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THE ROLE OF ANTAGONISTIC FUNGI AND BACTERIA LIMITING THE OCCURRENCE OF SOME PHYTOPATHOGENS INHABITING THE SOYBEAN SOIL ENVIRONMENT

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ABSTRACT

The object of the studies conducted in the years 1998-2000 was the rhizosphere soil of soybean, Mazovia cultivar, and the non-rhizosphere soil. The experimental plot was set in Czesławice near Nałęczów on grey-brown podzolic soil formed from loesses, which was the second complex of soil suitability (good wheat complex). The microbiological analysis showed a much greater number of microorganisms in the rhizosphere soil than in the non-rhizosphere soil. The proportion of pathogenic fungi in the non-rhizosphere soil was almost twice as high in comparison to the rhizosphere, and it was 48.4% and 25.6%, respectively. The dominating pathogenic fungi were *Fusarium* spp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum.* On the other hand, bacteria *Bacillus* spp. (81 isolates) and *Pseudomonas* spp. (231 isolates) as well as fungi *Trichoderma* spp. (64 isolates) were obtained among the microorganisms distinguished by their antagonistic effect towards phytopathogens. These antagonistic microorganisms considerably reduced the occurrence of pathogenic fungi in the soybean rhizosphere, which can testify to their biological activity contributing to the improvement of the phytosanitary condition of the soil.

Key words: pathogenic fungi, antagonistic fungi, *Bacillus* spp., *Pseudomonas* spp.

INTRODUCTION

Various reactions take place within the group of microorganisms and between microorganisms and plants. The highest activity is characteristic of rhizosphere soil. The composition of microorganisms in the root zone undergoes constant changes, for example under the influence of root exudates [17]. On the other hand, the chemical composition of the substances exudated by the roots is related to the genus, species, cultivar, the age of the plant as well as many other biotic and abiotic factors [13, 15, 18].

A huge role in limiting the occurrence of pathogenic fungi in the soil is played by antagonistic bacteria Bacillus spp. and Pseudomonas spp. as well as by fungi Gliocladium spp. and Trichoderma spp. [1, 6, 12].

Establishing the composition of antagonistic microorganisms towards soil-borne phytopathogens is especially important from the point of view of biological protection of plants. That is the reason why studies were undertaken whose purpose was to look for antagonistic microorganisms limiting the occurrence of pathogenic soil-borne fungi.

MATERIALS AND METHODS

The studies were conducted in the years 1998-2000 on the experimental plot located at Czesławice near Nałęczów. The object of the studies was the rhizosphere soil of soybean, Mazovia cultivar, and the non-rhizosphere soil formed from loesses, which was the second complex of soil suitability (good wheat complex).

In each year, samples of rhizosphere soil were taken at the anthesis of soybean plants. The samples of non-rhizosphere soil were taken from the belts mechanically maintained in black fallow. The manner of rhizosphere soil sampling and the laboratory microbiological analysis were in accordance with the method described by Martyniuk et al. [11]. The rhizosphere soil of soybean and the non-rhizosphere soil from the depth of 5-10 cm were taken to sterile Petri dishes. Next, a sterile soil solution with the dilutions from 10^{-1} to 10^{-7} was prepared in sterile laboratory conditions. Those dilutions were used to determine the total number of bacteria, the number of bacteria *Bacillus* spp. and *Pseudomonas* spp. as well as the total number of fungi in 1 g of d.w. of the examined soil samples [16].

The results concerning the number of bacteria and fungi were analyzed statistically, and the significance of differences was established on the basis of Tukey's confidence intervals [14].

In each year of the studies, the bacteria isolated (100 isolated from *Pseudomonas* spp. and 100 isolated from *Bacillus* spp.) as well as all the isolates of saprophytic fungi from the genera of *Gliocladium, Penicillium* and *Trichoderma* were used to determine their antagonistic effect towards such pathogenic fungi as *Botrytis cinerea, Fusarium culmorum, F. oxysporum* f. sp. *glycines, F. solani, Phoma exigua* var. *exigua, Rhizoctonia solani* and *Sclerotinia sclerotiorum* according to the methods described by Martyniuk et al. [11], Mańka and Mańka [10] and Pięta [17].

RESULTS AND DISCUSSION

The microbiological analysis showed that the number of bacteria in 1 g d.w. of the rhizosphere soil ranged from $4.52 \cdot 10^6$ to $6.38 \cdot 10^6$ colonies, and those of the non-rhizosphere soil from $3.16 \cdot 10^6$ to $4.18 \cdot 10^6$ colonies (tab. 1). The mean numbers of bacteria *Bacillus* spp. in the rhizosphere and non-rhizosphere soil were similar and they were $1.73 \cdot 10^6$ and $1.13 \cdot 10^6$ colonies, respectively. The number of *Pseudomonas* spp. in the soil was higher ($3.45 \cdot 10^6$ colonies, on average) as compared to the non-rhizopshere soil ($2.06 \cdot 10^6$ colonies, on average). The total number of fungi in the rhizopshere soil of soybean was twice as high (mean $78.95 \cdot 10^3$ colonies) as in the non-rhizosphere soil (mean $37.44 \cdot 10^3$ colonies) (tab. 1).

