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CHITOSAN AS A COMPOUND INHIBITING THE OCCURRENCE OF SOYBEAN DISEASES

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Abstract. Studies were conducted on a field of soybean monoculture at Czesławice near Nałęczów in the years 2002–2004. The object of the studies were soybean plants 'Polan' cv. and chitosan. A water solution of chitosan micro-gel at the concentration of 0.1% was used for studies. The studies tested the effect of this compound on the inhibition of soybean diseases caused by soil-borne pathogenic fungi. The solution of chitosan was used a few times, i.e. for seed dressing, seedling spraying, plant spraying at anthesis and in mixed combinations. During the experiment field observations were conducted in particular developmental stages when the number and healthiness of plants were established. Infected plants were submitted to mycological analysis. Results from the field observations and mycological analyses showed that chitosan used in a mixed combination (for seed dressing + seedling spraying + plant spraying at anthesis) was the most effective in protecting soybean from the infection of soil-borne pathogenic fungi. In those combinations the best density of soybean seedlings and plants at anthesis was obtained. The mycological analysis of infected parts of soybean seedlings and plants at anthesis showed that chitosan proved effective in inhibiting the infection of soybean plants from fungi from the genera of *Fusarium, Pythium, Phomopsis* and *Botrytis cinerea, Sclerotinia sclerotiorum* and *Rhizoctonia solani.*

Key words: chitosan, soybean, pathogenic fungi

INTRODUCTION

An increase of the number of ecological farms in the last decade is based on natural cultivation methods, i.e. without the use of chemicals to protect plants and the application of mineral fertilization. Introducing pro-ecological cultivations contributes to the development of studies on biological control of plant diseases.

Biopreparations used to control plant diseases are based on natural organic compounds and antagonistic microorganisms. The advantage of these biopreparations is

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high selectivity as well as effectiveness in controlling diseases. They can be applied directly before the harvest as they have no currency and prevention date. They do not inhibit the plants' growth and development and they are not phytotoxic [Wolski and Gliński 2001].

One of such compounds – at present fairly frequently used in biological control – is chitosan. It follows from earlier studies that chitosan used in the soil and on the leaves as well as to soak the plants' vegetative parts in caused reduced the occurrence of diseases of ornamental and vegetable plants caused by fungi, bacteria and viruses [Pospieszny et al. 1995, Orlikowski and Skrzypczak 1997, Wojdyła and Orlikowski 1997, Pastucha 2001b, Pięta et al. 2001, 2002, Pięta and Pastucha 2002, Ben-Shalomin et al. 2003, Mazur et al. 2003].

Studies conducted so far at the Department of Plant Pathology of the University of Life Science in Lublin on protecting soybean from diseases using chitosan have proved insufficient [Pięta et al. 1998, Pastucha 2001a, 2001b, Pięta and Pastucha 2002]. Due to the fact that soybean seeds are used by pharmaceutic industry (to obtain lecitin and other pharmacological substances) and food industry (e.g. infant nutritive foods), this raw material may not be contaminated by chemical preparations used for plant protection from diseases. Besides, soybean seeds may not be infected by toxin-forming fungi which contaminate them with myco-toxins [Chełkowski 1985]. Hence, the purpose of the studies was to determine the effectiveness of the protective effect of chitosan used at different stages of soybean growth and development against its diseases.

MATERIAL AND METHODS

Studies were conducted at the Experimental Station at Czesławice near Nałęczów in the years 2002–2004. Soybean seeds of cv. 'Polan' without visible disease symptoms and chitosan in the form of a gel (containing 2.76% polymer) were used to establish the experiment. The compound was obtained from the Institute of Chemical Fibres in Łódź. The experiment used a 0.1% aqueous solution of the compound.

