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Antagonistic activity of selected bacteria of the soil environment of carrot

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ABSTRACT

Patkowska E., Błażewicz-Woźniak M., Wach D. (2017): Antagonistic activity of selected bacteria of the soil environment of carrot. Plant Soil Environ., 63: 277–281.

The purpose of the present studies was to determine the antagonistic effect of bacteria *Bacillus* spp. and *Pseudomonas* spp. towards selected fungi from the soil environment of carrot. Oats (*Avena sativa* L.), tansy phacelia (*Phacelia tanacetifolia* Bentham) and vetch (*Vicia satica* L.) were used as cover crops in the cultivation of this plant. In the traditional cultivation of carrot the population of bacteria was the smallest, while after the application of oats it was the largest. Laboratory tests showed that cover crops promoted the development of antagonistic *Bacillus* spp. and *Pseudomonas* spp. Irrespective of the experimental treatment, those bacteria were the most effective in limiting the growth and development of *Fusarium oxysporum* and *Thanatephorus cucumeris* (syn. *Rhizoctonia solani*), while being less effective towards *Altenaria dauci* and *Alternaria radicina*, and the least towards *Sclerotinia sclerotiorum.* The best total antagonistic effect of *Pseudomonas* spp. and *Bacillus* spp. towards the tested fungi was found after using oats and vetch, while the worst – in the traditional cultivation of carrot.

Keywords: *Daucus carota* L.; microorganisms; root vegetables; fungal infection

Microorganisms occurring in the soil have a considerable effect on the growth, development and healthiness of plants. Antagonistic fungi and bacteria (especially *Pseudomonas* spp. and *Bacillus* spp.) limit the occurrence of different species of soil-borne fungi through antibiosis, competition and parasitism (Saravanan et al. 2013, Patkowska and Konopiński 2014a, Vanitha and Ramjegathesh 2014). This has an especially positive influence on the healthiness of underground parts of plants, including root vegetables (Reis and Nascimento 2011, Patkowska and Konopiński 2013b).

Cover crops, which – when left on the soil surface – make a natural mulch, are used increasingly often in the cultivation of root vegetables (Patkowska and Konopiński 2013c, 2014a,b, Kosterna 2014, Patkowska and Błażewicz-Woźniak 2014). Mulching the soil with cover crops and using living mulches protects it from water evaporation and surface runoffs in addition to enriching it with the organic substance and reducing weeds (Law et al. 2006, Olfati et al. 2008, Kołota and Adamczewska-Sowińska 2013). Cover crops can, therefore, affect the size and quality of the yield of vegetables (Samaila et al. 2011, Kosterna 2014). Moreover, they stimulate the development of microorganisms antagonistic towards plant pathogens (Patkowska and Konopiński 2014a,b, Patkowska et al. 2016).

The purpose of the studies was to establish the effect of cover crops (oats, tansy phacelia and vetch) used

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in carrot cultivation on the population of selected bacteria in the soil. The degree of the antagonistic effect of *Bacillus* spp. and *Pseudomonas* spp. on soil-borne fungi infecting the carrot roots was determined.

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 (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. The experiment used cover crops such as oats (*Avena sativa* L.), tansy phacelia (*Phacelia tanacetifolia*) and vetch (*Vicia satica* L.). The conventional cultivation, i.e. without any cover crops, was the control. The experiment was established in a split-plots scheme, in four replications.

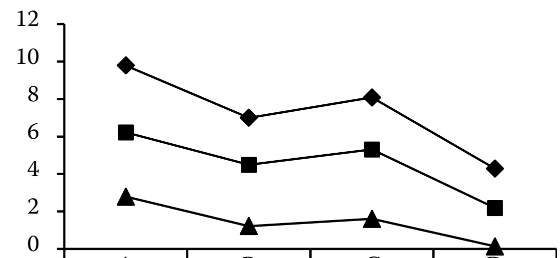
Laboratory analyses. Microbiological analysis was made according to the methods described by Czaban et al. (2007) and Patkowska and Konopiński (2014a). The soil was sampled from each experimental combination from four randomly chosen places. The total population of bacteria was marked on the nutrient 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. After the incubation, the number of bacteria was converted into CFU/g of soil DW (colony forming units/g dry weight (DW) of soil).

