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Antagonistic activity of selected fungi of the soil environment of carrot

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ABSTRACT

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The present studies aim at determining the antagonistic effect of selected fungi species occurring in the soil under carrot cultivation towards *Altenaria dauci* (J.G. Kühn) J.W. Groves & Skolko, *Alternaria radicina* Meier, Drechsler & E.D. Eddy, *Fusarium oxysporum* E.F. Sm & Swingle, *Rhizoctonia solani* J.G. Kühn and *Sclerotinia sclerotiorum* (Lib.) de Bary. The field experiment considered cover crops (oats, tansy phacelia and vetch). The control consisted of traditional carrot cultivation (without cover crops). Statistically, the smallest population of fungi was found in 1 g of soil dry weight after the application of oats, and a slightly bigger – after tansy phacelia and vetch. The largest population of fungi was obtained in the traditional cultivation of carrot. Cover crops contributed to the increase of the population of antagonistic *Clonostachys* spp., *Myrothecium* spp., *Penicillium* spp. and *Trichoderma* spp. in the soil. Regardless of the experimental treatment, those antagonistic fungi were the most effective in limiting the growth of *S. sclerotiorum*, *A. dauci* and *A. radicina.* The effect of those fungi was a little smaller towards *F. oxysporum* and *R. solani.* Oats as a cover crop had the best effect on the antagonistic activity of the studied fungi occurring in the soil environment of carrot.

Keywords: *Daucus carota* L.; soil structure; microorganism; soil pathogens; vegetable

Cover crops have a very positive effect on the soil environment. They improve soil structure, increase the content of organic substances and water retention, in addition to improving the nitrogen balance and protecting from the harmful influence of toxic substances (Kęsik et al. 2010). Besides, they considerably reduce the occurrence of weeds as they contain allelopathic substances, which inhibit the germination of their seeds (Adler and Chase 2007, Nouri et al. 2012, Gella et al. 2013). They increase the biological activity of the soil by stimulating the growth and development of microorganisms antagonistic towards different soil pathogens (Patkowska and Błażewicz-Woźniak 2014, Patkowska et al. 2015, 2016). In this way, they can improve the healthiness of plants and increase the size and quality of the yield (Borowy 2013, Patkowska and Konopiński 2013a, Kosterna 2014).

Such cover crops as rye, oats, common vetch, white mustard, tansy phacelia, vetch and sunflower can be used in the cultivation of different species of vegetables. (Borowy 2013, Patkowska et al. 2015). Those species increase the population of antagonistic fungi (*Clonostachys* spp. and *Trichoderma* spp.) and bacteria (*Bacillus* spp. and

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Pseudomonas spp.) occurring in the soil (Patkowska and Konopiński 2014a,b, Patkowska et al. 2016).

The purpose of the studies was to determine the effect of cover crops (oats, tansy phacelia and vetch) used in the cultivation of carrot on the fungi population in the soil. Moreover, the antagonistic effect of *Clonostachys* spp., *Myrothecium* spp., *Penicillium* spp. and *Trichoderma* spp. towards selected fungi pathogenic to carrot was established.

MATERIAL AND METHODS

Fieldwork. 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 (22°56'E, 51°23'N, Central Eastern Poland, 200 m a.s.l.), on grey brown podzolic soil made of loess formations lying on chalk marls with the mechanical composition corresponding to silty medium loams. The object of the studies was the soil sampled each year from a depth of 5–6 cm of the plough layer of the field where carrot (*Daucus carota* L.) cv. Flakkee 2 was cultivated. Four soil samples taken from four different interrows of a given plot (i.e. from 16 places for each experimental combination) made up the non-rhizosphere soil. 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). The experiment took into consideration cover crops such as oats, tansy phacelia and vetch. The conventional cultivation, i.e. without any cover crops, was the control. The experiment was established in a split-plots scheme, in four replications.

