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EFFECT OF MIEDZIAN 50 WP AND GRAPEFRUIT EXTRACT ON THE HEALTHINESS AND COMMUNITIES OF SOIL MICROORGANISMS OF PEA (*Pisum sativum* L.)

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Abstract. The present studies determined the effect of fungicide Miedzian 50 WP and grapefruit extract on the healthiness of pea and on the microorganism population in the rhizosphere of this plant. The number of plants grown from the seeds dressed with Grevit 200 SL was similar to, but no significantly, the number of plants obtained after the application of fungicide Miedzian 50 WP. The value of the infection index of plants was the lowest after the application of Grevit 200 SL, but not statistically different from that of Miedzian 50 WP. The following fungi were isolated from the infected plants: *Alternaria alternata*, *F. culmorum*, *F. oxysporum*, *F. solani*, *Phoma eupyrena*, *Pythium irregulare*, *Rhizoctonia solani* i *Sclerotinia sclerotiorum*. From the rhizosphere soil were isolated: *Alternaria alternata*, *Fusarium* spp., *Gliocladium* spp., *Mucor* spp., *Penicillium* spp., *R. solani*, *S. sclerotiorum* i *Trichoderma spp*. The population of rhizosphere bacteria in the combinations with Grevit 200 SL and Miedzian 50 WP was significantly higher than in the control treatment. A reverse relationship occurred for the fungi population, but significantly the lowest number of fungi were found with Grevit 200 SL. Antagonistic microorganisms dominated in the rhizosphere of plants grown from the seeds dressed with Grevit 200 SL.

Key words: Grevit 200 SL, fungicide, phytopathogens, antagonistic microorganisms

INTRODUCTION

One of the most popular methods to protect plants, including pea, against plant pathogens is based on using fungicides to dress seeds or to spray the plants' aboveground parts [Patkowska 2005, Brand et al. 2009, Scherm et al. 2009, Rezende and Juliatti 2010].

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However, it happens more and more frequently that the aim is to eliminate the nvironmentally-unfriendly substances since the abuse of pesticides creates a threat for man which is due to their residues in agricultural crops [Tomalak et al. 2010]. Besides, chemical preparations cause the appearance of resistant pathogen breeds and reduce the useful organisms while the costs of detecting them and introducing a new pesticide in the protection are still very high [Scherm et al. 2009, Tomalak et al. 2010]. One of the alternative methods used in plant protection is the biological method consisting in limiting the pathogens by means of antagonistic bacteria and fungi [Patkowska 2010, Patkowska and Pięta 2010, Patkowska and Błażewicz-Woźniak 2013]. Direct and indirect mechanisms of microorganisms affecting pathogens are used here. The direct effect is based on antibiosis, competition and parasitism [Weller 2007, Afsharmanesh et al. 2010, Farhan et al. 2010, Mohamed et al. 2010]. The indirect effect, on the other hand, is based on stimulating the growth and yielding of plants and on triggering a number of resistance reactions in plants [Patkowska 2009, Farhan et al. 2010, Tomalak et al. 2010].

In recent years, biotechnical preparations based on the substances of plant or animal origin have been introduced in plant protection. Their application mainly is based on dressing the seeds as well as watering or spraying the plants [Orlikowski and Skrzypczak 2003, Pięta et al. 2005, Kurzawińska and Mazur 2009, Patkowska 2009]. Grapefruit extract proves to be especially effective in limiting the occurrence of plant pathogens [Dakora 1995, Pięta et al. 2005, Patkowska 2009]. It contains, for example, endogenous flavonoids, citrate, limonene and glycosides which inhibit the germination of fungi spores, the growth of the germ tube, and vegetative hyphae through damaging the membrane system, as well as decreasing the activity of respiratory enzymes [Dakora 1995]. Thus, it has the ability to eliminate bacteria and fungi pathogenic towards plants [Dakora 1995, Pięta et al. 2005, Patkowska 2009]. It also facilitates the development of antagonistic bacteria occurring in the phyllosphere and the soil environment, thus having a good influence on the healthiness of plants [Patkowska 2009, 2012]. A important role in limiting the occurrence of soil-borne pathogenic fungi is played by *Pseudomonas* spp., *Bacillus* spp. [Weller 2007, Afsharmanesh et al. 2010, Farhan et al. 2010], *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. [Patkowska 2005, Mohamed et al. 2010, Patkowska and Pięta 2010].

