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Antagonistic fungi in the soil after *Daucus carota* L. cultivation

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Abstract: Field and laboratory studies determined the effect of intercrop plants (rye, buckwheat, white mustard and sunflower) used in carrot cultivation on the occurrence of *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. in the soil and their antagonistic activity. Rye and white mustard were the most effective in limiting the occurrence of soil-borne fungi. Those plants caused an increase of the population of the studied antagonistic fungi. Buckwheat and sunflower promoted the development of antagonists a little worse. The antagonistic activity of the aforementioned fungi was the highest after using rye and white mustard and slightly after buckwheat and sunflower. Those fungi were the most effective in inhibiting the growth of *Altenaria dauci*, *A. radicina* and *Sclerotinia sclerotiorum* pathogenic towards carrot.

Keywords: soil-borne pathogens; saprotrophic fungi; soil microorganisms; biotic effect; intercropping; root vegetable

Intercrop plants perform a significant protective and conserving role in vegetable cultivation. They protect the soil from the effect of unfavourable external factors, enriching it with the organic substance and mineral nutrients in addition to reducing weed infestation and occurrence of pests (Kołota and Adamczewska-Sowińska 2013, Orluchukwu and Udensi 2013, Jankowska and Wojciechowicz-Żytko 2016). As green fertilizers ploughed over before winter, they fertilize the soil, thereby increasing the yielding of plants (Borowy 2013, Asiimwe et al. 2016).

The most frequently used intercrop plants include oats, rye, vetch, phacelia, white mustard and fodder radish. These plants can improve the microbiological activity of the soils as they favour the development of antagonistic bacteria and fungi (Patkowska et al. 2015). Therefore, they limit the occurrence of soil pathogens, and they improve the plants' healthiness (Patkowska and Konopiński 2013b). The best antagonistic properties are shown by fungi *Trichoderma* spp. and *Clonostachys* spp. (Leelavathi et al. 2014, Reddy et al. 2014, Wu et al. 2018). They produce antibiotics, siderophores and chitinolytic enzymes, which cause decomposition of cell walls and lysis of the mycelium (Sarma et al. 2014, Smitha et al. 2014).

The purpose of the present studies was to determine the effect of intercrop plants (rye, buckwheat, white mustard and sunflower) used in carrot cultivation on the occurrence of *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. in the soil and their antagonistic activity.

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 Haplic Luvisol formed from silty medium loams. The object of the studies was selected fungi isolated from the soil sampled (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 was cultivated. The experiment took into consideration cover crops such as rye (*Secale cereale* L.), white mustard (*Sinapis alba* L.),

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buckwheat (*Fagopyrum esculentum* Moench.) and sunflower (*Helianthus annuus* L.). The conventional cultivation, i.e., without any cover crops, was the control. Completely randomized blocks method at four replications was used in the experiment.

Analysis of fungal community. The microbiological analysis of the soil was made according to the method described by Patkowska (2009a,b) and Patkowska and Konopiński (2014a). The soil was sampled at each experimental treatment from four randomly chosen places. Soil solutions from 10 g of soil with the dilutions from 10^{-1} to 10^{-4} were prepared in laboratory conditions from particular soil samples. Martin's medium was used to establish the 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), and the obtained isolates were determined to the species.

The obtained isolates of *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. served to determine their antagonistic effect towards the following fungi: *Altenaria dauci*, *A. radicina*, *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (isolated from the infected carrot roots). Estimation of the effect of *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. on the studied pathogenic fungi was carried out using the method described by Mańka and Mańka (1992). Most frequently occurring species that build up over 75% of the fungal community were taken into consideration. Their phytopathological function is expressed by individual biotic effect (IBE) that is the effect of one isolate of the given species on the pathogens. The IBE multiplied by the species frequency results in the general biotic effects (GBE), treated as the effect of all the component's isolates on the pathogen. After summarizing all the GBEs the summary biotic effect (SBE) will be obtained, providing the effect of the entire soil fungi community of the pathogen. The summary antagonistic effect of saprotrophic fungi from particular experimental combinations on the studied pathogenic fungi made it possible to determine their antagonistic activity in the soil environment of carrot.

