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ORIGINAL PAPER

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COVER CROPS AND SOIL-BORNE FUNGI DANGEROUS TOWARDS THE CULTIVATION OF *Daucus carota* L.

Elżbieta Patkowska✉

Department of Plant Protection, Leszczyńskiego 7, 20–069 Lublin, University of Life Sciences in Lublin, Poland

ABSTRACT

The principles of good agricultural and horticultural practice, which considers both environmental protection and high yielding of plants, require modern methods of cultivation. Cover crops are used as the previous cropping in agricultural plant technology, including root vegetables such as carrot. The purpose of field and laboratory studies was to determine the effect of selected cover crops on the healthiness of carrot (*Daucus carota* L.). The field experiment took into consideration cover crops such as spring rye, white mustard, buckwheat, fodder sunflower and two systems of soil tillage, i.e.: tillage before winter (ploughing) + spring tillage (a combined cultivator) and only spring tillage (a combined cultivator). In each experimental treatment, the number and healthiness of carrot seedlings were determined. A laboratory mycological analysis made it possible to determine the qualitative and quantitative composition of fungi infecting the underground parts of carrot. Good emergencies and the healthiness of carrot plants were observed in the objects with rye and white mustard as cover crops. The cultivation system had no significant effect on the proportion of diseased seedlings of carrot. Rye and white mustard were more effective than buckwheat and sunflower in limiting the occurrence of fungi pathogenic towards carrot. Diseased seedlings and roots after harvest of carrot were most frequently colonized by *Altenaria alternata*, *A. chartarum*, *A. dauci*, *A. radicina*, *Sclerotinia sclerotiorum*, *Rhizoctonia solani* and *Fusarium* spp.

Key words: carrot, pre-winter and spring tillage, soil pathogens, healthiness of plants

INTRODUCTION

The principles of good agricultural and horticultural practice, which considers both environmental protection and high yielding of plants, require modern methods of cultivation. The modern cultivation of horticultural plants uses, for example, cover crops and living mulches protecting the soil from degradation and the plants from plant pathogens and stress [Chavarría et al. 2016, Campanelli et al. 2019, Toom et al. 2019].

Cover crops (oats, rye, turnip rape, forage radish, hairy vetch, white mustard, berseem clover) are used in the cultivation of various plants, including root vegetables such as carrot, root celery or parsley [Błażewicz-Woźniak 2005, Patkowska et al. 2019, Toom et al. 2019]. They are cultivated between periods of cash crop production and can protect the soil from loss of nutrients [Thorup-Kristensen et al. 2003, Toom et al. 2019]. Moreover, cover crops can positively influence beneficial soil microorganisms and their microbial activity [Patkowska and Konopiński M. 2014a,b, Piotrowska-Długosz and Wilczewski 2014, Patkowska et al. 2015, Chavarría et al. 2016, Toom et al. 2019]. The beneficial effects also include the suppression of weeds [Campigila et al. 2012, Madsen et al. 2016] and the control of soil-borne phytopathogens [Dawadi et al. 2019, Patkowska et al. 2016, 2019, Toom et al. 2019].

✉ elzbieta.patkowska@up.lublin.pl

Carrot (*Daucus carota* L.) is a very important root vegetable plant. Information in the literature mainly concerns such carrot pathogens as: *Pectobacterium carotovorum* pv. *carotovorum*, *Xanthomonas campestris* pv. *carotae* [Nesha and Siddiqui 2013], *Streptomyces scabiei* [Lerat et al. 2009], *Alternaria alternata*, *Alternaria dauci*, *Alternaria radicina* [Mazur and Nawrocki 2007, Koutouan et al. 2018, Le Clerc et al. 2019], *Sclerotinia sclerotiorum* and *Rhizoctonia solani* [Lamichhane et al. 2017, Baturo-Cieśniewska et al. 2018, Siddiqui et al. 2019]. These fungi were isolated from diseased carrot plants.

The purpose of field and laboratory studies was to determine the effect of selected cover crops (white mustard, rye, buckwheat and fodder sunflower) on the healthiness of carrot (*Daucus carota* L.).

