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THE EFFECT OF CHITOSAN ON THE FORMATION OF MICROORGANISM COMMUNITIES IN THE RHIZOSPHERE SOIL OF SOYBEAN

Alina Pastucha
Agricultural University in Lublin

Abstract. The experiment was conducted in the years 1999–2001. The object of the studies was the rhizosphere soil of soybean 'Polan' cv., and 0.1% chitosan. This compound was used once (seed dressing), twice (seed dressing and seedling spraying), three times (seed dressing, seedling spraying and plant spraying at anthesis). The experiment also considered a control combination, i.e. without chitosan. The purpose of the studies was to determine the effect of chitosan on the qualitative and quantitative composition of microorganisms in rhizosphere soil with antagonists distinguished.
The microbiological analysis of samples of soybean rhizosphere soil taken from particular experimental combinations pointed to different numbers of bacteria and fungi. The greatest total number of bacteria colonies and the smallest number of fungi colonies were characteristic of the rhizosphere soil of soybean after using chitosan three times. This sample of soil contained the greatest number of bacteria and fungi with antagonistic effect towards plant pathogens.

Key words: chitosan, soybean, rhizosphere, antagonistic bacteria, antagonistic fungi

INTRODUCTION

Microorganisms belong to the elements of proper functioning of natural systems. Soil is the habitat of organisms living together and influencing each other. It is also the medium, the place where plants are attached and a reservoir of biogenous elements [Badura 2004]. Thanks to the existing microorganisms, soils are suppressive towards certain plant pathogens. Antagonistic microorganisms exudating metabolites with the properties of antibiotics or other toxic compounds are probably responsible for those properties [Pięta 1988, Parke 1990, Badura 2004].

Besides, compounds exudated by the roots affect the species composition of microorganisms and their number [Rovira 1969, Funck-Jensen and Hockenhull 1984]. The

Corresponding author – Adres do korespondencji: Alina Pastucha, Department of Phytopathology, Agricultural University in Lublin, ul. Leszczyńskiego 7, 20-069 Lublin, e-mail: alina.pastucha@ar.lublin.pl

number of particular populations of microorganisms as well as their biological activity in the soil depends, among other things, on the content of organic substances in the soil [Alexander 1977].

Chitin and its derivatives belongs to such organic compounds and it is more and more frequently introduced in cultivation and in plant protection [Transmo et al. 1993, Pospieszny 1997, Pięta et al. 2000, Orlikowski et al. 2001].

The purpose of the present paper was to determine the effect of chitosan on the quantitative and qualitative composition of microorganism populations in the rhizosphere soil of soybean.

MATERIAL AND METHODS

The studies were conducted in the years 1999–2001 and their object was the rhizosphere soil of soybean 'Polan' cv. The experiment was set during the first 10 days of May at the Experimental Station of Czesławice near Nałęczów on a field of soybean monoculture. It comprised three combinations, i.e. when 0.1% chitosan was used once (only for seed dressing), twice (for seed dressing + seedling spraying) and three times (for seed dressing + seedling spraying + plant spraying at the beginning of anthesis). Besides, the control combination was also considered, i.e. without using chitosan. Chitosan in the form of micro-crystalline gel was obtained from the Institute of Chemical Fibres in Łódź. Soil samples during the full anthesis of plants were sampled to sterile Petri dishes with the aim of conducting a microbiological analysis described by Martyniuk et al. [1991]. Soil solutions with the dilutions from 10^{-1} to 10^{-7} were prepared from particular soil samples in laboratory conditions. The total number of bacteria was determined on Nutrient Agar with the use of the solutions 10^{-5}, 10^{-6}, 10^{-7}. In the case of bacteria from genus *Bacillus* the medium Tryptic Soy Agar and the dilutions 10^{-4}, 10^{-5}, 10^{-6} were used, while for *Pseudomonas* spp. the studies used Pseudomonas Agar F and the dilutions 10^{-2}, 10^{-3} and 10^{-4}. The total number of fungi in each soil sample was established on Matrin's medium [1950] with the dilutions of 10^{-2}, 10^{-3}, 10^{-4}. The numbers of bacteria colonies and fungi were converted into 1 g of soil dry weight.

The obtained isolates of bacteria from genera *Bacillus* and *Pseudomonas* (200 isolates from each) and all isolates of fungi from genera *Gliocladium* and *Trichoderma* were used to determine their antagonistic effect towards *Botrytis cinerea, Fusarium culmorum, F. oxysporum, F. solani, Phoma exigua* var. *exigua, Pythium irregulare, Rhizoctonia solani* and *Sclerotinia sclerotiorum* according to the method described by Mańka [1974], Martyniuk et al. [1991], Pięta [1999].

