

THE EFFECT OF DRESSING ZAPRAWA OXAFUN T ON THE QUANTITATIVE AND QUALITATIVE COMPOSITION OF RHIZOSPHERE MICROORGANISMS OF RUNNER BEAN AND SOYBEAN

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Abstract. The studies were conducted in the years 2000–2002 at the Experimental Station of Czesławice near Nałęczów on a field of monocultures of runner bean and soybean. The subject of the studies was the rhizosphere soil of these plant species. The experiment of each plant included 2 combinations, i.e. with dressing the seeds with Zaprawa Oxafun T and without dressing the seeds (control).

Results of the microbiological analysis of the rhizosphere soil of runner bean and soybean showed that the plants grown of the seeds dressed with Zaprawa Oxafun T always gave a greater number of bacteria colonies, *Bacillus* spp. and *Pseudomonas* spp. but a smaller number of fungi colonies than in the control combination. In comparison to the rhizosphere soil of soybean, the rhizosphere soil of runner bean contained much more bacteria and a much lower number of fungi colonies.

Among the obtained saprotrophic macroorganisms, a more numerous group of antagonistic bacteria and fungi was achieved from the rhizosphere soil of runner bean than from the rhizosphere soil of soybean. Whatever the plant species, considerably more antagonistic bacteria and fungi occurred in the combination with Zaprawa Oxafun T as compared to the control.

The plant species as well as the introduction of Zaprawa Oxafun T into the soil together with the seeds had a considerable influence on the quantitative and qualitative composition of the populations of soil microorganisms.

Key words: runner bean, soybean, rhizosphere microorganisms, Zaprawa Oxafun T

INTRODUCTION

The soil environment is the habitat of a lot of microorganisms, both pathogenic and saprotrophic ones, among which a variety of relations take place.

The qualitative and quantitative composition of the communities of particular microorganisms and their biological activity in the soil are related to biotic and abiotic factors.

An important role for the microorganisms inhabiting the rhizosphere is played by the root exudates, whose quantitative and qualitative composition can change under the effect of soil or climatic factors. Increased temperature, decreased soil humidity [Rovira 1959], elevated level of mineral nitrogen [Liljeroth et al. 1990] or a lowered quantity of phosphorus or potassium [Krafczyk et al. 1984]. According to Gołębiowska [1986], a constant inflow of nutritive substrates from the root exudates causes that the rhizosphere soil contains much more microorganisms than the non-rhizosphere soil. An important role is also played by the crop residues introduced to the soil, which are the source of nutrition for the development of soil microorganisms [Myśków 1989, Pięta and Patkowska 2000, Pięta et al. 2001].

Among the abiotic factors a significant function in the formation of the populations of soil microorganisms is also played by mineral fertilizers and pesticides introduced into the soil. According to Borecki [1984], rational application of fungicidal and bactericidal preparations brings about changes in the composition of microorganism populations in the soil decreasing the number of propagation units of microorganisms harmful for the plants. Introducing fungicides into the soil on the surface of the dressed seeds decreased the plant infection by pathogenic fungi [Marcinkowska and Schollenberger 1979, Pięta and Pastucha 1993, Pięta and Pastucha 1994].

On the other hand, the literature of the subjects lacks information about the populations of rhizosphere microorganisms, which induced the author of the present paper to undertake the studies.

MATERIALS AND METHODS

The studies were conducted in the years 2000–2002 at the Experimental Station at Czesławice near Nałęczów on a monoculture field of runner bean and soybean.

The object of the studies was the rhizosphere soil of:

- the plants of runner bean grown from the seeds dressed with Zaprawa Oxafun T (2g/1kg seeds),
- the plants of runner bean grown from the seeds without (control),
- the plants of soybean grown from the seeds dressed with Zaprawa Oxafun T (2g/1kg seeds),
- the plants of soybean grown from the seeds without (control).

Each experimental combination included 4 repetitions (plots).

The rhizosphere soil was sampled in each year of the studies during the anthesis of runner bean and soybean plants. The soil taken for the microbiological analysis was prepared according to the method described by Martyniuk et al. [1991].

