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SOIL-BORNE MICROORGANISMS THREATENING CARROT CULTIVATED WITH THE USE OF COVER CROPS

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ABSTRACT

Cover crops are used in the cultivation of various plants. They properly modify the composition of soil microorganisms and can protect of plants from phytopathogens. The purpose of the field and laboratory studies was to determine the quantitative and qualitative composition of microorganisms in the soil under carrot cultivated with the use of oats, tansy phacelia and spring vetch as cover crops. The paper presents also studies on soil-borne fungi threatening the healthiness of carrot roots. In the conventional cultivation of carrot the population of bacteria (including *Pseudomonas* spp. and *Bacillus* spp.) was the smallest, while after the application of oats it was the largest. Oats and spring vetch were most effective in limiting the occurrence of soil-borne fungi. Those plants and tansy phacelia caused an increase of the population of saprotrophic fungi (*Albifimbria* spp., *Clonostachys* spp. and *Trichoderma* spp.) in the soil. Intercrop plants had a positive effect on the healthiness of carrot seedlings and roots. *Alternaria dauci*, *A. alternata*, *A. radicina*, *Fusarium oxysporum*, *Globisporangium irregulare*, *Neocosmospora solani*, *Phytophthora* sp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum* proved to be the most harmful towards the studied underground parts of carrot. Oats proved to be the most effective in inhibiting the occurrence of the pathogenic fungi for *Daucus carota* L.

Key words: *Daucus carota* L., healthiness of plants, intercrop plants, bacteria and fungi, soil pathogens, plant-microbial interactions

INTRODUCTION

Intercrop plants, cover crops and mulch are used in the cultivation of various plants, including root vegetables such as carrot, parsley, root celery, parsnip, red beet and high-inulin vegetables (root chicory, salsify, scorzonera) [Kęsik et al. 2000, Patkowska and Konopiński 2013a, c, Patkowska et al. 2016]. Cover crops, which fulfill an important protective and conserving function, are more frequently used in an integrated field cultivation [Borowy 2013, Orluchukwu and Udensi 2013, Patkowska 2018]. These plants can be used as green manure, which – after ploughing in – provide the organic mass and mineral elements to the soil. They increase the biological activity of the soil [Lemańczyk and Sadowski 2002, Patkowska and Konopiński 2014b], properly modify the composition of soil microorganisms [Mackiea et al. 2014, Chavarría et al. 2016, Schmidt et al. 2018, Hallama et al. 2019] and can protect cultivated plants from soil-borne phytopathogens [Patkowska and Konopiński 2013b]. Cover crops (rye, oats, spring vetch, fodder radish, white mustard, tansy phacelia) can increase the number antagonistic microorganisms in the soil and improve the quality of the plants yield [Kosterna 2014, Patkowska and Konopiński 2014 a, b, Himmelstein et al. 2016, Oliveira et al. 2016].

The best antagonistic properties are shown by fungi (*Trichoderma* spp., *Clonostachys* spp.) [Krauss et al. 2013, Aarti and Meenu 2015, Wu et al. 2018] and

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bacteria (*Pseudomonas* spp., *Bacillus* spp.) [Ma et al. 2015, Meng et al. 2015, Hernández-Hernández et al. 2018, Damiri et al. 2019]. The mechanisms underlying their antagonism for plant disease control involve mycoparasitism, antibiosis, competition with other microorganism, promotion of root and plant development, induction of plant disease resistance, inactivation of the pathogen's enzymes [Harman 2000, Sarma et al. 2014, Smitha et al. 2014, Aarti and Meenu 2015]. Therefore, they limit the occurrence of soil pathogens and they improve the plants' healthiness [Patkowska and Błażewicz-Woźniak 2014, Ma et al. 2015, Meng et al. 2015].

Carrot (*Daucus carota* L.) is a very important vegetable plant belongs to family *Apiaceae* cultivated for its edible roots. It is also well known for its medicinal purposes. Carrot became value food because of its rich source of the fat – soluble hydrocarbon, carotene, which is a precursor of vitamin A [Rachamallu 2016]. Besides the food value, different parts of carrot can be used for different medicinal purposes. Carrot roots are used as refrigerant and seeds are used as aromatic, stimulant and carminative [Rachamallu 2016].

Different cultivars of this plant can be infected by pathogenic microorganisms. Adams et al. [2014] report that the plants of carrot are infected by viruses: CYLV (Carrot yellow leaf virus), CtRLV (Carrot red leaf virus), CMoV (Carrot mottle virus), PYFV (Parsnip yellow fleck virus) and CtRLVaRNA (Carrot red leaf associated viral RNA). According to Nesha and Siddiqui [2013], the healthiness of this plant is decreased by *Xanthomonas campestris* pv. *carotae*, causing bacterial leaf blight and *Pectobacterium carotovorum* pv. *carotovorum*, causing bacterial soft rot. Rachamallu [2016] informs about *Agrobacterium rhizogenes* (updated scientific name: *Rhizobium rhizogenes*), causing hairy roots, while Lerat et al. [2009] report on *Streptomyces scabiei*, causing common scab on carrot. The cultivation of carrot is threatened by fungi: *Alternaria dauci*, *A. radicina*, *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* [Coles and Wicks 2003, Mazur and Nawrocki 2007, Nawrocki and Mazur 2011, Park et al. 2011, Aktaruzzaman et al. 2014, Koike et al. 2017, Zafar et al. 2017]. As reported by Naqvi [2004] and Boiteux et al. [2017], on the carrot leaves can also occur *Peronospora crustosa* and *Erysiphe heraclei*.

