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Pathogenicity of selected soil-borne microorganisms for scorzonera seedlings (*Scorzonera hispanica* L.)

Elżbieta Patkowska[1], *Mirosław Konopiński*[2]

[1]Department of Plant Pathology
Agricultural University in Lublin
Leszczyńskiego 7, 20-069 Lublin, Poland
e-mail: elzbieta.patkowska@ar.lublin.pl
[2] Department of Soil Cultivation and Fertilization of Horticultural Plants
Agricultural University in Lublin
Leszczyńskiego 58, 20-068 Lublin, Poland
e-mail: miroslaw.konopinski@ar.lublin.pl

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ABSTRACT

The pathogenicity of *A. alternata*, *F. culmorum*, *F. oxysporum*, *F. solani*, *P. irregulare,* and *R. solani* isolated from oats, spring vetch, and tancy phacelia mulch soil to *Scorzonera hispanica* L. in the conditions of a growth chamber was studied. The pathogenicity of these microorganisms was established on the basis of the number of emerged plants and their healthiness. The studied species considerably decreased the emergencies and healthiness of the seedlings, e.g. necrosis of sprouts and roots. Differentiated harmfulness of particular microorganisms isolates was observed within a given species. The isolates of *P. irregulare* and *F. oxysporum* proved to be the most severe for the seedlings, while *A. alternata* the least.

INTRODUCTION

Scorzonera hispanica L. is a little known and rare vegetable, which belongs to *Asteracae* family. In its wild state, it is a permanent perennial plant, while its cultivated form is a two-year plant. In the first year it forms a leaf rosette and a fleshy, edible black taproot, whereas in the second – an inflorescence stem, fruits and seeds (Kołota et al. 1994). In Poland, the root of this plant is usually utilized. It resembles asparagus in its taste. In other European countries, e.g. France or Spain, its leaves are also used (Chaux and Fouty 1994). It largely owes its nutritive and dietetic value to the big content of carbohydrates, mineral salts (potassium, sodium, calcium, magnesium, phosphorus, iron), vitamins (C, B_1, B_2), polyphenolic acids and glycosides, particularly inulin (Dolota and Dąbrowska 2004a, 2004b, Wierzbicka 2000). It can be used in the prophylaxis and treatment of the diseases of the alimentary and circulatory systems as well as diabetes. It is so because inulin stimulates the development of probiotic flora of the intestines, mainly bacteria *Lactobacillus acidophilus* and *Bifidobacterium* sp., which inhibit the development of putrefactive bacteria, i.e. *Clostridum perfringens*, *Escherichia coli* and *Salmonella* spp. (Gibson et al. 1995, Bouhnik et al. 1996, Kleessen et al. 1997). Apart from that, inulin can replace fats, sugar and enrich the food with fibre (Robertfroid 1993, Hofer and Jenewein 1999, Lutomski 2001).

Due to the rarity of scorzonera cultivation, only scarce information is available in literature on the occurrence of this plant's diseases. Loerakker (1984) reports that in Northern Europe the cultivation of scorzonera may be endangered by *Alternaria scorzonerae* (Aderhold). Many years of vegetable cultivation on the same site or the cultivation of different vegetable species one after another may result in poorer healthiness of plants and worse quality of the yield. Then the accumulation of the infection material increases in the soil, for example: *Alternaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Pythium irregulare*, and *Rhizoctonia solani.* They live in soil as saprotrophs, they are of polyphagous character, and they are common pathogens towards a number of species of root vegetables causing, for instance, seedling blights and root rot of older plants (Bralewski et al. 2004, Mazur et al. 2004, Nawrocki 2005).

Considerable valours of the taste, as well as dietetic and health values speak for the need to get to know this plant better, popularize its cultivation and protect it against pathogens in order to obtain a high quality yield. The purpose of the present studies was to determine the harmfulness of different species of microorganisms colonizing the soil environment of the cultivation of *Scorzonera hispanica* L. on the basis of pathogenicity tests.

