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THE ROLE OF RHIZOSPHERE ANTAGONISTIC MICROORGANISMS IN LIMITING THE INFECTION OF UNDERGROUND PARTS OF SPRING WHEAT

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ABSTRACT

The studies were conducted in 1998-2000 on an experimental field at Czesławice near Nałęczów. The field was sown with spring wheat, 'Sigma' cv. The purpose of the studies was to establish the species composition of fungi living on the underground parts of spring wheat, and to establish the quantity and quality composition of microorganisms developing in the rhizosphere of this plant. Besides, the studies established the effect of rhizosphere antagonistic bacteria and fungi on some pathogenic soil-borne fungi.

The mycological analysis of the infected roots and the stem base of spring wheat showed that the main cause of the occurrence of necrotic signs on the roots and the stem base of spring wheat were the fungi of *Rhizoctonia solani* and *Fusarium* spp., and above all, the species of *F. avenaceum, F. culmorum* and *F. oxysporum.*
The microbiological analysis of rhizosphere soil gave $7.16 \cdot 10^6$ bacteria colonies, totally. The number of *Bacillus* spp. was a little lower and amounted to $4.37 \cdot 10^6$ colonies, while *Pseudomonas* spp. constituted $3.98 \cdot 10^6$ colonies on average. The total number of fungi was $35.8 \cdot 10^3$ colonies. Within the pathogenic fungi isolated from the rhizosphere of the analyzed plant the dominating ones were *Fusarium* spp. and *Rhizoctonia solani*, while among saprophytic fungi those were *Penicillium* spp. and *Trichoderma* spp.

Key words: spring wheat, rhizosphere, pathogenic fungi, antagonistic microorganisms

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INTRODUCTION

One of the most frequently cultivated cereals in Poland is spring wheat. The habitat of this plant as a link in crop rotation is widely known. On the other hand, the information on the microbiological activity of bacteria and fungi formed in spring wheat rhizosphere is scarce. The dynamics of the population of microorganisms in the soil environment is related to the amount of crop residue. According to Pałys [24], the amount of root mass remaining after the harvest of this plant species was about 1 t ha^{-1}, depending on rainfalls and soil type.

It follows from the bibliography that rhizosphere soil is characterized by the highest biological activity. The quantitative and qualitative composition of microorganisms in the root zone undergoes constant changes under the effect of root exudates and compounds formed from the decomposition of shelling root cells [6, 28]. The substances including aminoacids, sugars soluble in water, elements and phenols exudated by the roots to the rhizosphere serve as the basic source of carbon and nitrogen for microorganisms [10, 13]. Differentiation of the chemical composition of root exudates is related to the genus, species, cultivar, age of the plant and a lot of biotic and abiotic factors [22, 25, 29].

A big role in limiting the occurrence of the population of pathogenic soil-borne fungi is played by microorganisms antagonistic towards phytopathogens. Such properties are exposed above all by bacteria from the genera of *Bacillus* and *Pseudomonas* and by fungi *Gliocladium* spp. and *Trichoderma* spp. [1, 5, 8, 11, 21]. Bacteria and antagonistic fungi considerably limit the number of phytopathogens through the exudated antibiotics or siderophores with fungistatic or fungicidal properties [4, 5, 7, 15].

The purpose of the studies was to establish the species composition of fungi occurring on underground parts of spring wheat and to determine the quantitative and qualitative composition of microorganisms developing in the rhizosphere of this plant. Besides, the effect of rhizosphere bacteria and antagonistic fungi on some pathogenic soil-borne fungi was determined.

MATERIALS AND METHODS

The studies were conducted in the years 1998-2000 on an experimental field at Czesławice near Nałęczów. The field was sown with spring wheat, ‘Sigma’ cv. During anthesis, the plants of the analysed species were sampled for mycological analysis, which was conducted according to the method described by Łacicowa and Pięta [14]. The procedure related to the fungi colonies grown around the fragments of the plant material was like in the studies concerning bean root diseases [26]. At that time the rhizosphere soil of spring wheat was also sampled in order to carry out a microbiological analysis in accordance with the method described by Martyniuk et al. [20]. The total number of bacteria in 1 g of dry weight of the examined soil was established on the medium “Nutrient agar”, using the soil solutions in the dilutions of 10^{-5}, 10^{-6} and 10^{-7}. In the case of bacteria from the genus of *Bacillus* the medium “Tryptic soy agar” and the dilutions of 10^{-4}, 10^{-5} and 10^{-6} were applied, while for *Pseudomonas* spp. the studies used the medium “Pseudomonas agar F” and the dilutions of 10^{-2}, 10^{-3} and 10^{-4}. The total number of fungi in 1 g of dry weight of rhizosphere soil was established on the medium of Martin [19] with the dilutions of the soil solution 10^{-2}, 10^{-3} and 10^{-4}.

