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EFFECTIVENESS OF GRAPEFRUIT EXTRACT AND *PYTHIUM OLIGANDRUM* IN THE CONTROL OF BEAN AND PEAS PATHOGENS

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Abstract: The purpose of the present work was to estimate the effectiveness of grapefruit extract and *Pythium oligandrum* in protection of common bean, runner bean and pea from soil-borne pathogenic fungi. The investigated preparations were used for seed dressing and spraying plants at the beginning of anthesis. The results pointed out that the applied products considerably improved emergence, healthiness and yielding of the examined plant species. Besides, Biosept 33 SL showed a better effect than Polyversum. Independently on the species, the fewest plants, with the greatest proportion of infected ones and the smallest yield of seeds were obtained from the untreated control. *Alternaria alternata, Fusarium* spp., *Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum* were frequently isolated from infected roots and stem bases as well as from seeds of bean and pea. *Fusarium oxysporum* tuned out to be dominant. The proportion of the above listed fungi in the treatments with Biosept 33 SL or Polyversum was smaller than in the control. At the same time, the role of those fungi in infecting the plants of common bean, runner bean and pea treated with Biosept 33 SL was only a little smaller than after using Polyversum.

Key words: bean, pea, grapefruit extract, *Pythium oligandrum*, control

INTRODUCTION

Biological protection of numerous plant species from pathogenic factors is being more and more frequently applied in practice, thanks to the fact that biological plant protection products based on antagonistic microorganisms or plant extracts have been registered and introduced into the market. Biosept 33 SL and Polyversum belong to such products and have been lately used in Poland.

Biosept 33 SL, containing 33% of grapefruit extract, and Polyversum, produced on the basis of *Pythium oligandrum* oospores, act directly on pathogenic factors and they induce plant resistance to some pathogens (Gołębniak and Jarosz 2003; Orlikowski et al. 2002; Sadowski et al. 2005; Saniewska 2001; Skrzypczak 2003). The compounds contained in grapefruit extract such as 7-geranoxycumarine, triclosan or benzetonine chloride can inhibit the development of bacteria and fungi (Angioni et al. 1998; Woedtke et al. 1999). The studies by Orlikowski (2001b) 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 the 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 (Orlikowski and Skrzypczak 2001). The studies conducted by Orlikowski and Skrzypczak (2001) 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 *P. oligandrum* on pathogens is differentiated. As stated by Benhamou et al. (1999), 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 the effect is antibiosis, which leads to dying out of filaments, despite the lack of a direct contact between the pathogen and the antagonist (Benhamou et al. 1999). According to Orlikowski et al. (2002), biopreparation Polyversum protected different species of ornamental plants from the fungi of genera *Pythium, Phytophthora* and species forms of *F. oxysporum.*

The author's own studies showed that both Biosept 33 SL and Polyversum were effective in inhibiting mycelial growth, sporulation and formation of sclerotia of fungi pathogenic towards bean and soybean (Pięta et al. 2004). Besides, seed dressing and spraying plants with these biopreparations protected them from infection by soil-borne fungi (Patkowska 2005a; Patkowska and Pięta 2004). As stated by Angioni et al. (1998) and Woedtke et al. (1999), in *in vitro* conditions extract from grapefruit very strongly inhibited the growth of *Bacillus subtilis, Candida maltosa, Escherichia coli, Serratia marcescens, Staphylococcus subtylis, Penicillium digitatum* and *P. italicum.*

The purpose of the present studies was to estimate the effectiveness of grapefruit extract and *P. oligandrum* in protection of common bean, runner bean and pea from soil-borne pathogenic fungi such as *Alternaria alternata, Botrytis cinerea, Fusarium* spp., *Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum.*

MATERIALS AND METHODS

The studies were conducted at the Experimental Station at Czesławice near Lublin in the years 2003–2004. The subject of studies were common bean cv. Narew, runner bean cv. Westa and pea cv. Sześciotygodniowy TOR, grown on the fields of monoculture with naturally accumulated infective material in the soil. Earlier studies (Patkowska 2005b; Pięta and Patkowska 2003) showed that in this soil environment the following fungi considered to be pathogenic are present: *A. alternata, B. cinerea, Fusarium* spp., *Phoma exigua, P. irregulare, R. solani* and *S. sclerotiorum* (unpublished results of pathogenicity test). To control these pathogens, the seeds of particular plant species were dressed with 0.2% Biosept 33 SL or Polyversum (in the amount 1cm^3 and 1 g/100 g seeds) before sowing. The experiment also included untreated control without seed dressing and it was performed in four replications. 100 seeds well formed and healthy without any visible spots on the seed cover were sown on each plot. At the beginning of anthesis plants were sprayed with the same preparations as those used for seed dressing.