In order to establish the number of bacteria from the genera of *Bacillus* and *Pseudomonas* the studies made use of the method of rubbing the soil solution of a definite dilution. A selective medium *Pseudomonas* agar F and dilutions of the soil solution 10^{-2} , 10^{-3} and 10^{-4} were used for the bacteria from the genus of *Pseudomonas*, while for the genus of *Bacillus* the medium Tryptic soy agar and the dilutions of 10^{-4} , 10^{-5} and 10^{-6} previously heated up to the temperature of 80° for 20 minutes were used.

In order to determine the total number of bacteria the studies used the medium Nutrient agar and the method of pouring the medium cooled to about 40°C over the dilutions of the soil solution of 10^{-5} , 10^{-6} , 10^{-7} . In order to obtain the bacteria colonies the dishes were stored for the period of 24 to 28 hours in a thermostat at the temperature of 26°C. After this time the bacteria colonies were counted and next they were split off to the test-tubes for the slants prepared from the Nutrient agar medium.

The total number of fungi was determined on the Martin's [1950] agar medium using the dilutions of the soil solution of 10^{-2} , 10^{-3} , 10^{-4} and the method of flooding with cooled medium.

The results concerning the numbers of bacteria and fungi were statistically analyzed and the significance of differences was established on the basis of Tukey's confidence intervals [Oktaba 1987].

The obtained bacteria isolates from the genera of *Bacillus* and *Pseudomonas* as well as the fungi from the genera of *Gliocladium* and *Trichoderma* were used to determine their antagonistic effect towards pathogenic fungi such as *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Phoma exigua* var. *exigua*, *Pythium irregulare*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*.

The study used the method described by Mańka and Kowalski [1968], Mańka [1974], Martyniuk et al. [1991] and Pięta [1999].

RESULTS

On the basis of the microbiological analysis of the rhizosphere soil of runner bean and soybean the studies observed differences in the numbers of particular microorganism populations (tab. 1).

Among the examined soil samples more colonies of bacteria in total in 1 g of d.w. were obtained from the rhizosphere soil of runner bean than from the rhizosphere soil of soybean. Regardless of the examined rhizosphere soil of runner bean or soybean the combination with Zaprawa Oxafun T always gave more bacteria colonies (tab. 1).

Results of the microbiological analysis showed that a similar number of colonies *Bacillus* spp. was achieved both from the rhizosphere soil of runner bean and soybean grown from the seeds dressed with Zaprawa Oxafun T. The number of colonies of those bacteria was also similar in 1 g of d.w. of the rhizosphere soil of runner bean and soybean growing on control plots (tab. 1). Comparing the number of *Bacillus* spp. in particular experimental combinations, significantly more of these bacteria were found in the rhizosphere soil with Zaprawa Oxafun T (tab. 1).

Whichever the experimental combination, more colonies of *Pseudomonas* spp. were obtained from 1 g d.w. of the rhizosphere soil of runner bean than from the rhizosphere soil of soybean. Both the rhizosphere soil of runner bean and soybean sampled from the combination with Zaprawa Oxafun T contained a greater number of *Pseudomonas* spp. as compared to the control (tab. 1).

The total number of fungi colonies was greater in the rhizosphere soil of soybean than in the rhizosphere soil of runner bean. The microbiological analysis of rhizosphere soils showed that the presence of Zaprawa Oxafun T contributed to a drop of the num-

Table 1. The number of bacteria and fungi in the rhizosphere of runner bean and soybean
 Tabela 1. Liczebność bakterii i grzybów w ryzosferze fasoli wielokwiatowej i soi