MATERIAL AND METHODS

The object of the studies were the seedlings scorzonera of 'Duplex' cultivar and soil-borne microorganisms such as *Alternaria alternata*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Pythium irregulare*, and *Rhizoctonia solani*. The enumerated species were obtained from the microbiological analysis of the soil (Martyniuk et al. 1991) taken from the plough layer of the field where scorzonera was cultivated in the years 2006 – 2007. The field experiment considered mulching the soil with inter-crop cover crops such as oat (*Avena sativa* L.), spring vetch (*Vicia sativa* L.) and phacelia (*Phacelia tanacetifolia* B.) managed in two ways: 1) pre-winter plough, or 2) spring plough. A traditional cultivation of scorzonera, i.e. without cover crops, was the control (Table 1).

Twelve randomly selected isolates of each species, given the numbers from 1 to 12, were considered in the studies on the pathogenicity of enumerated microorganisms (Table 2). The experiment was conducted in the conditions of growth chamber, where the temperature of the day, which lasted 16 hours, was 22-24°C (with irradiance of 27 µmol m^{-2} s^{-1}) and the temperature at night was 18-20°C. The relative humidity of the air ranged from 70 to 85% (Pięta and Kęsik 2007). The superficially disinfected scorzonera seeds were sown into pots filled with compost earth and overgrowth mycelium of an individual isolate of the studied microorganisms. One hundred seeds per pot were considered for each microorganisms isolate and for the control. Each experimental combination included 4 pots (four repetitions). The infection mixture of the microorganisms used in the experiment was prepared according to the method by Noll, described by Łacicowa (1969) and Patkowska (2005). To this aim, the compost earth with a 5% addition of barley grain was placed in Erlenmayer flasks of 3000 cm^3 volume. The flasks with the mixture were sterilized three times for 2 hours in an autoclave under the pressure of one atmosphere and at the temperature of 121°C. After sterilization, the earth in the flasks was inoculated with 14-day-old cultures of the above mentioned microorganisms, growing on PDA medium, considering 1 flask for each isolate. Next, the flasks with inoculum were kept at the temperature of 23°C for three weeks so that the microorganism would overgrow the earth, which could be confirmed macroscopically. The control were scorzonera seedlings grown from the seeds sown into sterile soil. Four weeks after the experiment was set, the number of grown seedlings was established, and after they were taken out from the soil, their healthiness was determined accepting a five-degree scale of infection (Pięta and Kęsik 2007), i.e. 0° – no disease symptoms, 1° – necrosis up to 10% of the root surface, 2° – necrosis up to 25% of the root surface, 3° – necrosis up to 50% of the root surface, and 4° – necrosis over 50% of the root surface. The degrees of infection served to calculate the index of plants' infection by the studied microorganisms isolates according to the formula by McKinney given by Łacicowa (1969):

$$\text{Disease index} = \frac{\sum a}{b} \times 100$$

∑ a – the sum of products of numerical scale index (infection degree) and corresponding number of plants,
b – total number of tested plants multiplied by the highest numerical scale index.

Next, the plant material was submitted to mycological analysis according to Koch's postulates.

The obtained results were statistically analyzed, and the significance of differences was established on the basis of Tukey's confidence intervals.

RESULTS AND DISCUSSION

The microbiological analysis of the soil taken from the plough layer of particular experimental combinations provided different populations of microorganisms the isolates of which were used in phytotron studies (Table 1). Among the examined species, *Fusarium oxysporum* was most frequently isolated, and its proportion was 41.9%. *Alternaria alternata* and *Fusarium culmorum* were also often isolated (totally 21.8% and 15.2% respectively). These fungi are of polyphagous character and, living in the soil, they constitute a big threat to a number of species of root vegetables, including scorzonera (Tylkowska and Van der Bulk 2001, Bralewski et al. 2004, Mazur et al. 2004). Most isolates of the studied microorganisms were obtained from traditionally cultivated soil (control) and after ploughing in the mulch of phacelia plants. The smallest number of isolates was obtained after mulching the soil with oat (Table 1).