In each study year, the obtained isolates of *Bacillus* spp. and *Pseudomonas* spp. (300 isolates from each genus) were used to establish their antagonistic effect towards fungi such as *Altenaria dauci*, *A. radicina*, *Fusarium oxysporum*, *Thanatephorus cucumeris* and *Sclerotinia sclerotiorum* (isolated from the infected carrot roots). While establishing the antagonistic effect of bacteria on pathogenic fungi, laboratory tests were conducted and the method and scale described by Martyniuk et al. (1991), Patkowska and Konopiński (2014a) and Patkowska and Błażewicz-Woźniak (2014) were used. It took into consideration 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.

Statistical analysis. The population of bacteria 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 Statistica program, version 6.0 (StatSoft, Krakow, Poland).

RESULTS AND DISCUSSION

The microbiological analysis of the soil showed that oats, tansy phacelia and vetch used in the cultivation of carrot promoted the development of the populations of the studied bacteria. In the traditional cultivation of carrot (without cover crops) the population of those microorganisms in the soil was the smallest and it differed in a statistically significant way from the population in the other experimental treatments. The total population of bacteria in the soil ranged from 4.28×10^6 to 9.79×10^6 CFU/g of soil DW (Figure 1). The population of *Bacillus* spp. was higher than the population of *Pseudomonas* spp. and it ranged from 2.18×10^6 to 6.22×10^6 CFU/g of soil DW. The population of *Pseudomonas* spp. ranged from 0.14×10^6 (in control) to 2.79×10^6 CFU/g of soil DW (after the application of oats). The highest



	A	B	C	D
B.	9.79	7	8.08	4.28
Bac.	6.22	4.49	5.31	2.18
Ps.	2.79	1.22	1.61	0.14

Figure 1. Total number of bacteria isolated from the soil in individual experimental years (means from the years 2010–2012). *means in verses 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 tansy phacelia cultivation; C – soil after vetch cultivation; D – soil without cover crops cultivation

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Table 1. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after oat cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria dauci*	*A. radicina*	*Fusarium oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2010							
Bacillus spp.	51	51	51	153	102	51	408
Pseudomonas spp.	75	150	225	375	300	75	1125
Total effect of antagonistic activity		201	276	528	402	126	1533
2011							
Bacillus spp.	42	84	126	168	126	84	588
Pseudomonas spp.	48	96	144	240	144	48	672
Total effect of antagonistic activity		180	270	408	270	132	1260
2012							
Bacillus spp.	30	60	60	90	60	30	300
Pseudomonas spp.	37	111	111	185	148	37	592
Total effect of antagonistic activity		171	171	275	208	67	892
Total effect of antagonistic activity		552	717	1211	880	325	3685

statistically significant population of bacteria was found after using oats, while being slightly lower after the application of tansy phacelia and vetch as cover crops. 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. A similar relationship was observed in the bacteria population while using those cover plants in the cultivation of salsify and root chicory (Patkowska and Konopiński 2013a).

As a result of laboratory tests, more antagonistic isolates of *Pseudomonas* spp. than those of *Bacillus* spp. were obtained from each experimental treatment (Tables 1–4). The highest total antagonistic effect of *Pseudomonas* spp. and *Bacillus* spp. on the tested fungi pathogenic towards carrot was observed after using oats and vetch; it was 3685 and 2195, respectively (Tables 1 and 3). Using tansy phacelia, a slightly smaller total antagonistic effect on the tested fungi was found out and it was 1316 (Table 2). In the traditional cultivation of carrot, the total antagonistic effect was the smallest (318) (Table 4). The antagonistic effect of *Pseudomonas* spp. and *Bacillus* spp. towards different species of pathogenic fungi was confirmed by a number of

Table 2. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after tansy phacelia cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria dauci*	*A. radicina*	*Fusarium oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2010							
Bacillus spp.	15	15	15	45	45	15	135
Pseudomonas spp.	24	24	48	72	96	24	264
Total effect of antagonistic activity		39	63	117	141	39	399
2011							
Bacillus spp.	18	18	36	36	36	18	144
Pseudomonas spp.	21	42	42	63	63	21	231
Total effect of antagonistic activity		60	78	99	99	39	375
2012							
Bacillus spp.	20	40	40	60	60	20	220
Pseudomonas spp.	23	46	69	92	69	46	322
Total effect of antagonistic activity		86	109	152	129	66	542
Total effect of antagonistic activity		185	250	368	369	144	1316