The purpose of the studies was to establish the effect of oats, spring vetch and tansy phacelia used in carrot cultivation on the population of soil-borne microorganisms and on the healthiness of underground parts of this vegetable.

MATERIAL AND METHODS

Field experiment. The field experiment was conducted in the years 2010–2012 at the Felin Experimental Station belonging to the University of Life Sciences in Lublin, district of Lublin (22º56'E, 51º23'N, Central Eastern Poland, 200 m a.s.l.), on Haplic Luvisol formed from silty medium loams. The object of the studies was the soil taken (every year during the first 10 days of July) from a depth of 5–6 cm of the plough layer of the field where carrot (*Daucus carota* L.) cv. 'Flakkee 2' (sown in the third 10 days of April) was cultivated. The experiment took into consideration cover crops such as oats (*Avena sativa* L.), spring vetch (*Vicia satica* L.), tansy phacelia (*Phacelia tanacetifolia* Benth.) and one system of soil tillage, i.e.: tillage before winter (ploughing) and spring tillage (a combined cultivator). The conventional cultivation, i.e. without any cover crops, was the control. Completely randomized blocks method at four replications was used in the experiment. The area of a single plot was 33m^2.

Analysis of microorganisms communities. Microbiological analysis of the soil was made according to the method described by Patkowska and Błażewicz-Woźniak [2014] and Patkowska and Konopiński [2013a, 2014a]. The soil was sampled from each experimental treatment from four randomly chosen places (i.e. from 16 places for each experimental combination). In sterile laboratory conditions the soil samples from the same experimental combination were mixed, then weighed in the quantities of 10 g and prepared for further analyses (4 repetitions for each experimental combination). Soil solutions from 10 g of soil with the dilutions from 10^{-1} to 10^{-6} were prepared in laboratory conditions. The total population of bacteria was marked on the Nutrient agar. In the case of bacteria from genus *Bacillus*, Tryptic soy agar were used, whereas Pseudomonas agar F was used for *Pseudomonas* spp. For isolation of *Bacillus* spp. soil dilutions were heated for 20 min at 80°C. Martin's medium was

used to establish fungi number. After the incubation, the number of microorganisms was converted into CFU/g of soil DW (colony forming units/g dry weight of soil), and the obtained isolates of fungi were determined to the species.

Laboratory mycological analysis. In each year of studies, the number and healthiness of carrot seedlings were determined. 10 seedlings with disease symptoms were taken from particular experimental treatments with the aim of conducting the mycological analysis of the infected roots. Besides, after the harvest (the first 10-days' period of October), 10 roots of carrot with necrotic signs chosen at random from each experimental treatments were submitted to the mycological analysis. The mycological analysis was conducted according to the method described by Patkowska and Konopiński [2013b, c] for chicory and scorzonera roots and by Patkowska and Krawiec [2016] for pea. This analysis made it possible to determine the quantitative and qualitative composition of fungi infecting the underground organs of carrot.

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 were examined for each of the experimental treatments.

Fungi isolated from the roots and soil were identified to the species using the available keys and monographs of different taxons given in the paper by Patkowska and Konopiński [2013c]. The fungi of *Fusarium* genus were identified on PDA and selected agar medium SNA by Leslie and Summerell [2006]. The malt and Czapek-Dox media were used for the fungi of *Penicillium* spp. [Ramirez 1982]. The other fungi were identified on the malt medium using the corresponding keys and monographic papers given in the publication by Patkowska and Krawiec [2016].

Statistical analysis. The total population of microorganisms, the emergencies and healthiness of carrot seedlings 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 6.0 (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

The total population of bacteria in the soil ranged from 4.51×10^6 to 9.99×10^6 CFU/g of soil DW (Fig. 1a). The population of *Bacillus* spp. and *Pseudomonas* spp. ranged from 2.37×10^6 to 6.31×10^6 and 0.2×10^6 to 2.91×10^6 CFU/g of soil DW, respectively. The highest statistically significant population of bacteria was found after using oats, while being slightly lower after the application of spring vetch and tansy phacelia as cover crops. Statistically, the smallest bacteria population occurred in the soil with the conventional cultivation of carrot. Other plants (rye, buckwheat, white mustard and sunflower) used in the cultivation of carrot limited the population of bacteria in the soil [Patkowska 2018]. The total population of fungi in the soil ranged from 34.65×10^3 to 89.67×10^3 CFU/g of soil DW (Fig. 1b). Much fewer colonies of fungi as compared to the control occurred in the soil sampled from the experimental treatment where oats, spring vetch or tansy phacelia were the ground cover plants. The smallest fungi population (34.65×10^3 CFU/g of soil DW) was observed after the use of oats in carrot cultivation and it differed in a statistically significant manner from the population in the other experimental treatments. The population of fungi in the soil after using spring vetch and tansy phacelia was 53.38×10^3 and 59.74×10^3 CFU/g of soil DW, respectively. Studies conducted by Patkowska and Konopiński [2014a, b] also confirmed the positive effect of oats, vetch and tansy phacelia on the communities of soil-borne bacteria and fungi in the cultivation of scorzonera. Pięta and Bełkot [2002] reported that ground cover crops as tansy phacelia, white mustard and winter wheat can stimulate the growth and development of bacteria in the soil. According to Oliveira et al. [2016], cover crops (palisade grass, millet and common bean) limit the occurrence of fungi in the soil. The ability of *Bacillus* spp. and *Pseudomonas* spp. to limit the growth of plant pathogens results from the production of antibiotics, siderophores and HNC hav-