Table 1. The number of bacteria and fungi in soybean rhizosphere and non-rhizosphere soil

Type of soil	Total number of bacteria (mln · g^{-1} d.w. of soil)				Number of bacteria of *Bacillus* genus (mln · g^{-1} d.w. of soil)				Number of bacteria of *Pseudomonas* genus (mln · g^{-1} d.w. of soil)				Total number of fungi (thous · g^{-1} d.w. of soil)			
	1998	1999	2000	mean	1998	1999	2000	mean	1998	1999	2000	mean	1998	1999	2000	mean
Rhizosphere of soybean	4.52[b*]	6.38[b]	5.76[b]	5.55[b]	1.16[a]	2.10[b]	1.95[b]	1.73[b]	3.05[b]	4.18[b]	3.14[b]	3.45[b]	96.72[b]	64.60[b]	75.54[b]	78.95[b]
Non-rhizosphere soil	3.16[a]	3.20[a]	4.18[a]	3.51[a]	1.08[a]	0.84[a]	1.47[a]	1.13[a]	1.97[a]	2.15[a]	2.05[a]	2.06[a]	43.16[a]	31.30[a]	37.86[a]	37.44[a]

[*]Means in columns differ significantly ($P \leq 0.05$), if they are not marked with the same letter

The proportion of pathogenic fungi obtained from the non-rhizosphere soil was almost twice as high as compared to the rhizosphere soil and it was 48.4% and 25.6%, respectively (figs. 1 and 2). *Fusarium* spp., *Rhizoctonia solani* and *Sclerotinia sclerotiorum* dominated within the group of pathogenic fungi. The genus *Fusarium* was represented by *F. culmorum, F. oxysporum* f. sp. *glycines* and *F. solani. Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp. were most often isolated in the group of saprophytic fungi, and they were isolated twice as frequently from the rhizosphere soil of soybean as from the non-rhizopshere soil (figs. 1 and 2).

Fig. 1. Participation of fungi isolated from soybean rhizosphere (total from the years 1998-2000): 1 – total of pathogenic fungi, 2 – *Fusarium* spp., 3 – *F. culmorum*, 4 – *F. oxysporum* f. sp. *glycines*, 5 – *F. solani,* 6 – *R. solani,* 7 – *Sclerotinia sclerotiorum*, 8 – *Gliocladium* spp., 9 – *Penicillium* spp., 10 – *Trichoderma* spp., 11 – other of saprophytic fungi

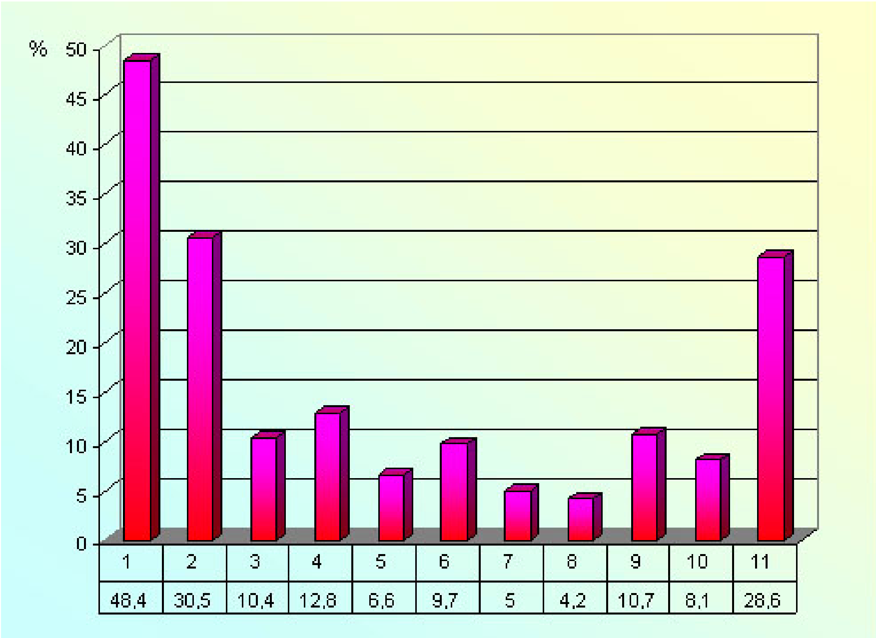
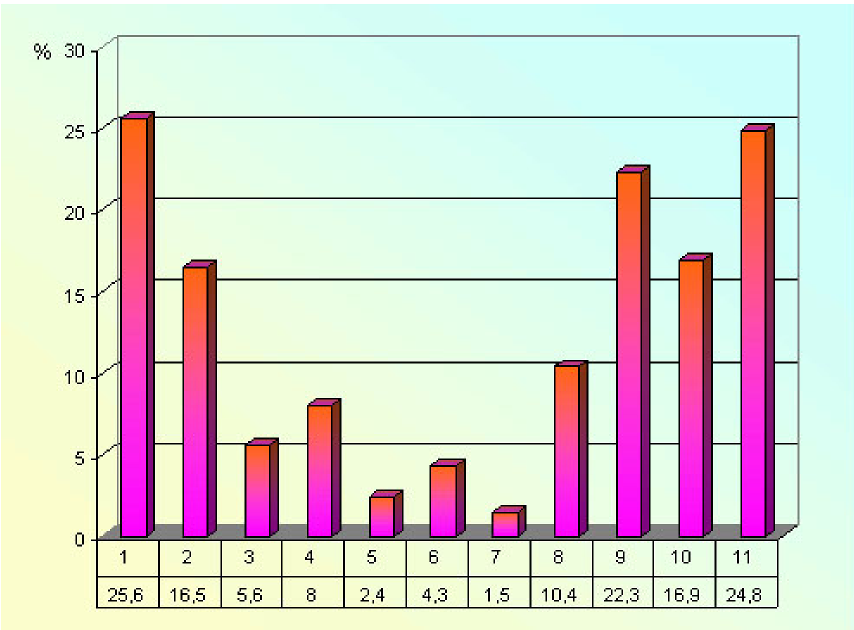


