

# QUANTITATIVE AND QUALITATIVE COMPOSITION OF RHIZOSPHERE MICROORGANISMS OF PEA (*Pisum sativum*. L) AFTER APPLYING BIOPREPARATIONS

**Alina Pastucha, Elżbieta Patkowska, Danuta Pięta**

*Department of Plant Pathology,  
University of Agriculture in Lublin,  
ul. Kr. Leszczyńskiego 7, 20-069 Lublin, Poland  
e-mail: alina.pastucha@ar.lublin.pl*

## 1. Introduction

Progress which has taken place in the last decade in the sphere of research on the use of biopreparations, antagonistic microorganisms, composts and plant extracts in controlling soil pathogens is especially significant. Compounds of plant and animal origin which are secondary metabolites play an increasing role both in the prophylaxis and direct control of plant pathogens. These compounds include phenols, polyphenols, flavonoids and tannins, occurring in a free or bound form. They occur for example in such biopreparations as Biochikol 020 PC, Biosept 33 SL, whereas Polyversum contains oospores of antagonistic fungus *Pythium oligandrum* Polyversum [1, 2].

Biopreparations Polyversum, Biochikol 020 PC, Biosept 33 SL, used as dressing for papilionaceous plants, or as foliar application inhibited the development of pathogens [3 - 6].

In literature there is no information concerning the effect of biopreparations on the composition of microorganism communities in the soil environment. Hence, the purpose of the present studies was to determine the effect of Polyversum, Biochikol 020 PC and Biosept 33 SL on the quantitative and qualitative composition of bacteria and fungi in the *Rhizosphere soil* of pea.

## 2. Material and methods

Field studies were conducted in the area of the Experimental Station at Czesławice near Nałęczów in the years 2005-2006. The object of the studies was the rhizosphere soil of pea cv. Sześciotygodniowy Tor, whose seeds were dressed before the sowing, while at anthesis they were sprayed with the solution of 2.5% Biochikol 020 PC, 0.2 % Biosept 33 SL and Polyversum (1 g × 100<sup>-1</sup> seeds). As a comparison, a combination with chemical dressing

with Zaprawa Oxafun T (2 g × kg<sup>-1</sup>seeds) and spraying the plants at anthesis with Bravo Plus 500 S.C. was also considered. Besides, the experiment took into consideration a control combination, i.e. without any protective treatment.

The microbiological analysis and the manner of sampling the *Rhizosphere soil* from particular experimental combinations were in accordance with the method described by Martyniuk et al. [7] and Patkowska [8].

The total number cfu of bacteria in 1 g of d.w. of soil was determined in soil solutions with the dilutions of 10<sup>-5</sup>, 10<sup>-6</sup> and 10<sup>-7</sup>, to this aim using the medium Nutrient Agar. In order to establish *Bacillus* spp. the dilutions of 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> as well as medium Tryptic Soy Agar were used. On the other hand, the dilutions of 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup> as well as medium *Pseudomonas* Agar F were used in order to isolate the colonies of *Pseudomonas* spp. Martin's medium [9] and the dilutions of 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup> were used to determine the total number cfu of fungi.

All the isolates of *Trichoderma* spp. and *Gliocladium* spp. as well as bacteria from genus *Bacillus* (500) strains and *Pseudomonas* (500) strains were tested towards pathogenic fungi according to Mańka [10] and Martyniuk et al. [7].

The obtained results concerning the total number cfu of bacteria, *Bacillus* spp., *Pseudomonas* spp. and fungi totally were statistically analyzed using Duncan's half-intervals [11].

### 3. Results and discussion

The microbiological analysis of the rhizosphere soil of pea showed that biopreparations had a positive effect on increasing the number cfu of bacteria totally as well as increasing the number cfu of *Bacillus* spp. and *Pseudomonas* spp.

It was stated on the basis of the mean number from the years 2005-2006 that the most cfu *Pseudomonas* spp. occurred in the rhizosphere soil of peas after the application of Polyversum, while the smallest number was observed in the control combination (Table 1). On the other hand, the highest number cfu of *Bacillus* spp. was characteristic of the rhizosphere soil of pea after using Biosept 33 SL, whereas the smallest number of those bacteria was found in the rhizosphere soil of control combinations (Table 1).