Experimental combination Kombinacja doświadczenia	Total number of bacteria (mln g ⁻¹ d.w. of soil) Ogólna liczebność bakterii mln g ⁻¹ s.m. gleby				Total number of <i>Bacillus</i> spp. (mln g ⁻¹ d.w. of soil) Liczebność <i>Bacillus</i> spp. mln g ⁻¹ s.m. gleby				Total number of <i>Pseudomonas</i> spp. (mln g ⁻¹ d.w. of soil) Liczebność <i>Pseudomonas</i> spp. mln g ⁻¹ s.m. gleby				Total number of fungi (thous g ⁻¹ d.w. of soil) Ogólna liczebność grzybów (tys. g ⁻¹ s.m. gleby)			
	2000	2001	2002	mean średnia	2000	2001	2002	mean średnia	2000	2001	2002	mean średnia	2000	2001	2002	mean średnia
	runner bean – fasola wielokwiatowa															
Dressed seeds with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	8.52 ^b	9.3 ^b	16.28 ^b	11.37 ^b	5.2 ^b	4.1 ^a	8.9 ^b	6.1 ^b	3.12 ^b	3.29 ^b	2.91 ^a	3.1 ^b	21.2 ^a	22.7 ^a	23.21 ^a	22.37 ^a
Control – Kontrola	6.8 ^a	7.3 ^a	15.78 ^a	9.96 ^a	4.1 ^a	3.75 ^a	3.28 ^a	3.71 ^a	2.12 ^a	2.58 ^a	2.89 ^a	2.53 ^a	28.9 ^b	26.6 ^b	33.19 ^b	29.56 ^b
soybean – soja																
Dressed seeds with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	8.11 ^b	8.53 ^b	15.18 ^b	10.6 ^b	3.11 ^b	5.23 ^b	8.7 ^b	5.68 ^b	0.89 ^b	2.6 ^b	2.66 ^b	2.05 ^b	67.09 ^a	73.98 ^a	30.5 ^a	57.19 ^a
Control – Kontrola	5.62 ^a	5.30 ^a	7.73 ^a	6.2 ^a	1.95 ^a	1.80 ^a	5.73 ^a	3.16 ^a	0.47 ^a	1.79 ^a	0.51 ^a	0.92 ^a	83.08 ^b	130.09 ^b	40.71 ^b	84.63 ^b

*Means for individual plant species in columns differ significantly ($P \leq 0.05$) if they are not marked with the same letter

*Średnie wartości dla określonego gatunku rośliny w kolumnach różnią się istotnie ($P \leq 0.05$), jeśli nie są oznaczone tą samą literą

ber of fungi colonies. In each year of the studies the numbers of fungi colonies in 1 g of d.w. of the rhizosphere soil of both species of plants growing in the control was significantly higher (tab. 1).

The species composition of fungi isolated from the rhizosphere soil of particular plant species and the corresponding combinations was different (tab. 2, 3). 907 colonies of fungi belonging to 16 species (tab. 2) were obtained from the rhizosphere soil of runner bean, while the rhizosphere soil of soybean gave 1367 colonies of fungi belonging to 33 species (tab. 3). In the rhizosphere soil of runner bean a little more fungi colonies were obtained from the control combination (487 colonies) than from the combination with Zaprawa Oxafun T (420 colonies) (tab. 2).

Among the pathogenic fungi isolated from the rhizosphere soil of runner bean sampled from the combination with Zaprawa Oxafun T the most numerously isolated species was *Fusarium oxysporum*, constituting 6.4% of all colonies. Besides, *Fusarium solani* was isolated constituting 2% of fungi colonies in this experimental combination (tab. 2). On the other hand, these species were more numerously isolated from the rhizosphere soil of runner bean from the control combination (*F. oxysporum* – 11.9%, *F. solani* – 5.7% of all colonies).

Table 2. Fungi isolated from rhizosphere of runner bean (sum colonies of 3 years)
Tabela 2. Grzyby wyizolowane z ryzosfery fasoli wielokwiatowej (suma kolonii z 3 lat badań)