In each year of the studies the obtained bacteria isolates (100 isolates of *Bacillus* spp. and 100 isolates from *Pseudomonas* spp.) and the isolates of saprophytic fungi obtained from the rhizosphere of the analyzed plant were used to determine their antagonistic effect on such pathogenic fungi as *Fusarium culmorum, F. oxysporum, F. solani, Pythium irregulare* and *Rhizoctonia solani.* A five-degree scale described by Martyniuk et al. [20] and the degrees of growth inhibition described by Pięta [27] were used to determine the antagonistic effect of bacteria on pathogenic fungi. The effect of saprophytic fungi on the studied pathogenic fungi was determined using the method of biotic rows [16, 18], and the individual antagonistic effect was established on the basis of the scale provided by Mańka and Kowalski [17].

RESULTS

286 fungi isolates were achieved as a result of the mycological analysis of the infected roots and the stem base of spring wheat (tab. 1). In each year of the studies almost five times as many fungi were isolated from the stem base as compared with the roots. *Fusarium* spp. was most frequently isolated; their proportion was 68.2% of all the fungi. This genus was represented by *F. avenaceum, F. culmorum, F. equiseti, F. graminearum, F. oxysporum, F. solani* and *F. sporotrichioides.* The first four of the enumerated fungi species as well as *F.solani* were isolated both from the roots and the stem base, and their proportion was 10.4%, 34.9%, 3.8%, 2.8% and 2.8% of all the fungi, respectively. On the other hand, *F. oxysporum* and *F. sporotrichioides* were isolated much more rarely and only from the infected stem base. The proportion of *F. oxysporum* in the infection of the analyzed plant was 10.5% (tab. 1).

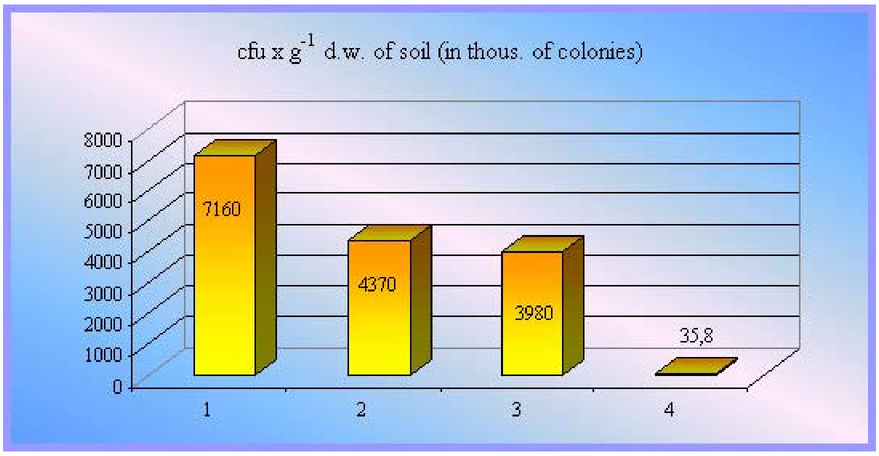
Table 1. Fungi isolated from infected roots (a) and stem base (b) of spring wheat