RESULTS

A laboratory microbiological analysis of particular soil samples pointed to a differentiated number of bacteria and fungi (tab. 1). The biggest total number of bacteria among the studied samples was characteristic of rhizosphere soil sampled from the combination after chitosan was used three times, i.e. for seed dressing, seedling spraying and plant spraying at the beginning of anthesis. The smallest total number of bacteria ($3.98 \cdot 10^6$ cfu) was found in the rhizosphere soil of soybean in the control combination (tab. 1). A similar number was observed for bacteria *Bacillus* spp. The number of colonies of these bacteria in 1 g of d.w. of soybean rhizosphere soil was the highest in soil samples after chitosan was used three times, while the smallest ($1.8 \cdot 10^6$ cfu) was found in the rhizsophere soil of the control combination (tab. 1). Bacteria from genus *Pseudomonas* were also isolated from the soil samples. The greatest number of these bacteria was obtained from the rhizosphere soil after chitosan was used once, i.e. when it was used only for seed dressing, whereas the lowest number of colonies was observed in the control soil (tab. 1).

Particular soil samples contained varying numbers of fungi colonies. The smallest number of fungi isolates, $30.06 \cdot 10^3$ cfu was obtained from the rhizosphere soil of soybean after chitosan was used three times (tab. 1). On the other hand, the highest number of fungi colonies in soybean rhizosphere soil was found in the control combination.

The species and quantitative composition of fungi from particular samples of soybean rhizosphere soil was variable (tab. 2). Saprobionts and fungi pathogenic towards soybean occurred within the isolated fungi colonies (unpublished results from infection tests).

The most frequently isolated pathogenic fungus was *Fusarium oxysporum,* whose isolates constituted about 13.5% of all the obtained fungi. This fungus was most frequently isolated from the soil sampled from the control combination and from the combination after using chitosan twice. This species was scarce in the rhizosphere soil of soybean after chitosan was used three times (tab. 2). Besides, *Fusarium solani, Pythium irregulare, Rhizoctonia solani* and *Scleroctinia sclerotiorum* were isolated from the studied samples of rhizosphere soil. In the case of the rhizosphere soil from the control combination, *Verticillium albo-atrum* was sporadically isolated.

The dominating saprobiontic fungus was *Trichoderma* spp. represented by *T. hamatum, T. harzianum, T. koningii, T. pseudokoningii* and *T. viride.* Those species were especially numerous in the rhizosphere of soybean after chitosan was used three times (tab. 2). *Gliocladium* spp. was isolated from the studied soil samples in smaller numbers. The species from this genus was mainly isolated from the rhizosphere soil of soybean after chitosan was used two and three times. Besides, fungi from the genera *Acremonium, Aspergillus* and *Penicillium* as well as *Aureobasidium pullulans, Cladosporium cladosporioides, Mucor hiemalis, Myrothecium verrucaria* were isolated from the soil samples (tab. 2).

Among *Bacillus* spp. and *Pseudomonas* spp. the greatest number of isolates with antagonistic effect towards pathogenic fungi was obtained from the rhizosphere soil of soybean whose seeds were dressed with chitosan. The smallest number of bacteria with such an effect was obtained from the rhizosphere soil in the control combination (tab. 3). The highest number of antagonistic fungi from genus *Gliocladium* occurred in the rhizosphere soil of soybean whose seeds were dressed with chitosan and the seedlings were sprayed, while in the case of genus *Trichoderma* – in the combination when chitosan was used three times (seed dressing, seedling spraying and plant spraying at the beginning of anthesis) (tab. 3).

Table 1. The number of bacteria and fungi isolated from the rhizosphere of soybean (1999–2001)
Tabela 1. Liczebność bakterii i grzybów wyizolowanych z ryzosfery soi (1999–2001)

Experimental combination Kombinacja doświadczenia	Total number of bacteria (mln · g^{-1} d.w. of soil) Ogólna liczba bakterii (mln · g^{-1} s. m. gleby)	Total number of *Bacillus* spp. (mln · g^{-1} d.w. of soil) Liczba bakterii *Bacillus* spp. (mln · g^{-1} s. m. gleby)	Total number of *Pseudomonas* spp. (mln · g^{-1} d.w. of soil) Liczba bakterii *Pseudomonas* spp. (mln · g^{-1} s. m. gleby)	Total number of fungi (thous. · g^{-1} d.w. of soil) Ogólna liczba grzybów (tys. · g^{-1} s. m. gleby)
Seed treated with chitosan Nasiona zaprawiane chitozanem	4.77*[ab]	1.89[a]	1.87[d]	38.58[b]
Seed treated wit chitosan + sprayed seedlings Nasiona zaprawiane chitozanem + opryskane siewki	5.22[b]	2.01[a]	1.3[c]	47.88[c]
Seed treated wit chitosan + sprayed seedlings + sprayed plants at anthesis Nasiona zaprawiane chitozanem + opryskane siewki + opryskane rośliny w fazie kwitnienia	6.82[c]	3.84[b]	0.87[b]	30.06[a]
Control – Kontrola	3.98[a]	1.80[a]	0.62[a]	73.55[d]