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Table 3. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after vetch cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria dauci*	*A. radicina*	*Fusarium oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2010							
Bacillus spp.	26	52	52	104	78	52	338
Pseudomonas spp.	39	117	117	156	156	78	624
Total effect of antagonistic activity		169	169	260	234	130	962
2011							
Bacillus spp.	20	20	40	80	60	20	220
Pseudomonas spp.	24	48	72	96	96	24	336
Total effect of antagonistic activity		68	112	176	156	44	556
2012							
Bacillus spp.	22	44	44	66	66	22	242
Pseudomonas spp.	29	58	87	116	116	58	435
Total effect of antagonistic activity		102	131	182	182	80	677
Total effect of antagonistic activity		339	412	618	572	254	2195

authors (Patkowska 2009, Alemu and Alemu 2013, Dalal and Kulkarni 2013, Saravanan et al. 2013, Patkowska and Konopiński 2014a, Vanitha and Ramjegathesh 2014). Oats and vetch as cover crops used in the cultivation of scorzonera also increased the antagonistic effect of the bacteria towards soil-borne fungi (Patkowska and Konopiński 2014a,b).

Irrespective of the experimental treatment, antagonistic *Bacillus* spp. and *Pseudomonas* spp. were the most effective in limiting the growth and development of *F. oxysporum* and *T. cucumeris*. After using oats, the value of the antagonistic effect of the studied bacteria towards those fungi was 1211 and 880, respectively (Table 1). After using tansy phacelia, the antagonistic effect was 368 and 369, respectively (Table 2), while after using vetch it was 618 and 572 (Table 3). The smallest antagonistic effect of *Bacillus* spp. and *Pseudomonas* spp. towards *F. oxysporum* and *T. cucumeris* (103 and 82) was observed for the control (Table 4). Antagonistic isolates of *Bacillus* spp. and *Pseudomonas* spp. obtained from particular experimental treatments also successfully limited the growth of *A. dauci* and *A. radicina.* The antagonistic effect of the studied bacteria towards the fungi species was the highest in the combination with oats (552 and 717, respectively) (Table 1) and

Table 4. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil without cover crops cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria dauci*	*A. radicina*	*Fusarium. oxysporum*	*Thanatephorus cucumeris*	*Sclerotinia sclerotiorum*	Total effect of antagonistic activity
2010							
Bacillus spp.	5	5	10	20	10	5	50
Pseudomonas spp.	6	12	12	24	18	12	78
Total effect of antagonistic activity		17	22	44	28	17	128
2011							
Bacillus spp.	2	2	2	6	6	2	18
Pseudomonas spp.	4	8	12	16	16	4	56
Total effect of antagonistic activity		10	14	22	22	6	74
2012							
Bacillus spp.	3	3	6	12	12	3	36
Pseudomonas spp.	5	10	15	25	20	10	80
Total effect of antagonistic activity		13	21	37	32	13	116
Total effect of antagonistic activity		40	57	103	82	36	318

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the lowest in the control (40 and 57, respectively) (Table 4). Regardless of the experimental treatment, antagonistic *Bacillus* spp. and *Pseudomonas* spp. had the worst effect in limiting the growth of *S. sclerotiorum* colony. After using oats, tansy phacelia and vetch, the antagonistic effect of bacteria towards this fungus species was 325, 144 and 254, respectively (Tables 1–3). A similar effect of the studied bacteria towards pathogenic fungi was shown by Patkowska and Konopiński (2014a) in the cultivation of scorzonera when cover crops were used. The ability of *Bacillus* spp. and *Pseudomonas* spp. to limit the growth of plant pathogens results from the production of antibiotics, siderophores and HNC having fungistatic and fungicidal properties (Vanitha and Ramjegathesh 2014). According to Dalal and Kulkarni (2013), *Bacillus* spp. and *Pseudomonas* spp. show the antagonistic effect towards, for example, *F. oxysporum*, *T. cucumeris*, *Macrophomina phaseolina* and *Alternaria alternata*, which are pathogenic towards soybean. It is reported by Alemu and Alemu (2013) that *Pseudomonas fluorescens* limits the growth and development of *Botrytis fabae*, which are pathogenic towards faba bean. The application of the cover crops is sufficient to ensure a drop in root vegetable fungal infections. On the other hand, pre-sowing dressing of vegetable seeds with biological and chemical preparations is recommended.

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