Fungus species	Number of isolates							
	1998		1999		2000		Total	
	a	b	a	b	a	b	a	b
Acremonium roseum (Oud.) W.Gams	-	1	-	-	-	-	-	1
Acremonium strictum W. Gams	-	1	-	2	-	2	-	5
Alternaria alternata (Fr.) Keissler	-	2	-	6	-	14	-	22
Aureobasidium pullulans (de Bary) Arnaud	-	6	-	2	-	3	-	11
Bipolaris sorokiniana (Sacc.) Shoern.	-	-	-	2	-	-	-	2
Epicoccum purpurascens Ehr. Ex Schl.	1	-	-	2	-	-	1	2
Fusarium avenaceum (Corda ex Fr.) Sacc.	2	7	3	6	3	9	8	22
Fusarium culmorum (W. G. Sm.) Sacc.	12	21	6	25	8	28	26	74
Fusarium equiseti (Corda) Sacc.	3	5	2	1	-	-	5	6
Fusarium graminearum Schwabe	-	-	3	-	-	5	3	5
Fusarium oxysporum Schl.	-	3	-	6	-	21	-	30
Fusarium solani (Mart.) Sacc.	-	1	1	2	-	4	1	7
Fusarium sporotrichioides Sherb.	-	3	-	-	-	5	-	8
Gliocladium catenulatum Gilman et Abbott	-	-	2	-	-	2	2	2
Rhizoctonia solani Kühn	-	8	1	15	1	13	2	36
Trichoderma koningii Oud.	-	-	-	-	-	2	-	2
Trichoderma viride Pers. ex S.F.Gray	-	1	-	-	1	1	1	2
Total	18	59	18	69	13	109	49	237

Rhizoctonia solani, whose isolates constituted 13.3% of all the isolates, also occurred within the pathogenic fungi obtained from the analyzed parts of spring wheat. On the other hand, the species from the genera of *Acremonium, Epicoccum, Gliocladium* and *Trichoderma* were isolated out of the saprophytic fungi (tab. 1).

The microbiological analysis of spring wheat rhizosphere soil showed that in each year of the studies the total quantity of bacteria, the total quantity of fungi and the quantity of *Bacillus* spp. and *Pseudomonas* spp. in 1 g of dry weight were similar; hence, fig. 1 contains mean results from the three studied years. The greatest number of bacteria totally (on average, $7.16 \cdot 10^6$ colonies) was obtained out of the analyzed samples of rhizosphere soil. The number of *Bacillus* spp. was slightly lower and it was $4.37 \cdot 10^6$ colonies, while *Pseudomonas* spp. constituted $3.98 \cdot 10^6$ colonies, on average. The total number of fungi was $35.8 \cdot 10^3$ colonies (fig. 1).

Fig. 1. The number of bacteria and fungi in spring wheat rhizosphere (mean from the years 1998-2000): 1 – total number of bacteria, 2 – number of bacteria *Bacillus* spp., 3 – number of bacteria *Pseudomonas* spp. 4 – total number of fungi



he proportion of pathogenic fungi obtained from the rhizosphere of spring wheat was 19.3% (fig. 2). *Fusarium* spp. and *Rhizoctonia solani* dominated within pathogenic fungi. *Fusarium* spp. was mostly represented by *F. culmorum* and *F. oxysporum,* whose proportion was 4.3% and 5.1% of all the fungi, respectively. *Penicillium* spp. (27.9%) and *Trichoderma* spp. (23.8%) dominated within the group of saprophytic fungi, while *Gliocladium*

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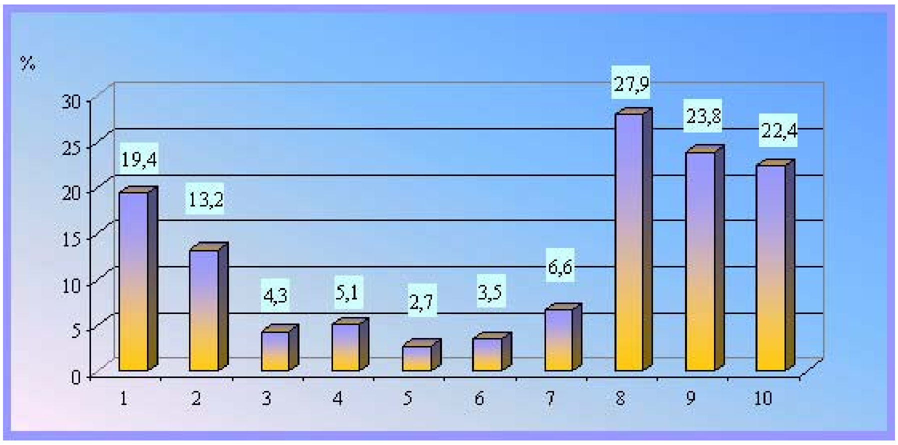
spp. was isolated much less frequently. The proportion of the remaining species of saprophytic fungi obtained from spring wheat rhizosphere was 22.4% (fig. 2).