The results obtained in phytotron studies indicated varying harmfulness of particular isolates of the studied species towards the seedlings of scorzonera (Fig. 1). This harmfulness was established on the basis of the number of emerged plants and their healthiness (Tables 2, 3, 4).

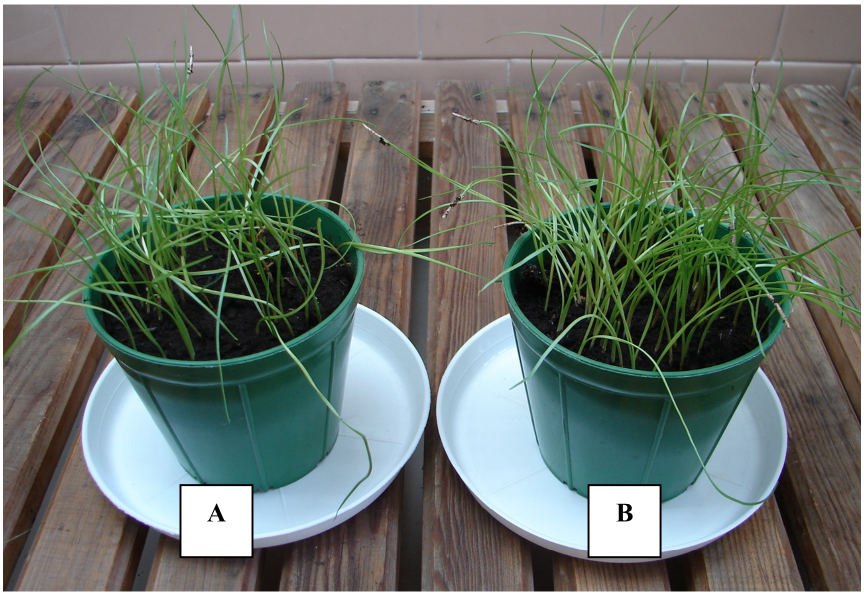

Fig. 1. 14-day-old scorzonera seedlings growing in soil with *Fusarium culmorum* (A) and control (B), (Phot. E. Patkowska)

Fig. 2. The necrosis of roots of 4-week-old scorzonera seedlings growing in growth chamber (Phot. E. Patkowska)

Table 1. Microorganisms isolated from the soil (total from the years 2006 – 2007)

Species	Experimental combination / Number of isolates							Total number (%)
	1*	2	3	4	5	6	7	
Alternaria alternata (Fr.) Keissler	2	3	7	8	11	12	20	63 (21.8)
Fusarium culmorum (W.G.Sm.) Sacc.	2	1	7	9	2	9	14	44 (15.2)
Fusarium oxysporum Schl.	5	9	13	16	13	23	42	121 (41.9)
Fusarium solani (Mart.) Sacc.	-	-	4	2	1	3	9	19 (6.6)
Pythium irregulare Baisman	-	2	8	-	1	4	11	26 (9.0)
Rhizoctonia solani Kühn	-	-	1	3	2	4	6	16 (5.5)
Total	9	15	40	38	30	55	102	289 (100)

*1 – oats mulch + spring ploughing, 2 – oats mulch + pre-winter ploughing, 3 – spring vetch mulch + spring ploughing, 4 – spring vetch mulch + pre-winter ploughing, 5 – tancy phacelia mulch + spring ploughing, 6 – tancy phacelia mulch + pre-winter ploughing, 7 – conventional cultivation

Table 2. Mean number of scorzonera seedlings emerged in particular experiment combinations