The number and healthy plants on particular plots was determined four weeks after sowing and at anthesis. Besides, at the same time plants were sampled from each treatment and laboratory mycological analysis was performed according to the method described by Łacicowa and Pięta (1998). After harvest the amount and quality of seed yield of each plant species were determined. For this purpose, 100 spot-free seeds and 100 seeds with spots on the seed cover were subjected to mycological analysis, which was done for each plant species and each experimental variant.

Results concerning the number of plants, their healthiness and yield were statistically analyzed on the basis of Tukey's confidence intervals (Oktaba 1987).

RESULTS

The results showed that both products effectively improved germination, number, healthiness of seedlings and yield of the examined plants. Field observations, performed at seedling phase and at anthesis, revealed a considerably greater number of healthy plants from seeds treated with both products (Table 1). The best emergencies were achieved after dressing pea seeds with Biosept 33 SL (96 seedlings, on average) or Polyversum (92 seedlings). The number of seedlings of common bean and runner bean grown from the seeds dressed with the biopreparations was slightly smaller (82 and 79 seedlings and 91 and 88 seedlings, respectively). On the other hand, the smallest number of seedlings emerged from not-dressed seeds in the control objects of all plant species (Table 1). The propor-

Table 1. The number and healthiness of bean and pea plants (means from the years 2003–2004)

Treatment	Concentration [%]	Seedlings		Plants at anthesis	
		number of seedlings on a plot	participation of diseased seedlings on a plot [%]	number of plants on a plot	participation of diseased plants on a plot [%]
Common bean					
Biosept 33 SL	0.2	82 b	1.7 a	78 b	2.3 a
Polyversum	0.1	79 b	2.2 a	79 b	2.9 a
Control	–	58.5 a	8.3 b	55 a	9.8 b
Runner bean					
Biosept 33 SL	0.2	91 b	1.5 a	90 b	1.9 a
Polyversum	0.1	88 b	1.7 a	87 b	2.2 a
Control	–	70.5 a	5.9 b	67.5 a	7.0 b
Pea					
Biosept 33 SL	0.2	96 b	0.5 a	93 b	0.75 a
Polyversum	0.1	92 b	3.0 b	90 b	3.5 a
Control	–	69 a	7.15 c	66.5 a	8.5 b

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

tion of infected seedlings ranged from 0.5% (in the case of pea) to 8.3% (in the case of common bean), in the treatment with Biosept 33 SL or Polyversum this being considerably smaller than in the control. The most strongly infected seedlings of common bean, runner bean and pea grew from the seeds that were not dressed and their proportion was 8.3%, 5.9% and 7.15%, respectively (Table 1).