MATERIAL AND METHODS

Field trials. The field experiment was carried out in 2009–2012 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 were carrot plants cv. 'Flakkese 2'. The seeding rate was 2.61 kg ha^{-1}. The experiment took into consideration cover crops such as spring rye (*Secale cereale* L.), white mustard (*Sinapis alba* L.), buckwheat (*Fagopyrum esculentum* Moench), fodder sunflower (*Helianthus annuus* L.) and two systems of soil tillage, i.e.: tillage before winter (ploughing) + spring tillage (a combined cultivator) and only spring tillage (a combined cultivator). Before sowing the cover crops, the soil contained 1.06–1.15% of humus in the 0–20 cm layer and was characterized by slightly acidic reaction (pH in 1 M KCl 5.76–5.90). The amount of available phosphorus, potassium, and magnesium was as follows: P – 146.8; K – 111.5; Mg – 102.9 mg kg^{-1} soil. Cultivation without cover crops was the control. The experiment was set up as a completely randomized block design in 4 replicates. The area of a single plot was $33m^2$. The weather conditions during the carrot growing season in the years 2010–2012 are shown

Phot. 1. The seedlings of *Daucus carota* infected by *Fusarium* spp. (photo by E. Patkowska)

Phot. 2. Sclerotia and mycelium of *Sclerotinia sclerotiorum* on the carrot roots (photo by E. Patkowska)

in Figure 1 (on the basis of the information obtained from the Chair of Agrometeorology of the University of Life Sciences in Lublin). Every year, the cover crops were sown on the same date, i.e. on August 1st.

Laboratory mycological analysis. In each year of studies, the number and healthiness of carrot seedlings were determined. 10 seedlings with disease symptoms (Phot. 1) 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 (Phot. 2) 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. 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 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

Field trials. Thermal conditions in the years of studies promoted the emergence of carrot plants. The mean May temperature in particular years of studies was higher (by 1.3°C–2°C) than the means of many years (Fig. 1A). Since the beginning of plants' vegetation, throughout the following months of the cultivation and until September, the mean temperatures of each month were higher than the mean temperatures of many years. The air temperature was lower (on average, by 0.4°C and 2.3°C, respectively) than the many-years' means only in September and October, 2010, i.e. during the root harvest.

Considering the precipitation, 2010 did not promote the emergence and further growth of carrot plants since the monthly sums of precipitation from May to September (except June) was far higher than the mean sums of many years (Fig. 1B). Such atmospheric conditions decreased the healthiness of carrot seedlings as in 2010 the proportion of infected seedlings (in all experimental combinations) was the highest (Tab. 1). In the successive two years the monthly sums of precipitation differed from the means of many years only slightly. It was only in July 2011 when very heavy rainfalls occurred (the sum of rainfalls was 189 mm), while September of that years was a dry month (the sum of rainfalls was only 5.4 mm). High temperatures and high rainfall are conditions conducive to the development of pathogenic fungi [Patkowska and Konopiński 2013b, Kurzawińska et al. 2019].

Regardless of the system of cultivation, good emergencies of carrot plants were observed in the objects with rye and white mustard, and slightly worse with buckwheat or sunflower as cover crops. The mean number of carrot seedlings in all experimental treatments ranged from 44.9 to 68.7 plants · m^{-2} (Tab. 1). The smallest density of plants was in control (on average, 44.9 in pre-winter + spring cultivation and 51.6 in spring cultivation) and it significantly differed from the density in the other experimental treatments. Domagała-Świątkiewicz et al. [2019] found out a positive effect of vetch and rye on the emergences and yielding of celery and zucchini. Studies conducted by Kęsik et al. [2000] and Błażewicz-Woźniak [2005] also pointed to a positive effect of rye, oats and tansy phacelia on the emergences, healthiness and yielding of carrot and root parsley.

Observations found out seedlings of inhibited growth and development on all the plots of particular experimental treatments. After digging out the roots, brown necrotic spots were visible (Phot. 1). Disease symptoms in the form of rot or dry necrosis with the mycelium hyphas were also observed on the roots after the harvest of carrot (Phot. 2). The reason for these disease symptoms might be, for example: *Alternaria alternata*, *A. dauci*, *A. radicina*, *Sclerotinia sclerotiorum*, *Fusarium* spp. [Mazur and Nawrocki 2007, Nawrocki and Mazur 2011, Ahmad and Siddiqui 2017, Que et al. 2019].

