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YIELDING AND HEALTHINESS OF PEA CV. 'SZEŚCIOTYGODNIOWY TOR' AFTER APPLYING BIOTECHNICAL PREPARATIONS

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Abstract. One of the method of plant protection, including the protection of pea, from phytopathogens is the biological one, especially applied in ecological cultivations. The effectiveness of biotechnical preparations such as Biosept 33 SL, Grevit 200 SL, Biochikol 020 PC, Bioczos BR and a fungicide Miedzian 50 WP in the protection of pea cv. 'Sześciotygodniowy TOR' from pathogenic fungi was studied. The greatest number of not infected plants was obtained after the application of Biosept 33 SL, Miedzian 50 WP and Grevit 200 SL. The highest yield was gathered from pea plants after the application of Biosept 33 SL and Grevit 200 SL. Plants in the combinations with Biochikol 020 PC and Bioczos BR were characterized by good yielding. It was obtained from infected pea seeds and plants of two stage 24–26 species of fungi from 13–17 genera. Among them most often occurred the following species: *Alternaria alternata*, *Boeremia exigua*, *Fusarium culmorum*, *F. oxysporum*, *Gibberella avenacea*, *Haematonectria haematococca*, *Peyronellaea pinodes*, *Pythium irregulare* and *Thanatephorus cucumeris*. Biosept 33 SL and Grevit 200 SL were most effective in improving the healthiness and yielding of the studied cultivar of pea.

Key words: *Pisum sativum*, biological control, mycological analysis, soil-borne fungi

INTRODUCTION

Throughout the period of vegetation pea plants can be infected, for example, by such pathogenic fungi as *Fusarium* spp., *Peyronellaea pinodes*, *Thanatephorus cucumeris*, *Sclerotinia sclerotiorum* [Pięta et al. 2005, Patkowska 2013]. One of the method of plant protection, including the protection of pea, from plant pathogens is the biological one, especially applied in ecological cultivations. Biotechnical preparations based on antagonistic microorganisms (*Bacillus* spp., *Pseudomonas* spp., *Clonostachys* spp.,

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Trichoderma spp.) and the substances of plant or animal origin have been used to dress the seeds or spray the plants [Kurzawińska and Mazur 2008, 2009, Patkowska 2009a, 2010, Rajeswardi and Kumari 2009, Zhang and Xue 2010, Patkowska and Błażewicz-Woźniak 2013]. The effectiveness of such methods in controlling fungi pathogenic for pea was shown by Pięta et al. [2005].

In recent years much attention has been devoted to the protective effect of such preparations as Biochikol 020 PC, Biosept 33 SL, Bioczos BR, Polyversum, Constans XX [Propagdee et al. 2007, Ye Li-min et al. 2009, Mazur et al. 2013, Patkowska 2013].

The active substance of Biochikol 020 PC is an organic compound of animal origin, namely chitosan (a deacetylated derivative of chitin). Through the contact with a plant, chitosan – as an elicitor – induces systemic acquired resistance (SAR) in plants, enhancing the activity of genes which trigger the formation of biochemical compounds with fungistatic or fungicidal effect, which is called plant immunization [Orlikowski and Skrzypczak 2003, Lee et al. 2005]. Biosept 33 SL, which contains grapefruit extract, acts directly towards pathogenic factors and it induces plants' resistance to certain pathogens [Kućmierz et al. 2010, Patkowska 2013]. Bioczos BR, which contains garlic pulp, owes its antimicrobiotic effect to allicin and ajoene, which are inhibitors for insects, viruses, bacteria and fungi [Kućmierz et al. 2010]. Polyversum, which contains oospores of parasite *Pythium oligandrum,* also acts directly on soil and phyllosphere pathogens and induces plants' resistance [Orlikowski and Skrzypczak 2003, Patkowska 2013]. Preparation Constans XX, which contains *Coniothyrium minitans*, is used in cultivation of vegetables [Tomalak et al. 2010]. *Coniothyrium minitans* has the ability to degrade oxalates secreted by the hyphae of mycelium and sclerotia of *Sclerotinia sclerotiorum* [Ren et al. 2010].

The purpose of the present studies was to establish the effect of biotechnical preparations such as Biosept 33 SL, Grevit 200 SL, Biochikol 020 PC, Bioczos BR and fungicide Miedzian 50 WP on the yielding and healthiness of pea.