The purpose of the present studies was to determine the effect of fungicide Miedzian 50 WP and grapefruit extract on the healthiness of *Pisum sativum* and on the microorganism communities in the rhizosphere of this plant.

MATERIAL AND METHODS

Field experiment. Field studies were conducted in the Experimental Station in Felin belonging to the University of Life Sciences in Lublin in the years 2010–2012 on the field of a five-years' monoculture of pea. The experiment was set up in a scheme of random blocks in four replications. The area of each of the four plots in a repetition was 3 m^2. 100 pea seeds were sown onto each plot, which included four rows. The spacing between the rows was 40 cm. Before sowing, the seeds of pea cv. 'Sześciotygodniowy TOR' were dressed with 0.2% Grevit 200 SL (grapefruit extract 200 $g \cdot dm^{-3}$) and fungi-

cide Miedzian 50 WP (50% oxychloride of copper) in the quantity of 2 g·kg^{-1} seeds. The seeds that were not dressed constituted the control.

Mycological analysis of plants. In each year of studies, the number of the grown plants and their healthiness was established four weeks after seed sowing and at anthesis. The healthiness of plants was estimated according to the five-degree scale, i.e. 0º – no disease symptoms, 1º – necrosis up to 10% of the root surface, 2º – necrosis up to 25% of the root surface, 3º – necrosis up to 50% of the root surface, 4º – necrosis over 50% of the root surface [Patkowska and Konopiński 2008]. The disease index was calculated according to McKinney's formula provided by Patkowska and Konopiński [2008].

$$\text{Disease index} = \frac{\sum a}{b} \cdot 100\%$$

where:

$\sum a$ – sum of products of numerical scale index (infection degree) and corresponding number of plants,

b – total number of tested plants multiplied by the highest numerical scale index.

Plants with the symptoms of necrosis on the roots and the stem base were taken for laboratory mycological analysis which was conducted according to the method described by Patkowska and Konopiński [2011].

Analysis of microbial community. Samples of the rhizosphere soil of pea were taken from particular experimental treatment in the phase of seedlings (after four weeks) and at anthesis (eight weeks after seed sowing). A microbiological analysis was conducted in the laboratory according to the method described by Patkowska [2009] and Patkowska and Konopiński [2011].

The manner of sampling the soil was in accordance with the method described by Martyniuk et al [1991]. Four whole plants were dug out from each plot of particular experimental combination (ie 16 plants from each combination). The soil directly adhering to the pea roots (rhizosphere soil) was shaken off onto sterile Petri dishes. In sterile laboratory conditions, the soil samples from the same experimental treatment were mixed, then weighed in the amount of 10 g and prepared for further analyses (4 repetitions for each experimental treatment). The total bacteria population was established on the medium Nutrient Agar. In the case of *Bacillus* spp. bacteria, the medium of Tryptic Soy Agar was used while for *Pseudomonas* spp. the medium Pseudomonas Agar F. The total fungi population in each sample was determined on Martin's medium. The population of culturable bacteria and fungi was next converted into colony forming units per gram of dry soil (cfu·g^{-1} d.w. of soil).

The obtained isolates of fungi *Gliocladium* spp. and *Trichoderma* spp. (all isolates) and bacteria *Bacillus* spp. and *Pseudomonas* spp. (300 isolates of each genus), from each experimental treatment were used to determine their antagonistic effect towards the following fungi pathogenic to pea: *Alternaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*. The interaction between these microorganisms was established according to the methods described by Pięta and Kęsik [2007] and Mańka and Mańka [1992]. They took into consideration the

degree of growth inhibition of plant pathogen colonies and the size of the inhibition zone with the common growth of those microorganisms. Laboratory tests determined the number of antagonistic bacteria isolates and fungi occurring in the rhizosphere soil.