The proportion of the infected seedlings of *Daucus carota* ranged, on average, from 14.7% to 42.3% (Tab. 1). The cultivation system, i.e. tillage before winter (ploughing) + spring tillage (a combined cultivator) and only spring tillage (a combined cultivator) had no significant effect on the proportion of diseased seedlings of carrot. In each year of studies the highest number of infected seedlings was observed in the control. Statistically, the smallest such seedlings was found after using rye and white mustard. The reasons for worse emergencies and healthiness of older plants can be different species of soil-borne fungi [Patkowska et al. 2016, Lamichhane et al. 2017]. Studies conducted by Patkowska and Konopiński [2013a, 2014a,b], Patkowska and Błażewicz-Woźniak [2014] and Dawadi et al. [2019] showed that cover plants (spring vetch, rye, oats, white mustard, tansy phacelia) significantly reduce the population of pathogenic fungi in the soil environment, thus positively affecting the healthiness of the cultivated plants.

Laboratory mycological analysis. A total of 1628 colonies of fungi and fungus-like organisms were isolated from diseased seedlings of carrot (Tab. 2). Of all the isolates, fungi *Alternaria* spp. and *Fusarium* spp. were decidedly the dominant pathogens. The genus of *Altenaria* was represented by the species of *Altenaria alternata*, *A. dauci*, *A. radicina* and their total proportion was 5.6%, 4.1% and 8.6% (Fig. 2). The genus of *Fusarium* included the species of *Fusarium avenaceum* (6.7%) and *F. oxysporum* (9.9%). In addition, the following considered as potential pathogens were

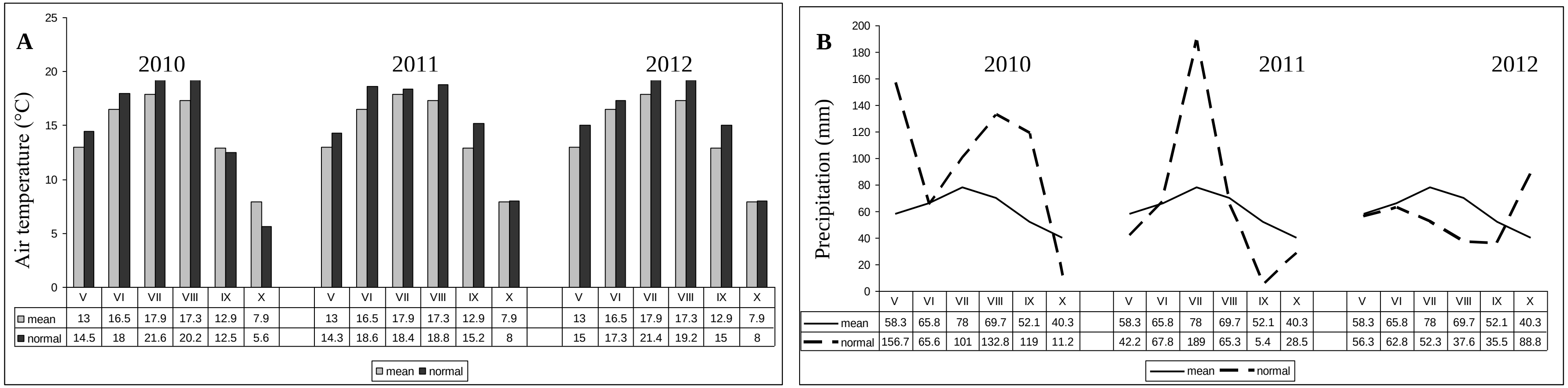

Fig. 1. Average temperatures (A) and sum of precipitation (B) in vegetation season of the experimental years on the background of the long-term average (1951–2000)