MATERIALS AND METHODS

Fieldwork. The field experiment was conducted in the years 2010–2012 in Felin Experimental Station belonging to the University of Life Sciences in Lublin, district of Lublin (22°56'E, 51°23'N, Central Eastern Poland, 200 m a.s.l.). The object of studies was pea plants (*Pisum sativum* L.) cv. 'Sześciotygodniowy TOR'. Before sowing, the seeds were dressed with such biotechnical preparations as 0.2% Biosept 33 SL (a.s. grapefruit extract 33%) produced by Cintamani Poland, 0.2% Grevit 200 SL (a.s. grapefruit extract 20%) produced by Cintamani Poland, 2.5% Biochikol 020 PC (a.s. chitosan 1.88%) produced by Gumitex Poll-Farm, Poland and 2.5% Bioczos BR (a.s. garlic pulp 20%) produced by Himal PPH, Poland. For a comparison, Miedzian 50 WP (a.s. 50% copper oxychloride) produced by Organika-Azot in Jaworzno, Poland, was used in the quantity of 2 g kg^{-1} seeds as well as the control combination, i.e. without any dressing. The seeds were treated by tested preparations for half an hour (100 ml preparation for 100 seeds). The second protective treatment was performed at the beginning of anthesis. At this stage the plants were sprayed with the same preparations and the same concentrations that were used for seed dressing. Preparations were applied by spraying with 0.04 dm^3 liquid for each 1 m². Each experimental treatment comprised 4 plots (4 replications) with the area of 3 m², where 100 seeds were sown on each.

During vegetation, observations were carried out twice – at the seedling phase (four weeks after seeds treatment) and at anthesis (seven weeks after seeds treatment) – determining the number of plants on the plots and assessing their healthiness.

Laboratory Analyses. Five plants with distinct signs of necrosis on the stem base were taken from each plot for a laboratory analysis. The mycological analysis was carried out according to the method described by Patkowska and Konopiński [2011, 2013]. This analysis made it possible to determine the quantitative and qualitative composition of fungi infecting the roots and stem base of pea. The infected parts of plants were rinsed for 30 minutes under running tap water, next were disinfected in 0.1% sodium hypochlorite. The plant material disinfected on the surface was rinsed three times in sterile distilled water. 3-milimetre fragments were made from so prepared plant material and 10 of them were put on each of the Petri dishes on solidified mineral medium with the following composition: 38 g saccharose, 0.7 g NH_4NO_3, 0.3 g KH_2PO_4, 0.3 g $MgSO_4 \times 7H_2O$, 20 g agar and trace quantities of $FeCl_3 \times 6\ H_2O$, $ZnSO_4 \times 7\ H_2O$, $CuSO_4 \times 7\ H_2O$ and $MnSO_4 \times 5\ H_2O$. 100 fragments of infected roots and stem base were examined for each of the experimental treatments.

The mycological analysis was also conducted after the harvest both on the seeds with spots and those without any spots on the seed cover (100 seeds in 4 replications for each experimental treatment). Fungi isolated from the stem base and the seeds were identified to the species using the available keys and monographs of different taxons given in the paper by Patkowska and Konopiński [2013]. The malt and Czapek-Dox media were used for the fungi of *Penicillium* spp. [Ramirez 1982]. The fungi of *Fusarium* genus were identified on PDA and selected agar medium SNA by Leslie and Summerell [2006]. The other fungi were identified on the malt medium using the corresponding keys and monographic papers [De Vries 1952, Gillman 1957, Barnett 1960, Raper et al. 1968, Rifai 1969, Domsch and Gams 1970, Booth 1971, Ellis 1976, Sałata and Rudnicka-Jezierska 1979, Marcinkowska 2003].

After the seeds were gathered and dried up, the dry mass yield of pea seeds was established. In addition, the ratio of infected seeds in the total yield was determined.