**Table 1.** Number of bacteria and fungi in the rhizosphere of pea (mean from the years 2005-2006); \* Mean in columns differ significantly ( $P \leq 0.05$ ), if they are not marked with the same letter.

Experimental combination	Total number cfu			
	of bacteria, mln x g <sup>-1</sup> d.w. of soil	<i>Bacillus</i> spp., mln x g <sup>-1</sup> d.w. of soil	<i>Pseudomonas</i> spp., mln x g <sup>-1</sup> d.w. of soil	of fungi, thous x g <sup>-1</sup> d.w. of soil
Polyversum	8.84 <sup>e*</sup>	2.53 <sup>b</sup>	2.70 <sup>d</sup>	24.15 <sup>b</sup>
Biochikol 020 PC	5.29 <sup>c</sup>	2.64 <sup>b</sup>	2.15 <sup>c</sup>	25.0 <sup>b</sup>
Biosept 33 SL	6.80 <sup>d</sup>	3.29 <sup>c</sup>	2.40 <sup>cd</sup>	17.54 <sup>a</sup>
Zaprawa Oxafun T + Bravo Plus 500 SC	3.59 <sup>b</sup>	2.27 <sup>b</sup>	0.77 <sup>b</sup>	33.58 <sup>c</sup>
Control	2.0 <sup>a</sup>	1.18 <sup>a</sup>	0.244 <sup>a</sup>	36.54 <sup>d</sup>

Particular soil samples differed from each other with the number cfu of fungi. The highest number cfu of fungi was characteristic of the rhizosphere soil of pea in the control (on average,  $36.54 \times 10^3$ ), while the smallest number cfu of fungi was found in the rhizosphere soil after applying Biosept 33 SL (on average,  $17.54 \times 10^3$ ) (Table 1).

The obtained results are close to the results concerning the plants studied by others [4, 12 - 14].

An increase of the number cfu of bacteria caused a decrease of the number cfu of fungi. A similar relationship was stated by Myśków [15].

Among the obtained colonies *Fusarium* spp., represented by *F.culmorum*, *F. equiseti*, *F. oxysporum* and *F. solani*, were most frequently isolated. Those species were isolated from all samples of the rhizosphere soil, and the smallest number was isolated from the soil after the application of Biosept 33 SL (Table 2, see page 208). Besides, *Alternaria alternata* and *Rhizoctonia solani* were isolated from all samples of rhizosphere soil. *Pythium irregulare* was not obtained from the combination after using Polyversum (Table 2). Biopreparation Polyversum containing biologically active oospores of *Pythium oligandrum* limits the development of the pathogen in direct contact with it [16, 17]. Antagonistic species represented by *Gliocladium* spp. and *Trichoderma* spp. were isolated from the soil samples. Those species were most numerous from the combination after using Biosept 33 SL and Biochikol 020 PC. According to Skrzypczak and Orlikowski [18], chitosan occurring in Biochikol 020 PC stimulates the development of antagonistic microorganisms, especially *Trichoderma* spp. Besides, it should be supposed that abundant occurrence of *Trichoderma* spp. and *Gliocladium* spp. limited the development of pathogenic fungi.

Laboratory tests showed that among *Bacillus* spp. and *Pseudomonas* spp. the greatest number of colonies having antagonistic effect towards plant pathogens was found in the rhizosphere soil after the application of Biochikol 020 PC. A little smaller number of antagonists among those enumerated earlier occurred in the combination after using Biosept 33 SL (Table 3). Antagonistic microorganisms – through the production of enzymes and toxic