Results concerning the number, healthiness of plants and population of microorganisms occurring in the rhizosphere of pea were statistically analyzed using variance analysis. The significance of differences between the means was established using Tukey's confidence intervals ($P < 0.05$). Statistical calculations were carried out using Statistica program, version 7.1.

RESULTS AND DISCUSSION

The number of plants grown from dressed seeds with Grevit 200 SL was similar to the number of plants obtained after applying fungicide Miedzian 50 WP, even exceeding it to reach, respectively, 94 and 89 seedlings (tab. 1). Seedlings with inhibited growth and yellowing leaves occurred on all plots. At anthesis, only a slight loss of plants and a small proportion of infected plants with distinct disease symptoms on the stem base and the roots were observed. The mean proportion of infected seedlings and older plants was the highest in the control and it was 11.25% and 15.25%, respectively. The smallest number of infected seedlings and plants at anthesis was found after the application of Grevit 200 SL (3.75 and 5.75%). The value of the infection index for seedlings ranged from 3.62 to 20.16, while for older plants from 3.92 to 27.94 and it was the lowest in the treatment with Grevit 200 SL, but statistically without differences in the treatment with Miedzian 50 WP (tab. 1).

Table 1. Number and healthiness of pea plants (mean from 2010–2012)

Experimental treatment	Seedlings			Plants at anthesis		
	a	b	c	a	b	c
Grevit 200 SL	94^{b*}	3.75^{a}	3.62^{a}	90^{b}	5.75^{a}	3.92^{a}
Miedzian 50 WP	89^{b}	4.50^{a}	5.17^{a}	87^{b}	6.75^{b}	5.46^{a}
Control	70^{a}	11.25^{b}	20.16^{b}	56^{a}	15.25^{c}	27.94^{b}

a – number of pea plants; b – mean percent of infected pea plants; c – disease index; *mean in columns differ significantly ($P \leq 0.05$), if they are not marked with the same letter

A very similar effect of another biotechnical preparation (Biosept 33 SL), containing grapefruit extract, on the emergence and healthiness of the *Fabaceae* plants was shown by other authors [Orlikowski and Skrzypczak 2003, Pięta et al. 2005, Patkowska 2012]. Fungicides used for seed dressing also protect the seeds from infection by soil-borne fungi, which has a positive effect on the number and healthiness of plants [Pięta et al. 2005, Patkowska 2010, 2012]. Studies conducted by Patkowska [2005] showed that fungicide Zaprawa Oxafun T had a similar effect on the emergences of *Pisum sativum*.

A laboratory mycological analysis of the infected seedlings and older pea plants gave totally 406 and 516 fungi isolates, respectively, in all experimental treatments (tab. 2). The smallest amount of fungi was isolated from plants after Miedzian 50 WP application (115 isolates from the seedlings and 139 isolates from older plants). The following species of fungi were isolated in higher number: *Alternaria alternata*, *Acremonium* spp., *Fusarium equiseti*, *F. culmorum*, *F. oxysporum*, *F. solani*, *Gliocladium* spp., *Penicillium* spp., *Phoma eupyrena*, *Pythium irregulare*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Trichoderma* spp. Among them *F. oxysporum*, *F. solani* and *R. solani* were pathogens of pea and *S. sclerotiorum* were also known pathogens the others may be weak pathogens in certain cases. Those fungi often occurred on plants growing in the control. Besides above fungi, the following species were isolated: *Gliocladium catenulatum*, *G. fimbriatum*, *G. roseum*, *Trichoderma aureoviride*, *T. harzianum*, *T. koningi*, *T. piluliferum* and *T. pseudokoningii*. Those fungi known as antagonistic were most frequently obtained from plants after the application of Grevit 200 SL and Miedzian 50 WP (tab. 2).

