

PROTECTIVE EFFECT OF ANTAGONISTIC MICROORGANISMS IN LIMITING THE OCCURRENCE OF CERTAIN PATHOGENIC SOIL-BORNE FUNGI

Elżbieta Patkowska

Department of Phytopathology, Agricultural University, Leszczyńskiego str. 7, 20-069 Lublin
e-mail: epatko@consus.ar.lublin.pl

Summary. The purpose of the present studies was to search for antagonistic microorganisms limiting the occurrence of certain pathogenic soil-borne fungi. The studies were carried out in the years 1999-2001 at Czesławice on an experimental field with naturally accumulated infection material in the soil. The object of the studies was the rhizosphere soil of soybean, cv. Polan. The isolates of bacteria obtained from the rhizosphere of soybean (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 *Fusarium culmorum*, *F. oxysporum* f. sp. *glycines*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. Laboratory studies showed that the growth and development of the tested phytopathogens were most effectively limited by antagonistic bacteria *Pseudomonas* spp. and antagonistic fungi *Penicillium* spp. and *Trichoderma* spp.

Key words: soil environment, phytopathogens, antagonistic microorganisms

INTRODUCTION

The soil is the habitat of fungi and bacteria that have a positive or harmful effect on the growth and development of plants. The greatest biological activity is characteristic of the rhizosphere soil [15]. The composition of microorganisms in the root zone undergoes constant changes, for example under the influence of root exudates [14]. On the other hand, the chemical composition of the substances exuded by the roots is related to the genus, species, cultivar, the age of the plant as well as many other biotic and abiotic factors [11, 13, 15].

It is especially important to establish the composition of antagonistic microorganisms towards the soil-borne phytopathogens because of the

phenomenon of soil resistance from the point of view of the biological protection of plants. That is the reason why studies were undertaken in order to look for the antagonistic bacteria and fungi limiting the growth and development of pathogenic fungi in the cultivation environment of soybean.

MATERIAL AND METHODS

The studies were carried out in the years 1999-2001 at Czesławice on an experimental field with naturally accumulated infection material in the soil. The object of the studies was the rhizosphere soil of soybean, cv. Polan.

The experiment was set on grey-brown podzolic soil formed from loesses. The soil constituted the second good wheat complex. Besides, there was the soil with granulometric composition of very fine sandy soil (34% of silt and clay). Its 0-30 cm-deep layer contained 1.4% of humus on average. The mean content of K_2O , P_2O_5 and MgO in 100 g of the soil was 35.8 mg, 35.2 mg and 6.8 mg, respectively. The soil reaction was acid ($pH = 5.9$). These data are presented on the basis of the results of analyses performed at the Chemical-Agricultural Station in Lublin.

In each year of the studies the rhizosphere soil was sampled at the anthesis of soybean in order to conduct a laboratory microbiological analysis according to the method described by Martyniuk *et al.* [9].

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 [12].

The isolates of bacteria (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 *Fusarium culmorum*, *F. oxysporum* f. sp. *glycines*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in accordance with the methods described by Martyniuk *et al.* [9] and Mańka and Mańka [8].

RESULTS AND DISCUSSION

The microbiological analysis of the rhizosphere soil of soybean showed that 1 g of d. w. of the soil contained the total number of bacteria of 9.36×10^6 colonies on average from three years of studies. The number of *Pseudomonas*

spp. was slightly lower and it was mean 5.05×10^6 colonies, while *Bacillus* spp. constituted the average of 3.48×10^6 colonies. The total number of fungi colonies was 132.75×10^3 colonies (Fig. 1).

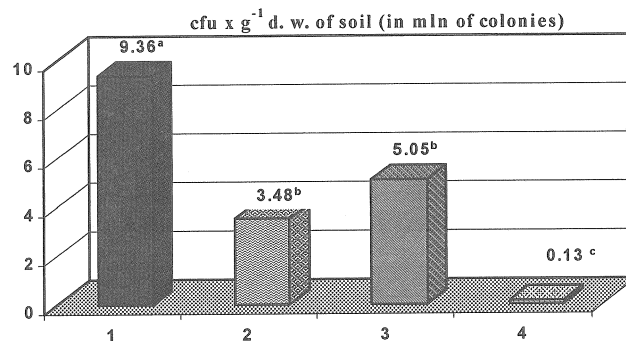


Fig. 1. The number of bacteria and fungi in soybean rhizosphere (mean from the years 1999-2001)
 1 – Total number of bacteria, 2 – Number of bacteria *Bacillus* spp., 3 – Number of bacteria *Pseudomonas* spp., 4 – Total number of fungi
 Means differ significantly ($P < 0.05$), if they are not marked with the same letter

The proportion of pathogenic fungi obtained from the rhizosphere of soybean was 36.5% (total from three years of studies). *Fusarium* spp. (15.4%), *Rhizoctonia solani* (7.5%) and *Sclerotinia sclerotiorum* (9.8%) dominated within the group of pathogenic fungi. The genus *Fusarium* was represented by *F. culmorum* (4%), *F. oxysporum* (8.9%) and *F. solani* (1.7%). Within the saprophytic fungi, *Penicillium* spp. (15.1%), *Trichoderma* spp. (13.9%) and *Gliocladium* spp. (10.6%) were most frequently isolated. Among the other saprophytic fungi there were also isolated *Acremonium* spp., *Cladosporium* spp., *Chaetomium* spp. and *Torula* spp., and their proportion was 23.9% (Fig. 2).