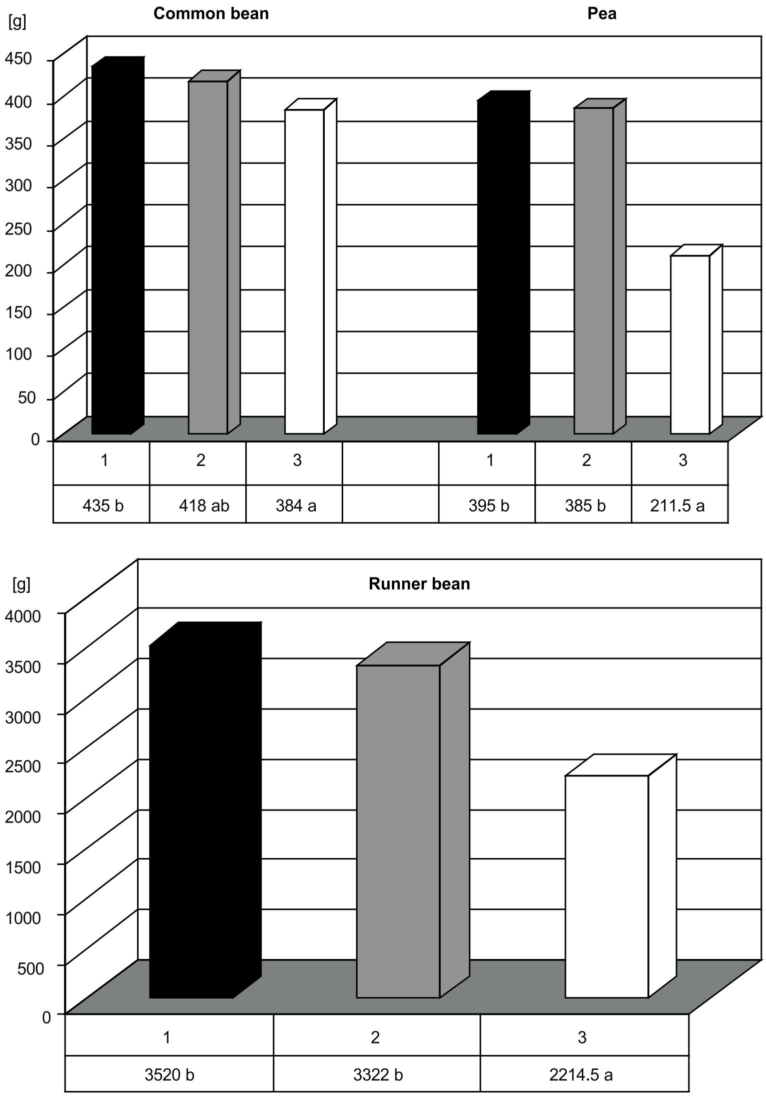

Fig. 1. Yield of bean and pea seeds in g on a plot (mean from the years 2003–2004)
1 – Biosept 33 SL, 2 – Polyversum, 3 – Control
Mean values for plant species marked with the same letter do not differ significantly at $p<0.05$

A similar relationship was observed during anthesis of the studied plant species. Then a slight decrease of the number of plants was observed and the increase of infected ones. The greatest proportion of infected plants of common bean, runner bean and pea was found in the control object, and it amounted to 9.8%, 7.0% and 8.5%, respectively (Table 1).

The size and quality of seed yield of a particular plant species were recorded after harvest. Plants of bean and pea in the treatments with Biosept 33 SL or Polyversum were characterized by better yielding than control plants (Fig. 1). The highest seed yield (3520 g from a plot, on average) was harvested from the plants of runner bean after Biosept 33 SL application. The smallest amount of seeds was harvested from the plants of common bean, pea and runner bean in the control (384 g, 211.5 g and 2214.5 g from a plot) (Fig. 1). The proportion of infected seeds of the studied species ranged from 1.75% (in the case of common bean) to 9.3% (in the case of pea) (Fig. 2). After applying Biosept 33 SL a slightly smaller amount of infected seeds was obtained as compared with the treatments with Polyversum. The proportion of infected seeds in the treatments with Biosept 33 SL was almost three times less than in the control. The most infected seeds of common bean, runner bean and pea were harvested from control plants, and their proportion was 6.5%, 8.75% and 9.3%, respectively (Fig. 2).