Statistical Analysis. The results concerning the number, healthiness and yielding of plants were statistically analyzed, and the significance of differences was determined on the basis of Tukey's confidence intervals ($P < 0.05$). Statistical calculations were carried out using Statistica program (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

The number of plants grown from the seeds dressed with biotechnical preparations was close to the number of plants obtained after the application of fungicide Miedzian 50 WP. The best emergences were observed on the plots where the seeds dressed with Biosept 33 SL, Grevit 200 SL and Miedzian 50 WP (mean about 90 seedlings) were sown (tab. 1). The number of seedlings for treatments with Biochikol 020 PC and Bioczos BR was slightly smaller. The smallest number of seedlings (mean 73) was observed for the control plots. Seedlings with disease symptoms were observed on each plot. The ratio of infected seedlings ranged, on an average, from 1.8 to 8.8%. The smallest number of infected seedlings was observed after the application of Biosept 33 SL, and slightly more for Grevit 200 SL and Miedzian 50 WP, whereas the biggest number was found in the control (tab. 1).

Table 1. The number and healthiness of pea plants

Experimental treatment	Seedlings								Plants at anthesis							
	A				B				A				B			
	2010	2011	2012	Mean	2010	2011	2012	mean	2010	2011	2012	mean	2010	2011	2012	mean
Biosept 33 SL	92 c	90 c	94 c	92 c	0.5 a	1.5 a	3.5 a	1.8 a	90 c	88 c	92 c	90 c	1.5 a	3.5 a	6.5 a	3.8 a
Biochikol 020 PC	85 b	83 b	87 b	85 b	4.5 c	5.5 c	7.5 c	5.8 c	84 b	81 b	85 b	83 b	5.5 c	7.5 c	10.5 c	7.8 c
Bioczos BR	85 b	82 b	86 b	84 b	5.0 c	6.0 c	8.0 c	6.3 c	84 b	80 b	84 b	82 b	6.0 c	8.0 c	11.0 c	8.3 c
Grevit 200 SL	87 b	90 c	94 c	90 c	1.5 b	2.5 b	4.5 ab	2.8 b	85 b	88 c	92 c	88 c	2.5 b	4.5 ab	7.5 ab	4.8 b
Miedzian 50 WP	92 c	89 c	93 c	91 c	2.0 b	3.0 b	5.0 b	3.3 b	90 c	87 c	91 c	89 c	3.0 b	5.0 b	8.0 b	5.3 b
Control	74 a	71 a	75 a	73 a	7.5 d	8.5 d	10.5 d	8.8 d	71 a	67 a	72 a	70 a	9.0 d	11.5 d	14.5 d	11.6 d

A – number of plants on the plot, B – ratio of infected plants on the plot (%)
* – means in columns followed by the same letter do not differ significantly at $P \leq 0.05$

When observation was done at the phase of anthesis, the greatest number of plants was obtained after the application of Biosept 33 SL, Miedzian 50 WP and Grevit 200 SL (mean 90, 89 and 88 plants, respectively) (tab. 1). The smallest number of plants were obtained in the control (mean 70 plants). In each treatment of the studies were observed plants with inhibited growth which did not form flower buds and did not set the pods. Clear signs of necrosis could be seen on the roots and the stem base of infected plants. The ratio of plants with disease symptoms ranged, on an average from 3.8%, after the application of Biosept 33 SL and 11.6 % for the control (tab. 1).

The positive effect of biotechnical preparations, especially Biosept 33 SL, but also Biochikol 020 PC, on the emergences and healthiness of other plant species from the family *Fabaceae* was confirmed in earlier studies conducted by Pięta et al. [2005] and Patkowska [2009b, 2013]. Those biotechnical preparations were also used to dress the tubers, bulbs and seeds as well as in watering or spraying the plants with the aim of improving the plants' healthiness [Orlikowski and Skrzypczak 2003, Kurzawińska and Mazur 2009, Mazur et al. 2013].

The yield of seeds ranged mean from 229 to 411g from a plot (tab. 2). The highest yield was noted from pea plants after the application of Biosept 33 SL and Grevit 200 SL (mean 411 and 402 g from a plot, respectively). Plants in the combinations with Biochikol 020 PC and Bioczos BR were characterized by good yielding. The lowest yield with the highest ratio of infected seeds (mean 14.5%) was obtained from control plants. The smallest amount of seeds with necrotic spots on the seed cover was observed after the application of Biosept 33 SL (mean 5.4%) and Grevit 200 SL (mean 7.1%). High effectiveness of chitosan and grapefruit extract as well as biopreparation Polyversum in increasing the yielding of different plant species was shown, for example, by Pięta et al. [2005], Propagdee et al. [2007], Kurzawińska and Mazur [2008, 2009], Patkowska [2009a] and Ye Li-min et al. [2009].