Laboratory tests showed that *Pseudomonas* spp. (87 isolates) and *Penicillium* spp. (54 isolates) dominated among the microorganisms antagonistic towards the examined pathogenic fungi in the rhizosphere of soybean (Fig. 3).

The presented studies showed that the great number of antagonistic isolates of *Bacillus* spp. (42 isolates), *Pseudomonas* spp. (87 isolates), *Gliocladium* spp. (54 isolates) and *Trichoderma* spp. (49 isolates) affected the reduction of the number of phytopathogens in the rhizosphere of soybean (Figs 2 and 3). This fact is confirmed in numerous items of literature [1, 4, 6, 10]. Besides, as stated by Księżniak and Kobus [5], siderophores formed by fluorescent *Pseudomonas* inhibit the development of pathogenic fungi in the soil. Antagonistic fungi and

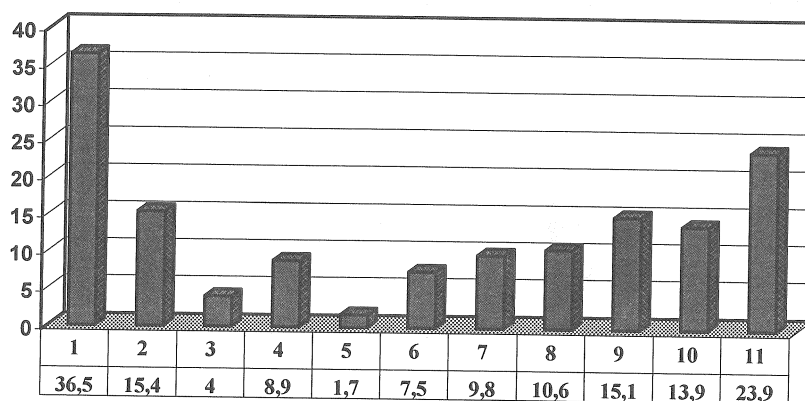


Fig. 2. Participation of fungi isolated from soybean rhizosphere (total from the years 1999-2001)
 1 – total of pathogenic fungi, 2 – *Fusarium spp.*, 3 – *F. culmorum*, 4 – *F. oxysporum*, 5 – *F. solani*,
 6 – *R. solani*, 7 – *S. sclerotiorum*, 8 – *Gliocladium spp.*, 9 – *Penicillium spp.*, 10 – *Trichoderma spp.*,
 11 – other of saprophytic fungi

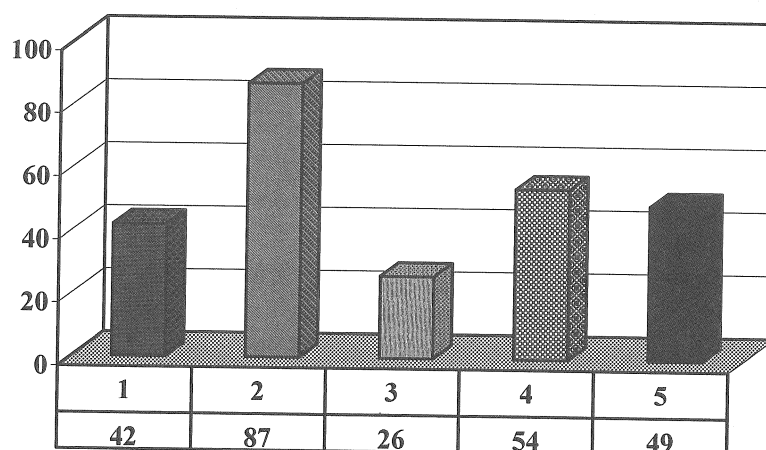


Fig. 3. Antagonistic microorganisms isolated from soybean rhizosphere (total from the years 1999-2001)
 1 – *Bacillus spp.*, 2 – *Pseudomonas spp.*, 3 – *Gliocladium spp.*, 4 – *Penicillium spp.*, 5 – *Trichoderma spp.*

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, 7]. 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, 4].

CONCLUSIONS

1. The rhizosphere soil of soybean contained such antagonistic bacteria as *Bacillus* spp., *Pseudomonas* spp. and fungi *Gliocladium* spp., *Penicillium* spp. and *Trichoderma* spp.

