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

Elżbieta PATKOWSKA*

Department of Plant Protection, University of Life Sciences in Lublin, Lublin, Poland
**Corresponding author: elzbieta.patkowska@up.lublin.pl*

ABSTRACT

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The studies determined the effect of bacteria *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after carrot cultivation on pathogenic fungi *Altenaria dauci*, *A. radicina*, *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* A field experiment on carrot cultivation considered different intercrop plants (rye, buckwheat, white mustard, sunflower). Rye and buckwheat were the most conducive to the growth of *Bacillus* spp. and *Pseudomonas* spp. Those bacteria were the most effective in inhibiting the growth of *F. solani*, *F. oxysporum* and *R. solani.* The antagonistic effect of soil-borne *Bacillus* spp. and *Pseudomonas* spp. towards the tested fungi was the largest after the application of rye and white mustard as intercrop plants in the cultivation of carrot. Buckwheat and sunflower showed a slightly smaller influence on the antagonistic activity of the studied bacteria. *Bacillus* ssp. had a significantly lesser antagonistic effect than *Pseudomonas* ssp.

Keywords: soil-borne microorganisms; plant pathogens; root vegetable; gram-positive and gram-negative bacterium

In recent years a lot of attention has been drawn to the possibility of using cover crops, intercrops and mulch in the cultivation of different plant species (Univer et al. 2009, Borowy 2013, Patkowska and Konopiński 2014a,b). They can have a positive effect on the microbiological activity of the soil and they enrich the soil with organic substances in addition to reducing weed infestation (Abdullahi et al. 2011, Patkowska and Konopiński 2013a,b, Orzech and Wanic 2014, Patkowska and Błażewicz-Woźniak 2014). Moreover, they increase the population of soil-borne antagonistic microorganisms, which has a positive effect on the health of the cultivated plants (Patkowska and Konopiński 2013c, Patkowska et al. 2015).

Antagonistic bacteria *Bacillus* spp. and *Pseudomonas* spp. have a considerable influence on the plants health. They inhibit the growth and development of plant pathogens living in the soil through antibiosis, competition and parasitism (Adhikari et al. 2013, Patkowska and Błażewicz-Woźniak 2013, Bhattacharjee and Dey 2014, Maurya et al. 2014). Intercrop plants (oats, vetch, tansy phacelia, buckwheat) can contribute to the development of antagonistic soil-borne bacteria and fungi in the cultivation of different plant species (Patkowska et al. 2015).

The purpose of the present studies was to determine the effect of rye, buckwheat, white mustard and sunflower used as intercrop plants in the cultivation of carrot on the soil populations of *Bacillus* spp. and *Pseudomonas* spp. and their antagonistic effect towards the soil-borne fungi.

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 objects of the studies were selected bacteria 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

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into consideration cover crops such as rye (*Secale cereale* L.), buckwheat (*Fagopyrum esculentum* Moench.), white mustard (*Sinapis alba* L.) and sunflower (*Helianthus annuus* 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 analysis. The microbiological analysis of the soil of particular experimental treatments was made according to the method described by Patkowska (2009a,b) and Patkowska and Konopiński (2014a). The soil was sampled from each experimental treatment from four randomly chosen places. It served to establish the total populations of bacteria, the populations of *Pseudomonas* spp. and the spores of *Bacillus* spp. in 1 g dry weight (DW) of the soil. After preparing the proper dilutions of the soil solution, the total number of bacteria was marked on the nutrient agar. Tryptic soy agar was used to determine the number of *Bacillus* spp. bacteria, and 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 studied 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*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (isolated from the infected carrot roots). The antagonistic effect of the studied bacteria on pathogenic fungi was established according to the method and scale described by Patkowska and Konopiński (2013a, 2014a) and Patkowska and Błażewicz-Woźniak (2014). It took into consideration five degrees, i.e. 1° – no inhibition zone; 2° – inhibition zone of 1–2 mm; 3° – inhibition zone of 3–5 mm; 4° – inhibition zone of 6–10 mm, 5° – 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 the Statistica program, version 6.0 (StatSoft Inc., Krakow, Poland).