Fig. 2. Participation of fungi isolated from non-rhizosphere soil (total from the years 1998-2000): 1 – total of pathogenic fungi, 2 – *Fusarium* spp., 3 – *F. culmorum*, 4 – *F. oxysporum* f. sp. *glycines*, 5 – *F. solani*, 6 – *R. solani*, 7 – *Sclerotinia sclerotiorum*, 8 – *Gliocladium* spp., 9 – *Penicillium* spp., 10 – *Trichoderma* spp., 11 – other of saprophytic fungi



The increase of the number of microorganisms, especially in the rhizosphere soil of soybean, could have taken place under the effect of this plant's root exudates. This fact is explained in numerous items of literature concerning the role of compounds exudated by the roots of different cultivated plants [4, 17].

Laboratory studies showed that the non-rhizosphere soil contained twice as few bacteria distinguished by their antagonistic effect towards the examined pathogenic fungi (103 isolates) as the rhizosphere soil (209 isolates) (tab. 2). Besides, almost three times as many antagonistic fungi were obtained from the soybean rhizosphere as from the non-rhizosphere soil. *G. catenulatum* spp., *Penicillium* spp. and *Trichoderma viride* dominated within the group of antagonistic fungi (tab. 2).

Table 2. Antagonistic microorganisms isolated from the rhizosphere of soybean and non-rhizosphere soil (mean from the years 1998-2000)

Bacteria and fungi	Number of isolates	
	Rhizosphere of soybean	non-rhizosphere soil
Bacillus spp.	53	28
Pseudomonas spp.	156	75
Total bacteria	**209**	**103**
Gliocladium catenulatum Gilman et Abbott	16	9
Gliocladium fimbriatum Gilman et Abbott	12	5
Gliocladium roseum Bainier	10	3
Penicillium spp.	46	18
Trichodrema hamatum (Bon.) Bain	3	
Trichoderma harzianum Rifai	9	4
Trichoderma koningii Oud.	18	6
Trichoderma pseudokoningii Rifai	7	1
Trichoderma viride Pers. ex S.F.Gray	21	10
Total fungi	**142**	**56**

It should be supposed that a considerable number of antagonistic isolates of *Bacillus* spp., *Pseudomonas* spp., *Gliocladium* spp. and *Trichoderma* spp. could have had a significant influence on the decrease of the number of phytopathogens in soybean rhizosphere. This fact is confirmed in numerous items of literature [1, 6, 8, 12]. As stated by Księżak and Kobus [7], siderophores formed by fluorescent *Pseudomonas* inhibit the development of pathogenic fungi in the soil. Besides, antagonistic fungi and bacteria considerably limit the growth and development of pathogenic fungi not only by the exudated siderophores but also by antibiotics with fungicidal and fungistatic effect [2, 3, 5, 9]. On the other hand, the species of *Trichoderma* spp. are characterized by highly antagonistic effect on phytopathogens, and this effect is based on antibiosis, competition and parasitism [1, 6].

CONCLUSIONS

1. The proportion of pathogenic fungi in the non-rhizopshere soil was almost twice as high as in the soybean rhizosphere.
2. Antagonistic microorganisms included bacteria *Bacillus* spp., *Pseudomonas* spp., and fungi *Gliocladium* spp., *Penicillium* spp., *Trichoderma* spp.
3. The big numbers of antagonistic microorganisms in the rhizosphere of soybean can testify to their considerable biological activity, which contributes to the improvement of the phytosanitary condition of the soil.

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