* mean in columns fellowed by the same letter do not differ significantly at $P \leq 0.05$
średnie wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy $P \leq 0,05$

Table 2. Fungi frequently in habited in the rhizosphere soil of soybean (sums isolates 1999–2001)
Tabela 2. Grzyby często wystepujące w glebie ryzosferowej soi (suma izolatów 1999–2001)

Fungus species Gatunek grzyba	Experimental combination / numer of isolates – Kombinacja doświadczenia / Liczba izolatów			
	seed treated with chitosan nasiona zaprawiane chitozanem	seed treated wit chitosan + sprayed seedlings nasiona zaprawianechitozanem + opryskane siewki	seed treated wit chitosan + sprayed seedlings + sprayed plants at anthesis nasiona zaprawiane chitozanem + opryskane siewki + opryskane rośliny w fazie kwitnienia	control kontrola
Acremonium murorum (Corda.) W. Gams	9	3	3	1
Acremonium roseum (Oud.) W. Gams	22	6	-	-
Alternaria alternata (Fr.) Keissler	4	4	1	5
Aspergillus fumigatus Fresenius	1	-	-	2
Aspergillus niger van Tieghem	-	-	2	-
Aureobasidium pullulans (de Bary) Arnaud.	2	55	8	-
Cladosporium cladosporioides (Fres.) de Vries	58	-	-	-
***Fusarium* spp.**	**39**	**108**	**3**	**171**
Fusarium equiseti (Corda.) Sacc.	2	-	-	3
Fusarium oxysporum Schl.	36	60	3	101
Fusarium solani (Mart.) Sacc.	1	48	-	67
***Gliocladium* spp.**	**23**	**58**	**43**	**6**
Gliocladium catenulatum Gilman et Abbott	6	15	28	4
Gliocladium fimbriatum Gilman et Abbott	4	-	-	-
Gliocladium roseum Bainier	13	43	15	2
Mucor hiemalis Wehmer	1	4	87	20
Myrothecium verrucaria Dietmar ex Fries	-	1	-	-
***Penicillium* spp.**	**20**	**100**	**68**	**115**
Penicillium canescens Scopp.	20	53	33	29
Penicillium nigricans (Bain.) Thom	-	44	35	74
Penicillium purpurogenum Stoll	-	2	-	4
Penicillium verrucosum Dierckx var. *cyclopium* (Westling) Samson, Stolk et Hadlok	-	-	-	5
Penicillium verrucosum Dierckx var. *verrucosum* Samson, Stolk et Hadlok	-	1	-	3
Pythium irregulare Buisman	16	-	-	18
Rhizoctonia solani Kühn	-	2	-	5
Sclerotinia sclerotiorum (Lib.) de Bary	17	3	-	7
***Trichoderma* spp.**	**68**	**82**	**196**	**7**
Trichoderma hamatum (Bon.) Bain	4	1	8	-
Trichoderma harzianum (Bonord.) Bain.	6	18	23	1
Trichoderma koningii Oud.	41	46	86	4
Trichoderma pseudokoningii Rifai	-	4	-	-
Trichoderma viride Pers. ex S. F. Gray	17	13	79	2
Verticillium albo-atrum Reinke, Berth	-	-	-	3
Total – Razem	**280**	**426**	**411**	**360**

Table 3. Antagonistic bacteria and fungi isolated from rhizosphere soil of soybean in particular experimental combinations
Tabela 3. Antagonistyczne bakterie i grzyby wyizolowane z gleby ryzosferowej soi w poszczególnych kombinacjach doświadczenia

Bacteria and fungi Bakterie i grzyby	Number of isolates – Liczba izolatów experimental combination – kombinacja doświadczenia			
	seed treated with chitosan nasiona zaprawiane chitozanem	seed treated wit chitosan + sprayed seedlings nasiona zaprawiane chitozanem + opryskane siewki	seed treated wit chitosan + sprayed seedlings + sprayed plants at anthesis nasiona zaprawiane chitozanem + opryskane siewki + opryskane rośliny w fazie kwitnienia	control kontrola
Bacillus spp.	19	1	6	4
Pseudomonas spp.	8	11	12	5
Total – Razem	**27**	**12**	**18**	**9**
***Gliocladium* spp.**	**23**	**58**	**43**	**6**
Gliocladium catenulatum	6	15	28	4
Gliocladium fimbriatum	4	-	-	-
Gliocladium roseum	13	43	15	2
***Trichoderma* spp.**	**68**	**82**	**196**	**7**
Trichoderma hamatum	4	1	8	-
Trichoderma harzianum	6	18	23	1
Trichoderma koningii	41	46	86	4
Trichoderma pseudokoningii	-	4	-	-
Trichoderma viride	17	13	79	2
Total – Ogółem	**91**	**140**	**239**	**13**

