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THE ROLE OF OATS, COMMON VETCH AND TANSY PHACELIA AS COVER PLANTS IN THE FORMATION OF MICROORGANISMS COMMUNITIES IN THE SOIL UNDER THE CULTIVATION OF ROOT CHICORY (*Cichorium intybus* var. *sativum* Bisch.) AND SALSIFY [*Tragopogon porrifolius* var. *sativus* (Gaterau) Br.]

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Abstract. The composition of microorganisms in the cultivation environment is extremely important since it affects the healthiness and hence the yielding of plants. The purpose of the present studies was to determine the effect of oats, common vetch and tansy phacelia as intercrop cover plants on the formation of microorganisms communities in the soil under the cultivation of root chicory and salsify. Before winter, cover plants formed an abundant green mass, which constituted the natural mulch on the surface of the land managed in two ways: 1) pre-winter ploughing, or 2) spring ploughing. The control was the conventional cultivation of these vegetables, i.e. without any cover plants. The microbiological analysis of the soil showed that regardless of the species of the studied vegetable, the total population of bacteria and the population of bacteria *Bacillus* spp. and *Pseudomonas* spp. were the highest when the soil was mulched with oats. A little fewer of those microorganisms occurred after using the mulch of common vetch or phacelia. On the other hand, the fewest bacteria were obtained as a result of the traditional cultivation of those vegetables. The total population of fungi in the soil mulched with oats was the lowest, whereas the most of fungi occurred in the control. The cultivation system, i.e. performing the spring or pre-winter ploughing, rather had no significant effect on the population of the studied microorganisms in the soil. Among the studied vegetables, the following were most frequently isolated: *Alternaria alternata, Fusarium culmorum, Fusarium oxysporum, Pythium irregulare, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. Laboratory tests showed that both the cultivation of root chicory and salsify which included cover crops promoted the development of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Glio-*

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cladium spp., *Penicillium* spp. and *Trichoderma* spp.) better than the traditional cultivation of those vegetables.

Key words: mulch, soil-borne bacteria and fungi, antagonistic microorganisms

INTRODUCTION

So-called cover crops used as mulch gain special importance both in integrated and ecological system of vegetable cultivation [Borowy and Jelonkiewicz 1999, Abdul-Baki et al. 2002, Adamczewska-Sowińska 2004, Błażewicz-Woźniak 2005, Patkowska and Konopiński 2011]. These plants, sown as aftercrop and next used in the form of green manure and destroyed mechanically or by means of herbicides, considerably affect the cultivation environment of vegetables [Smeda and Weller 1996, Adamczewska-Sowińska 2004]. Plant mulches influence the physical properties of the soil, the management of the organic matter and mineral elements, weed infestation, the microbiological activity of the soil and – indirectly – also the plants' healthiness and their yield [Leary and DeFrank 2000, Konopiński et al. 2001, Błażewicz-Woźniak 2005, Pięta and Kęsik 2007]. A big success in achieving the intended aim is attained after choosing the proper vegetable species, cover crops and the manner of managing them [Leary and DeFrank 2000, Adamczewska-Sowińska 2004].

So far, the studies have used a lot of cover crops, e.g. winter cereals (rye, wheat, barley), grasses (creeping fescue, meadow-grass, Italian rye-grass), papilionaceous plants (winter vetch, red and white clover, medick, cowpea), white mustard and phacelia [Kotliński 2001, Abdul-Baki et al. 2002, Błażewicz-Woźniak 2005, Patkowska and Konopiński 2011]. The literature provides a lot of information on the positive effect of mulch on vegetable production. The positive effect of rye on the yielding of onion cultivated from sowing was proved, for example, by Kęsik et al. [2000]. A similar effect of mulch on the yielding of cucumber, carrot, cabbage, celery and beet was observed by Borowy and Jelonkiewicz [1999] and Kotliński [2001]. On the other hand, Błażewicz-Woźniak [2005] informs on the positive effect of the mulch of oats and phacelia on the yielding of root parsley. The literature hardly gives any information on the role of cover crops in the formation of microorganisms communities in vegetable cultivation [Jamiołkowska and Wagner 2003, Pięta and Kęsik 2007]. The composition of microorganisms in the cultivation environment is extremely important since it affects the healthiness and hence the yielding of plants. It is known that depending on the species, and even the cultivar, the mulching plants – through their root exudates and products of decomposition of their organic substance – can reduce the development of plant pathogens and stimulate the growth and development of antagonistic microorganisms [Bending and Lincoln 2000, Smolińska 2000]. *Bacillus* spp. and *Pseudomonas* spp. isolates [Bacon and Hinton 2002, Patkowska 2005, Dehestani et al. 2010] and fungi *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. [Smith and Kirkegaard 2002, Pięta and Patkowska 2003, Sobowale et al. 2009, Mohamed et al. 2010] display the greatest ability of antagonistic effect.