Number of isolates	Pathogen species					
	A. alternata	*F. culmorum*	*F. oxysporum*	*F. solani*	*P. irregulare*	*R. solani*
1	96.0 c*	72.0 a	60.0 ab	95.0 d	63.0 b	80.0 d
2	92.5 bc	89.0 cd	64.5 b	89.5 bc	58.5 a	76.5 bc
3	88.0 b	94.5 d	60.5 a	94.0 cd	57.0 a	73.0 abc
4	91.0 bc	88.0 cd	59.0 a	85.5 ab	56.0 a	82.5 d
5	90.0 bc	76.0 a	61.0 ab	91.0 cd	59.0 ab	85.5 de
6	90.0 bc	90.5 c	71.5 c	83.0 a	67.0 c	72.0 ab
7	94.5 c	87.5 c	64.0 b	82.0 a	64.5 bc	78.5 cd
8	87.0 b	79.0 ab	65.5 b	87.5 ab	66.0 c	83.0 d
9	74.5 a	85.5 bc	59.5 ab	83.5 a	58.5 a	85.0 de
10	90.5 bc	91.0 c	72.0 c	93.0 cd	59.0 ab	90.0 e
11	86.5 b	86.0 bc	57.5 a	88.0 b	60.0 ab	71.5 a
12	91.0 bc	83.0 b	65.5 b	85.0 ab	59.5 ab	69.5 a
Mean	89.2 x	85.1 x	63.3 x	88.1 x	60.6 x	78.9 x
Control	100	100	100	100	100	100

*Values in columns followed by the same letter do not differ significantly at $p \leq 0.05$
x – Mean values in comparison to control differs significantly at $p \leq 0.05$

Table 3. Mean number of scorzonera seedlings infected in particular experiment combinations

Number of isolates	Pathogen species					
	A. alternata	*F. culmorum*	*F. oxysporum*	*F. solani*	*P. irregulare*	*R. solani*
1	2.5 a*	14.0 bcd	25.0 c	10.0 bc	14.0 a	15.0 b
2	8.0 bcd	16.5 cd	13.5 a	11.0 bc	30.0 bc	11.5 ab
3	4.5 ab	11.0 abc	28.0 cd	8.5 ab	29.0 bc	17.0 bc
4	5.5 ab	18.5 d	30.5 d	9.0 b	35.5 cd	9.0 a
5	10.5 cd	15.0 cd	18.0 ab	12.0 c	40.5 d	20.5 cd
6	9.0 cd	20.0 e	34.0 de	5.5 a	36.0 c	12.0 ab
7	11.5 cd	17.0 d	28.5 c	6.6 a	26.0 b	17.0 bc
8	7.0 bc	10.0 ab	31.0 d	14.0 c	18.0 a	14.5 b
9	12.0 d	14.5 bcd	42.5 f	7.0 ab	27.5 b	23.0 d
10	9.5 cd	10.5 ab	33.0 d	8.0 ab	16.0 a	16.0 bc
11	8.0 bcd	8.5 a	39.5 ef	10.5 bc	37.0 d	15.5 bc
12	4.5 ab	12.0 abc	23.0 bc	7.5 ab	41.5 d	16.5 bc
Mean	7.7	13.9	28.9	9.1	29.2	15.6
Control	0	0	0	0	0	0

*Values in columns followed by the same letter do not differ significantly at p ≤ 0.05

Table 4. Values of the disease index of scorzonera seedlings

Number of isolates	Pathogen species					
	A. alternata	*F. culmorum*	*F. oxysporum*	*F. solani*	*P. irregulare*	*R. solani*
1	3.6 a	11.4 a	32.0 e	10.1 b	23.2 ab	20.2 ab
2	11.0 cd	15.3 cd	27.0 de	12.7 bc	37.5 e	16.8 a
3	5.2 ab	12.5 abc	18.5 bc	14.0 cd	21.7 ab	21.5 bc
4	6.3 ab	10.2 ab	14.2 ab	11.2 bc	18.0 a	16.3 a
5	3.0 a	9.6 ab	15.4 ab	9.0 ab	25.3 bc	17.4 a
6	15.0 d	17.5 d	11.2 a	11.3 bc	38.4 e	22.8 bc
7	11.4 cd	14.3 b	16.6 ab	7.8 a	29.6 cd	26.8 c
8	5.6 ab	15.8 cd	24.1 cd	5.9 a	24.8 bc	18.3 ab
9	4.4 a	19.0 d	27.5 de	18.0 d	32.6 de	17.6 a
10	10.6 c	8.0 a	29.0 de	14.3 cd	21.8 ab	24.3 c
11	7.9 bc	7.6 a	31.2 de	15.0 cd	25.5 bc	26.4 c
12	5.5 a	10.4 ab	27.9 de	7.0 a	19.4 a	18.2 ab
Mean	7.4	12.6	22.9	11.3	26.5	20.5
Control	0	0	0	0	0	0