Table 2. Fungi isolated from infected pea plants (total from 2010–2012)

Fungus species	Number of isolates						Total
	seedlings			plants at anthesis			
	Grevit 200 SL	Miedzian 50 WP	control	Grevit 200 SL	Miedzian 50 WP	control	
Acremonium roseo-griseum (S.B. Saksena) W. Gams	1	2	4	2	3	6	18
Acremonium roseum (Oud.) W. Gams	1	3	6	2	3	7	22
Alternaria alternata (Fr.) Keisller	8	11	16	9	13	21	78
Epicoccum purpurascens Ehr. ex. Schl.	1	2	3	1	3	2	12
Fusarium culmorum (W.G.Sm.) Sacc.	6	8	13	7	9	15	58
Fusarium equiseti (Corda) Sacc.	2	3	8	3	4	10	30
Fusarium oxysporum Schl.	14	16	28	17	20	38	133
Fusarium solani (Mart.) Sacc.	3	6	11	5	8	16	49
Gliocladium catenulatum Gilman et Abbott	9	5	2	13	7	3	39
Gliocladium fimbriatum Gilman et Abbott	10	4	–	12	4	-	30
Gliocladium roseum Bainier	8	3	–	11	3	-	25
Mucor hiemalis Wehmer	1	2	4	2	2	6	17
Penicillium janthinellum Biourge	2	3	8	2	4	8	27
Penicillium nigricans (Bain.) Thom	–	1	4	–	3	7	15
Penicillium purpurogenum Stoll	2	2	5	3	5	9	26
Phoma eupyrena Sacc.	3	4	6	3	5	10	31
Pythium irregulare Buisman	8	10	18	–	–	-	36
Rhizoctonia solani Kühn	12	14	23	14	15	31	109
Sclerotinia sclerotiorum (Lib.) de Bary	–	–	–	6	8	17	31
Trichoderma aureoviride Rifai	5	4	–	8	5	1	23
Trichoderma harzianum Rifai	15	6	1	19	6	2	49
Trichoderma koningii Oud.	7	3	–	12	4	-	26
Trichoderma piluliferum Webster et Rifai	5	1	–	7	2	-	15
Trichoderma pseudokoningii Rifai.	8	2	–	10	3	-	23
Total	131	115	160	168	139	209	922
Total		406			516		922

The effectiveness of grapefruit extract in the protection of various plants was confirmed by, for example, Pięta et al. [2005], Mazur and Wojdyła [2010], Patkowska [2012]. The effect of Grevit 200 SL is related with the presence of flavonoids and 7-geranoxy-coumarin [Orlikowski and Skrzypczak 2003]. These compounds inhibit the growth and development of a number of fungi *in vitro* conditions [Orlikowski and Skrzypczak 2003, Patkowska 2012]. Studies by Orlikowski [2001] on the mechanism of grapefruit extract affecting *Phytophthora cryptogea* showed that the former limited the mycelium growth, inhibited the appearance of zoosporangia and the germination of zoospores. In laboratory conditions it inhibited the growth of *Phomopsis sojae* mycelium, causing its macro- and microscopic changes [Patkowska 2012]. It proved effective in protecting roses from powdery mildew (*Podosphaera pannosa*) and black spots (*Diplocarpon rosae*). Mazur and Wojdyła [2010] showed that a protective treatment consisting in spraying pedunculate oak with either Biosept 33 SL or Grevit 200 SL four times limited the infection of trees by *Erysiphe alphitoides*. Grevit 200 SL was used to protect Persian cyclamen against the pathogens infecting bulbs and roots (*A. alternata, B. cinerea*, *Cylindrocarpon radicicola* and *Fusarium* spp.) [Mazur and Kurzawińska 2010].