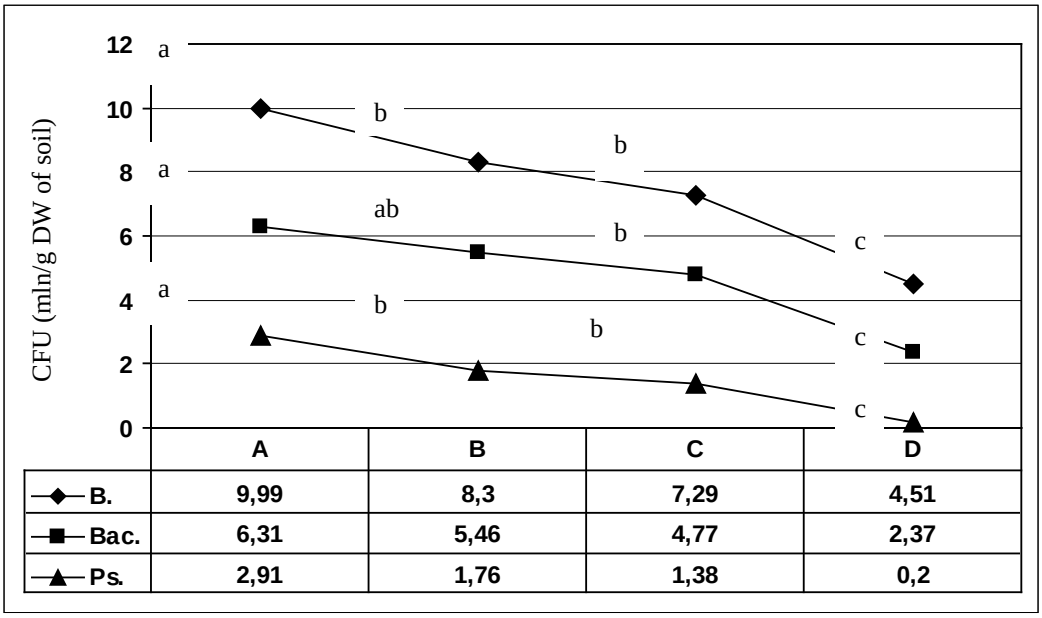


Fig. 1a. Total number of bacteria isolated from the soil in individual experimental treatment (means from the years 2010–2012). *means differ significantly ($P < 0.05$), if they are not marked with the same letter. B. – total bacteria, Bac. – *Bacillus* spp., Ps. – *Pseudomonas* spp. A – soil after oats cultivation; B – soil after spring vetch cultivation; C – soil after tansy phacelia cultivation; D – soil without cover crops cultivation; DW – dry weight

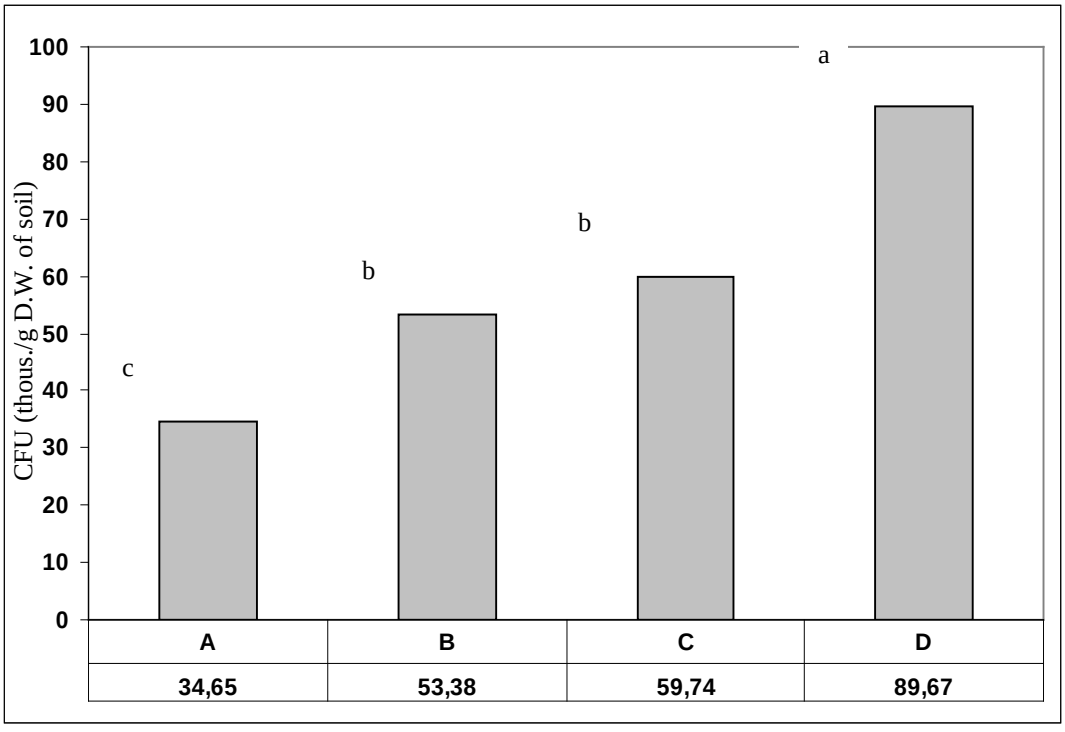


Fig. 1b. Total number of fungi isolated from the soil in individual experimental treatment (means from the years 2010–2012). *means differ significantly ($P < 0.05$), if they are not marked with the same letter. A – soil after oats cultivation; B – soil after spring vetch cultivation; C – soil after tansy phacelia cultivation; D – soil without cover crops cultivation; DW – dry weight