Laboratory analyses. Microbiological analysis was made according to the methods described by Czaban et al. (2007) and Patkowska and Konopiński (2014a). The soil was sampled from each experimental treatment from four randomly chosen places. Martin's medium was used to establish fungi number. After the incubation, the number of fungi was converted into CFU/g of soil DW (colony forming units/g dry weight of soil).

In each studied year, all fungi isolates from the genera of *Clonostachys*, *Myrothecium*, *Penicillium* and *Trichoderma*, obtained from particular experimental treatments, were used to establish their antagonistic effect towards such fungi as *Altenaria dauci*, *Alternaria radicina*, *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (isolated from the infected carrot roots). The effect of these saprotrophic fungi on the studied pathogenic fungi was estimated using the method described by Mańka and Mańka (1992). The individual antagonistic effect was determined on the basis of the scale provided by Mańka and Kowalski (1968) and the total antagonistic effect was calculated.

Statistical analysis. The population of fungi was statistically analysed, and the significance of differences was determined on the basis of the Tukey's confidence intervals ($P < 0.05$). Statistical calculations were carried out using the Statistica program, version 6.0 (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

The mean population of fungi obtained as a result of the microbiological analysis of the soil taken from particular experimental treatments ranged from 38.38×10^3 to 92.1×10^3 CFU/g of soil DW (Figure 1). The smallest population of fungi was obtained after using oats in carrot cultivation. Tansy phacelia and vetch as cover crops were more conducive to the development of fungi in the soil. The total population of fungi in 1 g of soil DW after using those plants was 62.43×10^3

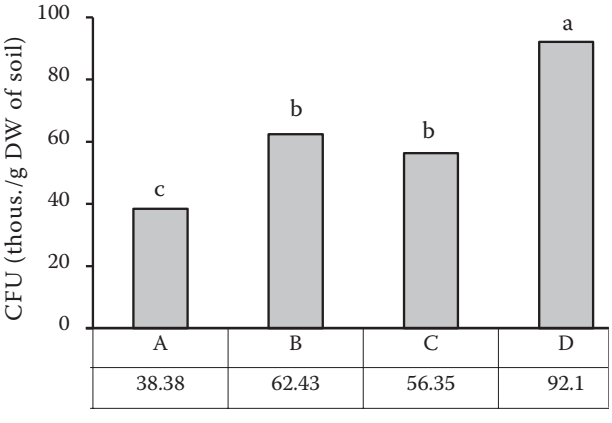

Figure 1. Average number of fungi isolated from the soil in individual experimental (means from the years 2010–2012). *means differ significantly ($P < 0.05$), if they are not marked with the same letter. A – soil after oats cultivation; B – soil after tansy phacelia cultivation; C – soil after vetch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

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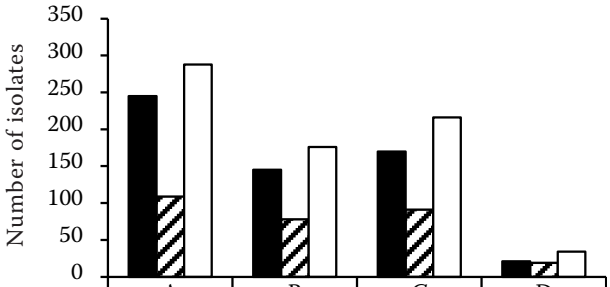

	A	B	C	D
C.spp.	245	145	170	21
P.spp.	109	78	91	19
T.spp.	288	176	216	34

Figure 2. Antagonistic fungi isolated from the soil in individual experimental (total from the years 2010–2012). C.spp. – *Clonostachys* spp. and *Myrothecium* spp., P.spp. – *Penicillium* spp., T.spp. – *Trichoderma* spp.; A – soil after oats cultivation; B – soil after tansy phacelia cultivation; C – soil after vetch cultivation; D – soil without cover crops cultivation

and 56.35 × 10^3 CFU/g, respectively and it was statistically different from the other experimental treatments. A statistically significantly greater population of fungi was obtained in the traditional cultivation of carrot (without cover crops). The positive effect of oats, tansy phacelia and vetch on decreasing the population of fungi occurring in the soil environment in the cultivation of scorzonera, root chicory and salsify was confirmed by Patkowska and Konopiński (2013b, 2014b,c). Studies conducted by Patkowska et al. (2016) showed that other species of cover crops (rye, white mustard, buckwheat and sunflower) can also limit the development of soil-borne fungi in the cultivation of carrot.

