	2006	mean	2005	2006	mean	2005	2006	mean
Polyversum	0.1	3.59^{d}*	3.00^{b}	3.29^{c}	1.95^{d}	1.83^{b}	1.89^{c}	0.02^{a}	0.13^{a}	0.07^{a}	10.33^{a}	17.77^{c}	14.05^{b}
Biochikol 020 PC	2.5	2.53^{c}	3.47^{c}	3.00^{bc}	0.73^{c}	2.06^{c}	1.39^{b}	1.66^{d}	0.24^{b}	0.95^{c}	10.13^{a}	15.28^{b}	12.70^{ab}
Biosept 33 SL	0.2	3.48^{d}	4.54^{d}	4.01^{d}	0.35^{b}	2.86^{e}	1.60^{bc}	1.27^{c}	0.45^{c}	0.86^{c}	9.14^{a}	10.21^{a}	9.68^{a}
Zaprawa Oxafun T + Bravo Plus 500SC	0.1	1.90^{b}	3.43^{c}	2.66^{b}	0.06^{a}	2.58^{d}	1.32^{b}	0.12^{ab}	0.24^{b}	0.18^{ab}	15.96^{b}	18.86^{d}	17.41^{c}
Control	-	1.00^{a}	1.66^{a}	1.33^{a}	0.08^{a}	1.26^{a}	0.67^{a}	0.24	0.15^{a}	0.20^{b}	24.79^{c}	21.27^{c}	23.03^{d}

* mean values in columns marked with the same letter do not differ significantly at $p \leq 0.05$.

16 plants from each combination). The soil directly adjoining the bean roots (i.e. the rhizosphere soil) was shaken off into sterile Petri dishes. Four soil samples taken from a depth of 5-10cm from four different interrows of a given plot (i.e. from 16 places for each experimental combination) made up the non-rhizosphere soil. In sterile laboratory conditions the soil samples from the same experimental combination were mixed, then weighed in quantities of 10g and prepared for further analyses (4 repetitions for each experimental combination).

Soil solutions from 10g of soil with dilutions from 10^{-1} to 10^{-7} were prepared in laboratory conditions from particular soil samples. The total number of bacteria was established on Nutrient Agar medium using the solutions of 10^{-5}, 10^{-6}, and 10^{-7}. In the case of *Bacillus* spp. bacteria, Tryptic Soy Agar medium and the dilutions of 10^{-4}, 10^{-5}, 10^{-6} were used, whereas Pseudomonas Agar F medium and the dilutions of 10^{-2}, 10^{-3}, 10^{-4} were used for *Pseudomonas* spp. The total number of fungi in each soil sample was established on Martin's medium [25] using the dilutions of 10^{-2}, 10^{-3}, and 10^{-4}. The population of bacteria and fungi colonies was calculated per 1 g of soil dry weight.

The obtained isolates of fungi *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. (all isolates) and bacteria *Bacillus* spp. and *Pseudomonas* spp. (500 isolates each) served to determine their antagonistic effects toward the following fungi: *Alternaria alternata, Botrytis cinerea, Fusarium culmorum, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani,* and *Sclerotinia sclerotiorum* (unpublished results of pathogenicity tests). The mutual effect of those microorganisms was determined according to the methods described by Martyniuk et al. [24] and Mańka and Mańka [26]. They considered the degree of growth inhibition of the colonies of plant pathogens and the size of the inhibition zone with common growth of those microorganisms. Laboratory tests made it possible to find the number of isolates of antagonistic bacteria and fungi occurring in non-rhizosphere and rhizosphere soil of the studied plant cultivated in particular experimental combinations.

Information referring to air temperature and precipitation in the area of the studies (Czesławice) was analyzed according to data from the Department of Agrometeorology of the University of Life Science in Lublin.

Results concerning the yield and population of microorganisms occurring in the soil under common bean were statistically analyzed using variance analysis. The significance of differences between the means was established using Tukey's confidence intervals [27]. Statistical calculations were carried out using the Statistica program, version 7.1.