Table 1. Fungi frequently isolated from the soil in individual experimental treatment (sum 2010–2012)

Fungus species	Experimental treatment/ Number of isolates				Total
	oats	spring vetch	tansy phacelia	control	
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	30	23	22	7	82
Alternaria alternata (Fr.) Keissl.	–	2	4	14	20
Alternaria chartarum Preuss	–	–	–	2	2
Alternaria dauci (J.G. Kühn) J.W. Groves & Skolko	3	7	8	20	38
Alternaria radicina Meier, Drechsler & E.D. Eddy	–	–	1	9	10
Boeremia exigua (Desm.) Aveskamp, Gruyter & Verkley	–	1	2	7	9
Cladosporium cladosporioides (Fresen.) G.A. de Vries	–		–	11	12
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	64	44	38	10	156
Cylindrocarpon didymum (Harting) Wollenw.	–	–	–	7	7
Epicoccum nigrum Link	–	1	2	15	18
Fusarium avenaceum (Fr.) Sacc.	1	3	5	16	25
Fusarium culmorum (W.G.Sm.) Sacc.	5	10	13	31	59
Fusarium oxysporum Schltdl.	9	13	16	49	87
Globisporangium irregulare (Buisman) Uzuhashi, Tojo & Kakish.	–	–	2	16	18
Neocosmospora solani (Mart.) L. Lombard & Crous	–	6	10	29	45
Mucor hiemalis Wehmer	–	–	–	7	7
Mucor racemosus Fresen.	–	–	2	4	6
Penicillium canescens Sopp.	6	5	5	2	18
Penicillium chrysogenum Thom	8	8	7	2	25
Penicillium aurantiogriseum Dierckx	16	14	12	4	46
Penicillium verrucosum var. *verrucosum* Dierckx	15	14	12	3	44
Rhizoctonia solani J.G. Kühn	4	7	10	24	45
Rhizopus stolonifer (Ehrenb.) Vuill.	–	–	–	5	5
Sclerotinia sclerotiorum (Lib.) de Bary	–	–	1	21	22
Trichoderma aureoviride Rifai	15	14	12	4	45
Trichoderma hamatum (Bonord.) Bainier	8	7	6	1	22
Trichoderma harzianum Rifai	15	12	10	3	40
Trichoderma koningii Oudem.	37	30	26	6	99
Trichoderma viride Pers.	39	29	27	12	107
Total	275	250	253	341	1119

ing fungistatic and fungicidal properties [Vanitha and Ramjegathesh 2014].

The species composition of fungi isolated from the soil of particular experimental treatments was varied (Tab. 1). Among the fungi considered to be pathogenic, species from the genera of *Alternaria*, *Boeremia*, *Fusarium*, *Globisporangium*, *Neocosmospora*, *Rhizoctonia* and *Sclerotinia* were isolated. *Fusarium oxysporum* proved to be the dominating one (total 87 isolates). The largest number of the enumerated fungi were isolated from the control and after tansy phacelia cultivation. Cover plants, which are used in the cultivation of vegetables and agricultural plants significantly limit the activity of pathogens in the soil [Lemańczyk and Sadowski 2002, Mazur and Nawrocki 2007, Nawrocki and Mazur 2011, Patkowska and Konopiński 2013a, b]. Saprotrophic fungi from the genera of *Albifimbria*, *Clonostachys* and *Trichoderma* were often isolated from the soil after oats, spring vecht and tancy phacelia cultivation. These fungi occurring in the soil play a positive role in the cultivation of root vegetables since they protect, for example, carrot from infection by *Alternaria alternata*, *A. dauci*, *A. radicina* [Mazur and Nawrocki 2007, Nawrocki and Mazur 2011]. The activity of soil-borne pathogens can be limited by the root exudates of phytosanitary plants which promote the development of antagonistic microorganisms [Li et al. 2013].

The average number of carrot seedlings in all experimental treatments ranged from 34.6 to 79.4 plants · m^{-2} (Tab. 2). Good emergencies of carrot plants were observed in the objects with the cultivation of oats (62.8), spring vetch (56.2) and tansy phacelia (55.2 plants · m^{-2}). Studies conducted by Patkowska and Konopiński [2013b, c] also pointed to a positive effect of oats, spring vetch and phacelia on the emergences and healthiness of scorzonera and root chicory. On the other hand, Borowy [2013] reports on the positive effect of mulch of white mustard on the emergences and yielding of root parsley. Kęsik et al. [2000] found out a positive effect of rye on the emergences and yielding of carrot and onion cultivated from sowing. In the presented studies the smallest density of plants (on average, 50.6) was in control. Nawrocki and Mazur [2011] report that pre-emergence blight and seedling necrosis of carrot were caused by different soil-borne fungi. They could also decrease the plant density in the field cultivation.

As reported by Rogers and Stevenson [2010] and Naqvi [2004], the emergences and the healthiness of older carrot plants can be significantly reduced as a result of infection by *Sclerotinia sclerotiorum* (Phot. 1) and *Alternaria radicina* (Phot. 2).