Table 2. Weight and quality of pea seeds yield

Experimental treatment	Yield of pea seeds in g from the plot				Percentage of infected seeds in the total yield			
	2010	2011	2012	mean	2010	2011	2012	mean
Biosept 33 SL	399 b	405 b	428 c	411 c	2.7 a	4.7 a	8.7 a	5.4 a
Biochikol 020 PC	343 b	351 b	373 b	356 b	5.7 c	7.7 c	11.5 bc	8.3 b
Bioczos BR	340 b	346 b	364 b	350 b	6.5 d	8.5 d	12.5 c	9.1 bc
Grevit 200 SL	386 b	400 b	419 bc	402 c	4.5 b	6.5 b	10.5 b	7.1 b
Miedzian 50 WP	317 b	324 b	345 b	329 b	7.0 d	9.0 d	13.0 c	9.6 c
Control	216 a	225 a	245 a	229 a	11.0e	14.0 e	18.5 d	14.5 d

* – means in columns followed by the same letter do not differ significantly at $P \leq 0.05$

Totally, 1394 isolates of fungi belonging to 13 genera were obtained as a result of the laboratory mycological analysis of the studied pea seedlings in all experimental treatments (tab. 3). The following fungi were often obtained from the infected seedlings: *Alternaria alternata*, *Gibberella avenacea*, *Fusarium culmorum*, *F. oxysporum*, *Haematonectria haematococca*, *Boeremia exigua*, *Peyronellaea pinodes*, *Pythium irregulare*

and *Thanatephorus cucumeris*. The most frequently isolated species were *F. oxysporum*, *P. irregulare*, *T. cucumeris* (182, 145 and 133 isolates, respectively) and *Fusarium culmorum*, *P. pinodes* and *B. exigua* (110, 106 and 105 isolates). The smallest amount of fungi were isolated from the infected roots and stems base of pea seedlings after the application of Grevit 200 SL and Biosept 33 SL. The greatest amount of fungi were obtained from the control treatment (378 isolates). It could be supposed that some of the enumerated species of fungi caused pathogenic symptoms on pea plants and decreased their healthiness. *F. oxysporum* f. sp. *pisi*, *Peyronellaea pinodes*, *Pythium irregulare*, *Alternaria alternata* and *Thanatephorus cucumeris*, among others, shows a pathogenic effect towards *Pisum sativum* [Marcinkowska 2008, Snowdon 2010, Patkowska 2013, 2014, Bani et al. 2014, Singh et al. 2014].

Totally, 1558 fungi isolates were obtained from the infected roots and the stem base of pea plants at anthesis (tab. 4). Their species composition was similar to that of the fungi isolated from the seedlings. *Fusarium oxysporum*, *T. cucumeris* and *Sclerotinia sclerotiorum* (208, 170 and 135 isolates, respectively) were most frequently isolated in all experimental treatments. *Peyronellaea pinodes* and *H. haematococca* (129 and 107 isolates) were also frequently isolated. Those fungi were isolated especially from the plants growing in the control combination. The enumerated fungi species are pathogenic towards pea, especially in field cultivations [Bogale et al. 2009, Snowdon 2010, Marinelli et al. 2012].