**Table 3.** The occurrence of antagonistic microorganisms in the rhizosphere of pea in the years 2005-2006.

Bacteria or fungus	Experimental combination Number of colonies				
	Polyversum	Biochikol 020 PC	Biosept 33 SL	Zaprawa Oxafun T + Bravo Plus 500 S.C.	Control
<i>Bacillus</i> spp.	26	54	40	22	11
<i>Pseudomonas</i> spp.	39	113	100	24	9
<b>Total</b>	<b>65</b>	<b>167</b>	<b>140</b>	<b>46</b>	<b>20</b>
<i>Gliocladium</i> spp.	8	12	13	4	1
<i>Trichoderma</i> spp.	12	18	18	3	3
<b>Total</b>	<b>20</b>	<b>30</b>	<b>31</b>	<b>7</b>	<b>4</b>
<b>Total</b>	<b>85</b>	<b>197</b>	<b>171</b>	<b>53</b>	<b>24</b>

Table 2. Fungi frequently inhabited in the rhizosphere soil of pea (sums colonies from the years 2005–2006).

Fungus species	Experimental combination/ Number of colonies						Total
	Polyversum	Biochikol 020 PC	Biosept 33 SL	Zaprawa + Bravo Plus 500 SC	Oxafun T	Control	
<i>Acremonium roseum</i> (Oud.) W. Gams	5	13	3	10	9	40	
<i>Alternaria alternata</i> (Fr.) Keissler	3	11	6	14	14	48	
<i>Aspergillus flavus</i> Link	11	7	-	4	2	24	
<i>Aspergillus fumigatus</i> Fresenius	4	16	9	12	13	54	
<i>Botryotrichum piluliferum</i> Sacc. et Marchal	3	-	-	1	-	4	
<i>Cladosporium cladosporioides</i> (Fres.) de Vries	19	15	41	17	22	114	
<i>Epicoecum purpurascens</i> (Ehr. ex. Schl.)	4	3	-	7	7	21	
<i>Fusarium culmorum</i> (W. G. Sm.) Sacc.	2	3	2	9	13	29	
<i>Fusarium equiseti</i> (Corda) Sacc.	2	4	3	15	11	35	
<i>Fusarium oxysporum</i> Schl.	32	31	20	56	74	213	
<i>Fusarium solani</i> (Mart.) Sacc.	21	22	14	29	43	129	
<i>Gliocladium catenulatum</i> Gilman Abbott	2	4	3	1	-	10	
<i>Gliocladium roseum</i> (Link) Bainier	6	8	10	3	1	28	
<i>Humicola grisea</i> Domsch	-	-	-	4	5	9	
<i>Mucor hiemalis</i> Wehmer	7	-	3	-	-	10	
<i>Penicillium expansum</i> Link ex S. F. Gray	6	9	1	7	12	35	
<i>Penicillium nigricans</i> (Bain.) Thom	5	-	3	7	4	19	
<i>Penicillium purpogenum</i> Stoll	2	6	-	9	8	25	
<i>Penicillium verrucosum</i> Dierckx var. <i>cyelopium</i> (West.) Samson et. al.	2	4	-	5	7	18	
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i> Samson et. al.	5	13	-	5	9	32	
<i>Pythium irregulare</i> Buisman	-	9	6	24	31	70	
<i>Rhizoctonia solani</i> Kühn	5	6	2	17	26	56	
<i>Rhizopus nigricans</i> Ehrenberg	13	4	10	9	5	41	
<i>Trichoderma aureoviride</i> Rifai	2	-	3	1	-	6	
<i>Trichoderma hamatum</i> (Bonord.) Bain.	3	4	-	-	1	8	
<i>Trichoderma harzianum</i> Rifai	1	5	6	2	-	14	
<i>Trichoderma koningi</i> Oud.	4	2	4	-	1	11	
<i>Trichoderma viride</i> Pers. ex S.F.Gray	2	7	5	-	1	15	
<b>Total</b>	171	206	154	268	319	1118	

compounds – together with metabolites produced by them cause inhibition of the growth and development of pathogenic fungi [19, 20].

It seems that the application of biopreparations in ecological agriculture can constitute a recommendable alternative for the chemical methods of plant protection.

#### 4. Conclusions

1. The application of biopreparations reduced the number cfu of fungi in the *Rhizosphere soil* of pea.
2. Biopreparations, and especially Biochikol 020 PC, increased the number of antagonistic microorganisms in the studied soil.

#### 5. References

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