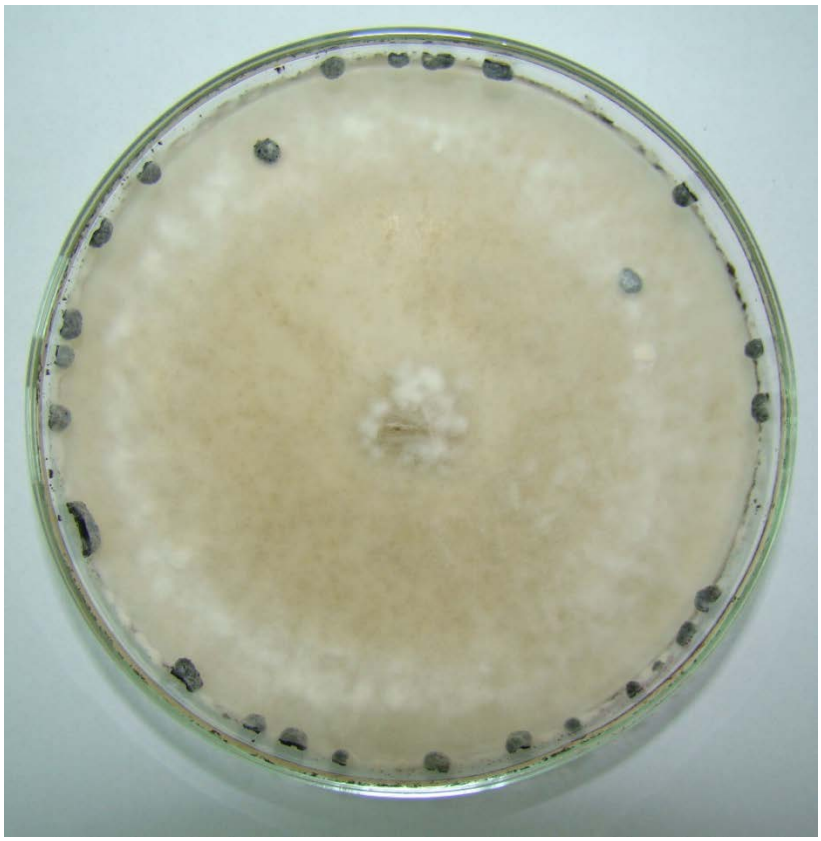
Seedlings with inhibited growth and brown necrotic spots on their roots occurred on all the plots of particular experimental treatments (Phot. 3, 4). Necrosis and the powder from the mycelium hyphas were also observed on the roots after the harvest of carrot (Phot. 5, 6).

The proportion of the diseased seedlings of carrot varied depending on the species of cover crops. It ranged, on average, from 12.3% to 40.7% (Tab. 2). In each year of studies the smallest number of infected seedlings was observed after oats. Information available in literature confirms the positive effect of mulch of cover crops on the healthiness and yielding of a lot

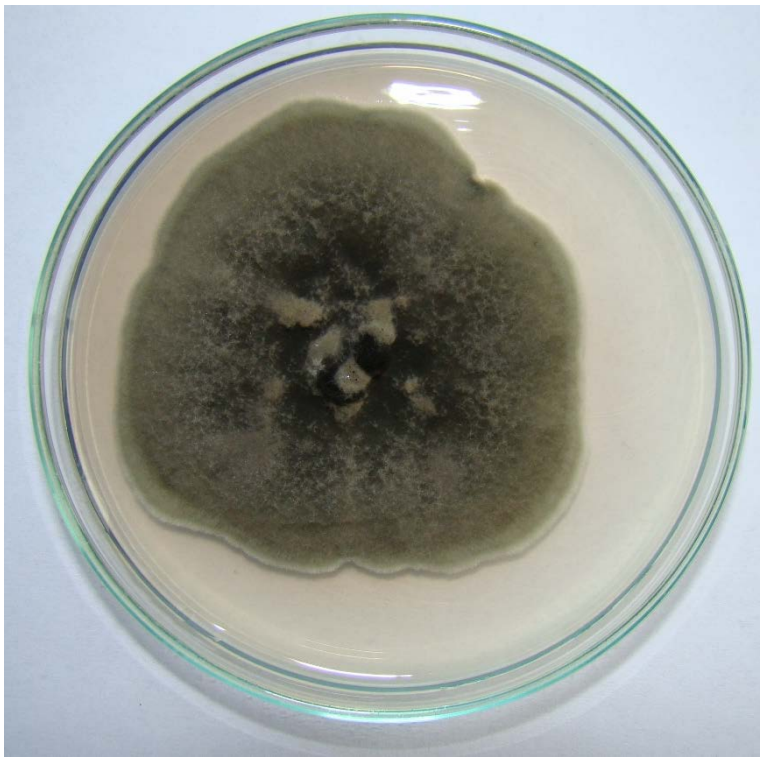
Table 2. Field stand and healthiness of carrot seedlings

Experimental treatment	Field stand per 1 m^2				Percentage of diseased seedlings (%)			
	2010	2011	2012	mean	2010	2011	2012	mean
Oats	79.4 a	73.6 a	35.4 a	62.8 a	14.4 c	10.3 c	12.0 b	12.3 c
Spring vetch	69.8 b	62.2 b	36.4 a	56.2 b	20.5 bc	16.2 b	17.5 b	18.1 b
Tansy phacelia	63.2 b	66.0 b	36.6 a	55.2 b	28.2 b	20.0 b	22.4 b	23.5 b
Conventional cultivation (control)	61.8 b	55.4 c	34.6 a	50.6 b	47.6 a	35.4 a	39.2 a	40.7 a

* mean values in columns marked with the same letter do not differ significantly at $P < 0.05$



Phot. 1. The 10-days colony of *Sclerotinia sclerotiorum* on the malt medium



Phot. 2. The 10-days colony of *Alternaria radicina* on the malt medium

Phot. 3. The seedlings of *Daucus carota* infected by *Fusarium* spp.

Phot. 4. The necrosis on the roots of carrot seedlings caused by *Fusarium* spp.