Statistical analysis. The total population of fungi was statistically analysed, and the significance of differences was determined based on 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

As a result of the microbiological analysis of particular soil samples, on average from 47.93 × 10^3 to 92.1 × 10^3 CFU of fungi/g of soil DW were obtained (Figure 1). The smallest fungi population was observed after the use of rye as the intercrop plant in carrot cultivation, and it differed in a statistically significant manner from the population in the other experimental treatments. White mustard, buckwheat and sunflower (as intercrop plants) caused a slight increase in the population of fungi in the soil. Their mean population was 71.43 × 10^3, 79.78 × 10^3 and 85.31 × 10^3 CFU/g of soil DW, respectively. Statistically, the highest fungi population occurred in the soil with the traditional cultivation of carrot (without intercrop plants). The use of oats, common vetch and tansy phacelia (as intercrop plants) in the cultivation of carrot also caused a decrease of the populations of soil-borne fungi and bacteria (Patkowska and Błażewicz-Woźniak 2014). Rye, white mustard, buckwheat, and sunflower used in the cultivation of carrot limited the population of bacteria in the soil (Patkowska 2018). Oats and tansy phacelia as cover plants used in the cultivation of root chicory inhibited the growth of soil-borne fungi population (Patkowska et al. 2015).

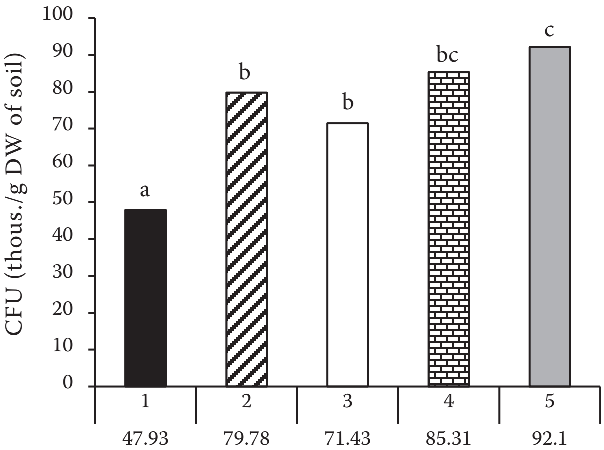

Figure 1. Total number of fungi isolated from the soil of particular experimental treatment (means from 2010–2012). 1 – soil after rye cultivation; 2 – soil after buckwheat cultivation; 3 – soil after white mustard cultivation; 4 – soil after sunflower cultivation; 5 – soil without cover crops cultivation. Means differ significantly ($P < 0.05$) if they are not marked with the same letter. CFU – colony-forming units; DW – dry weight

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

Fungus species	Average number of isolates (2010–2012)	General biotic effect					
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*F. solani*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams.	144	748	604	374	518	490	950
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	70	210	210	210	210	210	560
Penicillium aurantiogriseum Dierckx	36	72	72	36	72	72	72
P. canescens Sopp.	15	30	30	15	15	15	45
P. chrysogenum Thom	18	18	18	18	18	18	36
P. verrucosum Dierckx	33	66	66	33	66	66	66
Trichoderma aureoviride Rifai	32	256	256	192	160	128	256
T. hamatum (Bonord.) Bainier	17	136	119	136	102	85	136
T. harzianum Rifai	39	312	234	312	312	312	312
T. koningii Oudem.	86	688	688	516	430	602	688
T. viride Pers. ex. S.F. Gray	89	712	712	534	534	534	712
Number of isolates	579						
Summary biotic effect		3248	3009	2376	2437	2532	3833