RESULTS AND DISCUSSION

The studies showed that rye used as an intercrop plant in the cultivation of carrot had the most positive effect on the development of bacteria in the soil. White mustard, buckwheat and sunflower also showed a positive influence on the populations of the studied soil-borne bacteria. The total population of bacteria after the application of buckwheat was the highest (9.05×10^6 CFU/g of soil DW) and it differed in a statistically significant manner from the populations in the other experimental treatments (Figure 1). The smallest population of total

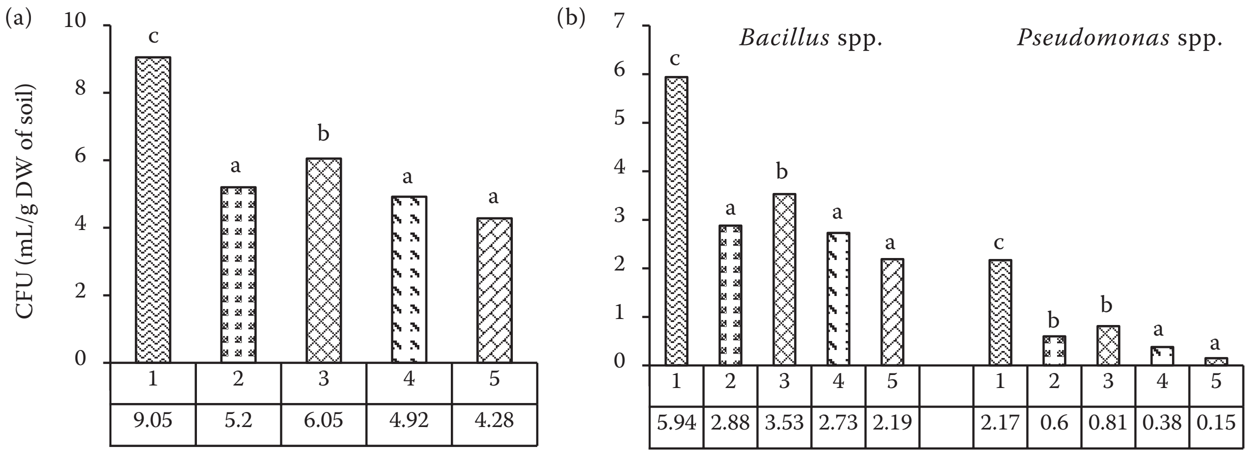

Figure 1. Total number of (a) bacteria and (b) *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil of particular experimental treatment (means from the years 2010–2012). 1 – soil after rye cultivation; 2 – soil after buckwheat cultivation; 3 – soil after white mustard cultivation; 4 – soil 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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bacteria was obtained from the control treatment (4.28×10^6 CFU/g of soil DW). More bacteria of *Bacillus* spp. as compared to *Pseudomonas* spp. were isolated from the studied soil samples. The largest population of those microorganisms was found in the soil with rye as an intercrop plant (5.94×10^6 and 2.17×10^6 CFU/g of soil DW, respectively) and it significantly differed from the population in the other experimental treatments. A little smaller populations of *Bacillus* spp. and *Pseudomonas* spp. were obtained after white mustard (3.53×10^6 and 0.81×10^6 CFU/g of soil DW, respectively), buckwheat (2.88×10^6 and 0.6×10^6 CFU/g of soil DW, respectively) and sunflower (2.73×10^6 and 0.38×10^6 CFU/g of soil DW, respectively) (Figure 1). A similar effect on the population of the studied soil-borne bacteria was shown by oats, tansy phacelia and vetch used as plant mulches in the cultivation of root chicory, carrot and scorzonera (Patkowska and Konopiński 2013a,b, Patkowska et al. 2017). These plant species also favoured the development of antagonistic microorganisms in carrot cultivation (Patkowska and Błażewicz-Woźniak