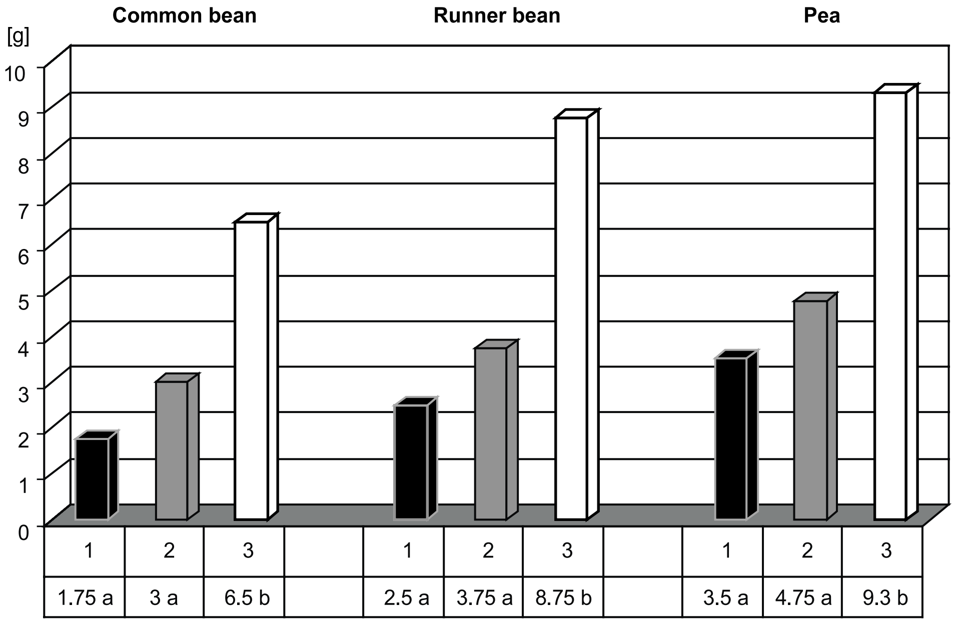

Fig. 2. Patricipation of infected bean and pea seeds (mean from the years 2003–2004)
1 – Biosept 33 SL, 2 – Polyversum, 3 – Control
Mean values for plant species marked with the same letter do not differ significantly at $p<0.05$

3253 fungal isolates (including 1 428 isolates from seedlings and 1 825 isolates from plants at anthesis), belonging to 19 genera, were obtained from infected roots and stem bases of all plant species in seedling phase and anthesis. Figures

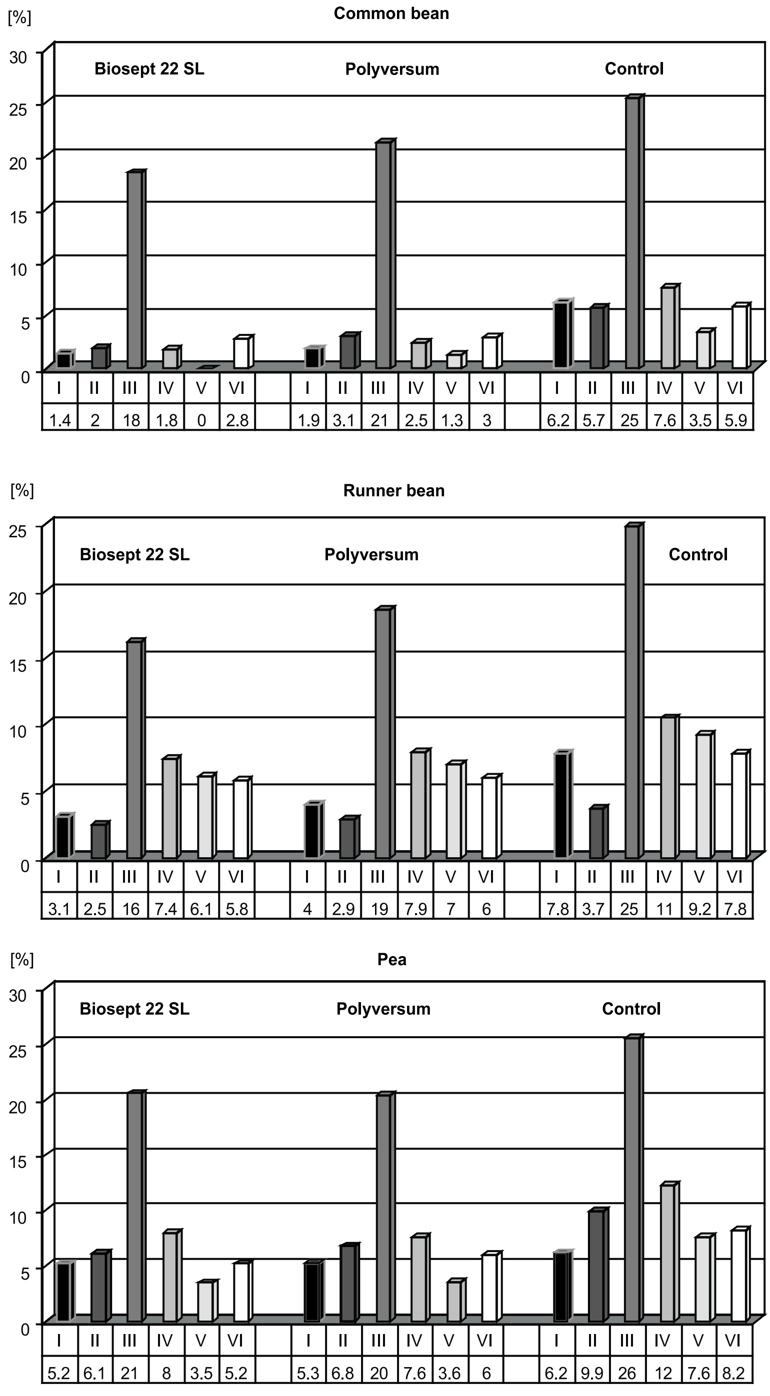

Fig. 3. Participation of pathogenic fungi isolated from bean and pea seedlings (mean from the years 2003–2004)
I – *A. alternata*, II – *F. culmorum*, III – *F. oxysporum*, IV – *F. solani*, V – *P. irregulare*, VI – *R. solani*

