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# ANTAGONISTIC BACTERIA AND FUNGI LIMITING POTATO INFECTION BY SOIL-BORNE PATHOGENIC FUNGI

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**Abstract**: The purpose of the presented studies was to determine the species composition of the fungi occurring on the underground parts of potato at anthesis and to establish the qualitative and quantitative composition of microorganisms living in the soil environment of this plant. Besides, the studies aimed at finding antagonistic microorganisms inhibiting potato infection by soil-borne phytopathogens. The mycological analysis showed that the symptoms of necrosis on the roots and the stem base of potato were caused by *Alternaria alternata, Fusarium* spp., *Colletotrichum coccodes* and *Rhizoctonia solani*. The microbiological analysis of the potato rhizosphere gave twice as many bacteria and fungi as from the non-rhizosphere soil. The dominating pathogenic fungi in the examined soil samples were *Fusarium* spp., *A. alternata* and *R. solani*. Three times as many antagonistic bacteria *Pseudomonas* spp. and *Bacillus* spp. and more than twice as many antagonistic fungi (*Penicillium* spp., *Trichoderma* spp., *Gliocladium* spp.) were obtained from the potato rhizosphere as compared to the non-rhizosphere soil.

**Key words:** potato, phytopathogens, *Bacillus* spp., *Pseudomonas* spp., *Trichoderma* spp., *Gliocladium* spp., *Penicillium* spp.

## INTRODUCTION

In Poland potato is one of the most important cultivated plants besides winter wheat (Pisarek et al. 2001). The role of this plant as a link in crop rotation cultivation is widely known, but there is little information on the relations between microorganisms occurring in the soil environment of potato.

The factors causing considerable reduction of the size and quality of the tuber yield of this plant include the diseases caused by soil-borne bacteria and fungi (Kapsa and Zachaj 1998; Kurzawińska 1997; Kurzawińska and Gajda 2001). Antagonistic microorganisms fulfil a positive function in limiting the pathogenic factors (Das and Hazarika 2000; Manwar et al. 2000; Pięta 1999; Saniewska 2000).

The purpose of the presented studies was to determine the species composition of the fungi occurring on the underground parts of potato at anthesis and to establish the qualitative and quantitative composition of microorganisms inhabiting the soil environment of this plant. Besides, the studies searched for the antagonistic microorganisms limiting the potato infection by soil-borne phytopathogens.

## MATERIALS AND METHODS

The studies were conducted in the years 1999–2001 in an experimental field at Czesławice near Nałęczów, where potato of cv. Bronka was cultivated. The plants of this cultivar at anthesis were submitted to laboratory mycological analysis according to the method described by Łacicowa and Pięta (1998). At the same time the potato rhizosphere and non-rhizosphere soil (from the belts mechanically maintained in black fallow) was sampled in order to conduct a microbiological analysis according to the method described by Martyniuk et al. (1991). The laboratory dilutions of the soil solution from $10^{-1}$ to $10^{-7}$ were used to determine the total number of bacteria, the number of *Pseudomonas* spp. and *Bacillus* spp. as well as the total number of fungi in 1 g. of d.w. of the examined soil samples (Patkowska 2001).

The results concerning the number of microorganisms obtained in the microbiological analysis were statistically analyzed, and the significance of differences was determined on the basis of Tukey's confidence intervals (Oktaba 1987).

In each year of the studies the isolates of saprophytic fungi and the isolates of bacteria *Pseudomonas* spp. and *Bacillus* spp. (100 isolates from each bacteria genus) isolated from the potato rhizosphere and the non-rhizosphere soil were used in order to determine their antagonistic effect towards pathogenic fungi such as *Fusarium culmorum, F. oxysporum, F. solani, Pythium irregulare* and *Rhizoctonia solani* in accordance with the methods described by Mańka (1974), Mańka and Mańka (1992) and Pięta (1999).

## RESULTS

The mycological analysis of the infected roots and the stem base of potato obtained 409 fungi isolates belonging to 10 genera (Tab. 1). In each year, more fungi were isolated from the stem base than from the roots. The most frequently isolated fungi belonged to the genus *Fusarium* (152 isolates) and *Alternaria alternata* (69 isolates), *Colletotrichum coccodes* (48 isolates) and *R. solani* (45 isolates). The genus *Fusarium* was represented by *F. oxysporum* (82 isolates), *F. culmorum* (39 isolates) and *F. solani* (31 isolates). Among the saprophytic fungi the species from the genera *Acremonium, Epicoccum, Gliocladium* and *Penicillium* were isolated (Tab. 1).