DISCUSSION

The results showed that the quantitative and qualitative composition of the communities of bacteria and fungi in the rhizosphere soil of soybean varied. Introducing chitosan in the form of gel into the soil probably stimulated the growth and development of bacteria and fungi. Those results were confirmed by the earlier studies conducted by Pięta et al. [2001]. The highest number of bacteria colonies occurred in the rhizosphere soil of soybean after chitosan was used three times (seed dressing, seedling spraying and plant spraying at the beginning of anthesis). On the other hand, the fewest cfu of fungi were observed in this sample of rhizosphere soil. According to Myśków [1989], there are definite proportions between microorganism populations. It means that intensive multiplication of bacteria is accompanied by poorer development of fungi and vice versa. This fact was fully confirmed in the present studies. Chitosan used three times considerably increased the population of *Bacillus* spp., which occurred in the smallest numbers in the control combination. Besides, *Gliocladium* spp. and *Trichoderma* spp., which showed antagonistic effect, were isolated in big numbers from the samples of rhizosphere soil after using chitosan. The smallest number of fungi was obtained from the control combination soil. According to Teichgraber et al. [1991], the application of chitosan causes the stimulation of chitinolytic microorganisms, which are *Trichoderm* spp. Transmo et al. [1993], Pospieszny and Struszczyk [1994], Orlikowski et al. [2001] observed that chitosan stimulates the development of microorganisms of antagonistic effect, which cause considerable reduction of plant pathogens. The development of microorganisms in the soil might have been caused not only by the introduction of chitosan but also by the effect of soybean through root exudates. Abundant occurrence of saprobionts in the rhizosphere should be ascribed to the synergistic effect of chitosan and root exudates.

Plants, through their roots, exudate compounds which are a rich nutritious substrate for microorganisms consisting of aminoacids, sugars, organic acids or metal ions [Funck-Jensen and Hockenhull 1984, Pięta 1988]. Root exudates contain considerable amounts of aminoacids and sugars, the basic source of C and N for rhizosphere microorganisms [Patkowska 2001]. The presence of nutritious elements in rhizosphere and the stimulating effect on the growth and development of antagonistic microorganisms can affect the proper development of microorganisms and plants.

CONCLUSIONS

1. Introducing chitosan into the soil caused an increase of saprobiontic microorganisms with antagonistic effect towards pathogenic fungi.
2. Using chitosan three times caused a significant increase of the occurrence of antagonists in rhizosphere soil.
3. Using chitosan caused reduction of the populations of pathogenic fungi such as *Fusarium* spp., *Pythium irregulare, Rhizoctonia solani and Sclerotinia sclerotiorum* in rhizosphere soil.

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WPŁYW CHITOZANU NA KSZTAŁTOWANIE SIĘ ZBIOROWISK MIKROORGANIZMÓW W GLEBIE RYZOSFEROWEJ SOI

Streszczenie. Doświadczenie prowadzono w latach 1999–2001. Przedmiotem badań była gleba ryzosferowa soi odmiany 'Polan' oraz 0,1% chitozan. Związek ten użyto jednorazowo (zaprawianie nasion), dwukrotnie (zaprawianie nasion i oprysk siewek), trzykrotnie (zaprawianie nasion, oprysk siewek i oprysk roślin w fazie kwitnienia). W doświadczeniu uwzględniono również kombinacje kontrolną, tj. bez użycia chitozanu. Celem badań było określenie wpływu chitozanu na skład jakościowy i ilościowy populacji mikroorganizmów w glebie ryzosferowej z wyszczególnieniem antagonistów.
Przeprowadzona analiza mikrobiologiczna prób gleby ryzosferowej soi pobranej z poszczególnych kombinacji doświadczenia wskazała na różną liczebność bakterii oraz grzybów. Największą ogólną liczbą kolonii bakterii, a najmniejszą liczbą kolonii grzybów charakteryzowała się gleba ryzosferowa soi po trzykrotnym zastosowaniu chitozanu. W tej próbie gleby wystąpiło najwięcej bakterii i grzybów o antagonistycznym oddziaływaniu względem fitopatogenów

Słowa kluczowe: chitozan, soja, ryzosfera, bakterie antagonistyczne, grzyby antagonistyczne

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