Table 1. 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	
	a*	b	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Rye	88.4a	76.8a	72.6a	70.1a	45.2a	45.0a	68.7a	64.0a	16.3c	19.5c	13.5c	15.2c	14.3c	15.5c	14.7c	16.7c
White mustard	82.2a	80.4a	68.4a	61.3b	44.5a	43.2a	65.0a	61.6a	30.4ab	33.2b	22.6b	24.0b	25.6b	27.8b	26.2b	28.3b
Buckwheat	68.4b	66.2b	62.2b	58.8b	40.5ab	36.3b	57.0b	53.8b	35.7ab	37.0b	28.3ab	29.6ab	30.0ab	32.1ab	31.3ab	32.9b
Sunflower	65.5b	62.3b	58.5b	50.2c	42.8ab	35.4bc	55.6b	49.3b	40.5a	42.4a	30.1a	32.4a	34.5a	36.5a	35.0a	37.1ab
Conventional cultivation (control)	61.8b	50.6c	55.4b	51.6c	37.6b	32.6c	51.6c	44.9c	47.6a	49.3a	35.4a	37.0a	39.2a	40.5a	40.7a	42.3a

* mean values in columns marked with the same letter do not differ significantly at $P \leq 0.05$; *a – pre-winter and spring cultivation, b – spring cultivation

Table 2. Microorganisms isolated from diseased seedlings of carrot (sum 2010–2012)

Microorganisms	Experimental treatment / Number of isolates										
	Rye		White mustard		Buck-wheat		Sunflower		Control		Total
	a*	b	a	b	a	b	a	b	a	b	
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	14	12	10	6	7	5	6	4	2	–	66
Alternaria alternata (Fr.) Keissler	2	4	5	6	7	10	9	12	17	19	91
Alternaria dauci (J.G. Kühn) J.W. Groves	1	–	5	6	8	10	8	10	8	11	67
Alternaria radicina Meier Drechsler & E.D. Eddy	3	5	10	14	12	16	15	18	22	25	140
Athelia arachnoidea (Berk.) Jülich	–	–	–	–	3	5	7	9	9	15	48
Cladosporium cladosporioides (Fresen.) G.A. de Vries	1	2	4	6	5	8	7	10	10	14	67
Cylindrocarpon didymum (Harting) Wollenw.	–	–	5	8	7	10	8	10	10	15	73
Epicoccum nigrum Link	–	2	6	8	9	12	10	12	11	16	86
Fusarium avenaceum (Fr.) Sacc.	2	5	9	12	11	13	12	14	14	17	109
Fusarium oxysporum Schltdl.	3	6	13	15	15	18	16	20	26	30	162
Globisporangium irregulare (Buisman) Uzuhashi, Tojo & Kakish.	2	5	10	12	12	14	12	17	15	20	119
Mucor hiemalis Wehmer	–	–	3	4	5	6	6	10	8	12	54
Neocosmospora solani (Mart.) L. Lombard & Crous	5	7	8	9	10	9	11	12	14	20	105
Penicillium canescens Sopp.	–	–	–	–	6	8	8	9	11	14	56
Penicillium verrucosum Dierckx	1	2	3	5	5	7	6	8	8	11	56
Phytophthora sp.	–	–	5	6	7	8	7	9	7	11	60
Rhizoctonia solani J.G. Kühn	5	7	12	10	14	12	15	18	18	24	135
Rhizopus stolonifer (Ehrenb.) Vuill.	–	–	2	3	3	4	4	5	6	10	37
Trichoderma aureoviride Rifai	8	6	6	4	4	2	4	2	3	1	40
Trichoderma viride Pers.	15	8	9	5	7	3	6	2	2	–	57
Total	62	71	125	139	157	180	177	211	221	285	1628