The microbiological analysis of the potato rhizosphere and non-rhizosphere soil showed that in each studied year the numbers of the examined microorganisms in 1 g of d.w. of soil were similar, hence, figure 1 presents the mean results of three years. The total number of bacteria in the rhizosphere of potato was twice as big as in the non-rhizosphere soil and it was $7.42 \times 10^6$ and $3.56 \times 10^6$ colonies, respectively. The number of bacteria *Pseudomonas* spp. and *Bacillus* spp. in the rhizosphere soil was also bigger ($2.60 \times 10^6$ and $3.21 \times 10^6$ colonies, respectively) than in the non-rhizosphere soil ($1.28 \times 10^6$ and $2.13 \times 10^6$ colonies, respectively). The num-

Table 1. Fungi isolated from infected plants of potato

| Fungus species | Number of isolates | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1999 | | | 2000 | | | 2001 | | | Total | | |
| | a | b | sum | a | b | sum | a | b | sum | a | b | sum |
| *Acremonium kiliense* Grutz | 1 | 3 | 4 | – | 2 | 2 | 1 | 2 | 3 | 2 | 7 | 9 |
| *A. roseum* (Oud.) W. Gams | 2 | 5 | 7 | 1 | 3 | 4 | 2 | 3 | 5 | 5 | 11 | 16 |
| *A. strictum* W. Gams | 4 | 7 | 11 | 2 | – | 2 | 2 | 4 | 6 | 8 | 11 | 19 |
| *Alternaria alternata* (Fr.) Keissler | 11 | 14 | 25 | 9 | 12 | 21 | 10 | 13 | 23 | 30 | 39 | 69 |
| *Amauroascus niger* Schroeter | 1 | – | 1 | 2 | – | 2 | 1 | – | 1 | 4 | – | 4 |
| *Colletotrichum coccodes* (Wallr.) Hughes | 6 | 9 | 15 | 7 | 10 | 17 | 5 | 11 | 16 | 18 | 30 | 48 |
| *Epicoccum purpurascens* Ehr. ex Schl. | – | 3 | 3 | 1 | 2 | 3 | – | 2 | 2 | 1 | 7 | 8 |
| *Fusarium culmorum* (W.G.Sm.) Sacc. | 5 | 11 | 16 | 3 | 7 | 10 | 5 | 8 | 13 | 13 | 26 | 39 |
| *F. oxysporum* Schl. | 12 | 17 | 29 | 11 | 15 | 26 | 10 | 17 | 27 | 33 | 49 | 82 |
| *F. solani* (Mart.) Sacc. | 4 | 8 | 12 | 3 | 6 | 9 | 4 | 6 | 10 | 11 | 20 | 31 |
| *Gliocladium catenulatum* Gilman et Abbott | 7 | 4 | 11 | – | 2 | 2 | 3 | 4 | 7 | 10 | 10 | 20 |
| *Penicillium verrucosum* Dierckx var. *cyclopium* Westling, Samson, Stolk et Hadlok | 2 | 6 | 8 | 2 | – | 2 | 3 | 2 | 5 | 7 | 8 | 15 |
| *Rhizoctonia solani* Kühn | 7 | 10 | 17 | 4 | 9 | 13 | 5 | 10 | 15 | 16 | 29 | 45 |
| *Talaromyces flavus* (Klocker) Stolk, Samson | 2 | – | 2 | 1 | – | 1 | 1 | – | 1 | 4 | – | 4 |
| **Total** | **64** | **97** | **161** | **46** | **68** | **114** | **52** | **82** | **134** | **162** | **247** | **409** |

a – root, b – stem base

ber of fungi in the non-rhizosphere soil was almost twice as small (mean $45.12 \times 10^3$ colonies) as in the rhizosphere soil of potato (mean $92.84 \times 10^3$ colonies) (Fig. 1).

The proportion of pathogenic fungi obtained from the potato rhizosphere was more than twice as big as in the non-rhizosphere soil, and it was 37.2% and 16.4%, respectively (Figs. 2, 3). Among the pathogenic fungi, *Fusarium* spp., *A. alternata* and *R. solani* were most frequently obtained from the studied soil samples. The species *F. oxysporum* was isolated most frequently within the fungi from the genus *Fusarium*, and its proportion constituted 12.6% in the potato rhizosphere and 5.9% in the non-rhizosphere soil. *Penicillium* spp. (25.4%), *Trichoderma* spp. (20.7%) and *Gliocladium* spp. (12.3% of all isolations) dominated among the saprophytic fungi in the potato rhizosphere (Fig. 2). On the other hand, the proportion of these saprophytic fungi in the non-rhizosphere soil was slightly lower and it was 18.1%, 14.2% and 8.5% of all the fungi, respectively (Fig. 3).