Laboratory tests found the biggest populations of antagonistic *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. in the soil after the use of rye (579) and white mustard (357 isolates) (Tables 1 and 3). Slightly smaller populations of antagonistic fungi were found in the treatments with buckwheat and sunflower (286 and 232 isolates, respectively) and the smallest in control (74 isolates) (Tables 2, 4, 5). Oats, vetch and tansy phacelia as intercrop plants used in carrot cultivation also caused an increase of antagonistic fungi in the soil (Patkowska and Błażewicz-Woźniak 2014). Those species used as cover crops also

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

Fungus species	Average number of isolates (2010–2012)	General biotic effect					
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*F. solani*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams.	67	307	268	201	201	201	335
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	36	108	108	72	72	144	216
Penicillium aurantiogriseum Dierckx	19	19	38	198	38	19	38
P. canescens Sopp.	8	24	16		–8	8	16
P. chrysogenum Thom	10	20	10	–20	10	10	20
P. meleagrinum Biourge	3	3	6	–3	3	6	6
P. verrucosum Dierckx	17	34	34	17	17	17	34
Trichoderma aureoviride Rifai	15	90	90	60	75	75	90
T. hamatum (Bonord.) Bainier	8	48	56	40	48	48	56
T. harzianum Rifai	18	144	144	144	144	90	144
T. koningii Oudem.	40	200	200	280	160	280	200
T. viride Pers. ex. S.F. Gray	45	225	180	225	225	270	270
Number of isolates	286						
Summary biotic effect		1212	1150	1043	985	1168	1425

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

Fungus species	Average number of isolates (2010–2012)	General biotic effect					
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*F. solani*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams.	85	440	425	205	220	305	475
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	45	135	135	135	135	135	315
Penicillium aurantiogriseum Dierckx	22	44	22	22	44	22	44
P. canescens Sopp.	10	10	20	10	–20	–10	30
P. chrysogenum Thom	13	13	26	13	13	13	26
P. meleagrinum Biourge	6	6	12	6	6	6	12
P. verrucosum Dierckx	21	21	42	21	21	21	42
Trichoderma aureoviride Rifai	18	126	108	90	108	72	126
T. hamatum (Bonord.) Bainier	10	70	60	50 168	60	50	80
T. harzianum Rifai	21	168	168		168	168	168
T. koningii Oudem.	53	318	371	265	265	212	318
T. viride Pers. ex. S.F. Gray	53	318	318	265	371	318	318
Number of isolates	357						
Summary biotic effect		1669	1707	1250	1391	1312	1954

promoted the development of antagonistic fungi in the cultivation of root chicory, scorzonera and salsify (Patkowska and Konopiński 2013a, 2014b, Patkowska et al. 2015). The activity of soil-borne pathogens can be limited by the root exudates (phenolic compounds, amino acids, organic acids, sugars, vitamins, metal ions) of phytosanitary plants (oats, rye, vetch, tansy phacelia) which promote the development of antagonistic microorganisms (Li et al. 2013). Cover crops produce phytoncides that affect soil microorganisms and limit the occurrence of pathogens (Kołota and Adamczewska-Sowińska 2013).

Table 4. Activity of selected saprotrophic fungi isolated from soil after sunflower cultivation towards pathogenic fungi

Fungus species	Average number of isolates (2010–2012)	General biotic effect					
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*F. solani*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams.	54	234	201	135	145	168	257
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	31	124	93	62	93	62	124
Penicillium aurantiogriseum Dierckx	13	13	26	13	13	13	26
P. canescens Sopp.	7	7	14	7	7	14	14
P. chrysogenum Thom	9	18	9	9	9	9	18
P. verrucosum Dierckx	11	22	22	–22	11	22	11
Trichoderma aureoviride Rifai	10	60	60	40	50	40	70
T. hamatum (Bonord.) Bainier	4	20	16	16	24	20	24
T. harzianum Rifai	17	136	136	136	136	119	136
T. koningii Oudem.	37	222	185	148	185	185	222
T. viride Pers. ex. S.F. Gray	37	185	222	148	148	148	185
Number of isolates	232						
Summary biotic effect		1041	984	692	821	800	1087