Laboratory tests made possible to determine the population of soil-borne fungi that are antagonistic towards the studied phytopathogens. Regardless of the experimental treatment, the greatest population of antagonistic fungi was found within genus *Trichoderma* (Figure 2), slightly smaller in *Clonostachys* spp. and *Myrothecium* spp., and the smallest in *Penicillium* spp. Antagonistic fungi most frequently occurred in the soil after using oats (245, 109 and 288 isolates, respectively), and the most rarely in the control (21, 19 and 34 isolates, respectively). Tansy phacelia and vetch used in the cultivation of carrot also caused an increase of the population of antagonistic soil-borne fungi species as compared to the control. An increased population of antagonistic soil-borne fungi species was also observed in the cultivation of scorzonera and root chicory where cover crops were used (Patkowska and Konopiński 2014b, Patkowska et al. 2015).

Table 1. Activity of selected saprotrophic fungi isolated from soil after oat cultivation towards pathogenic fungi

Fungus species	Mean number of isolates (2010–2012)	General biotic effect					Summary biotic effect
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	
Clonostachys compacta Petch	106	636	530	318	318	636	2438
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	61	244	183	183	183	244	1037
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	78	312	312	234	234	390	1482
Penicillium aurantiogriseum Dierckx	40	40	40	–40	–40	40	40
Penicillium canescens Sopp.	14	14	14	28	14	28	98
Penicillium chrysogenum Thom	20	40	40	–40	–20	60	80
Penicillium verrucosum Dierckx	35	70	70	35	35	35	245
Trichoderma aureoviride Rifai	35	245	245	175	210	245	1120
Trichoderma hamatum (Bonord.) Bainier	19	114	133	114	114	133	608
Trichoderma harzianum Rifai	41	328	328	287	287	328	1558
Trichoderma koningii Oudem	92	736	552	460	552	736	3036
Trichoderma viride Pers. ex. S.F. Gray	101	606	505	505	505	707	2828
Number of isolates	642						
Summary biotic effect		3385	2952	2259	2392	3582	14 570

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Table 2. Activity of selected saprotrophic fungi isolated from soil after tansy phacelia cultivation towards pathogenic fungi

Fungus species	Mean number of isolates (2010–2012)	General biotic effect					Summary biotic effect
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	
Clonostachys compacta Petch	54	162	162	108	108	216	756
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	38	76	76	76	76	152	456
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	53	212	106	106	106	159	689
Penicillium aurantiogriseum Dierckx	27	54	27	–27	27	27	108
Penicillium canescens Sopp.	11	11	33	–22	–22	11	11
Penicillium chrysogenum Thom	14	14	28	14	–14	28	70
Penicillium lividum Westling	3	6	3	3	3	6	21
Penicillium verrucosum Dierckx	23	23	23	23	23	46	138
Trichoderma aureoviride Rifai	24	168	168	96	144	168	744
Trichoderma hamatum (Bonord.) Bainier	11	66	55	66	55	77	319
Trichoderma harzianum Rifai	24	168	144	168	168	144	792
Trichoderma koningii Oudem	57	228	22	228	342	399	1219
Trichoderma viride Pers. ex. S.F. Gray	60	300	300	240	300	300	1440
Number of isolates	399						
Summary biotic effect		1488	1353	1079	1316	1733	696