Similar effectiveness of biotechnical preparations, especially of Biosept 33 SL and Biochikol 020 PC, in protecting vegetables or ornamental plants was shown by Orlikowski and Skrzypczak [2003], Kućmierz et al. [2010], Mazur et al. [2013]. This effectiveness results from the direct effect on plant pathogens of active substances contained in those preparations. It is now known that chitosan has anti-virus, anti-bacterial and anti-fungal properties. It mobilizes the plants to a fast resistance reaction to the pathogen's attack by means of elicitors, which are resistance inductors [Orlikowski and Skrzypczak 2003]. After the application of chitosan, enhanced lignification takes place and phytoalexins and hydrolytic enzymes, which are the factors of induced resistance, are produced. Chitosan limits the mycelium growth and the formation of endospore forms of fungi pathogenic towards the plants from the *Fabaceae* [Pięta et al. 2004]. It effectively protected soybean plants from infection by *A. alternata*, *F. culmorum*, *F. oxysporum*, *H. haematococca*, *T. cucumeris* and *S. sclerotiorum* [Pięta et al. 2007]. On the other hand, the effect of Biosept 33 SL is related to grapefruit extract containing endogenous flavonoids, citrate, limonene and glycosides, which include naringenin rut*i*noside, isosacurentine, hesperidin, kaempferol, dihydrokaemferol, quercetin, apigenin rutinoside and nobiletin [Kędzia 2001]. These compounds inhibit spore germination, the growth of the germ tube and vegetative hyphae through damaging the membrane systems and they inhibit the activity of respiratory enzymes. As stated by Caccioni et al. [1998], aliphatic aldehydem, monoterpenes and nootkatone dominate among the numerous components of this extract. Those compounds can show synergism in inhibiting the development of a definite pathogenic factor or stimulate the germination of fungi spores. 7-geranoxycoumarin, triclosan or benzethonium chloride, which are present in grapefruit extract, inhibit the development not only of fungi but of bacteria as well [Woedtke et al. 1999, Szopińska et al. 2007].

Table 3. Fungi isolated from infected seedlings of pea (sums of isolates from the years 2010–2012)

Fungus species	Experimental treatment / Number of isolates														Total
	Biosept 33 SL		Biochikol 020 PC		Bioczos BR		Grevit 200 SL		Miedzian 50 WP		control		total		
	r	sb	r	sb	r	sb	r	sb	r	sb	r	sb	r	sb	
Acremonium rutilum W. Gams	–	1	–	2	1	–	2	1	2	3	1	5	6	12	18
Alternaria alternata (Fr.) Keissler	1	1	5	2	2	4	3	1	4	6	14	2	29	16	45
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	1	3	6	7	8	9	2	3	11	13	18	24	46	59	105
Clonostachys rosea f. *catenulata* (Gilman et Abbott) Schroers	13	15	7	7	6	5	11	12	4	–	–	–	41	39	80
Epicoccum nigrum Link	–	–	1	–	1	–	–	–	2	2	3	7	7	9	16
Fusarium culmorum (W. G. Sm.) Sacc.	3	5	4	9	8	11	5	4	15	13	22	13	57	53	110
Fusarium oxysporum Schl.	6	6	12	8	14	15	8	7	21	18	37	30	98	84	182
Gibberella avenacea R.J. Cook	1	1	–	3	1	4	2	1	4	6	5	9	13	24	37
Haematonectria haematococca (Berk. et Broome) S. Rossman	2	1	4	5	4	4	3	1	6	12	12	17	31	40	71
Mucor hiemalis Bainier	–	–	–	4	1	1	5	3	4	4	8	10	18	22	40
Myrothecium verrucaria (Alb. et Schwein) Ditmar	6	5	3	2	2	1	4	4	–	1	–	–	15	13	28
Penicillium aurantiogriseum Dierckx	3	4	2	3	2	3	3	3	–	–	–	–	10	13	23
Penicillium canescens Scopp.	7	7	1	5	4	5	3	3	5	2	7	2	27	24	51
Penicillium chrysogenum Thom	2	–	–	4	5	3	2	–	–	–	3	–	12	7	19
Peyronellaea pinodes (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley	2	3	4	9	7	9	3	5	10	13	18	23	44	62	106
Pythium irregulare Buisman	4	4	17	5	13	10	6	7	18	17	23	21	81	64	145
Thanatephorus cucumeris (A.B. Frank) Donk	2	5	7	9	10	13	5	5	19	15	27	16	70	63	133
Trichoderma harzianum Rifai	10	9	6	5	5	4	8	7	–	–	–	–	29	25	54
Trichoderma koningii Oud.	6	8	4	2	1	2	5	4	2	–	–	–	18	16	34
Trichoderma viride Pers. ex. S. F. Gray	19	14	10	8	9	7	15	13	1	–	1	–	55	42	97
Total	88	90	93	99	104	110	95	84	128	125	199	179	707	687	1394

r – root, sb – stem base

Table 4. Fungi isolated from infected plants of pea at anthesis (sums of isolates from the years 2010–2012)