Results

Results of the laboratory microbiological analysis of the rhizosphere soil of common bean showed that the total population of bacteria in 1g of the soil dry weigh ranged from 1.33×10^6 to 4.01×10^6 cfu, and the smallest number of total bacteria was found in the control combination (Table 1).

Table 2. The number of bacteria and fungi in the non-rhizosphere soil.

Treatment	Concentration	Total number of bacteria [cfu·g^{-1} DW of soil] · 10^6			Total number of *Bacillus* spp. [cfu·g^{-1} DW of soil] · 10^6			Total number of *Pseudomonas* spp. [cfu·g^{-1} DW of soil] · 10^6			Total number of fungi [cfu·g^{-1} DW of soil] · 10^3		
	(%)	2005	2006	mean	2005	2006	mean	2005	2006	mean	2005	2006	mean
Polyversum	0.1	2.52^{c}*	2.14^{b}	2.33^{c}	1.45^{d}	1.47^{b}	1.46^{d}	0.04^{a}	0.09^{ab}	0.06^{a}	8.12^{b}	14.39^{b}	11.25^{b}
Biochikol 020 PC	2.5	1.88^{b}	2.39^{b}	2.13^{c}	0.53^{c}	1.81^{c}	1.17^{c}	1.12^{d}	0.15^{c}	0.63^{b}	7.86^{b}	12.37^{b}	10.11^{b}
Biosept 33 SL	0.2	2.32^{c}	3.38^{c}	2.85^{d}	0.29^{b}	2.03^{d}	1.16^{c}	0.84^{c}	0.38^{d}	0.61^{b}	5.78^{a}	8.54^{a}	7.16^{a}
Zaprawa Oxafun T + Bravo Plus 500SC	0.1	0.82^{a}	2.36^{b}	1.59^{b}	0.04^{a}	1.57^{b}	0.80^{b}	0.08^{ab}	0.11^{bc}	0.09^{a}	9.20^{b}	13.22^{b}	11.21^{b}
Control	-	0.57^{a}	0.76^{a}	0.66^{a}	0.06^{a}	0.64^{a}	0.35^{a}	0.17^{b}	0.05^{a}	0.11^{a}	22.84^{c}	19.53^{c}	21.18^{c}

* mean values in columns marked with the same letter do not differ significantly at $p \leq 0.05$.

The most *Bacillus* spp. occurred in the rhizosphere of common bean after the application of Polyversum (mean 1.89 x 10^6 cfu), while the most *Pseudomonas* spp. were observed in the combination with Biochikol 020 PC or Biosept 33 SL (respectively, on average, 0.95 x 10^6 and 0.86 x 10^6 cfu). The total population of fungi in 1g of the rhizosphere soil of common bean growing in combinations with Biosept 33 SL or Biochikol 020 PC was the smallest (on average, 9.68 x 10^3 and 12.70 x 10^3 cfu, respectively). Slightly more fungi occurred in the rhizosphere of common bean after the application of Polyversum or Zaprawa Oxafun T + Bravo Plus 500 SC, and the most in the control combination (23.03 x 10^3 cfu) (Table 1).

In the non-rhizosphere soil of common bean the population of the studied microorganisms was slightly smaller than in the rhizosphere of this plant (Table 2). However, in particular experimental combinations the studies found a similar relation in the populations of the examined bacteria and fungi as in the rhizosphere of *Phaseolus vulgaris*. The total population of bacteria in 1g of dry weight of the non-rhizosphere soil ranged, on average, from 0.66 x 10^6 to 2.85 x 10^6 cfu. The most total bacteria occurred in 1g of dry weight of the non-rhizosphere soil after the use of Biosept 33 SL. The most *Pseudomonas* spp. was found in the non-rhizosphere soil of common bean after the application of Biochikol 020 PC or Biosept 33 SL (respectively, 0.63 x 10^6 and 0.61 x 10^6 cfu·g^{-1} DW of soil, on average). The most *Bacillus* spp., on average, occurred in the combination after Polyversum applying, whereas the least in the control. The total population of fungi in the non-rhizosphere soil in particular studied years ranged from 5.78 x 10^3 to 22.84 x 10^3 cfu·g^{-1} DW of soil (depending on the experimental combination). The least fungi in 1g of non-rhizosphere soil was observed after using Biosept 33 SL, and the most in the control combination (Table 2).