Fungus species Gatunek grzyba	The number of colonies Liczba kolonii		Total Razem
	Experimental combination Kombinacja doświadczenia		
	Zaprawa Oxafun T	Control Kontrola	
<i>Alternaria alternata</i> (Fr.) Keissler	11	39	50
<i>Aspergillus niger</i> van. Tieghm	8	37	45
<i>Cladosporium cladosporioides</i> (Fres) de Vries	9	22	31
<i>Fusarium oxysporum</i> Schl.	27	58	85
<i>Fusarium solani</i> (Mart.) Sacc.	9	28	37
<i>Gliocladium catenulatum</i> Gilman et Abbott	50	30	80
<i>Humicola grisea</i> Domsch	8	18	26
<i>Mucor hiemalis</i> Wehmer	5	38	43
<i>Penicillium canescens</i> Sopp.	7	33	40
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling) Samson, Stolk et Hadlok	5	24	29
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i> Samson, Stolk et Hadlok	6	14	20
<i>Rhizopus nigricans</i> Ehrenberg	9	30	39
<i>Talaromyces flavus</i> (Klocker) Stolk, Samson	8	38	46
<i>Trichoderma hamatum</i> (Bon.) Bain.	40	12	52
<i>Trichoderma koningii</i> Oud	145	54	199
<i>Trichoderma viride</i> Pers. ex S.F.Gray	73	12	85
Total – Razem	420	487	907

Both the rhizosphere soil of runner bean and the rhizosphere soil of soybean contained the fungi from the genus *Fusarium* represented by *F. oxysporum* and *F. solani* (tab. 3). The proportion of those species in the rhizosphere soil of soybean after Zaprawa Oxafun T was applied constituted 10.25% and 3.03% of all the colonies, respec-

tively, while in the control combination it was 26.01% and 11.62% of all the colonies (tab. 3). Besides, the species of *Rhizoctonia solani* and *Verticillium albo-atrum* were scarcely isolated from the rhizosphere soil of soybean, mainly from the control combination (tab. 3).

The fungi from the genera of *Trichoderma* spp. and *Gliocladium* spp. dominated among the saprotrophic fungi obtained from the combination with Zaprawa Oxafun T from the rhizosphere soil of runner bean and soybean. These fungi occurred in the rhizosphere soil of both plant species being less numerous in the control combination than in the combination with Zaprawa Oxafun T (tab. 2, 3).

Table 3. Fungi isolated from rhizosphere of soybean (sum colonies of 3 years)
Tabela 3. Grzyby wyizolowane z ryzosfery soi (suma kolonii z 3 lat badań)

Fungus species Gatunek grzyba	The number of colonies Liczba kolonii		Total Razem
	Experimental combination Kombinacja doświadczenia		
	Zaprawa Oxafun T	Control Kontrola	
<i>Acremonium murorum</i> (Corda) W.Gams	6	10	16
<i>Acremonium roseum</i> (Oud.) W.Gams	2	6	8
<i>Alternaria alternata</i> (Fr.) Keissler	7	10	17
<i>Aspergillus niger</i> van. Tieghm	3	8	11
<i>Aureobasidium pullulans</i> (de Bary) Arnoud	1	-	1
<i>Botryotrichum piluliferum</i> Sacc. et Marchal	2	3	5
<i>Cladosporium cladosporioides</i> (Fres) de Vries	5	15	20
<i>Fusarium equiseti</i> (Corda) Sacc.	2	13	15
<i>Fusarium oxysporum</i> Schl.	44	244	288
<i>Fusarium solani</i> (Mart.) Sacc.	13	109	122
<i>Gliocladium catenulatum</i> Gilman et Abbott	30	13	43
<i>Gliocladium fimbriatum</i> Gilman et Abbott	30	5	35
<i>Gliocladium roseum</i> Bainier	24	10	34
<i>Humicola grisea</i> Domsch	5	12	17
<i>Mucor hiemalis</i> Wehmer	8	90	98
<i>Myrothecium verrucaria</i> Dietmar ex Fries	1	4	5
<i>Penicillium canescens</i> Sopp.	8	27	35
<i>Penicillium lividum</i> Westling	1	9	10
<i>Penicillium Miczyński</i> Zal.	1	4	5
<i>Penicillium nigricans</i> Bainier ex Thom	15	144	159
<i>Penicillium verrucosum</i> Dierckx var. <i>cyclopium</i> (Westling) Samson, Stolk et Hadlok	7	24	31
<i>Penicillium verrucosum</i> Dierckx var. <i>verrucosum</i> Samson, Stolk et Hadlok	5	27	32
<i>Rhizoctonia solani</i> Kühn	-	3	3
<i>Rhizopus nigricans</i> Ehrenberg	22	74	96
<i>Talaromyces flavus</i> (Klocker) Stolk, Samson	5	15	20
<i>Trichoderma aureoviride</i> Rifai	20	4	24
<i>Trichoderma hamatum</i> (Bon.) Bain.	38	5	43
<i>Trichoderma harzianum</i> Rifai	28	10	38
<i>Trichoderma koningii</i> Oud	30	2	32
<i>Trichoderma pseudokoningii</i> Rifai	11	7	18
<i>Trichoderma polysporum</i> (Link ex Pers.) Rifai	12	4	16
<i>Trichoderma viride</i> Pers. ex S.F.Gray	40	6	46
<i>Verticillium albo-atrum</i> Reinke & Berth	3	21	24
Total – Razem	429	938	1367