Fungus species	Experimental treatment / Number of isolates														Total
	Biosept 33 SL		Biochikol 020 PC		Bioczos BR		Grevit 200 SL		Miedzian 50 WP		control		total		
	r	sb	r	sb	r	sb	r	sb	r	sb	r	sb	r	sb	
Acremonium roseogriseum (S. B. Saksena) W. Gams	–	2	–	3	1	–	2	2	3	4	2	6	8	17	25
Acremonium rutilum W. Gams	–	–	1	–	2	–	–	–	2	2	3	2	8	4	12
Alternaria alternata (Fr.) Keissler	2	2	7	6	5	8	2	4	7	13	21	11	44	44	88
Botrytis cinerea Pers.	–	3	–	6	–	7	–	4	–	9	–	12	–	41	41
Cladosporium cladosporioides (Fres.) de Vries	–	–	3	3	2	–	1	–	6	5	7	11	19	19	38
Clonostachys rosea f. *catenulata* (Gilman et Abbott) Schroers	4	10	3	3	4	2	6	5	2	1	1	–	20	21	41
Fusarium culmorum (W. G. Sm.) Sacc.	2	3	2	8	3	10	3	4	10	10	14	11	34	46	80
Fusarium oxysporum Schl.	8	7	16	8	17	16	10	9	23	21	41	32	115	93	208
Gibberella avenacea R.J. Cook	2	2	1	5	3	6	2	3	6	8	8	11	22	35	57
Haematonectria haematococca (Berk. et Broome) S. Rossman	2	4	7	5	9	6	5	3	10	14	21	21	54	53	107
Mucor globosus Fischer	–	–	1	2	–	–	–	2	2	3	6	4	9	11	20
Mucor hiemalis Bainier	–	–	–	–	–	–	1	–	4	2	6	5	11	7	18
Myrothecium verrucaria (Alb. et Schwein) Ditmar	16	11	7	4	6	2	11	8	1	2	–	–	41	27	68
Penicillium aurantiogriseum Dierckx	–	2	3	3	4	2	–	3	6	6	7	6	20	22	42
Penicillium chrysogenum Thom	–	3	–	–	2	1	1	1	–	3	2	4	5	12	17
Penicillium purpurogenum Stoll	2	3	2	1	2	2	2	2	2	1	2	1	12	10	22
Penicillium verrucosum Dierckx var. *verrucosum* Samson et al.	3	3	–	2	2	3	3	2	–	1	–	–	8	11	19
Peyronellaea pinodes (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley	3	4	5	12	9	9	4	9	12	15	20	27	53	76	129
Sclerotinia sclerotiorum (Lib.) de Bary	3	2	9	10	11	15	6	5	14	17	20	23	63	72	135
Thanatephorus cucumeris (A.B. Frank) Donk	5	4	11	11	14	17	7	9	24	19	31	18	92	78	170
Trichoderma hamatum (Bon.) Bain	8	6	5	3	5	2	7	5	–	–	–	–	25	16	41
Trichoderma harzianum Rifai	4	3	2	2	2	1	3	2	–	–	–	–	11	8	19
Trichoderma koningii Oud.	16	16	10	5	6	6	12	11	2	1	–	1	46	40	86
Trichoderma viride Pers. ex. S. F. Gray	14	9	7	6	6	5	13	11	3	–	1	–	44	31	75
Total	94	99	102	108	115	120	101	104	139	157	213	206	764	794	1558

r – root, sb – stem base

Table 5. Fungi isolated from seeds of pea (sums of isolates from the years 2010–2012)