Laboratory studies showed that the rhizosphere of potato contained almost three times as many bacteria *Pseudomonas* spp. and *Bacillus* spp. (66 and 49 isolates, respectively) which had an antagonistic effect on the studied pathogenic fungi as in the non-rhizosphere soil (24 and 16 isolates, respectively) (Fig. 4). Also, twice as many antagonistic fungi (*Penicillium* spp. – 86 isolates, *Trichoderma* spp. – 70 isolates, *Gliocladium* spp. – 41 isolates) were obtained from the potato rhizosphere in comparison to the non-rhizosphere soil (42, 33 and 19 isolates, respectively) (Fig. 4). Within the antagonistic fungi, *Penicillium verrucosum* var. *cyclopium*, *P. verrucosum*

var. *verrucosum, Trichoderma viride, T. koningii, T. pseudokoningii, T. hamatum, Gliocladium catenulatum* and *G. fimbriatum* dominated.

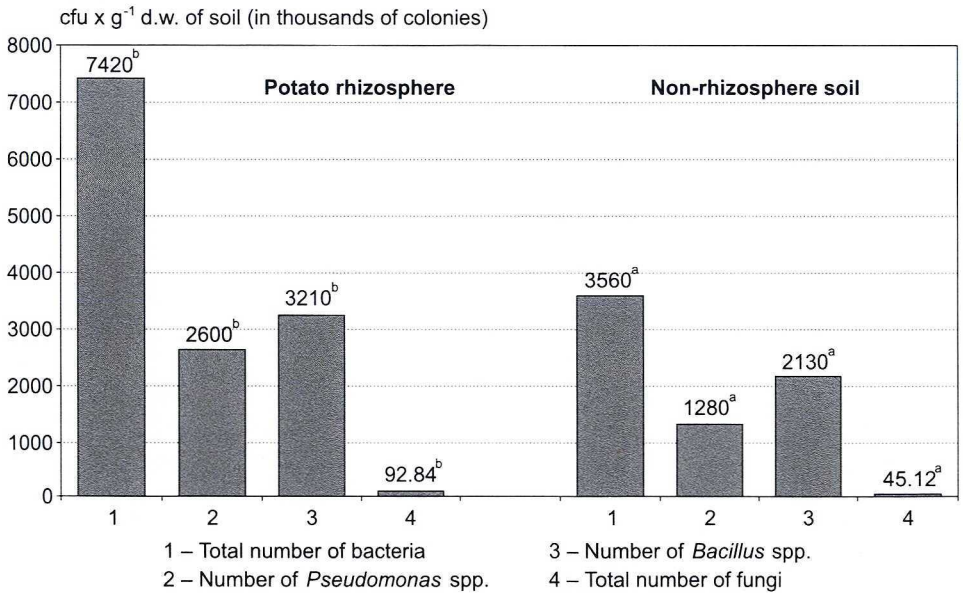


Fig. 1. Mean number of bacteria and fungi in the studied samples of soil

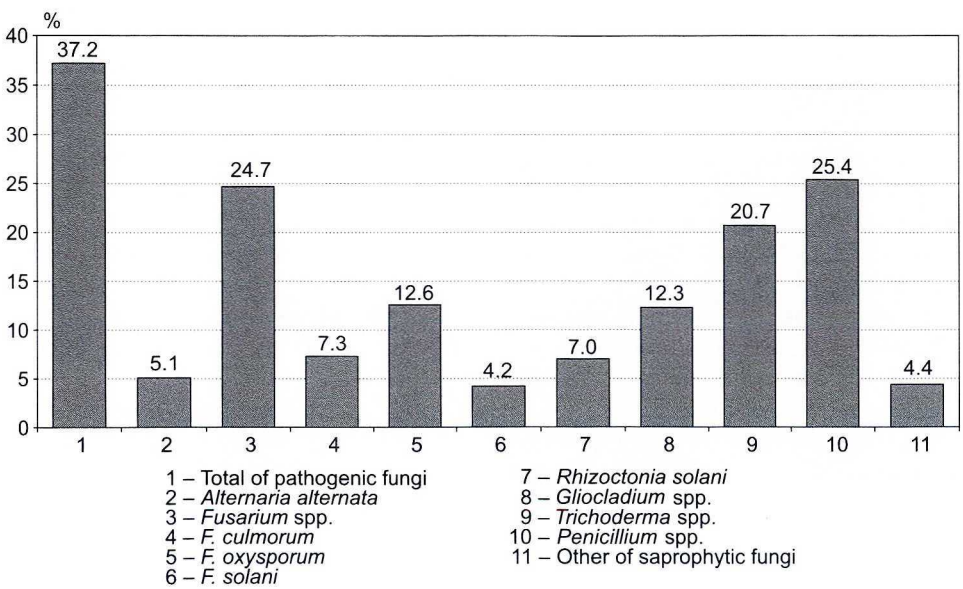


Fig. 2. Participation of fungi isolated from rhizosphere of potato

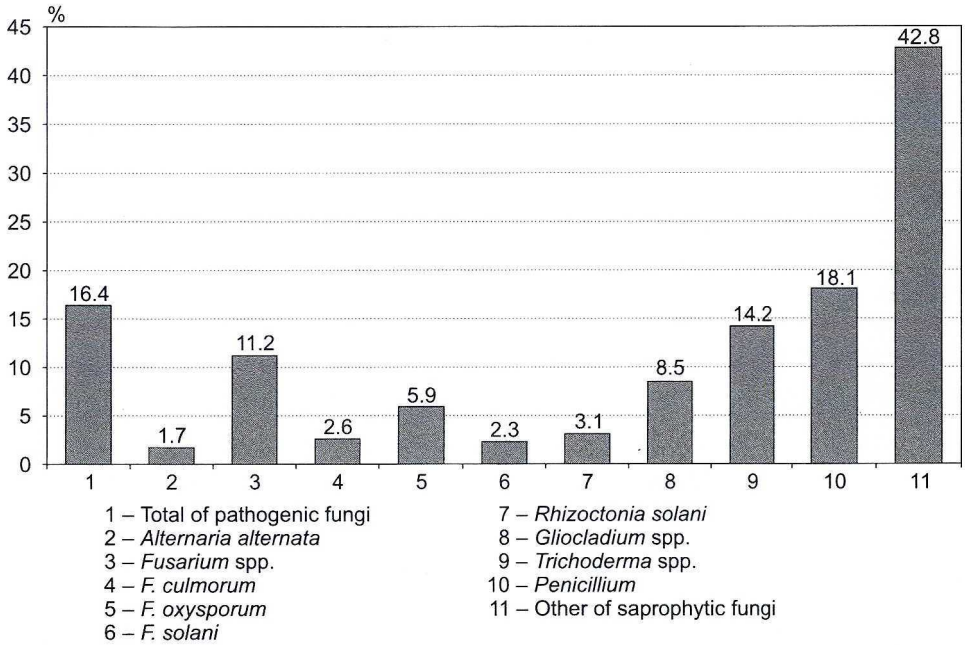


Fig. 3. Participation of fungi isolated from non-rhizosphere soil

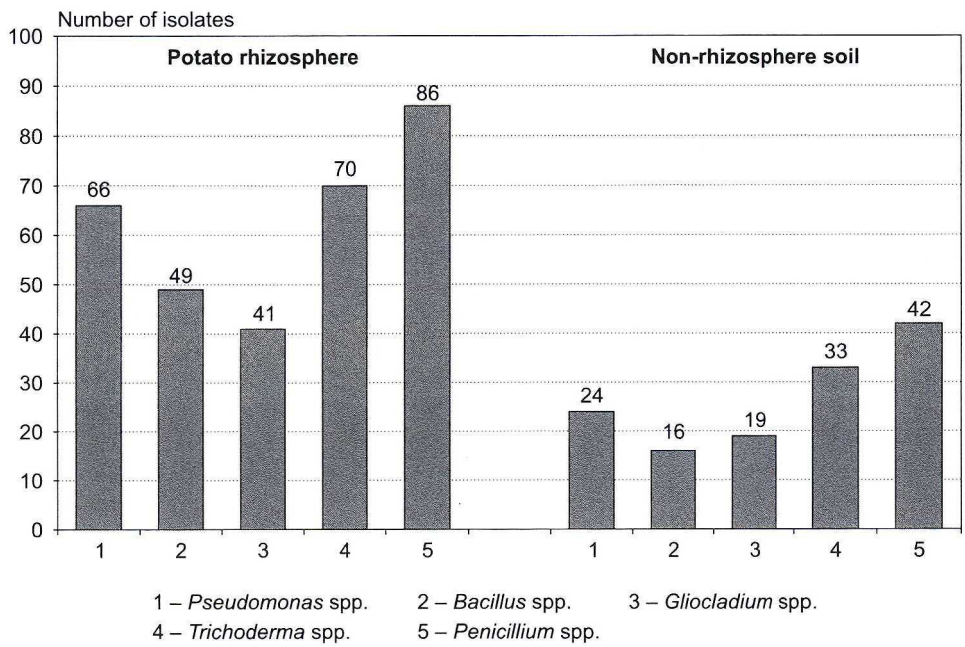


Fig. 4. Occurrence of antagonistic bacteria and fungi in the studied samples of soil