Fig. 2. Participation of fungi isolated from rhizosphere of spring wheat (total from the years 1998-2000): 1 – total of pathogenic fungi, 2 – *Fusarium* spp., 3 – *F. culmorum*, 4 – *F. oxysporum*, 5 – *F. solani*, 6 – *R. solani*, 7 – *Gliocladium* spp., 8 – *Penicillium* spp., 9 – *Trichoderma* spp., 10 – other of saprophytic fungi



There were 88 isolates of *Bacillus* spp. and 158 isolates of *Pseudomonas* spp. in the group of bacteria isolated from spring wheat rhizosphere which were distinguished by their antagonistic effect towards the studied pathogenic fungi (tab. 2). Those bacteria were poor antagonists towards *F. culmorum* and *R. solani;* the value of the antagonistic effect of the three studied years was +156 and +51, respectively. On the other hand, the analyzed bacteria were most effective in limiting the growth and development of *P. irregulare* and *F. solani,* since the value of the antagonistic effect was totally +950.5 and +633, respectively. *F. oxysporum* was limited in varying degrees, and the value of the antagonistic effect ranged from +83 to +191.5 (tab. 2).

Table 2. Activity of selected bacteria *Bacillus* spp. and *Pseudomonas* spp. isolated from rhizosphere of spring wheat towards pathogenic fungi

Genus of bacteria	Number of antagonistic isolates	*F. culmorum*		*F. oxysporum*		*F. solani*		*P. irregulare*		*R. solani*	
		1	2	1	2	1	2	1	2	1	2
1998											
Bacillus spp.	20	+1	+20	+1.5	+30	+1	+20	+2	+40	+1	+20
Pseudomonas spp.	38	0	0	+2	+76	+6	+228	+5	+190	0	0
Total effect of antagonistic activity			+20		+106		+248		+230		+20
1999											
Bacillus spp.	31	+1	+31	+1	+31	+1	+31	+1.5	+46.5	+1	+31
Pseudomonas spp.	52	0	0	+1	+52	+1	+52	+5	+260	0	0
Total effect of antagonistic activity			+31		+83		+96		+306.5		+31
2000											
Bacillus spp.	37	+1	+37	+1.5	+55.5	+1	+37	+2	+74	0	0
Pseudomonas spp.	68	+1	+68	+2	+136	+4	+272	+5	+340	0	0
Total effect of antagonistic activity			+105		+191.5		+309		+414		0

1 – individual effect of antagonistic activity
2 – total effect of antagonistic activity

Within the saprophytic fungi isolated from the rhizosphere soil of spring wheat, 242 isolates of different species of fungi were separated (*Gliocladium* spp. – 22, *Penicillium* spp. – 117, *Trichoderma* spp. – 75 colonies and *Acremonium kiliense* -1, *Torula herbarum* – 5 and *Verticillium tenerum* – 1 colony). They were distinguished by their antagonistic effect towards the studied phytopathogens (tab. 3). Such an effect of the enumerated fungi was the weakest in the case of *R. solani* (+554), and the strongest for *F. solani* (+1389) and *F. oxysporum* (+1219 – the total antagonistic effect). A little lower value was found out for *F. culmorum* and *P. irregulare* (tab. 3).

Table 3. Activity of saprophytic fungi isolated from rhizosphere of spring wheat towards pathogenic fungi