*a – pre-winter and spring cultivation, b – spring cultivation

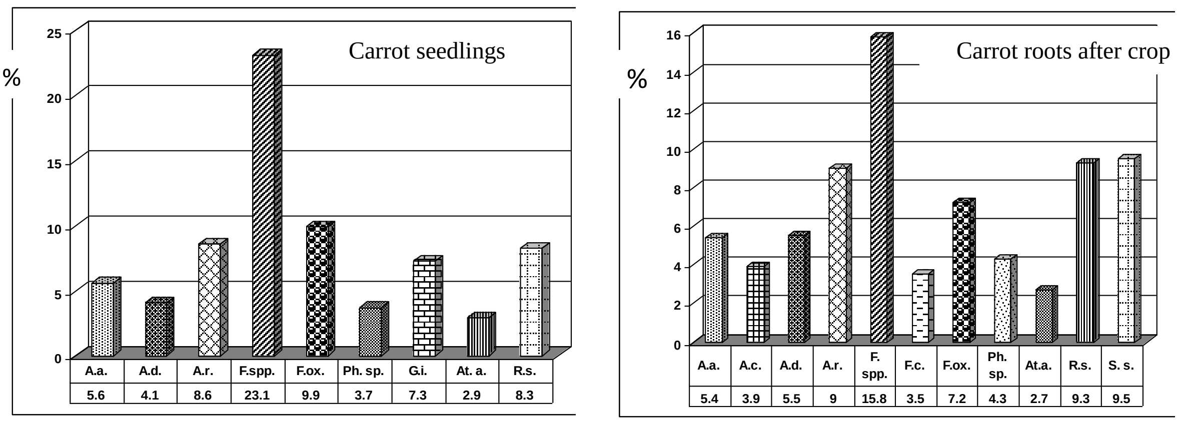

Fig. 2. Total participation of selected microorganisms isolated from carrot plants (mean from the years 2010–2012)

A.a. – *Alternaria alternata*, A.c. – *Alternaria chartarum*, A.d. – *Alternaria dauci*, A. r. – *Alternaria radicina*, F.spp. – *Fusarium* spp., F.c. – *Fusarium culmorum*, F.ox. – *Fusarium oxysporum*, Ph.sp. – *Phytophthora* sp., G.i. – *Globisporangium irregulare*, At.a. – *Athelia arachnoidea*, R.s. – *Rhizoctonia solani*, S.s. – *Sclerotinia sclerotiorum* isolated from the diseased seedlings of carrot: *Rhizoctonia solani* (8.3%), *Globisporangium irregulare* (7.3%), *Phytophthora* sp. (3.7%) and *Athelia arachnoidea* (2.9%). The least of these fungi was obtained from the infected seedlings after using rye and white mustard as cover crops. *Alternaria* spp. are common, and the pathogenic species cause diseases of various plants [Mangwende et al. 2018, Ding et al. 2019, Kurzawińska et al. 2019]. Mazur and Nawrocki [2007], Ahmad and Siddiqui [2017], Le Clerc et al. [2019] found out high harmfulness of *A. alternata*, *A. dauci*, *A. radicina* towards the seedlings and roots of carrot. As reported by Kathe et al. [2017], *A. radicina* is a fungal pathogen that causes the black rot disease of

Table 3. Microorganisms isolated from diseased roots of carrot after harvest (sum 2010–2012)

Microorganisms	Experimental treatment / Number of isolates										Total
	Rye		White mustard		Buckw-heat		Sunflower		Control		
	a*	b	a	b	a	b	a	b	a	b	
Albifimbria verrucaria (Alb. & Schwein.) L. Lombard & Crous	9	6	7	5	6	3	4	2	2	–	44
Alternaria alternata (Fr.) Geissler	2	4	8	10	9	12	10	14	23	27	119
Alternaria chartarum Preuss	–	–	4	6	6	8	9	11	12	16	72
Alternaria dauci (J.G. Kühn) J.W. Groves	1	2	7	10	9	12	11	14	17	20	103
Alternaria radicina Meier, Drechsler & E.D. Eddy	2	4	12	15	15	18	16	19	31	35	167
Athelia arachnoidea (Berk.) Jülich	–	–	2	3	4	7	5	9	8	12	50
Cladosporium cladosporioides (Fresen.) G.A. de Vries	–	–	6	8	7	9	9	11	9	12	71
Cylindrocarpon didymum (Harting) Wollenw.	–	1	7	9	8	10	8	12	12	15	82
Epicoccum nigrum Link	–	2	5	5	6	8	8	10	10	14	68
Fusarium culmorum (Wm.G.Sm.) Sacc.	–	–	4	6	7	8	8	10	10	12	65
Fusarium oxysporum Schltdl.	7	8	12	14	13	15	14	16	16	19	134
Mucor hiemalis Wehmer	–	–	–	–	4	6	5	7	7	10	39
Neocosmospora solani (Mart.) L. Lombard & Crous	–	–	8	9	10	11	10	15	11	21	95
Penicillium canescens Sopp.	2	3	4	5	6	8	6	9	8	11	62
Penicillium verrucosum Dierckx	–	–	–	2	3	5	4	6	6	8	34
Phytophthora sp.	–	–	6	7	8	10	9	12	11	17	80
Rhizoctonia solani J.G. Kühn	8	10	15	17	17	20	18	20	22	25	172
Rhizopus stolonifer (Ehrenb.) Vuill.	–	–	5	6	7	9	8	10	12	16	73
Sclerotinia sclerotiorum (Lib.) de Bary	6	9	14	16	16	18	17	21	26	34	177
Trichoderma harzianum Rifai	10	8	6	4	4	3	4	2	2	–	43
Trichoderma koningii Oudem.	22	16	14	12	11	8	10	6	6	2	107
Total	69	73	146	169	176	208	193	236	260	327	1857