Totally, 815 isolates of fungi frequently occurring in the soil were obtained from the rhizosphere of the common bean and they belonged to 15 genera. The most frequently isolated fungi belonged to the genera of *Alternaria, Fusarium, Rhizoctonia, Sclerotinia and Gliocladium, Penicillium* and *Trichoderma. Fusarium* spp. proved to be the dominating one (Fig. 1). This genus was represented by *F. culmorum, F. oxysporum* and *F. solani*. Among the saprophytic fungi, *Cladosporium* spp., *Epicoccum* spp., *Mucor* spp. and *Rhizopus* spp. were isolated, but the dominating ones were *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. (Fig. 2). Such species as *G. fimbriatum* and *G. roseum* occurred within *Gliocladium*, while genus *Trichodemra* was represented by *T. aureoviride* and *T. harzianum.* The proportion of *Alternaria alternata, Fusarium* spp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum* was the lowest in the rhizosphere of common bean after the application of Biosept 33 SL, and it was 6.6%, 17.3%, 3.3% and 1%, respectively (Fig. 1). The highest proportion of *Fusarium* spp. was found in the control combination (41.7%). The proportion of *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. was higher in the rhizosphere of common bean in the combinations with biopreparations than in the combination with Zaprawa

Oxafun T + Bravo Plus 500 SC or in the control (Fig. 2). The highest proportion of *Gliocladium* spp. was observed in the rhizopshere after the application of Biosept 33 SL or Polyversum (respectively, 18.2% and 12.7%). The proportion of *Trichoderma* spp. was the highest in the rhizosphere of common bean after the introduction of Biochikol 020 PC into the environment (16.1%), slightly lower in the combinations with Polyversum (11.1%) or Biosept 33 SL (11.6%), and the lowest in the combination with Zaprawa Oxafun T + Bravo Plus 500 SC or in the control (respectively, 2.2% and 2.8%) (Fig. 2).

The qualitative composition of fungi isolated from the non-rhizosphere soil of common bean cultivated in particular experimental combinations was close to the qualitative composition of fungi obtained from the rhizosphere of the studied plant. Totally, 490 isolates of fungi belonging to 14 genera and frequently occurring in the soil were obtained from the non-rhizopshere soil. Among the fungi considered to be pathogenic, *Fusarium* spp. most frequently occurred in the non-rhizosphere soil of particular experimental combinations (Fig. 3). The proportion of fungi of this genus was the highest in the control and it constituted 45.1%, whereas the smallest was found in the combination with Biosept 33 SL (19.4%) (Fig. 3). The proportion of *Alternaria alternata*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in the non-rhizosphere soil was the highest in the control, and it was 11.5%, 15.4% and 6.0%, respectively (Fig. 3). The proportion of saprophytic fungi from genera *Gliocladium*, *Penicillium* and *Trichoderma* was the lowest after the application of Zaprawa Oxafun T + Bravo Plus 500 SC, and it was 1.0%, 8.6% and 1.5%, respectively (Fig. 4). The genera of saprophytic fungi mentioned above were obtained much more frequently from the non-rhizopshere soil in the combinations with the studied biopreparations as compared to the control or after the use of fungicides.