The microbiological analysis of the rhizosphere soil of pea showed that the population of bacteria in 1 g d.w. of soil in the treatment with Grevit 200 SL and Miedzian 50 WP, both in the seedling phase and at anthesis, was significantly higher than in the control treatment (tab. 3). A reverse relationship occurred for the fungi population. The total amount of bacteria ranged, on an average, from 2.15×10^6 to 3.57×10^6 cfu·g^{-1} d.w. of soil at the seedling phase and from 2.85×10^6 to 4.96×10^6 cfu at anthesis. The total amount of fungi was, on an average, from 4.34×10^3 to 8.86×10^3 cfu at the seedling phase and from 5.12×10^3 to 9.98×10^3 cfu at anthesis. In all experimental treatments, bacteria *Bacillus* spp. were more frequently isolated from the rhizosphere of pea than *Pseudomonas* spp. The population of *Bacillus* spp., depending on the experimental combination and the studied phase of plants, ranged from 1.03×10^6 to 3.01×10^6 cfu. The population of *Pseudomonas* spp. ranged from 1.02×10^6 to 1.75×10^6 cfu. More studied microorganisms were isolated from the rhizosphere of pea at anthesis than in the phase of seedlings (tab. 3).

Table 3. Number of bacteria and fungi isolates in the rhizosphere of pea plants (mean from 2010–2012)

Experimental treatment		All bacteria (10^6 cfu·g^{-1} d.w. of soil)	*Bacillus* spp. (10^6 cfu·g^{-1} d.w. of soil)	*Pseudomonas* spp. (10^6 cfu·g^{-1} d.w. of soil)	All fungi (10^3 cfu·g^{-1} d.w. of soil)
Seedlings	Grevit 200 SL	3.57a*	2.10a	1.12a	4.34c
	Miedzian 50 WP	3.13a	1.20b	1.11a	6.25b
	control	2.15b	1.03b	1.02a	8.86a
Plants at anthesis	Grevit 200 SL	4.96a	3.01a	1.75a	5.12c
	Miedzian 50 WP	4.33a	2.67a	1.51a	7.96b
	control	2.85b	1.25b	1.12b	9.98a

*mean for seedlings and plants at anthesis differ significantly ($P < 0.05$) if they are not marked with the same latter

The qualitative and quantitative composition of fungi isolated from the rhizosphere of pea is presented in table 4. Totally, 1306 isolates belonging to 15 genera were obtained. The smallest amount of fungi considered to be pathogenic were isolated after the application of Grevit 200 SL, slightly more – in the treatment with Miedzian 50 WP, and the most in the control. A reverse relationship was observed in the case of saprophytic fungi. Fewer fungi isolates were obtained from the rhizosphere in the seedling phase than at anthesis. *F. oxysporum* and *R. solani* were most frequently isolated (totally, 182 and 130 isolates, respectively). Besides, the following fungi were often isolated: *A. alternata, Botrytis cinerea, F. culmorum, F. solani, S. sclerotiorum, Mucor* spp., *Rhizopus nigricans, Acremonium strictum. Gliocladium* spp. and *Trichoderma* spp. dominated among saprophytic fungi in the rhizosphere of pea, and they were more abundant in the treatment with Grevit 200 SL and Miedzian 50 WP as compared to the control. The species *G. fimbriatum* and *G. roseum* occurred within *Gliocladium* spp.

Table 4. Fungi isolated from rhizosphere of pea (total from 2010–2012)