The purpose of the studies was to determine the effect of oats, common vetch and phacelia as intercrop cover plants on the formation of microbial populations in the soil under the cultivation of the above enumerated high-inulin root vegetables.

MATERIAL AND METHODS

Field experiments were conducted in the years 2007–2008 at the Experimental Station of Felin belonging to the University of Life Science in Lublin. The object of the studies was the soil, wich was sampled every year in the second 10-days' period of June from the depth of 6 cm of the plough layer of the field where root chicory cv. 'Polanowicka' and salsify cv. 'Mamut' were grown. For each of the vegetable species, soil mulching with intercrop cover plants such as oats (*Avena sativa* L.), common vetch (*Vicia sativa* L.) and tansy phacelia (*Phacelia tanacetifolia* B.) was tested. Before winter, those plants formed an abundant yield of the green mass, which constituted the natural mulch on the surface of the field which was managed in a two-fold manner: 1) pre-winter ploughing, or 2) spring ploughing. The control was the conventional cultivation of those plants, i.e. without any cover crops.

The microbiological analysis of the soil sampled from particular experimental combinations was conducted according to the method described by Martyniuk et al. [1991] and Patkowska [2009]. 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 (3 repetitions for each experimental combination). Soil solutions from 10g of soil with the 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}, 10^{-7}. In the case of *Bacillus* spp., 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 using the dilutions of 10^{-2}, 10^{-3}, 10^{-4}. 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 data of microbial counts were transformed by logarithm base 10.

The isolates of bacteria *Pseudomonas* spp. and *Bacillus* spp. obtained so (500 isolates from each genus) and fungi of genera *Gliocladium* (100 isolates), *Penicillium* (100 isolates) and *Trichoderma* (100 isolates), from each experimental combinations were used to determine their antagonistic effect towards *Altenaria alternata*, *Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The antagonistic effect of *Pseudomonas* spp. and *Bacillus* spp. was established according to the method described by Martyniuk et al. [1991]. The scale of the antagonistic effect of bacteria comprised five degrees, i.e. 0° – no inhibition zone, 1° – inhibition zone of 1–2 mm, 2° – inhibition zone of 3–5 mm, 3° – inhibition zone of 6–10 mm, 4° – inhibition zone of over 10 mm. In order to fully determine the effect of bacteria on the pathogenic fungus, the studies also used the degrees of growth inhibition of plant pathogens as provided by Pięta and Kęsik [2007]. It comprised the following: 0° – no fungus growth inhibition, 1° – colony growth inhibited to 20%, 2° – colony growth inhibited to 50%, 3° – colony growth inhibited to 80%, 4° – colony growth inhibited to 100%. The effect of *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. on the studied pathogenic fungi was estimated according to the method described by Mańka and Mańka [1992].

Results concerning the population of microorganisms 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, version 7.1.