As a result of laboratory tests, 174 total bacteria isolates (*Bacillus* spp. and *Pseudomonas* spp.) and 189 fungi isolates (*Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.) were obtained, which had an antagonistic effect on the tested pathogenic fungi (Table 3). The greatest number of antagonistic bacteria and fungi was obtained from the rhizopshere of common bean after the introduction of biopreparations Biochikol 020 PC or Biosept 33 SL, while the smallest number after dressing the seeds with Zaprawa

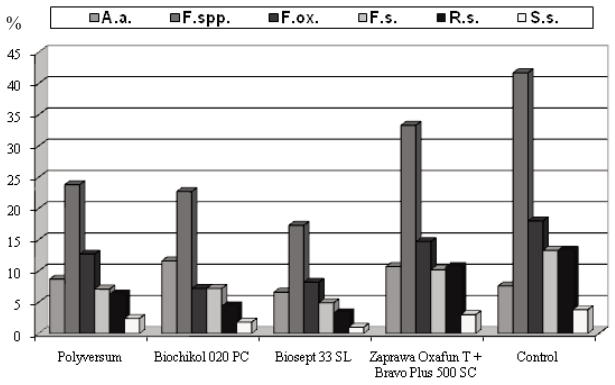

Fig. 1. Participation of pathogenic fungi isolated from the rhizosphere of common bean (mean from the years 2005-06).
A.a. - *Alternaria alternata*, F.spp. - Total *Fusarium* spp., F.ox. - *Fusarium oxysporum*, F.s. - *Fusarium solani*, R.s. - *Rhizoctonia solani*, S.s. - *Sclerotinia sclerotiorum*

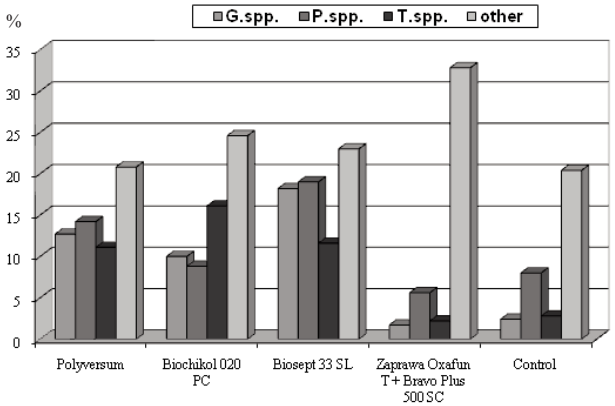

Fig. 2. Participation of saprophytic fungi isolated from the rhizosphere of common bean (mean from the years 2005-06).
G.spp. - *Gliocladium* spp., P.spp. - *Penicillium* spp., T.spp. - *Trichoderma* spp., other - other saprotrophic fungi

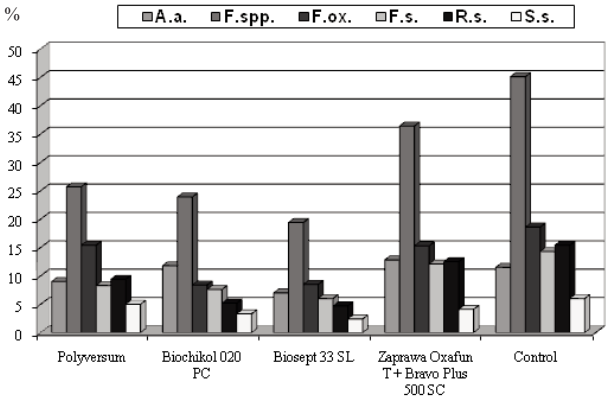

Fig. 3. Participation of pathogenic fungi isolated from the non-rhizospheric soil (mean from the years 2005-06).
A.a. - *Alternaria alternata*, F.spp. - Total *Fusarium* spp., F.ox. - *Fusarium oxysporum*, F.s. - *Fusarium solani*, R.s. - *Rhizoctonia solani*, S.s. - *Sclerotinia sclerotiorum*