*Values in columns followed by the same letter do not differ significantly at p ≤ 0.05

In the control combination healthy seedlings were obtained from all scorzonera seeds. The mean number of the grown seedlings, depending on the species of pathogen present in the soil, ranged from 60.6 to 89.2 (Table 2). The smallest number of scorzonera seedlings grew in the experimental combinations where the soil overgrown with *P. irregulare* was used (60.6 seedlings, on average) or *F. oxysporum* (mean 63.3 seedlings). The best emergencies were obtained in the combination with *A. alternata* and *F. solani*, mean 89.2 and 88.1 seedlings (Table 2).

According to Loerakker (1984), fungi from genus *Alternaria*, especially the species *Alternaria scorzonerae*, may, however, threaten the field cultivation of scorzonera. The harmfulness of *Alternaria dauci* towards root vegetables, mainly carrot, was also observed in the studies by Santos et al. (2000) and Ben-Noon et al. (2001).

However, differentiated harmfulness of particular isolates within a given species was observed. Between 74.5 and 96.0 seedlings grew in the combination with *A. alternata*, between 72.0 and 94.5 seedlings from the combination with *F. culmorum*, 82.0 to 95.0 from the combination with *F. solani*, and between 69.5 and 90.0 scorzonera seedlings grew in the combination with *R. solani*. The poorest emergencies were found out after sowing the scorzonera seeds into an infection mixture with *P. irregulare* (from 56.0 to 67.0 seedlings) or *F. oxysporum* (from 57.5 to 72.0 seedlings, depending on the isolate present in the soil) (Table 2). Reports from literature point to *P. irregulare*, *R. solani*, *F. culmorum*, *F. solani* and *F. oxysporum* as the causal agents for the worse emergencies of a lot of cultivated plants, for example as a result of infection of the underground parts. Pięta and Kęsik (2007), among the other authors, inform about the harmfulness of these species towards the seeds and the seedlings of onion. Patkowska (2005), on the other hand, found out considerable pathogenicity of the enumerated soil-borne fungi towards soybean seedlings.

Seedlings of inhibited growth, with the symptoms of necrosis on the roots, occurred in each experimental combination (Fig. 2). The greatest number of infected scorzonera seedlings was obtained in the soil with different isolates of *P. irregulare* (mean 29.2 diseased seedlings) or *F. oxysporum* (mean 28.9 diseased seedlings) (Table 3). The sprouting seeds and the roots of scorzonera were infected by *A. alternata* and *F. solani* in the smallest degree since the mean number of 7.7 and 9.1 diseased seedlings occurred in those experimental combinations. The number of seedlings with necrotic symptoms caused by particular species was also related to the studied isolates within a given species (Table 3). As reported by Nawrocki (2005), fungi from genera *Fusarium* (mainly *F. oxysporum* and *F. avenaceum*) as well as *Alternaria* (*A. alternata* and *A. radicina*) colonized the seeds in great numbers and caused the necrosis of sprouts and seedlings of different cultivars of parsley root. Similar results were obtained by Nowicki (1997), who found out that fungi from genera *Alternaria* and *Fusarium* could be the cause of parsley seedlings roots necrosis.