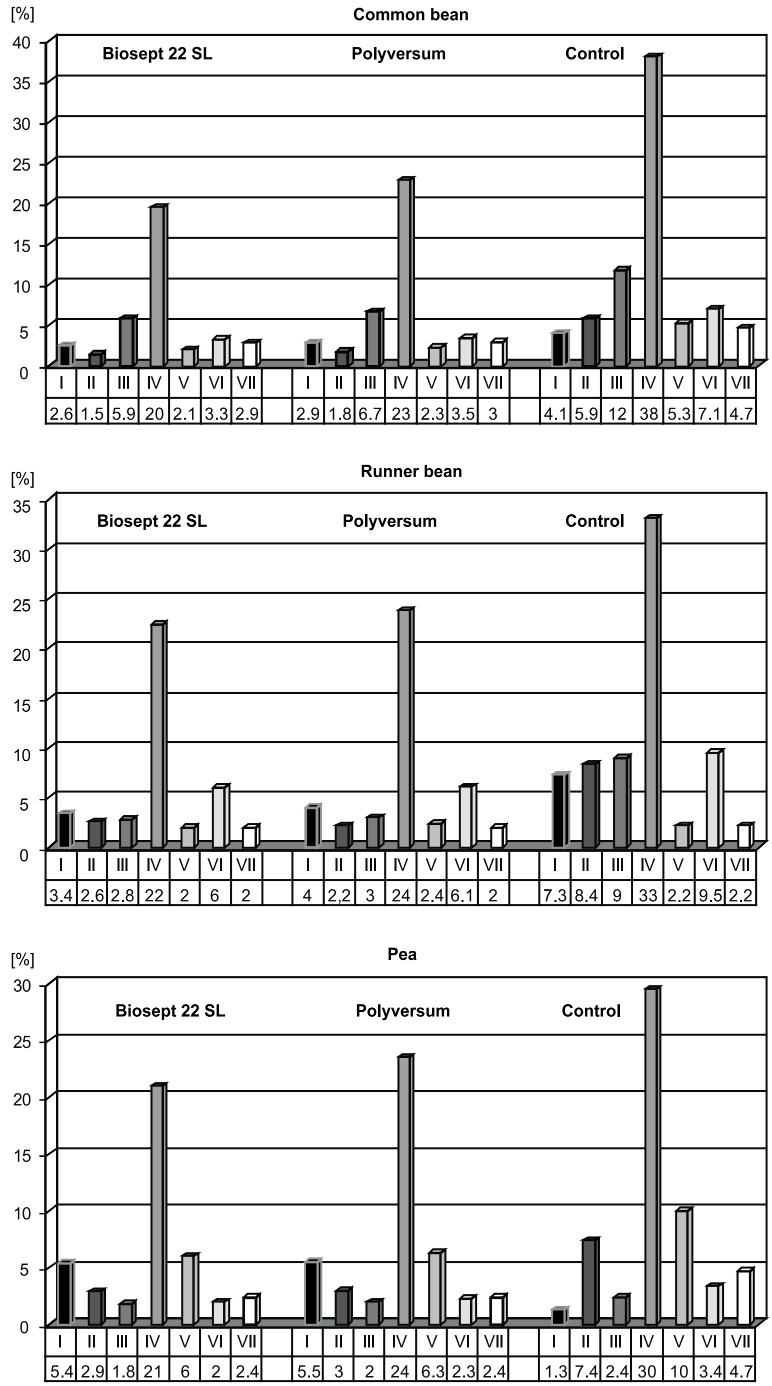

Fig. 4. Participation of pathogenic fungi isolated from bean and pea plants at anthesis (mean from the years 2003–2004)
I – *A. alternata*, II – *B. cinerea*, III – *F. culmorum*, IV – *F. oxysporum*, V – *F. solani*, VI – *R. solani*, VII – *S. sclerotiorum*

Table 2. Fungi isolated from seeds of bean and pea (mean from the years 2003–2004)