Fungus species	Experimental treatment / Number of isolates														Total
	Biosept 33 SL		Biochikol 020 PC		Bioczos BR		Grevit 200 SL		Miedzian 50 WP		control		total		
	a	b	a	b	a	b	a	b	a	b	a	b	a	b	
Alternaria alternata (Fr.) Keissler	5	1	10	2	10	4	7	1	13	5	17	9	62	22	84
Aspergillus niger van Tiegh	–	–	3	1	4	1	–	1	3	1	4	1	14	5	19
Botrytis cinerea Pers.	1	–	4	2	5	1	1	–	9	3	17	6	37	12	49
Chaetomium globosum Kunze ex. Fr.	–	–	3	1	3	2	–	1	5	2	8	3	19	9	28
Cladosporium cladosporioides (Fres) de Vries	2	1	4	1	4	–	3	2	6	4	11	3	30	11	41
Clonostachys rosea f. *catenulata* (Gilman et Abbott) Schroers	6	10	3	6	2	7	4	6	–	2	2	–	17	31	48
Epicoccum nigrum Link	2	–	3	1	6	2	2	1	11	2	19	7	43	13	56
Fusarium culmorum (W. G. Sm.) Sacc.	1	–	3	1	4	2	2	1	5	2	7	3	22	9	31
Fusarium oxysporum Schl.	10	3	18	7	24	11	14	4	30	13	40	19	136	57	193
Fusarium poae (Peck.) Wollenw.	2	–	5	1	6	2	3	–	8	4	11	3	35	10	45
Gibberella intricans Wollenw.	4	1	6	2	8	3	5	1	9	3	10	3	42	13	55
Gliomastix murorum (Corda) S. Hughes	–	–	3	1	5	2	1	–	6	2	8	3	23	8	31
Humicola grisea Domsch	–	–	–	–	–	–	1	–	6	–	13	5	20	5	25
Mucor globosus Fischer	–	–	–	–	–	–	1	–	2	–	5	1	8	1	9
Mucor hiemalis Bainier	1	–	3	1	3	1	2	–	4	2	8	3	21	7	28
Myrothecium verrucaria (Alb. et Schwein) Ditmar	4	2	3	2	2	1	3	2	2	1	–	–	14	8	22
Penicillium aurantiogriseum Dierckx	4	1	6	2	7	3	4	–	11	4	15	5	47	15	62
Penicillium canescens Scopp.	2	3	1	2	1	2	2	3	–	–	–	–	6	10	16
Penicillium purpurogenum Stoll	3	–	4	1	5	1	3	1	11	3	15	6	41	12	53
Penicillium verrucosum Dierckx var. *verrucosum* Samson et al.	3	2	2	1	2	1	3	2	2	–	4	–	16	6	22
Peyronellaea pinodes (Berk. & A. Bloxam) Aveskamp, Gruyter & Verkley	1	–	10	1	8	2	4	1	14	4	20	10	57	18	75
Sclerotinia sclerotiorum (Lib.) de Bary	13	3	18	8	19	9	15	3	24	13	32	18	121	54	175
Thanatephorus cucumeris (A.B. Frank) Donk	12	3	17	6	20	10	13	5	23	11	34	17	119	52	171
Trichoderma harzianum Rifai	4	7	3	4	3	3	4	6	–	2	–	–	14	22	36
Trichoderma koningii Oud.	4	7	2	4	3	4	4	7	–	1	–	–	13	23	36
Trichoderma viride Pers. ex S.F. Gray	10	15	4	9	5	8	9	12	–	–	–	–	28	44	72
Total	94	59	138	67	159	82	110	60	204	84	300	125	1005	477	1482

a – seeds with spots, b – seeds without spots

As a result of the mycological analysis of pea seeds, 1482 fungi isolates belonging to 17 genera were obtained in all experimental treatments (tab. 5). More fungi were isolated from the seeds with discolorations and necrotic spots on the seed cover as compared to the seeds that were well formed and properly coloured. The smallest amount of fungi was obtained after the application of Grevit 200 SL and Biosept 33 SL, slightly more after the application of Biochikol 020 PC and Bioczos BR and the most in the control treatment. The dominating species were: *F. oxysporum*, *S. sclerotiorum* and *T. cucumeris*. Besides, fungi from genera *Mucor*, *Penicillium* and *Trichoderma*, as well as 8 following species: *Alternaria alternate*, *Aspergillus niger*, *Botrytis cinerea*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Clonostachys rosea*, *Epicoccum nigrum*, *Humicola grisea*, were isolated from the studied seeds.