RESULTS AND DISCUSSION

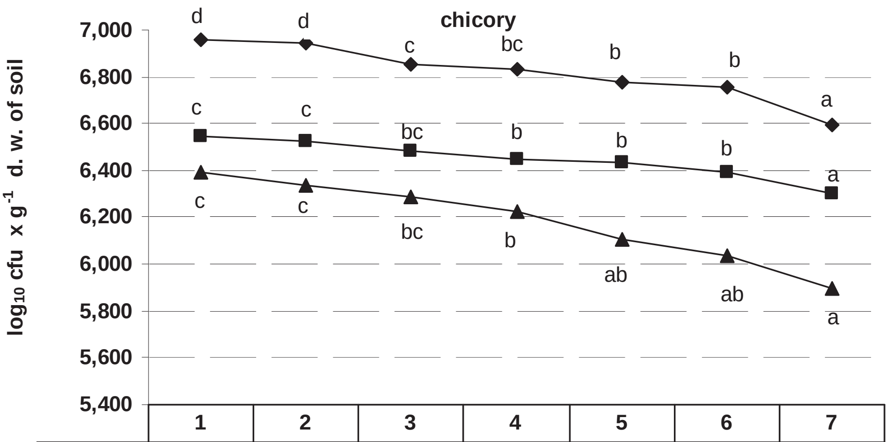
The laboratory microbiological analysis of the soil showed that both in the cultivation of root chicory and salsify the quantitative and qualitative composition of soil-borne microorganisms was differentiated (figs 1 and 2). Regardless of the species of the studied vegetable, the total population of bacteria, the population of bacteria *Bacillus* spp. and *Pseudomonas* spp. were the highest when the soil was mulched with oats. Also independently of the species of the studied vegetable, the smallest total population of fungi occurred after ploughing in the oat mulch, a little more after managing the mulch of common vetch or phacelia, and the most in the traditional cultivation (fig. 2). Moreover, it was observed that the population of the studied soil-borne microorganisms was slightly bigger in the cultivation of root chicory as compared to that of salsify.

The total population of bacteria in the soil under the cultivation of root chicory ranged from 6.598 to 6.957 ($\log_{10}$ CFU), and under the cultivation of salsify it ranged from 6.486 to 6.826 ($\log_{10}$ CFU) (fig. 1). The population of *Bacillus* spp. in the cultivation of root chicory and salsify, respectively, ranged from 6.299 to 6.547 and from 5.949 to 6.493 ($\log_{10}$ CFU). In the cultivation of root chicory the population of *Pseudomonas* spp. ranged from 5.898 to 6.393 ($\log_{10}$ CFU), and in the case of salsify from 5.477 to 6.230 ($\log_{10}$ CFU) (fig. 1). It was observed in the present study that intercrop plants had a positive effect on the formation of bacteria populations in the soil. An increase in the population of bacteria, especially after mulching the soil with oats, could have occurred under the effect of root exudates of this plant. This fact is explained in numerous literature items concerning the role of compounds exudated by the roots of different cultivated plants [Pięta 1999, Pięta and Patkowska 2001]. Moreover, as reported by Elliott and Lynch [1984], Pięta and Kęsik [2007] and Smeda and Weller [1996], cereals can stimulate the growth and development of bacteria.

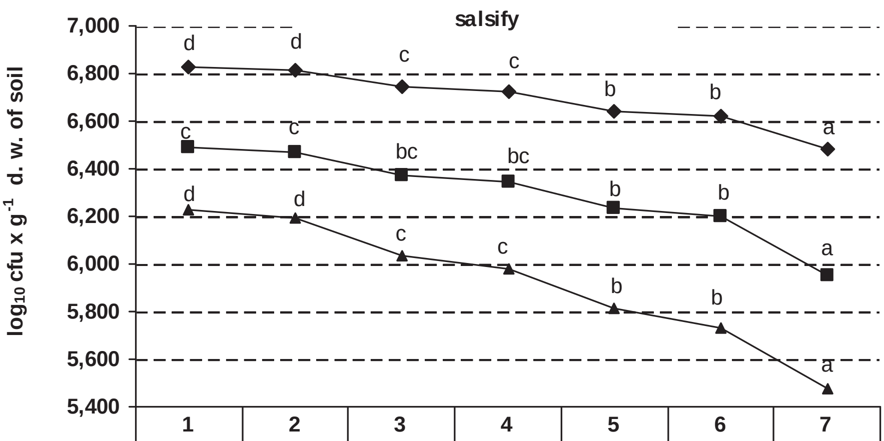
The total population of fungi in the soil mulched with oats was 4.450 ($\log_{10}$ CFU) and 4.451 ($\log_{10}$ CFU) in the case of root chicory, and 4.264 ($\log_{10}$ CFU) and 4.302 ($\log_{10}$ CFU) in the cultivation of salsify, depending on the way of managing the cover plant (fig. 2). In the control, i.e. the cultivation of root chicory and salsify without any cover crops, the total population of fungi was respectively, 4.760 ($\log_{10}$ CFU) and 4.663 ($\log_{10}$ CFU) (fig. 2). Similar results were obtained by Pięta and Kęsik [2007] using rye and common vetch for soil mulching in the cultivation of onion. The small population of fungi in the soil after the cultivation of oats could have been caused by the effect of root exudates of this plant. Earlier studies conducted by Patkowska and Kono-