Phot. 5. *Sclerotinia sclerotiorum* on the carrot roots

Phot. 6. Sclerotia and mycelium of *Sclerotinia sclerotiorum* on the carrot roots

Table 3. Fungi isolated from diseased seedlings of carrot (sum 2010–2012)

Fungus species	Experimental treatment / Number of isolates										Total (%)
	Oats		Spring vetch		Tansy phacelia		Control		Total		
	a*	b	a	b	a	b	a	b	a	b	
Acremonium murorum (Corda) W. Gams	1	–	2	–	3	1	6	4	12	5	17 (3.1)
Alternaria alternata (Fr.) Keissl.	–	1	3	–	1	4	8	9	12	14	26 (4.7)
Alternaria dauci (J.G. Kühn) J.W. Groves	–	–	–	2	–	3	2	6	2	11	13 (2.4)
Alternaria radicina Meier, Drechsler	1	2	1	4	2	6	10	12	14	24	38 (6.9)
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	10	8	8	5	6	4	2	–	26	17	43 (7.9)
Cylindrocarpon didymum (Harting) Wollenw.	–	–	–	1	1	2	8	2	9	5	14 (2.5)
Epicoccum nigrum Link	–	–	1	1	3	2	7	4	11	7	18 (3.3)
Fusarium avenaceum (Fr.) Sacc.	2	–	4	2	5	2	10	4	21	8	29 (5.3)
Fusarium oxysporum Schltdl.	3	1	5	2	7	4	15	11	30	18	48 (8.8)
Globisporangium irregulare (Buisman) Uzuhashi, Tojo & Kakish.	1	–	3	2	5	4	9	6	18	12	30 (5.5)
Neocosmospora solani (Mart.) L. Lombard	1	1	3	1	4	2	8	6	16	10	26 (4.7)
Penicillium simplicissimum (Oudem.) Thom	–	–	–	–	3	1	5	3	8	4	12 (2.2)
Penicillium aurantiogriseum Dierckx	1	–	3	–	4	1	9	5	17	6	23 (4.2)
Phytophthora sp.	–	–	–	–	3	1	7	4	10	5	15 (2.8)
Rhizoctonia solani J.G. Kühn	3	–	5	1	7	3	10	8	25	12	37 (6.8)
Rhizopus stolonifer (Ehrenb.) Vuill.	–	–	1	–	3	–	9	6	13	6	19 (3.5)
Trichoderma harzianum Rifai	16	11	14	10	10	7	2	–	42	28	70 (12.8)
Trichoderma koningii Oudem.	15	14	12	11	9	7	1	–	37	32	69 (12.6)
Total	54	38	65	42	76	54	128	90	323	224	547(100.0)
Total	92		107		130		218		547		

a* – root; b – head of root

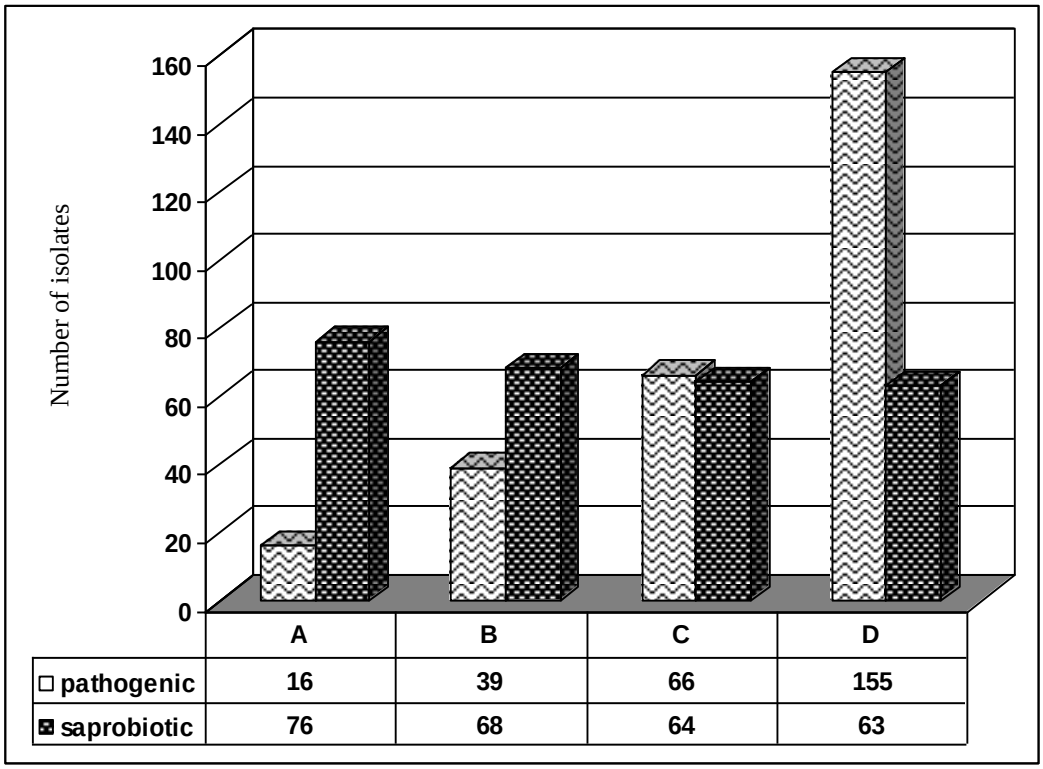


Fig. 2a. The occurrence of possible pathogenic and saprobiotic fungi on carrot seedlings in individual experimental treatment (sum from the years 2010–2012). A* – oats; B – spring vetch; C – tansy phacelia; D – conventional cultivation (control)