*a – pre-winter and spring cultivation, b – spring cultivation carrot. Alternaria leaf blight, caused by *A. dauci*, is the most damaging foliar disease affecting carrots [Koutouan et al. 2018].

After the harvest, 1857 colonies of microorganisms belonging to 15 genera were obtained from the roots of *Daucus carota* (Tab. 3). *Altenaria radicina* (9%), *A. dauci* (5.5%), *A. alternata* (5.4%), *A. chartarum* (3.9%), *Fusarium* spp. (15.8%), *Sclerotinia sclerotiorum* (9.5%), *Rhizoctonia solani* (9.3%), *Phytophthora* sp. (4.3%) and *Athelia arachnoidea* (2.7%) were also isolated from the roots of carrot after the harvest (Fig. 2). The greatest amount of these fungi were obtained from roots of carrot cultivated without cover crops. According to Baturo-Cieśniewska et al. [2018] and Siddiqui et al. [2019], carrot cultivation may be threatened by *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. As reported by Lamichhane et al. [2017], *Athelia arachnoidea* is a corticioid fungus that causes the carrot rhizoctoniosis (black rot).

The system of soil tillage including tillage before winter (ploughing) + spring tillage (a combined cultivator) showed a more psoitive effect on the healthiness of carrot plants, while the system consisting only of spring tillage (a combined cultivator) resulted in slightly greater infection of the studied plant species by pathogenic fungi. In addition, regardless of the system of soil cultivation, rye and white mustard limited the occurrence of fungi pathogenic towards *Daucus carota* in a greater degree than buckwheat and sunflower. Earlier studies [Patkowska et al. 2016] also confirmed big effectiveness of cover crops (rye and white mustard) in limiting the population of soil-borne fungi in carrot cultivation.

Within saprotrophic fungi, *Albifimbria* spp., *Cladosporium* spp., *Epicoccum* spp., *Mucor* spp., *Penicillium* spp. and *Trichoderma* spp. were isolated from the diseased seedlings and roots of carrot after the harvest (Tab. 2 and 3). *Trichoderma* spp. were often isolated from carrot roots cultivated after rye and white mustard as a cover crops, while slightly less after buckwheat and sunflower. Saprotrophic fungi (*Clonostachys* spp., *Trichoderma koningii*, *T. harzianum*, *T. viride*) were also often isolated from root chicory and scorzonera cultivated after oats, tansy phacelia and spring vetch [Patkowska and Konopiński 2013b, c]. These fungi can limit the development of pathogenic fungi [Abbas et al. 2017, Nygren et al. 2018, Patkowska et al. 2019, Singh et al. 2019] and have a positive influence on the healthiness of various plants.

CONCLUSIONS

1. The healthiness of *Daucus carota* was differentiated and it depended on the species of cover crops.
2. Rye and white mustard were more effective than buckwheat and sunflower in limiting the occurrence of fungi pathogenic towards carrot.
3. Diseased seedlings and roots after harvest of 'Flakkese 2' carrot were most frequently colonized by *Alternaria* spp. and *Fusarium* spp.
4. Other fungi frequently isolated from the diseased underground organs of carrot included: *Sclerotinia sclerotiorum*, *Rhizoctonia solani*, *Phytophthora* sp. and *Athelia arachnoidea*.
5. The system of soil tillage including tillage before winter (ploughing) + spring tillage (a combined cultivator) showed a more positive effect on the healthiness of carror plants than only spring tillage (a combined cultivator).

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