Phot. 1. Four-weeks' seedlings of root chicory growing in a field experiment (photo by E. Patkowska)

Phot. 2. Four-weeks' seedlings of salsify growing in a field experiment (photo by E. Patkowska)



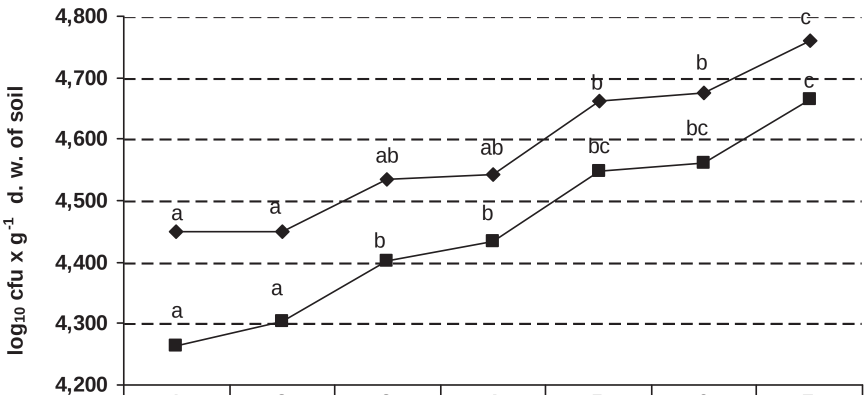
	1	2	3	4	5	6	7
B.	6,957	6,946	6,853	6,833	6,777	6,755	6,598
Bac.	6,547	6,526	6,480	6,450	6,436	6,391	6,299
Ps.	6,393	6,338	6,290	6,225	6,104	6,033	5,898



	1	2	3	4	5	6	7
B.	6,826	6,812	6,742	6,723	6,639	6,620	6,486
Bac.	6,493	6,467	6,371	6,342	6,236	6,201	5,949
Ps.	6,230	6,190	6,037	5,978	5,813	5,732	5,477

*means in lines differ significantly ($P<0.05$), if they are not marked with the same letter

Fig. 1. Total number of bacteria isolated from the soil in individual experimental (means from the years 2007–2008 in $\log_{10}$ CFU · g^{-1} d.w. of soil): B. – total bacteria, Bac. – *Bacillus* spp., Ps. – *Pseudomonas* spp. 1 – oats mulch + spring ploughing, 2 – oats mulch + pre-winter ploughing, 3 – spring vetch mulch + spring ploughing, 4 – spring vetch mulch + pre-winter ploughing, 5 – tansy phacelia mulch + spring ploughing, 6 – tansy phacelia mulch + pre-winter ploughing, 7 – conventional cultivation



	1	2	3	4	5	6	7
chicory	4,450	4,451	4,534	4,543	4,661	4,674	4,760
salsify	4,264	4,302	4,402	4,433	4,548	4,560	4,663

*means in lines differ significantly ($P < 0.05$), if they are not marked with the same letter

Fig. 2. Total number of fungi isolated from the soil in individual experimental (means from the years 2007–2008 ($\log_{10}$ CFU · g^{-1} d.w. of soil): 1 – oats mulch + spring ploughing, 2 – oats mulch + pre-winter ploughing, 3 – spring vetch mulch + spring ploughing, 4 – spring vetch mulch + pre-winter ploughing, 5 – tansy phacelia mulch + spring ploughing, 6 – tansy phacelia mulch + pre-winter ploughing, 7 – conventional cultivation

piński (unpublished data) showed that the mulch of oats and common vetch in scorzonera cultivation improved the biological activity of the soil by decreasing the population of soil-borne fungi.