Fungal species	Treatment/Number of isolates																		Total
	Common bean						Runner bean						Pea						
	Biosept 33 SL		Polyversum		control		Biosept 33 SL		Polyversum		control		Biosept 33 SL		Polyversum		Control		
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Acremonium roseum (Oud.)	–	–	1	–	3	1	–	–	1	–	2	–	–	–	–	–	–	–	8
Alternaria alternata (Fr.) Keissler	1	–	2	1	3	1	6	2	10	5	19	9	2	1	2	2	5	2	73
Ascochyta pisi Libert	–	–	–	–	–	–	–	–	–	–	–	–	2	–	3	2	4	1	12
Aspergillus niger van Tiegh	–	–	1	1	4	2	1	1	2	1	3	2	1	1	–	–	–	–	20
Botrytis cinerea Pers.	2	–	3	2	8	4	5	2	7	4	18	8	3	1	3	–	10	6	86
Cladosporium cladosporioides (Fres.) de Vries	–	–	1	–	3	1	–	–	1	–	1	–	2	–	2	1	4	2	18
Cladosporium herbarum (Pers.) Link	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	3
Epicoccum purpurascens (Ehr. ex. Schl.)	1	1	1	–	2	2	4	–	5	2	14	1	–	–	–	1	3	1	38
Fusarium avenaceum (Corda ex. Fr. Sacc.)	–	–	–	–	–	–	3	1	3	–	8	4	–	–	–	–	–	–	19
Fusarium culmorum (W. G. Sm.) Sacc.	4	4	8	3	11	5	4	2	6	2	19	8	3	2	3	1	9	4	98
Fusarium oxysporum Schl.	7	3	9	5	13	5	11	7	15	9	33	11	7	3	8	2	12	2	162
Fusarium solani (Mart.) Sacc.	–	–	2	–	4	2	2	–	5	1	12	8	1	–	1	–	–	–	38
Fusarium sporotrichioides Sherb.	–	–	2	–	5	2	–	–	–	–	–	–	–	–	–	–	–	–	9
Gliocladium catenulatum Gilman Abbott	–	2	–	1	–	1	–	1	–	2	–	–	2	2	–	3	–	–	14
Humicola grisea Domsch	–	–	2	–	–	–	1	–	3	1	11	3	–	–	–	–	–	–	21

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mucor hiemalis Wehmer	–	–	1	–	2	–	2	1	3	1	11	5	–	–	2	1	2	3	34
Papulaspora sphaerosperma (Persoon) von Höhnel	–	–	–	1	2	–	–	–	–	–	–	–	–	–	3	2	4	1	13
Penicillium canescens Thom	2	–	3	–	3	1	–	–	3	1	6	1	1	–	–	2	–	–	23
Penicillium expansum Link ex S. F. Gray	–	–	2	1	3	1	2	1	2	2	4	3	–	–	2	–	3	–	26
Penicillium notatum Westling	–	–	2	–	4	–	2	1	2	–	3	1	–	–	–	–	–	–	15
Penicillium nigricans (Bain.) Thom	1	–	2	2	4	–	1	1	2	1	5	3	–	–	–	–	–	–	22
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	1	–	1	–	3	1	–	–	–	–	–	–	2	–	2	1	3	2	16
Phoma exigua Desm.	1	–	1	2	4	2	1	–	1	–	3	1	–	–	–	–	4	–	20
Rhizoctonia solani Kühn	2	–	3	1	8	1	3	1	3	2	7	4	4	1	5	2	17	5	69
Rhizopus nigricans Ehrenberg	–	–	–	–	–	–	–	–	1	–	–	–	1	–	1	–	1	1	5
Sclerotinia sclerotiorum (Lib.) de Bary	2	–	2	1	6	2	6	3	8	3	21	7	–	–	2	–	5	2	70
Trichoderma hamatum (Bonord.) Bain.	–	2	–	1	–	–	–	3	–	2	1	–	–	4	–	2	–	–	15
Trichoderma koningii Oud.	–	3	1	2	–	1	–	1	–	1	–	2	–	2	1	1	–	1	16
Torula herbarum Link	–	–	–	–	–	–	1	–	2	–	–	–	–	–	1	–	3	1	8
Total	24	15	51	24	95	35	55	28	85	40	201	81	31	17	41	24	90	34	971