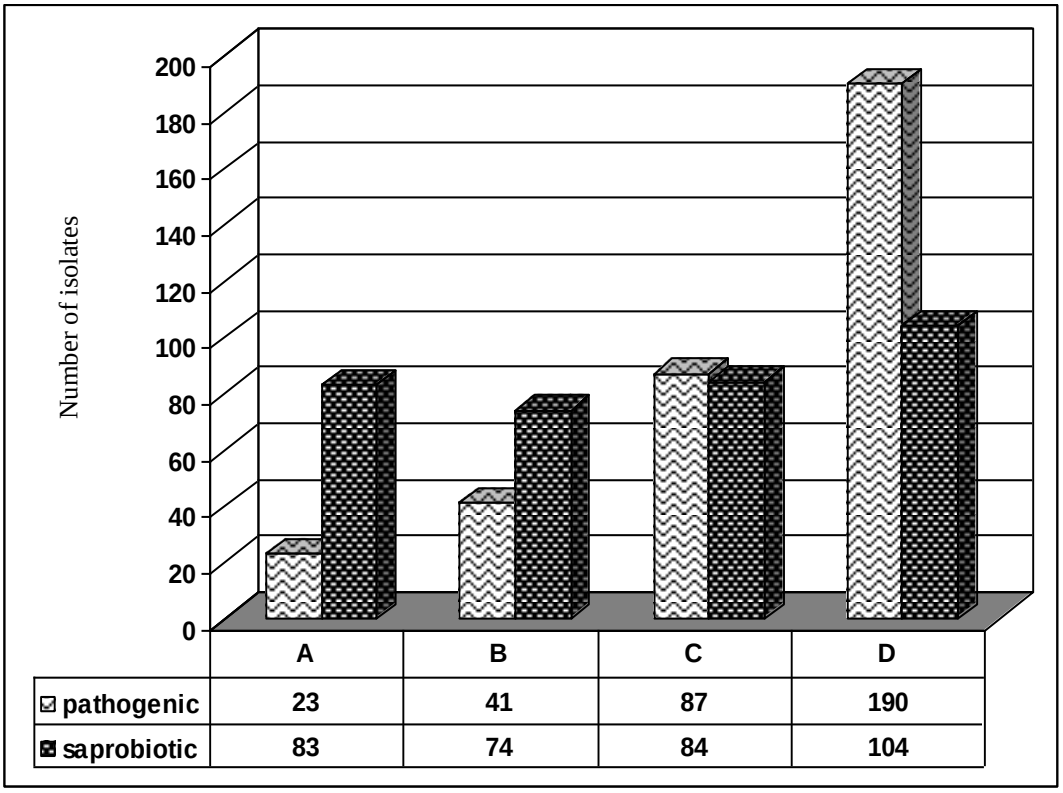


Fig. 2b. The occurrence of possible pathogenic and saprobiotic fungi on carrot roots after harvest in individual experimental treatment (sum from the years 2010–2012). A* – oats; B – spring vetch; C – tansy phacelia; D – conventional cultivation (control)

Table 4. Fungi isolated from diseased roots of carrot after harvest (sum 2010–2012)

Fungus species	Experimental treatment / Number of isolates										Total (%)
	oats		spring vetch		tansy phacelia		control		total		
	a*	b	a	b	a	b	a	b	a	b	
Acremonium murorum (Corda) W. Gams	–	–	–	–	3	–	4	2	8	2	10 (1.5)
Alternaria alternata (Fr.) Keissl.	1	–	3	–	5	2	16	6	25	8	33 (4.8)
Alternaria chartarum Preuss	–	–	–	–	3	–	9	3	12	3	15 (2.2)
Alternaria dauci (J.G. Kühn) J.W. Groves & Skolko	1	–	1	–	4	2	11	6	17	8	25 (3.6)
Alternaria radicina Meier, Drechsler & E.D. Eddy	1	–	1	–	6	4	17	14	25	18	43 (6.3)
Cladosporium cladosporioides (Fresen.) G.A. de Vries	–	–	–	2	2	3	6	3	8	8	16 (2.3)
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams	11	9	9	7	6	3	–	–	26	19	45 (6.6)
Cylindrocarpon didymum (Harting) Wollenw.	–	–	–	–	3	2	8	4	11	7	18 (2.6)
Epicoccum nigrum Link	–	–	1	–	2	2	7	3	10	5	15 (2.2)
Fusarium avenaceum (Fr.) Sacc.	–	–	–	–	2	2	6	4	8	6	14 (2.0)
Fusarium oxysporum Schltdl.	4	2	6	4	8	4	10	6	28	16	44 (6.4)
Neocosmospora solani (Mart.) L. Lombard & Crous	–	–	2	2	3	5	8	3	13	10	23 (3.3)
Penicillium aurantiogriseum Dierckx	2	1	3	1	4	2	8	4	17	8	25 (3.6)
Penicillium chrysogenum Thom	–	–	–	1	2	1	5	3	7	5	12 (1.7)
Penicillium meleagrinum Biourge	–	–	–	–	3	2	9	5	12	7	19 (2.8)
Penicillium simplicissimum (Oudem.) Thom	–	–	–	–	1	–	6	4	7	4	11 (1.6)
Phytophthora sp.	2	–	2	1	4	2	6	5	16	8	24 (3.4)
Rhizoctonia solani J.G. Kühn	5	3	7	4	8	6	12	10	32	23	55 (8.0)
Rhizopus stolonifer (Ehrenb.) Vuill.	2	3	3	2	8	3	16	8	29	16	45 (6.5)
Sclerotinia sclerotiorum (Lib.) de Bary	2	2	5	3	7	5	14	12	28	22	50 (7.3)
Trichoderma harzianum Rifai	7	5	6	4	5	3	2	–	20	12	32 (4.6)
Trichoderma koningii Oudem.	14	12	12	10	10	9	3	3	39	34	73 (10.6)
Trichoderma viride Pers.	10	7	8	5	6	4	2	–	26	16	42 (6.1)
Total	62	44	69	46	105	66	186	108	422	264	686 (100.0)
Total	106		115		171		294		686		

a* – root; b – head of root

of cultivated plants [Kęsik et al. 2000, Orluchukwu and Udensi 2013]. The highest proportion of infected seedlings of carrot was found out in the conventional cultivation (47.6%, 35.4% and 39.2%, depending on the year of studies).