The cultivation system, i.e. performing the spring or pre-winter ploughing, rather had no significant effect on the population of the studied microorganisms in the soil.

Similar fungi species were isolated from the soil under the studied vegetables growing in particular experimental cultivations (fig. 3). The most frequently isolated fungi included *Alternaria alternata*, *Fusarium culmorum*, *Fusarium oxysporum*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Among the enumerated species, *F. oxysporum*, *R. solani*, *S. sclerotiorum* and *A. alternata* proved to be dominating. Their proportion in the soil in the cultivation of salsify and root chicory was, respectively, 9.5% and 8.7%, 7.8% and 7.2%, 7.1% and 6.2%, and 6.7% and 6.6% (fig. 3). According to Abdul-Baki et al. [2002] and Lemańczyk and Sadowski [2002], cover crops used in vegetable cultivation not only decrease weed infestation and reduce the costs of production but they also reduce the activity of pathogens in the soil. Moreover, studied conducted by Jamiołkowska and Wagner [2003] showed that the mulch of field pea and rye remarkably decreased the population of *Fusarium oxysporum* in the soil environment of tomato.

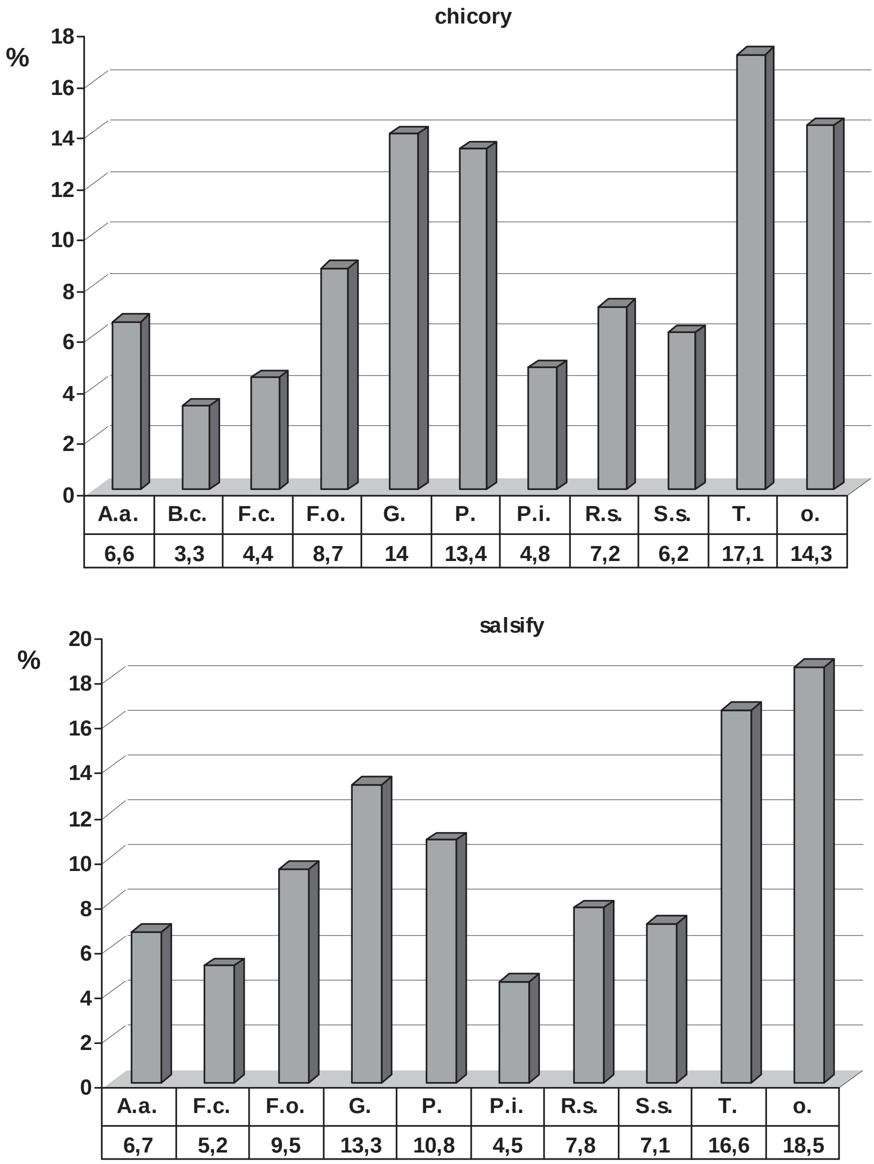


Fig. 3. Total participation of fungi isolated from the soil after chicory and salsify cultivation (total from the years 2007–2008): A.a. – *A. alternata*, B.c. – *B. cinerea*, F.c. – *F. culmorum*, F.o. – *F. oxysporum*, G. – *Gliocladium* spp., P. – *Penicillium* spp., P.i. – *P. irregulare*, R.s. – *R. solani*, S.s. – *S. sclerotiorum*, T. – *Trichoderma* spp., o. – other of saprophytic fungi

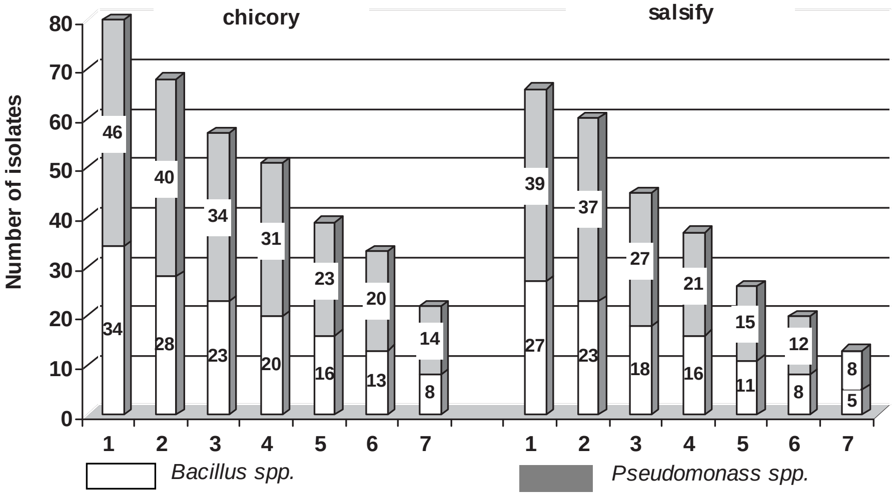