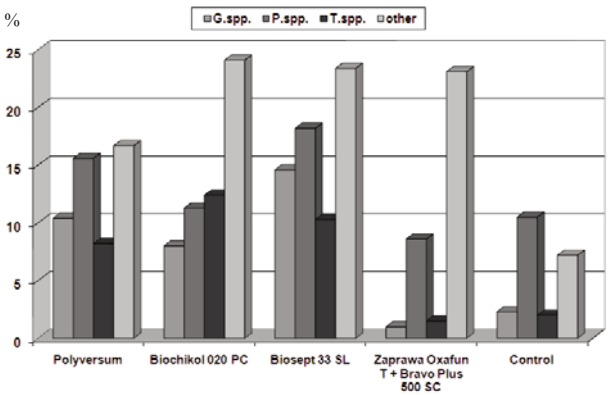

Fig. 4. Participation of saprophytic fungi isolated from the non-rhizospheric soil (mean from the years 2005-06).
G.spp. - *Gliocladium* spp., P.spp. - *Penicillium* spp., T.spp. - *Trichoderma* spp., other - other saprophytic fungi

Table 3. The number of antagonistic bacteria and fungi in the rhizosphere of common bean (mean from 2005-06).

Antagonistic bacteria and fungi	Treatment / Number of isolates				
	Polyversum	Biochikol 020 PC	Biosept 33 SL	Zaprawa Oxafun T	Control
Bacillus spp.	18	31	22	9	4
Pseudomonas spp.	19	33	27	7	4
Total bacteria	37	64	49	16	8
Gliocladium fimbriatum Gilman et Abbott	8	15	12	3	1
Gliocladium roseum (Link) Bainier	8	12	10	-	4
Penicillium spp.	11	13	16	2	7
Trichoderma aureoviride Rifai	6	15	8	3	-
Trichoderma harzianum Rifai	8	14	6	1	6
Total fungi	41	69	52	9	18
Total	78	133	101	25	26

Table 4. The number of antagonistic bacteria and fungi in the non-rhizosphere soil (mean from 2005-06).

Antagonistic bacteria and fungi	Treatment / Number of isolates				
	Polyversum	Biochikol 020 PC	Biosept 33 SL	Zaprawa Oxafun T	Control
Bacillus spp.	7	15	12	4	2
Pseudomonas spp.	10	16	13	3	2
Total bacteria	17	31	25	7	4
Gliocladium fimbriatum Gilman et Abbott	4	7	5	2	1
Gliocladium roseum (Link) Bainier	3	4	4	-	2
Penicillium spp.	5	7	9	1	3
Trichoderma aureoviride Rifai	3	6	5	1	-
Trichoderma harzianum Rifai	5	6	4	-	2
Total fungi	20	30	27	4	8
Total	37	61	52	11	12

Oxafun T and spraying the plants with Bravo Plus 500 SC fungicide and from the control combination (Table 3).

Laboratory tests showed that the non-rhizopshere soil of particular experimental combinations contained about twice less antagonistic bacteria and fungi than in the rhizopshere of the studied plant (Table 4). Totally, 84 isolates of antagonistic *Bacillus* spp. and *Pseudomonas* spp. and 89 isolates of antagonistic *Gliocladium* spp., *Penicillium* spp and *Trichoderma* spp. were obtained from all experimental combinations. The smallest amount of antagonistic bacteria and fungi occurred in the non-rhizopshere soil after the application of Zaprawa Oxafun T + Bravo Plus 500 SC and in the control. The greatest amount of the studied antagonistic microorganisms was obtained after introducing biopreparations Biochikol 020 PC or Biosept 33 SL into the environment (Table 4).

After common bean harvesting, the seed yield from particular experimental combinations was established (Fig. 5). In particular years of studies yields ranged from 424g to 952g from a plot. The highest yield was gathered after the application of Biosept 33 SL (on average 894g from a plot). A positive effect on seed yield was also found out after using other biopreparations (Polyversum and Biochikol 020 PC) and fungicides (Zaprawa Oxafun T and Bravo Plus 500 SC). The smallest amount of seeds was obtained from plants growing in the control (mean 460g from a plot) (Fig. 5).