The studied antagonistic fungi isolated from the soil after using oats were the most effective in limiting the growth of *S. sclerotiorum*, *A. dauci* and *A. radicina*. The values of their antagonistic effect

Table 3. Activity of selected saprotrophic fungi isolated from soil after vetch cultivation towards pathogenic fungi

Fungus species	Mean number of isolates (2010–2012)	General biotic effect					Summary biotic effect
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	
Clonostachys compacta Petch	65	390	260	130	195	325	1300
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	48	240	192	96	144	240	912
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	57	285	228	171	114	342	1140
Penicillium aurantiogriseum Dierckx	32	32	32	32	32	32	160
Penicillium canescens Sopp.	12	24	12	–12	12	12	48
Penicillium chrysogenum Thom	19	19	38	19	–38	19	57
Penicillium verrucosum Dierckx	28	28	28	28	28	56	168
Trichoderma aureoviride Rifai	29	232	203	174	174	232	1015
Trichoderma hamatum (Bonord.) Bainier	15	105	90	75	90	105	465
Trichoderma harzianum Rifai	31	217	217	186	186	248	1054
Trichoderma koningii Oudem	69	414	414	345	483	483	2139
Trichoderma viride Pers. ex. S.F. Gray	72	360	432	432	432	504	2160
Number of isolates	477						
Summary biotic effect		2346	2146	1676	1852	2598	10 618

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Table 4. Activity of selected saprotrophic fungi isolated from soil without cover crops cultivation towards pathogenic fungi

Fungus species	Mean number of isolates (2010–2012)	General biotic effect					Summary biotic effect
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	
Clonostachys compacta Petch	7	21	21	14	21	21	98
Clonostachys rosea (Link) Schroers, Samuels, Seifert et W. Gams	4	20	16	16	8	12	72
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	10	30	20	20	30	40	140
Penicillium aurantiogriseum Dierckx	9	9	18	–18	–18	9	0
Penicillium canescens Sopp.	2	2	2	2	2	2	10
Penicillium chrysogenum Thom	4	8	8	–8	–4	8	12
Penicillium verrucosum Dierckx	4	4	4	4	4	8	24
Trichoderma aureoviride Rifai	4	24	20	16	16	28	104
Trichoderma hamatum (Bonord.) Bainier	1	6	6	5	5	6	28
Trichoderma harzianum Rifai	5	20	20	30	35	35	140
Trichoderma koningii Oudem	9	45	45	54	54	46	244
Trichoderma viride Pers. ex. S.F. Gray	15	75	60	60	60	75	330
Number of isolates	74						
Summary biotic effect		264	240	195	213	290	1202

were in total 3582, 3385 and 2952, respectively (Table 1). A slightly smaller total biotic effect of those antagonists was found towards *F. oxysporum* and *R. solani* (2259 and 2392, respectively). A similar relationship was observed after using tansy phacelia and vetch as well as in the traditional cultivation of carrot (Tables 2–4). The highest antagonistic effect of *Clonostachys* spp., *Myrothecium* spp., *Penicillium* spp. and *Trichoderma* spp. was found for *S. sclerotiorum* and it was in total 1733 (after tansy phacelia), 2598 (after vetch) and 289 (in control) (Tables 2–4). The total antagonistic effect of fungi towards *A. dauci* was 1488 (after tansy phacelia), 2346 (after vetch) and 264 (in control) (Tables 2–4). The smallest total effect of antagonists was observed towards *F. oxysporum* and it was 1079 (after tansy phacelia), 1676 (after vetch) and 195 (in control). A similar effect of the studied fungi antagonistic towards different species of pathogenic fungi was shown by Patkowska and Konopiński (2014b) in the cultivation of scorzonera where cover crops were used. Banaay et al. (2012), Teshome et al. (2013) and Krauss et al. (2013) report that the ability of *Trichoderma* spp., *Clonostachys* spp. and *Penicillium* spp. to inhibit the growth and development of plant pathogens is based on antibiosis, competition and parasitism.

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