Fig. 4. Antagonistic bacteria isolated from the soil after chicory and salsify cultivation (sum from the years 2007–2008): 1 – oats mulch + spring ploughing, 2 – oats mulch + pre-winter ploughing, 3 – spring vetch mulch + spring ploughing, 4 – spring vetch mulch + pre-winter ploughing, 5 – tansy phacelia mulch + spring ploughing, 6 – tansy phacelia mulch + pre-winter ploughing, 7 – conventional cultivation

Different species of saprophytic fungi were also isolated from the examined soil samples, and the dominating were *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. (fig. 3).

Laboratory tests made possible to determine the population of antagonistic microorganisms in the soil in the cultivation of those vegetables towards the pathogens (figs. 4 and 5). Both the cultivation of root chicory and salsify with cover crops promoted the development of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.) better than the traditional cultivation. The most of antagonistic *Bacillus* spp. and *Pseudomonas* spp. occurred after mulching the soil with oats. The fewest antagonistic bacteria were found in the cultivation of root chicory and salsify without any cover crops, respectively, 8 and 14, and 5 and 8 isolates (fig. 4). Regardless of the species of the studied vegetable, the number of antagonistic *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. was the highest after ploughing in the oat mulch, slightly lower after ploughing common vetch, and the lowest in the traditional cultivation. Moreover, a little more antagonistic fungi were found in the soil of all experimental combinations with chicory cultivation as compared to the cultivation of salsify (fig. 5). It can be stated on the basis of the obtained results and information from the literature that cereals have a positive effect on the growth and development of antagonistic bacteria and fungi. A big number of propagation units of *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp., *Penicillium* spp. and *Trichoderma*