Weather conditions, apart from the soil microorganisms, had an effect on common bean yields. May and June

Table 5. Meteorological data for May–September of 2005 and 2006 in comparison to the mean from the period 1963-92.

Months	Mean from the period 1963-92		2005		2006	
	Mean temp. (ºC)	Precipitation total (mm)	Mean temp. (ºC)	Precipitation total (mm)	Mean temp. (ºC)	Precipitation total (mm)
May	13.3	60.9	13.0	146.9	13.3	68.1
June	16.4	78.3	15.6	48.0	16.9	23.2
July	17.8	77.9	19.8	55.8	21.1	26.6
August	17.3	69.3	17.0	46.2	17.4	202.5
September	13.1	56.0	14.7	23.1	15.1	10.1

of 2006 were especially favourable to the seed germination and seedling growth. At that time the air temperature was equal to, or even 0.5ºC higher, than the means of long-term period (Table 5). Humidity conditions in May 2006 were also conducive to seed germination, since the amount of precipitation was similar to the mean of the long-term period. On the other hand, May of 2005 was especially wet, and the amount of precipitation exceeded the norm of many years by 141%. In July 2005 and 2006, i.e. at anthesis, the air temperature was higher than the long-term period' means by 2ºC and 3.3ºC, and the amount of precipitation in those months was lower than the norm. During seed harvest, air temperature was higher than the means of long-term period and the amount of precipitation was considerably lower than the norm (Table 5).

Discussion

The present studies showed that biopreparations (Polyversum, Biochikol 020 PC and Biosept 33 SL) used for seed dressing and spraying of *Phaseolus vulgaris* plants had a positive effect on the communities of bacteria and fungi in the soil under the cultivation of this plant. The number of cfu of the studied microorganisms in the non-rhizosphere soil was slightly lower than in the rhizosphere.

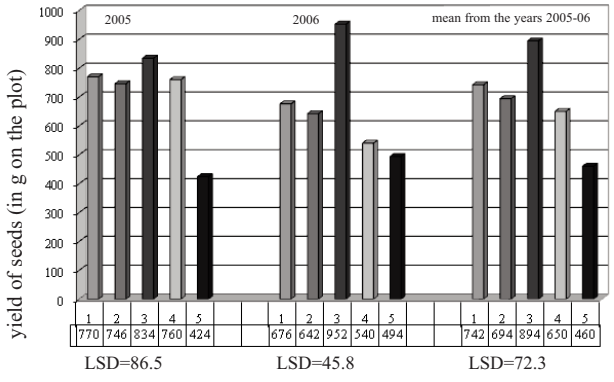

Fig. 5. Yield of common bean seeds in g on the plot in 2005-06. 1 - Polyversum, 2 - Biochikol 020 PC, 3 - Biosept 33 SL, 4 - Zaprawa Oxafun T + Bravo Plus 500 SC, 5 - Control

Biochikol 020 PC and Biosept 33 SL increased the number of cfu of bacteria *Bacillus* spp. and *Pseudomonas* spp. and decreased the population of soil-borne fungi.

A similar relation in the formation of rhizosphere microorganism communities was found after introducing the enumerated preparations into the soybean cultivation environment [9]. Besides, a smaller population of fungi in the soil after the application of biopreparations could have been caused by the composition of the root exudates of the studied plant. This fact also finds explanation in numerous items of literature concerning the role of compounds exudated by the roots of different cultivated plants [8, 28, 29]. Besides, it can be supposed that the biopreparations introduced into the soil had a positive effect on the composition of microorganism communities in the rhizosphere of *Phaseolus vulgaris* since – as reported by Myśków [30] – proper proportions occur between the populations of microorganisms in the soil. The development of fungi is weakened by the numerous occurrences of bacteria, and vice versa.