The mean value of the disease index of scorzonera seedlings, calculated on the basis of a five-degree scale, ranged in particular experimental combinations from 7.4 to 26.5 (Table 4). The highest mean values of the disease index were observed in the same experimental combinations as in the case of the highest number of infected seedlings, i.e. with the isolates of *P. irregulare* (26.5) or *F. oxysporum* (22.9) present in the soil. The lowest value of the disease index was characteristic for scorzonera seedlings grown in soil with *A. alternata.* The value of the disease index of scorzonera seedlings by this species of fungus ranged from 3.0 to 15.0, depending on the isolate present in the soil. Slightly bigger and similar to each other values of the disease index were obtained in the combinations with different isolates of *F. culmorum* and *F. solani* (from 7.6 to 19.0 and from 5.9 to 18.0, respectively). On the other hand, the highest value of the disease index was calculated for the seedlings infected by *F. oxysporum* or *P. irregulare* (from 11.2 to 32.0 and from 18.0 to 38.4 respectively, depending on the isolates of pathogens present in the soil) (Table 4). Numerous authors report the threat from the soil-borne pathogens towards different species of root vegetables. Considerable pathogenicity of *F. oxysporum*, *P. irregulare* and *R. solani* towards parsley seedlings was found out, among the other authors, by Nawrocki (2005), on the basis of field and glasshouse studies. On the other hand, Tylkowska and Van der Bulk (2001) drew attention in their studies to considerable harmfulness of *A. radicina* as the cause of infection of carrot seedlings. Apart from that, another species, *A. dauci* can pose a serious threat in the period of seed sprouting and emergencies of carrot, parsley and celery, causing a typical infection of the seedlings (Mazur et al. 2004). It can also infect the roots and the leaves of older plants. Scorzonera seedlings grown in the present phytotron experiment exhibited inhibited growth and clear necrotic spots on the roots. Microorganisms reisolation from the infected seedlings according to Koch's postulates confirmed colonization of those plants by *A. alternata*, *F. culmorum*, *F. oxysporum*, *F. solani*, *P. irregulare*, *R. solani* with the morphological features identical to the isolates used for inoculation.

The obtained results make it possible to consider the studied microorganism species, particularly *P. irregulare* and *F. oxysporum* pathogenic towards scorzonera seedlings. Positive results of pathogenicity tests and isolation of their from the infected plant material confirm this finding.

CONCLUSIONS

– The studied microorganisms species (*A. alternata*, *F. culmorum*, *F. oxysporum*, *F. solani*, *P. irregulare*, *R. solani*) significantly decreased the emergencies and healthiness of scorzonera seedlings growing in the conditions of a growth chamber.
– Pathogenicity tests pointed to considerable harmfulness of soil-borne pathogens towards *Scorzonera hispanica* L.
– The isolates of *P. irregulare* and *F. oxysporum* proved to be the most harmful towards scorzonera seedlings, while those of *A. alternata* – the least.

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PATOGENICZNOŚĆ WYBRANYCH MIKROORGANIZMÓW ODGLEBOWYCH DLA SIEWEK SKORZONERY (*SCORZONERA HISPANICA* L.)

Streszczenie: W fitotronie badano patogeniczność dla *Scorzonera hispanica* L. wprowadzonych do ziemi: organizmu grzybopodobnego *P. irregulare* i grzybów *A. alternata, F. culmorum, F. oxysporum, F. solani, R. solani,* wyizolowanych z gleby mulczowanej owsem, wyką jarą i facelią. Patogeniczność tych mikroorganizmów określono na podstawie liczby wyrosłych roślin oraz ich zdrowotności. Badane gatunki znacznie pogorszyły wschody oraz zdrowotność siewek, powodując m.in. nekrozę kiełków i korzeni. Zaobserwowano zróżnicowaną szkodliwość poszczególnych izolatów w obrębie danego gatunku. Najbardziej szkodliwymi dla siewek skorzonery okazały się izolaty *P. irregulare* i *F. oxysporum*, a najmniej *A. alternata.*

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