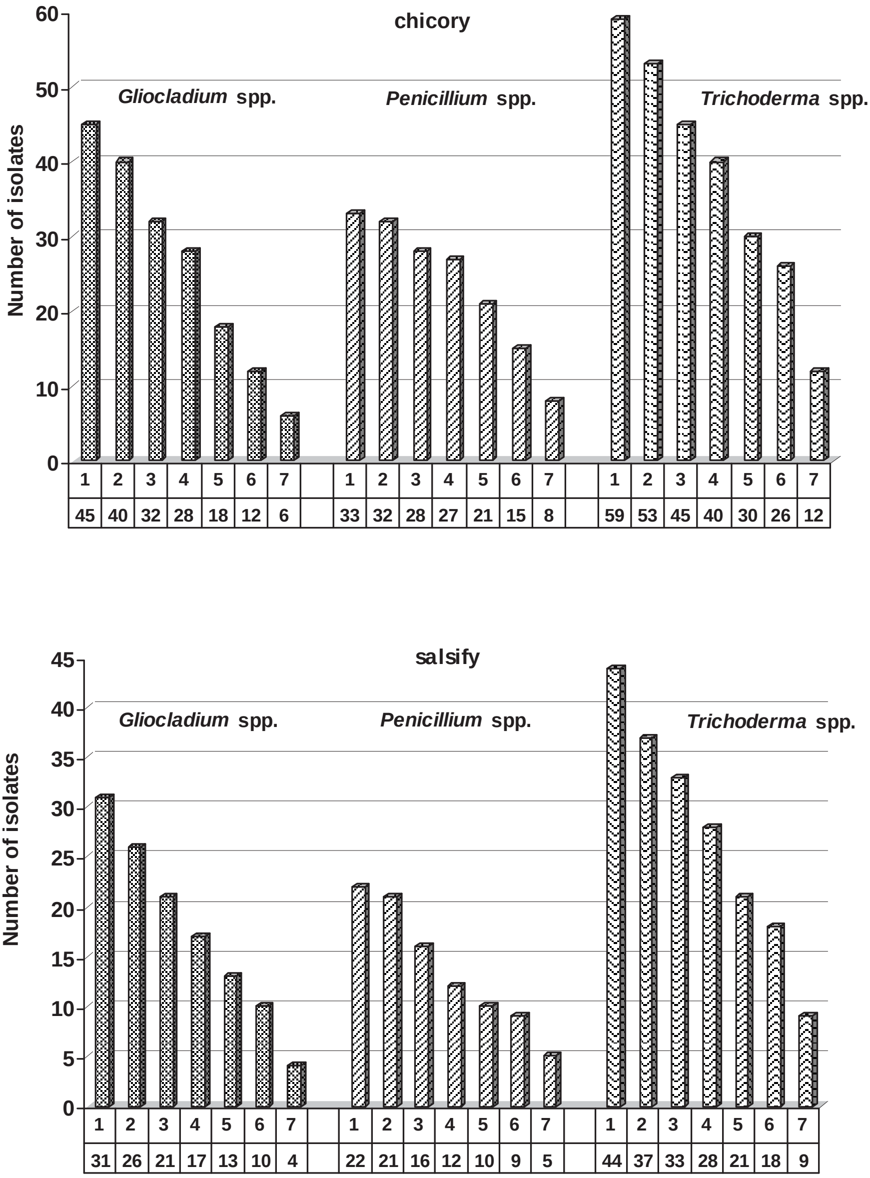


Fig. 5. Antagonistic fungi isolated from the soil after chicory and salsify cultivation (sum from the years 2007–2008): 1 – oats mulch + spring ploughing, 2 – oats mulch + pre-winter ploughing, 3 – spring vetch mulch + spring ploughing, 4 – spring vetch mulch + pre-winter ploughing, 5 – tansy phacelia mulch + spring ploughing, 6 – tansy phacelia mulch + pre-winter ploughing, 7 – conventional cultivation