Fungus species	Number of isolates						Total
	seedlings			plants at anthesis			
	Grevit 200 SL	Miedzian 50 WP	control	Grevit 200 SL	Miedzian 50 WP	control	
Acremonium strictum W. Gams	3	7	8	5	8	10	41
Alternaria alternata (Fr.) Keissler	6	9	12	8	12	16	63
Aspergillus flavus Link	2	4	7	2	5	9	29
Botrytis cinerea Pers.	–	3	6	1	4	8	22
Cladosporium cladosporioides (Fres.) de Vries	1	4	7	2	4	10	28
Epicoccum nigrum Link.	–	–	5	1	2	7	15
Fusarium culmorum (W.G.Sm.) Sacc.	11	15	18	13	16	22	95
Fusarium oxysporum Schl.	20	26	38	24	30	44	182
Fusarium solani (Mart.) Sacc.	6	9	14	7	13	20	69
Gliocladium fimbriatum Gilman et Abbott	4	1	–	5	2	–	12
Gliocladium roseum Bainier	9	3	1	11	5	2	31
Mucor mucedo Mich. ex St. Am.	7	8	11	8	10	15	59
Mucor hiemalis Wehmer	5	9	14	6	11	17	62
Penicillium janczewskii Zaleski	3	5	7	4	6	11	36
Penicillium purpurescens (Sopp) Raper et Thom	2	3	6	2	5	10	28
Penicillium verruculosum Peyronel	–	–	4	1	3	7	15
Rhizoctonia solani Kűhn	14	19	25	16	24	32	130
Rhizopus nigricans Ehrenberg	5	7	12	7	9	15	55
Sclerotinia sclerotiorum (Lib.) de Bary	8	12	20	9	13	26	88
Torula herbarum (Pers.) Link	–	–	5	1	2	8	16
Trichoderma aureoviride Rifai	7	4	2	9	6	3	31
Trichoderma harzianum Rifai	26	12	4	28	15	5	90
Trichoderma koningii Oud.	14	8	3	16	10	3	54
Trichoderma piluliferum Webster et Rifai	11	6	–	14	7	–	38
Trichoderma pseudokoningii Rifai.	5	2	–	7	3	–	17
Total	169	176	229	207	225	300	1306

and genus *Trichoderma* was represented by *T. aureoviride*, *T. harzianum*, *T. koningii*, *T. piluliferum* and *T. pseudokoningii*. Other species of saprophytic fungi belonged to the genera of *Acremonium*, *Aspergillus*, *Cladosporium*, *Epicoccum*, *Mucor*, *Penicillium*, *Rhizopus* and *Torula* (tab. 4).

The increase of cfu of bacteria and the decrease of cfu of soil-borne fungi in the rhizosphere of pea as compared to the control can be explained by the effect of Miedzian 50 WP, formed on the basis of copper oxychloride and grapefruit extract contained in Grevit 200 SL. Besides, the composition of rhizosphere organism communities is shaped under the effect of secondary metabolites exudated by those microorganisms, the root exudates and the compounds formed from the decay of the decorticating root cells [Koo et al. 2005, Steinkelner et al. 2007, 2008, Jaroszuk-Ściseł et al. 2008, 2009]. The factor shaping microorganism populations colonizing the root zone and directly or indirectly participating in interactions between plants and soil microorganisms are, for example the root border cells – RBC [Jaroszuk-Ściseł et al. 2008, 2009]. These are alive cells released during the root growth from the most outer layer of the cap to the soil. They were earlier known as dead, decorticated cells of the cap. According to Hirsch et al. [2003], Bais et al. [2004] and Tamás et al. [2005], metabolites exudated by RBC to the environment stimulate or inhibit the growth of rhizosphere organisms.

Table 5. Number of antagonistic bacteria and fungi isolated from pea rhizosphere (total from 2010–2012)

Bacteria and fungi	Number of isolates						Total
	seedlings			plants at anthesis			
	Grevit 200 SL	Miedzian 50 WP	control	Grevit 200 SL	Miedzian 50 WP	control	
Bacillus sp.	24	13	4	31	15	6	93
Pseudomonas sp.	32	20	7	37	24	9	129
Total bacteria	56	33	11	68	39	15	222
Gliocladium fimbriatum Gilman et Abbott	3	1	–	4	1	–	9
Gliocladium roseum Bainier	8	2	–	10	3	1	24
Trichoderma aureoviride Rifai	7	4	2	9	6	3	31
Trichoderma harzianum Rifai	26	12	4	28	15	5	90
Trichoderma koningii Oud.	14	8	3	16	10	3	54
Trichoderma piluliferum Webster et Rifai	11	6	–	14	7	–	38
Trichoderma pseudokoningii Rifai.	5	2	–	7	3	–	17
Total fungi	74	35	9	88	45	12	263
Total bacteria and fungi	130	68	20	156	84	27	485