1 – seeds with spots, 2 – seeds without spots

3 and 4 present the proportion (percentage) of only a few fungal species that were most often isolated from the examined plant material. Among the fungi considered to be pathogenic the following were frequently isolated: *A. alternata, F. culmorum, F. oxysporum, F. solani, R. solani, P. irregulare* (only from seedlings) as well as *B. cinerea* and *S. sclerotiorum* (only from plants at anthesis). Independently on plant species, the proportion of the above mentioned fungal species was greater in the control object than in the treatments with Biosept 33 SL or Polyversum (Fig. 3 and 4). Besides, the proportion of these fungi in the object with Biosept 33 SL was only slightly smaller than in the treatment with Polyversum. *F. oxysporum* was most frequently isolated from infected plants and its proportion in seedling phase ranged from 16% to 26% (Fig. 3), and from 20% to 38% at anthesis (Fig. 4). Besides, among saprophytic fungi different species from genera *Acremonium, Aspergillus, Cladosporium, Epicoccum, Mucor, Penicillium, Rhizopus* and fungi considered to be antagonistic (*Gliocladium* spp. and *Trichoderma* spp.) were often isolated.

971 fungal isolates belonging to 29 species were obtained as a result of mycological analysis of the seeds of common bean, runner bean and pea in all experimental objects (Table 2). The number of fungi isolated from well developed and properly coloured seeds was twice smaller than from spotted seeds. Independently on the species of examined plant, the smallest number of fungi was obtained from the seeds after applying Biosept 33 SL, slightly more after applying Polyversum and the highest number from the object without biological control (in the control). Dominating fungi were *F. culmorum, F. oxysporum, A. alternata, B. cinerea, R. solani* and *S. sclerotiorum. Fusarium* spp. (*F. avenaceum, F. culmorum, F. oxysporum, F. solani, F. sporotrichioides*) and they constituted the greatest proportion, which was in total 33.6% for all plant species. Besides, fungi from genera *Cladosporium, Epicoccum, Humicola* and *Penicillium* were often obtained from the seeds of studied plants (Table 2).

DISCUSSION

The present studies showed that both Biosept 33 SL and Polyversum used for seed dressing and spraying plants of common bean, runner bean and pea considerably improved their emergence, healthiness and yielding. The best emergence were obtained after dressing pea seeds with Biosept 33 SL. Regardless of the species, the smallest number of plants with the greatest proportion of infected ones and the smallest yield of seeds were obtained from the control objects. A similar protective effect was observed in the case of dressing pea and bean seeds with chitosan (Pięta et al. 2005). The effect of this preparation was comparable to the effect of Zaprawa Oxafun T since the number of pea seedlings obtained after the application of chitosan or fungicide was similar (Pięta et al. 2005). Earlier studies (Patkowska and Pięta 2004; Pięta et al. 2005) as well as the presented ones make it possible to suppose that the grapefruit extract and *P. oligandrum* can effectively protect germinating seeds of bean and pea as well as seedlings from soilborne fungi, which present a considerable danger, and especially from such species as *A. alternata, B. cinerea, F. culmorum, F. oxysporum, F. solani, P. irregulare, P. exigua* and *R. solani.*

The proportion of such fungi as *A. alternata, Fusarium* spp., *P. irregulare, R. solani* and *S. sclerotiorum*, which were isolated from roots and stem bases of the studied plants, was considerably smaller in the treatments with Biosept 33 SL and Polyversum than in the control. It can be supposed that reduced development of the above listed fungi took place as a result of the effect of the studied preparations. 7-geranoxicumarin, found in grapefruit extract, can have inhibiting effect on pathogenic fungi (Orlikowski and Skrzypczak 2003) and may induce resistance of gerbera to *P. cryptogea* (Orlikowski 2001b). The protective effect of Biosept 33 SL is also connected with the presence of endogenous flavonoids, glycosides, citrate and limenon in this preparation (Saniewska 2002). Study of Orlikowski and Skrzypczak (2001) showed that grapefruit extract has a wide range of activity. Applied as soil drench strongly decreased population density of *F. oxysporum* f. sp. *dianthi* and *Pythium ultimum*. The application of grapefruit extract as a plant spray inhibited the development of willow rust, *Myrothecium* leaf spot of diffenbachia and grey mould of tulip (Orlikowski and Skrzypczak 2001). As observed by Orlikowski (2001b) amendment of potato-dextrose agar with grapefruit extract at the dose 40 $\mu g/cm^3$ inhibited linear growth of *P. cryptogea* by about 50%. Besides, at such concentration of the product in soil leachate formation of zoosporangia was inhibited by about 95% (Orlikowski 2001b). The product inhibited at least by 50% the development of *Phytophthora* foot rot of gerbera when applied at 165 $\mu g/cm^3$ of peat (Orlikowski 2001b).