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Table 5. Activity of selected saprotrophic fungi isolated from soil without cover crops cultivation towards pathogenic fungi

Fungus species	Average number of isolates (2010–2012)	General biotic effect					
		Altenaria dauci	*A. radicina*	*Fusarium oxysporum*	*F. solani*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*
Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams.	11	37	29	26	33	36	44
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	10	30	30	30	20	20	30
Penicillium aurantiogriseum Dierckx	9	18	9	9	9	9	18
P. canescens Sopp.	2	4	4	2	2	–4	4
P. chrysogenum Thom	4	4	8	4	–8	4	8
P. verrucosum Dierckx	4	4	4	4	8	8	4
Trichoderma aureoviride Rifai	4	24	20	12	16	20	24
T. hamatum (Bonord.) Bainier	1	4	4	6	5	4	4
T. harzianum Rifai	5	40	35	30	30	35	35
T. koningii Oudem.	9	45	45	63	63	36	36
T. viride Pers. ex. S.F. Gray	15	90	75	60	60	+60	120
Number of isolates	74						
Summary biotic effect		300	263	246	238	228	327

Antagonistic activity of selected saprotrophic fungi towards the tested pathogens varied and was dependent on the species of the intercrop plant. The value of the antagonistic effect of *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. of the studied plant pathogens was the highest after using rye and white mustard in the treatments with buckwheat and sunflower (Tables 1–5). Oats, tansy phacelia and vetch as an intercrop and cover plants in the cultivation of carrot and root chicory also showed a positive effect on the antagonistic activity of *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. towards different species of soil-borne fungi (Patkowska et al. 2015, 2018).

Antagonistic fungi were the most effective in inhibiting the growth of *A. dauci*, *A. radicina*, and *S. sclerotiorum.* After using rye, the value of their total antagonistic effect towards the pathogens was the highest and reached 3248, 3009 and 3833, respectively (Table 1). Slightly smaller values of this index were found after using buckwheat, white mustard, and sunflower. The lowest value of the antagonistic effect towards the enumerated fungi was observed in control (300, 263 and 327, respectively) (Table 5). Antagonistic *Clonostachys* spp., *Albifimbria* spp., *Trichoderma* spp. and *Penicillium* spp. inhibited the growth of *F. oxysporum* and *H. haematococca* the worst. Their values of the total biotic effect ranged from 2376 and 2437 (after the use of rye) to 246 and 238 (in control) (Tables 1–5). The present studies enable to state that the use of cover crops (especially rye and white mustard) in the cultivation of *Daucus carota* L. had a positive effect on the antagonistic activity of soil-borne fungi. A big number of isolates of antagonistic fungi could improve the phytosanitary conditions of the soil. As reported by Gamliel et al. (2000), the development of antagonistic microorganisms could be supported by secondary metabolites introduced into the soil of cover crops. Examples of such compounds are glucosinolates, which – after enzymatic hydrolysis – change into different sulphuric compounds (Gamliel et al. 2000). Those compounds are produced inside the plant tissue, and after the microbiological decomposition of plant residues, they are freed into the soil. Isothiocyanates, which appear in the soil during the decomposition of plants roots, are a product of decomposition of glucosinolates (Vig et al. 2009). According to Bending and Lincoln (2000), those compounds are toxic towards plant pathogens, and they can have a synergistic effect on the communities of microorganisms, through which they can favour the development of antagonistic microorganisms.

The studied antagonistic fungi were also effective towards different plant pathogens in the cultivation of root chicory with cover crops (Patkowska et al.

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2015). Other authors reported high antagonistic activity of *Clonostachys* spp. and *Trichoderma* spp. against bacteria and fungi (Ondráčková et al. 2013, Leelavathi et al. 2014, Reddy et al. 2014, Wu et al. 2018).

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