It is worth mentioning that the studied preparations contributed to a decreased number of fungal species. A similar relationship in studies on biological methods of protecting common bean and runner bean was found by Pięta et al. [2005] and Patkowska [2009a]. Biosept 33 SL, Grevit 200 SL, Biochikol 020 PC and Bioczos BR., however, contributed to an increased population of *Trichoderma* spp. on the examined organs of pea in comparison to the control combination. It should be supposed that those fungi also had a positive influence on the healthiness and yielding of pea. Information available in literature indicates high antagonistic activity of *Trichoderma* spp. towards a number of plant pathogens [Shovan et al. 2008, Patkowska and Konopiński 2014, Patkowska et al. 2015]. It consists of antibiosis, competition and mycoparasitism [Mohamed et al. 2010, Muhammad et al. 2010, Schuster and Schmoll 2010]. Besides, those fungi are characterized by an ability to fast growth and abundant sporulation as well as survival in hard conditions [Wojtkowiak-Gębarowska 2006]. Probably, different species from genus *Trichoderma* decreased the occurrence of fungi lowering the healthiness of the examined cultivar of pea, thus increasing the yielding of this plant.

Results of the present studies and abundant information from literature [Orlikowski and Skrzypczak 2003, Pięta et al. 2007, Propagdee et al. 2007, Kurzawińska and Mazur 2009, Rajeswardi and Kumari 2009, Zhang and Xue 2010, Mazur et al. 2013, Patkowska 2013] make it possible to state that tested biotechnical preparations can also be useful in integrated protection of pea cv. 'Sześciotygodniowy TOR' against pathogenic fungi. Application of biopesticides is, however, troublesome and more difficult and their effect is worse as compared to chemical agents [Martyniuk 2012]. Moreover, they should be applied preventively and frequently during the plant vegetation. The procedure of their registration is difficult and costly. That is why it should be supposed that the application of both the studied biological preparations and others in integrated protection of pea will not be very economical.

CONCLUSIONS

1. Biotechnical preparations, especially Grevit 200 SL and Biosept 33 SL, can improve the healthiness and yielding of pea cv. 'Sześciotygodniowy TOR'.

2. Respectively, 24 and 26 species of fungi belonging to, respectively, 13 and 17 genera were obtained from the infected underground organs and seeds of the studied cultivar of pea.

3. Among fungi most often occurred the following species: *Fusarium oxysporum*, *Thanatephorus cucumeris*, *Peyronellaea pinodes*, *F. culmorum*, *Alternaria alternata*, *Haematonectria haematococca*, *Gibberella avenacea*, *Pythium irregulare* and *Boeremia exigua*.

4. The tested biotechnical preparations can be useful in integrated protection of pea cv. 'Sześciotygodniowy TOR' against pathogenic fungi.

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PLONOWANIE I ZDROWOTNOŚĆ GROCHU ODM. 'SZEŚCIOTYGODNIOWY TOR' PO ZASTOSOWANIU PREPARATÓW BIOTECHNICZNYCH

Streszczenie. Jedną z metod ochrony roślin, w tym również grochu, przed fitopatogenami jest metoda biologiczna, wykorzystywana zwłaszcza w uprawach ekologicznych. Badano skuteczności preparatów biotechnicznych, takich jak Biosept 33 SL, Grevit 200 SL, Biochikol 020 PC, Bioczos BR oraz fungicydu Miedzian 50 WP w ochronie grochu odm. 'Sześciotygodniowy TOR' przed grzybami chorobotwórczymi. Najwięcej nieporażonych roślin uzyskano po zastosowaniu Bioseptu 33 SL, Miedzianu 50 WP i Grevitu 200 SL. Największy plon nasion zebrano z roślin grochu po zastosowaniu Bioseptu 33 SL i Grevitu 200 SL. Dobrym plonowaniem charakteryzowały się również rośliny w kombinacjach z Biochikolem 020 PC i Bioczosem BR. Z porażonych nasion i roślin grochu razem uzyskano 24–26 gatunków grzybów należących do 13–17 rodzajów. Wśród nich najczęściej występowały gatunki: *Alternaria alternata*, *Boeremia exigua*, *Fusarium culmorum*, *F. oxysporum, Gibberella avenacea*, *Haematonectria haematococca*, *Peyronellaea pinodes*, *Pythium irregulare* and *Thanatephorus cucumeris*. Biosept 33 SL I Grevit 200 SL najskuteczniej poprawiły zdrowotność i plonowanie badanej odmiany grochu.

Słowa kluczowe: *Pisum sativum*, biokontrola, analiza mykologiczna, grzyby odglebowe

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