Grapefruit extract showed a remarkable effectiveness in controlling powdery mildew of rose (*Sphaerotheca pannosa* var. *rosae*), black spot (*Diplocarpon rosae*) (Wojdyła 2001), grey mould (*B. cinerea*), powdery mildew of pansy (*Sphaerotheca humuli*) and oak (*Microsphaera alphitoides*) (Wojdyła 2004a). According to Orlikowski (2001a), this preparation also inhibited the development of *Phytophthora cryptogea, P. cinnamoni* and *F. oxysporum* f. sp. *cyclaminis.* On the other hand, according to Wojdyła (2004b), grapefruit extract protected chrysanthemums from the infection by *Puccinia horiana*, and willow – from *Melampsora epitea.*

A good effectiveness of Polyversum was observed for example in protection of common China-aster from *F. oxysporum* f. sp. *callistephi* (Saniewska 2001). Adding the biopreparation to the medium significantly reduced the number of propagation units of that pathogen. Besides, the activity of mycoparasite *P. oligandrum* had a positive effect on healthiness of common China-aster (Saniewska 2001). Dressing the roots of aster seedlings with Polyversum before planting had the influence on earlier development of fluorescence buds and more intensive green colour of leaves (Saniewska 2001).

According to Orlikowski and Jaworska-Marosz (2002), at least 3 modes of *P. oligandrum* action on plant pathogens are known: mycoparasitisms mediated by intimate hyphae interactions, antibiosis, enhancement of plant. The fungus *P. oligandrum* produces a protein metabolite – oligandrin, inducing plants resistance. Besides, *P. oligandrum* colonizes the root zone of plants, in this way protecting it from the infection by pathogenic fungi (Vesely and Kocova 2001). Study by Orlikowski and Jaworska-Marosz (2002) showed that mycoparasite *P. oligandrum* may decrease population density of *F. oxysporum* f. sp. *dianthi* and suppressed the spread of *Fusarium* wilt of carnation. *In vitro* study showed an intimate contact of *F. oxysporum* f. sp. *dianthi* and *P. oligandrum* hyphae (Orlikowski and Jaworska-Marosz 2002). It is possible that a similar mycoparasitic activity occurs toward studied pathogens. This issue calls for further studies.

CONCLUSIONS

1. Biosept 33 SL and Polyversum were characterized by a considerable protective effect in protecting germinating seeds, roots and stem bases of seedlings and older plants of common bean, runner bean and pea from soil-borne pathogenic fungi.
2. Among the studied biopreparations, Biosept 33 SL was distinguished by a better protective effectiveness.
3. The tested biopreparations significantly improved emergence, healthiness of plants and their yielding.

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POLISH SUMMARY

SKUTECZNOŚĆ DZIAŁANIA WYCIĄGU Z GREJPFRUTA I *PYTHIUM OLIGANDRUM* W OCHRONIE ROŚLIN FASOLI I GROCHU PRZED PATOGENAMI

Celem niniejszej pracy była ocena skuteczności działania wyciągu z grejpfruta oraz *Pythium oligandrum* w ochronie fasoli zwykłej, fasoli wielokwiatowej oraz grochu przed grzybami patogenicznymi przeżywającymi w glebie. Biopreparaty takie jak Biosept 33 SL i Polyversum zostały użyte do zaprawiania nasion oraz opryskiwania roślin na początku ich kwitnienia. Uzyskane wyniki wykazały, że

zastosowane biopreparaty znacznie poprawiły wschody, zdrowotność i plonowanie badanych gatunków roślin. Poza tym lepszą efektywność działania wykazał Biosept 33 SL, aniżeli Polyversum. Bez względu na gatunek rośliny najmniej roślin, z największym udziałem porażonych oraz najmniejszy plon nasion uzyskano z kombinacji kontrolnych. Z porażonych korzeni i podstawy łodygi oraz nasion fasoli i grochu często izolowano *A. alternata, Fusarium* spp., *P. irregulare, R. solani, S. sclerotiorum.* Dominującym okazał się *F. oxysporum.* Udział wymienionych grzybów w kombinacjach z Bioseptem 33 SL lub Polyversum był mniejszy, aniżeli w kontroli. Jednocześnie udział tych grzybów w porażaniu roślin fasoli zwykłej, fasoli wielokwiatowej i grochu traktowanych Bioseptem 33 SL był tylko nieznacznie mniejszy, aniżeli po zastosowaniu Polyversum.