2. A high number 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.

REFERENCES

1. Ahmed A. S., Sánchez C., Candela E. M.: Evaluation of induction of systemic resistance in pepper plants (*Capsicum annuum*) of *Phytophthora capsici* using *Trichoderma harzianum* and its relation with capsidiol accumulation. European Journal of Plant Pathology, 106, 9, 817 – 824, 2000.
2. El – Tarabily K. A., Soliman M. H., Nassar A. H., Al – Hassani H. A., Sivasithamparan K., Mc Kenna F., Hardy G. E. ST. J.: Biological control of *Sclerotinia minor* using a chitinolytic bacterium and actinomycetes. Plant Pathol., 49, 5, 573-583, 2000.
3. Fiddman P.J., O'Neill T.M., Rossall S.: Screening of bacteria for the suppression of *Botrytis cinerea* and *Rhizoctonia solani* on lettuce (*Lactuca sativa*) using leaf disc bioassays. Annals of Applied Biology 137, 3, 223 – 235, 2000.
4. Kredics L., Dóczy L., Antal Z., Manczinger L.: Effect of heavy metals on growth and extracellular enzyme activities of mycoparasitic *Trichoderma* strains. Bulletin of Environmental Contamination and Toxicology, 66, 2, 249 – 252, 2000.
5. Księżniak A., Kobus J.: The proportion of microorganisms of the rhizosphere of wheat, barley and oat in the production of siderophores. (in Polish). Pam. Puławski – Prace IUNG, 102, 77-90, 1993.
6. Łacicowa B., Pięta D.: The harmful effect of fungi from the genera of *Trichoderma* and *Gliocladium* for certain pathogens of bean. (in Polish). Zesz. Probl. Post. Nauk Roln, 374, 235 – 242, 1989.
7. Manwar A.V., Vaiganker P.D., Bhonge L.S., Chincholkar S.B.: *In vitro* suppression of plant pathogens by siderophores of fluorescent *pseudomonas*. Indian Journal of Microbiology 40, 2, 109 – 112, 2000.
8. Mańka K., Mańka M.: A new method for evaluating interaction between soil inhibiting fungi and plant pathogen. Bull. OILB/SROP, XV, 73-77, 1992.
9. Martyniuk S., Masiak D., Stachyra A., Myśków W.: Populations of microorganisms of the root zone of different grasses and their antagonism towards *Gaeumannomyces graminis* var. *tritici*. (in Polish). Pam. Puł. Pr. IUNG, 98, 139-144, 1991.
10. McQuilken M.P., Gemmell J., Lahdenpera M.L.: *Gliocladium catenulatum* as a potential biological control agent of damping off in bedding plants. Journal of Phytopathology, 149, 3/4, 171 – 178, 2001.
11. Odham G., Tunlid A., Valeur A., Sundin P., White D. C.: Model system for studies of microbial dynamics at exuding surfaces such as the rhizosphere. Appl. Environ. Microbiol., 52, 191-196, 1986.

12. **Oktaba W.:** Methods of mathematical statistics in experimental studies. (in Polish). PWN, Warszawa, 488 pp, 1987.
13. **Parke J. L.:** Root colonization by indigenous and introduced microorganisms. In: The Rhizosphere and Plant Growth. D. L. Keister & P. B. Grogan, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands, 33-42, 1990.
14. **Pięta D.:** Initial studies of populations of fungi and bacteria in the soil under influence of the cultivation of spring wheat and winter wheat in a growth chamber. *Acta Agrobot.*, 52, 1-2, 161-166, 1999.
15. **Schoruvitz R., Zeigler H.:** Interaction of maize roots and rhizosphere microorganisms. *Z. Pflanzenkrankh. Bodenb.*, 152, 217-222, 1989.

OCHRONNE DZIAŁANIE MIKROORGANIZMÓW
ANTAGONISTYCZNYCH W OGRANICZANIU WYSTĘPOWANIA
NIEKTÓRYCH GRZYBÓW CHOROBTWÓRCZYCH
PRZEŻYWAJĄCYCH W GLEBIE

Elżbieta Patkowska

Akademia Rolnicza, Katedra Fitopatologii, ul. Leszczyńskiego 7, 20-069 Lublin,
e-mail: epatko@consus.ar.lublin.pl

Streszczenie. Celem prezentowanych badań było poszukiwanie mikroorganizmów antagonistycznych ograniczających występowanie niektórych grzybów chorobotwórczych przeżywających w glebie. Badania przeprowadzono w latach 1999-2001 na polu doświadczalnym w Czesławicach z naturalnie nagromadzonym w glebie materiałem infekcyjnym. Przedmiotem badań była gleba ryzosferowa soi odm. Polan.

Uzyskane z ryzosfery soi izolaty bakterii *Bacillus* spp. i *Pseudomonas* spp. oraz izolaty grzybów *Gliocladium* spp., *Penicillium* spp. i *Trichoderma* spp. użyto do określenia ich antagonistycznego oddziaływania względem takich fitopatogenów glebowych jak *Fusarium culmorum*, *F. oxysporum* f.sp. *glycines*, *F. solani*, *Rhizoctonia solani* i *Sclerotinia sclerotiorum*.

Badania laboratoryjne wykazały, że wzrost i rozwój testowanych fitopatogenów najskuteczniej hamowały antagonistyczne bakterie z rodzaju *Pseudomonas* oraz grzyby z rodzajów *Trichoderma* i *Penicillium*.

Słowa kluczowe: środowisko glebowe, fitopatogeny, mikroorganizmy antagonistyczne