From the infected seedlings of carrot were obtained 547 colonies of different species of fungi (Tab. 3). In each experimental treatment, slightly more fungi isolates were isolated from the roots as compared to the root heads (hypocotyle). The fewest fungi (92 isolates) were obtained from the infected seedlings of carrot cultivated after oats, and slightly more after spring vetch (107 isolates) or tansy phacelia (130 isolates). The greatest amount of fungi were obtained from carrot seedlings cultivated without cover crops (218 isolates). The following fungi considered as potential pathogens were obtained from the diseased seedlings of carrot: *Alternaria dauci*, *A. alternata*, *A. radicina*, *Fusarium oxysporum*, *Globisporangium irregulare*, *Neocosmospora solani*, *Phytophthora* sp. and *Rhizoctonia solani*. Studies by Mazur and Nawrocki [2007], Nawrocki and Mazur [2011], Koike et al. [2017], Zafar et al. [2017] also pointed to considerable harmfulness of these fungi towards carrot plants. Species of the genus *Alternaria* such as *Alternaria dauci*, *A. radicina*, *A. carotiincultae*, *A. petroselini*, have been reported on *Daucus carota* for several countries [Farrar et al. 2004, Tülek and Dolar 2015]. The most of fungi were obtained from carrot seedlings cultivated conventionally (Tab. 3, Fig. 2a). Fungi considered saprotrophic such as *Acremonium murorum*, *Clonostachys rosea*, *Epicoccum nigrum*, *Penicillium* spp. and *Trichoderma* spp. were also isolated from the seedlings of carrot.

After the harvest of carrot, among fungi considered to be pathogenic, species from the genera of *Alternaria*, *Fusarium*, *Phytophthora*, *Rhizoctonia* and *Sclerotinia* were isolated from diseased roots (Tab. 4). A little more the enumerated fungi were isolated from the infected roots of carrot cultivated conventionally or after tansy phacelia as a cover crops, while the least after oats. These fungi, especially *Altenaria dauci*, poses a threat towards carrot [Santos et al. 2000, Ben-Noon et al. 2001, Mazur and Nawrocki 2007, Koike et al. 2017]. According to Coles and Wicks [2003], Rogers and Stevenson [2010], Zafar [2017], fungi, especially *Alternaria dauci*, *A. radicina*, attacked carrots at all stages, causing damping-off, and rotting of roots, crowns, seedlings, petioles, leaves and crowns of maturing carrots.

Saprotrophic fungi of the genera *Clonostachys* (*C. rosea*) and *Trichoderma* (*T. harzianum*, *T. koningii*, *T. viride*) were often isolated from carrot roots cultivated after oats, spring vetch and tansy phacelia (Tab. 4, Fig. 2b). These plants inhibited the infection of underground organs of carrot by soil-borne pathogenic fungi. *Trichoderma* spp. and *Clonostachys* spp. can limit the occurrence of soil pathogens and they improve the plants' healthiness [Sarma et al. 2014, Smitha et al. 2014]. It should be supposed that those fungi also had a positive influence on the healthiness of carrot. Information available in literature indicates high antagonistic activity of *Trichoderma* spp. towards a number of plant pathogens [Patkowska and Konopiński 2014b, Sarma et al. 2014, Aarti and Meenu 2015].

Cover crops used in the cultivation of vegetables stimulate the development of saprophytic bacteria and fungi, which have an antagonistic effect on pathogens [Patkowska and Konopiński 2013b, d, 2014a, Patkowska and Błażewicz-Woźniak 2014]. They have a positive effect on the healthiness of root vegetables by considerably decreasing the infection of the roots of the seedlings and later older plants by *Alternaria alternata*, *Fusarium oxysporum*, *F. culmorum*, *Thanatephorus cucumeris*, *Sclerotinia sclerotiorum* [Patkowska and Konopiński 2011, 2013b].

The present studies confirmed the positive effect of cover crops on the growth and healthiness of *Daucus carota.* Oats, tansy phacelia and spring vetch inhibited the occurrence and development of soil-borne fungi and – consequently – improved the healthiness of the examined plant.

CONCLUSIONS

1. Using a cover crops had a positive effect on the communities of soil microorganisms in the cultivation of carrot.

2. Oats and spring vetch were most effective in limiting the occurrence of soil-borne fungi.

3. *Alternaria dauci*, *A. alternata*, *A. radicina*, *Fusarium oxysporum*, *Globisporangium irregulare*, *Neocosmospora solani*, *Phytophthora* sp., *Rhizoctonia solani*, *Sclerotinia sclerotiorum* proved to be the most

harmful towards the carrot seedlings and roots after harvest.

4. Oats proved to be the most effective in inhibiting the occurrence of the pathogenic fungi for *Daucus carota*, especially *Alternaria* spp., *Neocosmospora solani* and *Sclerotinia sclerotiorum*.

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