The qualitative composition of fungi isolated from the non-rhizosphere soil of common bean cultivated in particular experimental combinations was close to the qualitative composition of fungi obtained from the rhizosphere of the studied plant. Different species were isolated within the fungi and they belonged to the following genera: *Altenaria, Fusarium, Rhizoctonia, Sclerotinia* and *Gliocladium, Penicillium* and *Trichoderma*. A similar effect of the biopreparations used in the experiment on the formation of qualitative composition in the rhizosphere of other papilionaceous plants was established in earlier studies [9, 23]. Besides, the obtained results confirmed the information on the protective effect of biopreparations against soil-borne plant pathogens [10, 12-14, 15, 23]. Their effectiveness results from the direct effect of active substances contained in those preparations on pathogenic microorganisms. As reported by Benhamou et al. [21], the effect of *P. oligandrum* on pathogens is mycoparasitism consisting of direct contact between the pathogenic species and *P. oligandrum*, which results in destructive changes in the host's hypha. Chitosan present in Biochikol 020 PC – as resistance elicitor – enhances the activity of genes through contact with a plant, and these genes mobilize the formation of biochemical compounds of fungistatic and fungicidal effect [31].

On the other hand, grapefruit extract – through endogenous flavonoids – inhibited mycelium growth, the formation of conidial spores and chlamydospores of *F. oxysporum* f. sp. *dianthi* and the formation of zoosporangium and the germination of zoospores of *Phytophthora cryptogea* [12, 14].

The most antagonistic bacteria and fungi were obtained after introducing Biochikol 020 PC or Biosept 33 SL biopreparations. The smallest number of antagonists were found in the soil after dressing the bean seeds with Zaprawa Oxafun T and spraying the plants with Bravo Plus 500 SC fungicide and in the control combination. It can be supposed that numerous occurrences of antagonists can reduce the growth and development of plant pathogens. This fact is confirmed by abundant information in the literature [3, 7, 15, 32-34].

Biopreparations used in the present studies must have formed the populations of antagonistic bacteria and fungi, which could develop under the effect of root exudates of common bean. As reported by Pięta and Patkowska [29], exudates of papilionaceous and cereal plants stimulate the activity of antagonistic microorganisms (*Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp., and *Trichoderma* spp.). High acidic aminoacid and sugar content in root exudates stimulate the development of plant pathogens. On the other hand, alkaline aminoacids, aromatic aminoacids, hemicellulose and cellulose have a negative effect on the growth and development of pathogenic fungi, which results in increased populations of antagonistic microorganisms [29, 35].

The applied biopreparations (Polyversum, Biochikol 020 PC and Biosept 33 SL) and fungicides (Zaprawa Oxafun T and Bravo Plus 500 SC) had a positive effect on *Pheseolus vulgaris* yield. Studies conducted by Borkowski et al. [10] and Patkowska et al. [23], for example, also proved the inhibiting effect of the tested biopreparations on plant pathogens and, consequently, the positive effect on the yield of different plants.

Conclusions

1. The use of biopreparations in the cultivation of *Phaseolus vulgaris* had a positive effect on the formation of bacteria and fungi communities in the rhizosphere of this plant.
2. The number of cfu of the studied microorganisms in the non-rhizosphere soil was slightly smaller than in the rhizosphere of this plant.
3. Biochikol 020 PC and Biosept 33 SL increased the number of cfu of bacteria *Bacillus* spp. and *Pseudomonas* spp. and they caused a decrease in the number of cfu of soil-borne fungi.
4. The most antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.) were obtained from the soil after introducing Biochikol 020 PC or Biosept 33 SL biopreparations, and the least after dressing the bean seeds with Zaprawa Oxafun T and spraying the plants with Bravo Plus 500 SC fungicide and from the control combination.
5. The applied biopreparations and fungicides had a positive effect on *Phaseolus vulgaris* yield.

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