Seven combinations were considered in the experiment:

1 – seeds dressed with chitosan, 2 – dressed seeds + seedlings sprayed with chitosan, 3 – dressed seeds + sprayed seedlings + plants at the beginning of anthesis sprayed with chitosan , 4 – seedlings sprayed with chitosan, 5 – plants at the beginning of anthesis sprayed with chitosan, 6 – seeds dressed with Zaprawa Oxafun T, 7 – control (without any treatments).

In each year of studies the experiment was established in the first 10 days of May. 100 seeds were sown in four repetitions onto micro-plots of the area of 1.25 m^2 (4 plots). Field observations were carried out on particular plots in each year of the experiment. Each observation was performed 8 days after spraying the plants. The first was performed six weeks after establishing the experiment, whereas the second – at full anthesis. During those observations, the number and healthiness of plants in particular developmental stages were determined. After the harvest, the size and quality of soybean seeds yield was determined from particular plots. The obtained results were statis-

tically analyzed and the significance of differences were presented by means of Tukey's confidence intervals [Oktaba 1987].

The seedlings and the plants with disease symptoms in the form of necrosis on the roots and the stem base as well as those that were characterized by yellowing leaves and weakened growth were taken for a laboratory mycological analysis, in the quantity of 5 from each experimental combination. Preparation of the plant material and the seeds as well as the mycological analysis were conducted according to the method described by Pastucha [1997].

Meteorological data for the region of the studies were obtained from the Department of Agrometeorology of the University of Life Sciences in Lublin.

RESULTS

During the studies, weather conditions for particular years varied (tab. 1). The vegetation period in the years 2002 and 2003 should be regarded as a warm one as the mean air temperature from May till August was higher by + 0.6°C to +3.4°C. It was colder only in September of 2002 since the air temperature was lower by –0.1°C as compared to the means of many years calculated from 30-years' data. The period of vegetation in 2004 was chilly, since the mean air temperature was lower than the mean of many years, with an exception of August, when the temperature was higher by +1.1°C than the many-years' means. The precipitation during the soybean plants' growth and seeds' maturation in 2002 was scarce and the rainfalls constituted from 60% to 101% of the norm of rainfalls that was adopted as the means of 30 years. On the other hand, the rainfalls in 2003 constituted from 44% (May) to 138% (September) (tab. 1).

Table 1. Wheather conditions in the region of experiment in the years 2002 – 2004 on the backgroun of long-term period

Tabela 1. Warunki pogodowe w rejonie prowadzonych badań w latach 2002-2004 na tle średnich wieloletnich

Month Miesiąc	Means from the years 1963–1992 Średnie wieloletnie za okres 1963–1992		Difference of mean temperature in comparison with long-term period Różnica temperatury powietrza w porównaniu ze średnią wieloletnią			Percentage of the average annual rainfalls Procent normy opadów		
	air temperatures temperatura powietrza °C	rainfalls opady deszczu mm	2002	2003	2004	2002	2003	2004
May – Maj	13.3	60.9	+ 3.4	+ 2.6	- 1.7	75	109	44
June – Czerwiec	16.4	78.3	+ 0.6	+ 1.0	- 0.9	101	47	84
July – Lipiec	17.8	77.9	+ 3.4	+ 1.6	0.0	67	141	124
August – Sierpień	17.3	69.3	+ 2.9	+ 1.0	+ 1.1	60	34	83
September – Wrzesień	13.1	56.0	- 0.1	0.0	- 0.6	81	69	138

The obtained results from the first field observation pointed to a differentiated number and healthiness of seedlings on particular plots (tab. 2). The most – 87.7 seedlings, on average – were found out in the combination where chitosan was used for seed dressing + seedling spraying + plants sprayed at the beginning of anthesis, whereas the fewest in the control combination (on average, 69.6 seedlings on a plot).

Seedlings with distinct disease symptoms occurred in all experimental combinations. The highest number of such seedlings was observed in the control combination – on average, 7.8% – and the lowest – on plots in the combination where chitosan was used for seed dressing and seedling spraying. The combination after using the chemical plant protection with Zaprawa Oxafun T was also characterized by good seedling density and healthiness (tab. 2).