Other saprotrophic species isolated from the rhizosphere soil of runner bean in both experimental combinations were *Aspergillus niger*, *Cladosporium cladosporioides*, *Mucor hiemalis*, *Rhizopus nigricans* as well as the fungi from the genus of *Penicillium*. The rhizosphere soil of soybean sampled in both experimental combinations contained *Acremonium* spp., *Botryotrichum* spp., *Cladosporium* spp. and *Penicillium* spp. These colonies of saprotrophic fungi, in the rhizosphere soil of both runner bean and soybean, occurred in smaller numbers in the combination with Zaprawa Oxafun T as compared to the control (tab. 2, 3).

Among the obtained saprotrophic microorganisms, the most numerous group of antagonistic bacteria and fungi was obtained from the rhizosphere soil of runner bean (tab. 4).

Table 4. The occurrence of antagonistic microorganisms in the rhizosphere of plants in the years 2000–2001

Tabela 4. Występowanie mikroorganizmów antagonistycznych w glebie ryzosferowej roślin w latach 2000–2001

Fungus or bacteria Grzyb lub bakteria	Number of colonies in 1 g of soil Liczba kolonii	
	Zaprawa Oxafun T	Control Kontrola
The rhizosphere soil of runner bean		
<i>Bacillus</i> spp.	154	136
<i>Pseudomaonas</i> spp.	112	93
Total – Razem	266	229
The rhizosphere soil of soybean		
<i>Gliocladium</i> spp.	50	30
<i>Trichoderma</i> spp.	258	78
Total – Razem	308	108
The rhizosphere soil of soybean		
<i>Bacillus</i> spp.	49	45
<i>Pseudomaonas</i> spp.	28	15
Total – Razem	77	60
The rhizosphere soil of soybean		
<i>Gliocladium</i> spp.	84	28
<i>Trichoderma</i> spp.	179	38
Total – Razem	263	66

1 g of d.w. of the rhizosphere soil of runner bean in the combination with Zaprawa Oxafun T contained 266 colonies of bacteria and 308 colonies of fungi antagonistic towards pathogenic fungi. On the other hand, the control combination gave 229 colonies of bacteria and 108 colonies of antagonistic fungi (tab. 4). The rhizosphere soil of soybean in the combination with Zaprawa Oxafun T gave 77 colonies of bacteria and 263 colonies of antagonistic fungi, while the control combination contained 60 colonies of bacteria and 66 colonies of antagonistic fungi (tab. 4).

DISCUSSION

The field studies showed that the plant species as well as application of Zaprawa Oxafun T for seed dressing had a effect on the quantitative and qualitative composition of the population of soil microorganisms. As a result of the microbiological analysis of the rhizosphere soil of runner bean and soybean in the combinations with Zaprawa Oxafun T a greater total number of bacteria, *Bacillus* spp., *Pseudomonas* spp. and a much lower total number of fungi colonies were obtained. This fact can be explained by the activity of Zaprawa Oxafun T based on a two-element active substance [Borecki 1984].