## DISCUSSION

The presented studies confirmed that the occurrence of necrotic symptoms on the roots and the stem base of potato is caused by such fungi as *A. alternata, Fusarium* spp., *Colletotrichum coccodes* and *R. solani*. This fact is also confirmed in other publications (Kapsa and Zachaj 1998; Kurzawińska 1997; Reszel and Reszel 1997). In the case of *R. solani* the necrosis of the underground parts can appear under the effect of phytotoxic compounds exudated by this fungus, for example m-metoxyphenyloacetylene acid and m-hydroxyphenyloacetylene acid (Orellana and Mondava 1983). Moreover, the phenols exudated by potato not only limit the number of microorganisms in the soil but they also have a negative effect on the size of the tuber yield (Bajus et al. 1986; Reszel and Reszel 1997).

Results of the microbiological analysis of the examined soil samples emphasized the positive effect of potato on the growth of microorganisms, and especially antagonistic bacteria and fungi. The increase of the number of microorganisms in the potato rhizosphere could have been caused by the root exudates of this plant (Funck-Jensen and Hockenhull 1984). Besides, the fact is known that the rhizosphere soil has the greatest biological activity (Schoruvitz and Zeigler 1989).

The proportion of pathogenic fungi in the potato rhizosphere was much smaller than the proportion of antagonistic fungi *Gliocladium* spp., *Trichoderma* spp. and *Penicillium* spp. According to Shihuang et al. (2000), *Trichoderma* spp., owing to their intensive growth around the root top, can effectively protect it from infection by soil-borne pathogenic fungi. Besides, the species *T. harzianum, T. koningii* and *T. viride* are recognized antagonists used in the protection of plants from different phytopathogens (Das and Hazarika 2000; Kovach et al. 2000; Mesta and Amaresh 2000; Prasad and Rangeshwaran 2000; Weber et al. 2000). On the other hand, the species from the genus *Gliocladium* are slightly poorer antagonists towards the pathogenic fungi (Łacicowa and Pięta 1989; McQuilken et al. 2001; Sarmah 1999).

Antagonistic bacteria *Pseudomonas* spp. and *Bacillus* spp. could have also affected the reduction of the number of phytopathogens in the potato rhizosphere and hence the reduction of the infection of the underground parts by pathogenic fungi. These microorganisms limit the growth and development of pathogenic fungi through the exudation of antibiotics and siderophores, which have fungistatic and fungicidal effects (El-Tarabily et al. 2000; Goel et al. 2000; Manwar et al. 2000; Saniewska 2000; Sobiczewski 2002; Yeole and Dube 2000).

The results obtained in the present studies as well as abundant information from literature on the subject point to a big role of antagonistic microorganisms inhibiting the infection of plants by soil-borne pathogenic fungi.

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## POLISH SUMMARY

### ANTAGONISTYCZNE BAKTERIE I GRZYBY OGRANICZAJĄCE PORAŻENIE ZIEMNIAKA PRZEZ GRZYBY CHOROBOTWÓRCZE PRZEŻYWAJĄCE W GLEBIE

Celem prezentowanych badań było określenie składu gatunkowego grzybów występujących na organach podziemnych ziemniaka w okresie kwitnienia oraz ustalenie składu jakościowego i ilościowego mikroorganizmów zasiedlających glebowe środowisko uprawne tej rośliny. Ponadto poszukiwano mikroorganizmów antagonistycznych ograniczających porażenie ziemniaka przez fitopatogeny przeżywające w glebie.

Analiza mikologiczna wykazała, że nekroza na korzeniach i podstawie łodygi ziemniaka wywołana była przez *Alternaria alternata, Fusarium* spp., *Colletotrichum coccodes, R. solani.*

W wyniku analizy mikrobiologicznej z ryzosfery ziemniaka uzyskano dwukrotnie więcej bakterii i grzybów chorobotwórczych, aniżeli z gleby pozaryzosferowej. W badanych próbach gleby dominowały *Fusarium* spp., *A. alternata* i *Rhizoctonia solani.*

Z ryzosfery ziemniaka uzyskano prawie trzykrotnie więcej antagonistycznych bakterii *Pseudomonas* spp. i *Bacillus* spp., oraz ponad dwukrotnie więcej antagonistycznych grzybów (*Penicillium* spp., *Trichoderma* spp., *Gliocladium* spp.), aniżeli z gleby pozaryzosferowej.