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

Genus of bacteria	Number of antagonistic isolates	*Altenaria dauci*		*A. radicina*		*Fusarium oxysporum*		*F. solani*		*Rhizoctonia solani*		*Sclerotinia sclerotiorum*		Total effect of antagonistic activity
		1*	2*	1	2	1	2	1	2	1	2	1	2	
Soil after rye cultivation towards pathogenic fungi														
2010														
Bacillus spp.	37	2	74		111	4	148	5	185	4	148	2	74	740
Pseudomonas spp.	56	3	168		224	4	224	5	280	5	280	2	112	1288
Total effect of antagonistic activity			242		335		372		465		428		186	2028
2011														
Bacillus spp.	33	1	33	2	66	3	99	4	132	3	99	1	33	462
Pseudomonas spp.	37	3	111	3	111	4	148	5	185	4	148	2	74	777
Total effect of antagonistic activity			144		177		247		317		247		107	1239
2012														
Bacillus spp.	26	2	52	3	78	4	104	5	130	4	104	1	26	494
Pseudomonas spp.	31	3	93	4	124	5	155	5	155	4	124	2	62	713
Total effect of antagonistic activity			145		202		259		285		228		88	1207
Total effect of antagonistic activity			531		714		878		1067		903		381	4474
Soil after buckwheat cultivation towards pathogenic fungi														
2010														
Bacillus spp.	9	1	9	2	18	2	18	4	36	2	18	1	9	108
Pseudomonas spp.	15	2	30	2	30	3	45	4	60	3	45		30	240
Total effect of antagonistic activity			39		48		63		96		63		39	348
2011														
Bacillus spp.	12	2	24	2	24	3	36	4	48	3	36	1	12	180
Pseudomonas spp.	16	2	32		48	3	48	5	80	4	64	2	32	304
Total effect of antagonistic activity			56		72		84		128		100		44	484
2012														
Bacillus spp.	13	2	26	2	26	2	26	3	39	3	39	1	13	169
Pseudomonas spp.	14	2	28	2	28	3	42	4	56	3	42	1	14	210
Total effect of antagonistic activity			54		54		68		95		81		27	379
Total effect of antagonistic activity			149		174		215		319		244		110	1211

*1 – effect of antagonistic activity; *2 – total effect of antagonistic activity (*2 = number of antagonistic isolates × *1)

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Table 2. Antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. isolated from soil after white mustard cultivation towards pathogenic fungi, from soil after sunflower cultivation towards pathogenic fungi and from soil without cover crops cultivation towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*Altenaria dauci*		*A. radicina*		*Fusarium oxysporum*		*F. solani*		*Rhizoctonia solani*		*Sclerotinia sclerotiorum*		Total effect of antagonistic activity
		1*	2*	1	2	1	2	1	2	1	2	1	2	
Soil after white mustard cultivation towards pathogenic fungi														
2010														
Bacillus spp.	15	2	30	2	30	3	45	4	60	3	45	1	15	225
Pseudomonas spp.	23	2	46	3	69	4	92	5	115	4	92	2	46	460
Total effect of antagonistic activity			76		99		137		175		137		61	685
2011														
Bacillus spp.	15	2	30	3	45	3	45	5	75	4	60	2	30	295
Pseudomonas spp.	18	3	54	4	72	4	72	5	90	4	72	2	36	396
Total effect of antagonistic activity			84		117		117		165		132		66	681
2012														
Bacillus spp.	15	1	15	1	15	2	30	4	60	3	45	1	15	180
Pseudomonas spp.	16	2	32	3	48	3	48	4	64	4	64	2	32	288
Total effect of antagonistic activity			47		63		78		124		109		47	468
Total effect of antagonistic activity			207		279		332		464		378		174	1834
Soil after sunflower cultivation towards pathogenic fungi														
2010														
Bacillus spp.	11	1	11	1	11	3	33	3	33	2	22	1	11	121
Pseudomonas spp.	13	2	26	2	26	3	39	4	52	3	39	1	13	195
Total effect of antagonistic activity			37		37		72		85		61		24	316
2011														
Bacillus spp.	9	1	9	2	18	2	18	3	27	2	18	1	9	99
Pseudomonas spp.	11		22	2	22	3	33	3	33	3	33	2	22	165
Total effect of antagonistic activity			31		40		51		60		51		31	264
2012														
Bacillus spp.	12	2	24	2	24	2	24	3	6	3	36	2	24	168
Pseudomonas spp.	14	2	8	2	28	3	42	4	56	3	42	2	28	224
Total effect of antagonistic activity			52		52		66		92		78		52	392
Total effect of antagonistic activity			120		129		189		237		190		107	972
Soil without cover crops cultivation towards pathogenic fungi														
2010														
Bacillus spp.	5	1	5	1	5	2	10	3	15	2	10	1	5	50
Pseudomonas spp.	6	1	6	2	12	2	12	4	24	2	12	1	6	72
Total effect of antagonistic activity			11		17		22		39		22		11	122
2011														
Bacillus spp.	2	1	2	2	4	2	4	3	6	3	6	1	2	24
Pseudomonas spp.	3	2	6	2	6	3	9	4	12	3	9	2	6	48
Total effect of antagonistic activity			8		10		13		18		15		8	72
2012														
Bacillus spp.	3	1	3	1	3	2	6	3	9	3	9	1	3	33
Pseudomonas spp.	5	2	10	2	10	3	15	3	15	3	15	1	5	70
Total effect of antagonistic activity			13		13		21		24		24		8	103
Total effect of antagonistic activity			32		40		56		81		61		27	297

*1 – effect of antagonistic activity; *2 – total effect of antagonistic activity (*2 = number of antagonistic isolates × *1)

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2014). The earlier studies showed that oats, vetch and tansy phacelia promoted the development of *Bacillus* spp. and *Pseudomonas* spp. and were the most effective in limiting the growth and development of *F. oxysporum* and *R. solani* (Patkowska et al. 2017).