spp., which are distinguished with their antagonistic effect towards soil-borne pathogenic fungi, becomes a biological barrier making it difficult for plant pathogens to develop and infect the plants. According to Patkowska and Konopiński [unpublished data], oats, common vetch and phacelia as mulching plants in the cultivation of scorzonera played a positive role in the formation of microorganisms communities having an antagonistic effect on plant pathogens. Studies conducted by Pięta and Kęsik [2007] also pointed to the positive effect of spring rye on the populations of antagonistic bacteria and fungi in onion cultivation.

CONCLUSIONS

1. The use of cover crops in the cultivation of root chicory and salsify increase the population of total bacteria, *Pseudomonas* spp. and *Bacillus* spp. of the soil. This effect was higher using oat a cover crops than using common vetch or phacelia.

2. The use of cover crops, especially the oats, reduce the presence of fungi in the soil under cultivation of both root chicory and salsify.

3. Both the cultivation of root chicory and salsify including cover crops promoted the development of antagonistic bacteria (*Bacillus* spp. and *Pseudomonas* spp.) and fungi (*Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.) more than the conventional cultivation of those vegetables.

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ROLA OWSA, WYKI SIEWNEJ I FACELII BŁĘKITNEJ JAKO ROŚLIN OKRYWOWYCH W KSZTAŁTOWANIU SIĘ ZBIOROWISK MIKROORGANIZMÓW W GLEBIE SPOD UPRAWY CYKORII KORZENIOWEJ (*Cichorium intybus* L. var. *sativum* Bisch.) I SALSEFII [*Tragopogon porrifolius* var. *sativus* (Gaterau) Br.]

Streszczenie. Skład mikroorganizmów w środowisku uprawnym jest niezmiernie ważny, oddziałuje on bowiem na zdrowotność, a tym samym na plonowanie roślin. Celem prezentowanych badań było określenie wpływu owsa, wyki siewnej i facelii, jako międzyplonowych roślin okrywowych, na kształtowanie się populacji mikroorganizmów w glebie spod uprawy cykorii korzeniowej i salsefii. Rośliny okrywowe wytworzyły przed zimą obfitą zieloną masę, stanowiącą naturalny mulcz na powierzchni roli, który zagospodarowano w dwojaki sposób: 1) orką przedzimową lub 2) orką wiosenną. Kontrolę stanowiła tradycyjna uprawa tych warzyw, tj. bez roślin okrywowych. Analiza mikrobiologiczna gleby wykazała, że bez względu na gatunek badanego warzywa ogólna liczebność bakterii oraz liczebność bakterii *Bacillus* spp. i *Pseudomonas* spp. była największa wówczas, gdy glebę mulczowano owsem. Nieco mniej tych mikroorganizmów wystąpiło po zastosowaniu mulczu z wyki siewnej lub facelii. Natomiast najmniej bakterii uzyskano w wyniku tradycyjnej uprawy tych warzyw. Ogólna liczebność grzybów w glebie mulczowanej owsem była najmniejsza, natomiast najwięcej grzybów wystąpiło w kontroli. System uprawy, tj. wykonanie orki wiosennej lub przedzimowej, nie miał raczej istotnego wpływu na liczebność badanych mikroorganizmów w glebie. Spod uprawy badanych warzyw najczęściej izolowano *Alternaria alternata, Fusarium culmorum, Fusarium oxysporum, Pythium irregulare, Rhizoctonia solani, Sclerotinia sclerotiorum* oraz *Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp. Testy laboratoryjne wykazały, że zarówno uprawa cykorii korzeniowej, jak i salsefii z uwzględnieniem roślin okrywowych bardziej sprzyjała rozwojowi antagonistycznych bakterii (*Bacillus* spp. i *Pseudomonas* spp.) i grzybów (*Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp.) niż tradycyjna uprawa tych warzyw.

Słowa kluczowe: mulcz, bakterie i grzyby przeżywające w glebie, mikroorganizmy antagonistyczne

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