Fungus species	Mean number of isolates in 1998-2000	*F. culmorum*		*F. oxysporum*		*F. solani*		*P. irregulare*		*R. solani*	
		1	2	1	2	1	2	1	2	1	2
Acremonium kiliense	1	+2	+2	+3	+3	+3	+3	+2	+2	0	0
Gliocladium catenulatum	9	+5	+45	+5	+45	+7	+63	+6	+54	+2	+18
Gliocladium roseum	13	+5	+39	+7	+91	+8	+104	+7	+91	+5	+39
Penicillium brevi-compactum	21	+3	+63	+4	+84	+5	+105	+4	+84	-1	-21
Penicillium decumbens	19	+1	+19	+3	+57	+4	+76	+4	+76	+1	+19
Penicillium funiculosum	17	+1	+17	+3	+51	+3	+51	+2	+34	+1	+17
Penicillium janthinellum	30	+2	+60	+3	+90	+4	+120	+1	+30	-4	-120
Penicillium meleagrinum	2	+1	+2	+2	+4	+4	+8	+1	+2	-4	-8
Penicillium nigricans	5	+1	+5	+3	+15	+4	+20	+2	+10	0	0
Penicillium purpurogenum	3	+1	+3	+3	+9	+4	+12	+2	+6	+2	+6
Penicillium roseo-purpureum	2	+1	+2	+2	+4	+5	+10	+3	+6	+2	+4
Penicillium velutinum	11	+3	+33	+3	+33	+5	+55	+2	+22	-3	-33
Penicillium verrucosum var. cyclopium	6	+3	+18	+6	+36	+7	+42	+4	+24	+1	+6
Penicillium verrucosum var. verrucosum	22	+2	+44	+4	+88	+5	+110	+2	+44	+1	+22
Torula herbarum	5	0	0	+1	+5	+1	+5	0	0	-1	-5
Trichoderma hamatum	14	+8	+122	+8	+112	+8	+112	+8	+112	+8	+112
Trichoderma harzianum	18	+8	+144	+8	+144	+8	+144	+8	+144	+8	+144
Trichoderma koningii	17	+8	+136	+8	+136	+8	+136	+8	+136	+8	+136
Trichoderma pseudokoningii	10	+8	+80	+8	+80	+8	+80	+8	+80	+8	+80
Trichoderma viride	16	+8	+128	+8	+128	+8	+128	+8	+128	+8	+128
Verticillium tenerum	1	+3	+3	+4	+4	+5	+5	+2	+2	+2	+2
Total effect of antagonistic activity			+955		+1219		+1389		+1087		+554

1 – individual effect of antagonistic activity
2 – total effect of antagonistic activity

DISCUSSION

The studies showed that the main cause of the occurrence of necrotic signs on the roots and the stem base of spring wheat were the fungi of *Rhizoctonia solani* and *Fusarium* spp., and first of all the species of *F. avenaceum, F. culmorum* and *F. oxysporum.* Information from the bibliography also points out that spring wheat is widely infected by these species [2, 3, 9]. In the case of *R. solani,* necrosis of the underground parts can take place under the effect of phytotoxic compounds released by this fungus such as m-hydroxyphenylacetic and m-methoxyphenylacetic acids [23].

In the presented studies the infection of the analyzed plant by *R. solani* was relatively small. It can be supposed that the populations of bacteria and saprophytic fungi having antagonistic properties and numerously occurring in the root zone could have an inhibiting effect on the development of pathogenic fungi. The on-going microbiological studies of rhizosphere point out to this.

The microbiological analysis of the examined soil suggests a stimulating effect of spring wheat on the development of bacteria, especially *Pseudomonas* spp., which have an antagonistic effect on pathogenic fungi. Besides, the proportion of pathogenic fungi, mainly *Fusarium* spp., was smaller in the rhizosphere of the analyzed plant than the proportion of antagonistic *Penicillium* spp. and *Trichoderma* spp. An ability of fast growth and development of antagonistic fungi in the rhizosphere of plants has a positive effect on the healthiness of the underground parts. Shihuang et al. [30] proved that the fungi species from the genus of *Trichoderma* are effective in protecting it from pathogens through a very intensive growth around the root top.

It should be supposed that a considerable number of antagonistic isolates *Pseudomonas* spp., *Trichoderma* spp. and *Penicillium* spp. could have affect the smaller numbers of phytopathogens in the rhizosphere of spring wheat in a significant manner. According to Księżniak and Kobus [12], siderophores created by fluorescent

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Pseudomonas clearly inhibit the development of pathogenic fungi in the soil. Besides, such an effect of siderophores on the development of wheat pathogens was confirmed in the studies by Manwar et al. [15].

Abundant information from bibliography and the results obtained in the presented studies emphasize a great role of bacteria and antagonistic fungi in limiting the development of soil-borne phytopathogens.

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