Laboratory tests showed that *Bacillus* spp. and *Pseudomonas* spp. obtained from the soil after the application of rye and white mustard as intercrop plants had the highest antagonistic effect on all studied fungi pathogenic towards carrot. The values of this effect were 4474 and 1834 (Tables 1 and 2). A slightly smaller antagonistic effect was shown by the bacteria isolated from the soil after the cultivation of buckwheat and sunflower (1211 and 972, respectively) (Tables 1 and 2). The smallest antagonistic effect (297) was found for the control (Table 2). Other species (tansy phacelia, vetch, oats) used as cover crops in the cultivation of scorzonera and salsify had a positive effect on the antagonistic activity of *Bacillus* spp. and *Pseudomonas* spp. towards pathogenic fungi, especially *F. oxysporum* and *R. solani* (Patkowska and Konopiński 2014a,b). The earlier studies showed that these bacteria inhibited the growth of polyphagous pathogens *F. oxysporum* and *R. solani* more than *Alternaria dauci* and *A. radicina* typical for carrots (Patkowska et al. 2017).

Antagonistic *Bacillus* spp. and *Pseudomonas* spp. limited the growth of *F. solani* the most. The value of the antagonistic effect of bacteria towards this pathogen ranged from 1067 (after the application of rye) to 81 (control) (Tables 1–2). The studied bacteria also effectively limited the growth of *F. oxysporum* and *R. solani.* The antagonistic effect towards those fungi ranged from 878 to 56 and from 903 to 61, respectively (Tables 1–2). All experimental treatments revealed a slightly smaller antagonistic effect of *Bacillus* spp. and *Pseudomonas* spp. towards *A. dauci* and *A. radicina*. The tested bacteria worst inhibited the growth of *S. sclerotiorum*. The values of the antagonistic effect of bacteria towards this fungus in the treatments after the application of rye, white mustard, buckwheat and sunflower were 381, 174, 110 and 107, respectively (Tables 1–2). Antagonistic *Bacillus* spp. and *Pseudomonas* spp. isolated from the soil after the cultivation of oats, tansy phacelia and vetch limited the growth of the tested pathogenic fungi in a similar manner (Patkowska and Konopiński 2014a,b,c). The earlier studies showed that the antagonistic effect of soilborne *Bacillus* spp. and *Pseudomonas* spp. towards the tested fungi in the cultivation of carrot was the largest after the application of oats and vetch as intercrop plants (Patkowska et al. 2017).

In each experimental treatment, the antagonistic effect of *Bacillus* spp. towards the tested fungi was significantly smaller than the antagonistic effect of *Pseudomonas* ssp.

A great effect of the studied bacteria towards *Fusarium* spp., *Rhizoctonia solani* and *Alternaria alternata* was confirmed also by other authors (Killani et al. 2011, Adhikari et al. 2013, Jankiewicz et al. 2013, Bhattacharjee and Dey 2014, Maurya et al. 2014). The earlier studies showed the high harmfulness and significance of the tested pathogens, especially *Altenaria* ssp., *F. oxysporum*, *R. solani* and *S. sclerotiorum*, for root vegetables (Patkowska and Konopiński 2013c,d, 2014b). These fungi infected the roots of seedlings and older plants of salsify, root chicory, scorzonera and carrot (Mazur and Nawrocki 2007, Patkowska and Konopiński 2008a,b, 2011). According to Pięta and Kęsik (2007), antagonistic saprotrophic microorganisms occurring in the soil, especially in the root zone, protect plant roots from infection by pathogenic fungi and bacteria, which is very important in the cultivation of root vegetables. They can be successfully used to protect carrot against alternariosis, fusariosis, rhizoctoniosis and white mould (Mazur and Nawrocki 2007, Patkowska and Błażewicz-Woźniak 2014).

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