It was found out on the basis of the results that the examined soil samples differed in their quantitative and qualitative composition of pathogenic and saprotrophic fungi in particular experimental combinations. A much smaller number of pathogenic fungi as compared to the control was obtained from the combination with Zaprawa Oxafun T, no matter what the plant species was. This phenomenon can be explained on the basis of earlier studies *in vitro*, which observed a fungicidal effect of Zaprawa Oxafun T towards *Botrytis cinerea*, *Fusarium culmorum*, *F. oxysporum*, *F. solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* [Łacicowa and Sułek-Pięta 1981, Pięta and Łabuda 1994].

The present studies found out a more numerous occurrence of saprotrophic microorganisms in the combination with Zaprawa Oxafun T than in the control. Within the saprotrophic microorganisms occurring in the rhizosphere soil of runner bean and soybean the isolated species included those from the genera of *Trichoderma* and *Gliocladium*, which had antagonistic effect on phytopathogens. The rhizosphere soil of both plant species contained much more propagation units of bacteria and antagonistic fungi after applying Oxafun T than in the control. This fact can be explained by considerable tolerance of *Trichoderma* and *Gliocladium* to the effect of chemical substances [Papavizas 1985]. Besides, it is supposed that the place of limited growth and development of pathogens is taken by saprotrophic microorganisms, including those with antagonistic effect. This phenomenon is explained by Myśków [1989], stating that there are definite proportions between microorganisms, where inhibition of one microorganism is followed by the development of another.

It follows from abundant information that both antagonistic fungi – *Trichoderma* spp. and *Gliocladium* spp. as well as bacteria *Bacillus* spp. and *Pseudomonas* spp. inhibit the growth and development of pathogenic fungi living in the soil [Pięta and Patkowska 2000, Pięta et al. 2001].

It can be supposed that a numerous occurrence of antagonistic microorganisms in the soil could have been, apart from the chemical preparation, the cause of a decreased number of propagation units of phytopathogens in this environment.

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ODDZIAŁYWANIE ZAPRAWY OXAFUN T NA SKŁAD ILOŚCIOWY I JAKOŚCIOWY MIKROORGANIZMOW RYZOSFEROWYCH FASOLI WIELOKWIATOWEJ ORAZ SOI

Streszczenie. Badania prowadzono w latach 2000–2002 w Gospodarstwie Doświadczalnym w Czesławicach k.Nałęczowa na polu monokultury fasoli wielokwiatowej oraz soi.. Przedmiotem badań była gleba ryzosferowa wymienionych gatunków roślin. Doświadczenie dla każdej rośliny obejmowało po 2 kombinacje, tj. z zaprawianiem nasion Zaprawą Oxafun T oraz bez zaprawiania nasion (kontrolę).

Wyniki analizy mikrobiologicznej gleby ryzosferowej fasoli wielokwiatowej i soi wykazały, że z roślin wyrosłych z nasion zaprawianych Zaprawą Oxafun T uzyskiwano zawsze większą ogólną liczbę kolonii bakterii, *Bacillus* spp. i *Pseudomonas* spp., a mniejszą liczbę kolonii grzybów, aniżeli w kombinacji kontrolnej. W glebie ryzosferowej fasoli występowało znacznie więcej bakterii, a mniej kolonii grzybów w porównaniu do gleby ryzosferowej soi.

Spośród uzyskanych mikroorganizmów saprotroficznych liczniejszą grupę antagonistycznych bakterii i grzybów uzyskano z gleby ryzosferowej fasoli wielokwiatowej aniżeli soi. Bez względu na gatunek rośliny znacznie więcej antagonistycznych bakterii i grzybów wystąpiło w kombinacji z Zaprawą Oxafun T w porównaniu do kontroli.

Gatunek rośliny, a także wprowadzenie do gleby Zaprawy Oxafun T wraz z nasionami miało istotny wpływ na skład ilościowy i jakościowy populacji mikroorganizmów glebowych.

Słowa kluczowe: fasola wielokwiatowa, soja, mikroorganizmy ryzosferowe, Zaprawa Oxafun T

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