Laboratory tests showed that in the rhizosphere soil of pea growing in particular experimental treatments were occurred antagonistic fungi and bacteria (tab. 5). Totally, 263 isolates of antagonistic *Gliocladium* spp. and *Trichoderma* spp. and 222 isolates of antagonistic *Bacillus* spp. and *Pseudomonas* spp. were obtained from all experimental

treatments. The fewest antagonistic bacteria and fungi were obtained from the rhizosphere of seedlings and older plants of pea grown from the seeds that were not dressed (11 and 15, and 9 and 12 isolates, respectively), and a little more after the application of Miedzian 50 WP (33 and 39, and 35 and 45 isolates, respectively). The highest number of antagonistic microorganisms were obtained on dressed seeds with Grevit 200 SL (tab. 5).

The occurrence of microorganisms considera to be antagonists reduced the growth and development of plant pathogens, thus positively affecting the healthiness of pea. This is confirmed by numerous information in literature on the role of antagonistic bacteria and fungi towards the pathogens of other cultivated plants [Patkowska 2009, Pedersen et al. 2010, Zhang and Xue 2010]. The populations of those microorganisms could have been affected by the root exudates which is also confirmed by other authors [Koo et al. 2005, Tamás et al. 2005, Steinkelner et al. 2007, 2008].

CONCLUSIONS

1. Grevit 200 SL and Miedzian 50 WP can be effective in protecting pea from soil plant pathogens.

2. The tested preparations stimulate the development of antagonistic *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp. and *Gliocladium* spp., at the same time limiting the occurrence of pathogenic soil-borne fungi.

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WPŁYW MIEDZIANU 50 WP I WYCIĄGU Z GREJPFRUTA NA ZDROWOTNOŚĆ I ZBIOROWISKA MIKROORGANIZMÓW GLEBOWYCH GROCHU (*Pisum sativum* L.)

Streszczenie. W badaniach określono wpływ ekstraktu z grejpfruta i fungicydu Miedzian 50 WP na zdrowotność grochu oraz na kształtowanie się populacji mikroorganizmów w ryzosferze tej rośliny. Liczba roślin wyrosłych z nasion zaprawianych Grevitem 200 SL była zbliżona, a nawet przewyższała, ale nie statystycznie istotnie, liczbę roślin uzyskanych po zastosowaniu fungicydu Miedzian 50 WP. Wartość indeksu porażenia roślin była najmniejsza po wykorzystaniu Grevitu 200 SL, ale statystycznie nie różniła się od tej dla Miedzianu 50 WP. Z porażonych roślin izolowano m. in. *Alternaria alternata*, *F. culmorum*, *F. oxysporum*, *F. solani*, *Phoma eupyrena*, *Pythium irregulare*, *Rhizoctonia solani* i *Sclerotinia sclerotiorum.* Z ryzosfery izolowano m. in. *Alternaria alternata*, *Fusarium* spp., *Gliocladium* spp., *Mucor* spp., *Penicillium* spp., *R. solani*, *S. sclerotiorum* i *Trichoderma spp.* Liczebność bakterii ryzosferowych w kombinacjach z Grevitem 200 SL i Miedzianem 50 WP była istotnie większa, aniżeli w kombinacji kontrolnej. Odwrotna zależność wystąpiła w przypadku liczebności grzybów, ale statystycznie mniej grzybów wystąpiło w kombinacji z Grevitem 200 SL. Mikroorganizmy antagonistyczne dominowały w ryzosferze roślin wyrosłych z nasion zaprawianych Grevitem 200 SL.

Słowa kluczowe: Grevit 200 SL, fungicyd, fitopatogeny, mikroorganizmy antagonistyczne

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