Table 2. Number and healthiness of soybean seedlings
Tabela 2. Liczebność i zdrowotność siewek soi

Experimental combination Kombinacja doświadczenia	Number of seedlings of field Liczba siewek na poletku				Percentage of infected seedlings Procent porażonych siewek			
	2002	2003	2004	mean średnia	2002	2003	2004	mean średnia
Seeds dressed with chitosan Nasiona zaprawiane chitozanem	80	88	85	84.3^{b}*	3.0	6.8	4.5	4.8^{d}*
Seeds dressed + seedlings sprayed with chitosan Nasiona zaprawiane + siewki opryskane chitozanem	83	86	87	85.3^{b}	1.0	6.9	1.75	2.2^{a}
Seeds dressed + seedlings sprayed + plants at the beginning of anthesis sprayed with chitosan Nasiona zaprawiane + siewki opryskane + rośliny opryskane chitozanem na początku kwitnienia	85	88	90	87.7^{b}	0.2	5.7	1.0	2.3^{a}
Seedlings sprayed with chitosan Siewki opryskane chitozanem	79	92	80	83.6^{b}	0.5	5.4	2.5	2.8^{ab}
Plants at the beginning of anthesis sprayed with chitosan Rośliny opryskane chitozanem na początku kwitnienia	60	75	75	70.0^{a}	0.5	8.2	3.0	3.9^{c}
Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	74	98	82	84.6^{b}	1.5	4.0	3.5	3.0^{b}
Controll – Kontrola	56	80	73	69.6^{a}	6.0	11.0	6.5	7.8^{e}

* mean in columns fellowed by the same letter do not differ significantly at $p \leq 0.5$
* średnie wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy ($p \leq 0,05$)

The results obtained from the second observation performed at full anthesis showed that plant density on particular plots of the experiment slightly decreased, while the proportion of plants with disease symptoms increased (tab. 3). The smallest density of soybean plants was characteristic of control plots (67 plants on a plot, on average), with the highest proportion of infected plants (8.2%, on average). The best plant density, with the smallest proportion of infected plants was observed in the combination with

a multiple application of chitosan, i.e. for seed dressing + seedling spraying + plant spraying at the beginning of anthesis. The density of plants and their healthiness was good on the plots after the application of Zaprawa Oxafun T for seed dressing (tab. 3).

Table 3. Number and healthiness of soybean plants at anthesis
Tabela 3. Liczebność i zdrowotność roślin soi w fazie kwitnienia

Experimental combination Kombinacja doświadczenia	Number of plants of field Liczba roślin na poletku				Percentage of infected plants of field Procent porażonych roślin			
	2002	2003	2004	mean średnia	2002	2003	2004	mean średnia
Seeds dressed with chitosan Nasiona zaprawiane chitozanem	78	87	83	82.6[b]*	2.0	7.0	5.0	4.6[b]*
Seeds dressed + seedlings sprayed with chitosan Nasiona zaprawiane + siewki opryskane chitozanem	81	84	86	83.6[b]	1.0	6.9	2.0	3.3[a]
Seeds dressed + seedlings sprayed + plants at the beginning of anthesis sprayed with chitosan Nasiona zaprawiane + siewki opryskane + rośliny opryskane chitozanem na początku kwitnienia	85	88	88	87.0[b]	0.5	5.8	1.5	2.6[a]
Seedlings sprayed with chitosan Siewki opryskane chitozanem	78	92	80	83.3[b]	0.5	5.8	2.7	3.0[a]
Plants at the beginning of anthesis sprayed with chitosan Rośliny opryskane chitozanem na początku kwitnienia	58	72	62	64.0[a]	0.5	8.5	3.2	4.0[b]
Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	74	94	78	82.0[b]	1.0	8.2	4.2	4.5[b]
Controll – Kontrola	54	78	69	67.0[a]	6.0	11.2	7.5	8.2[c]

* mean in columns fellowed by the same letter do not differ significantly at $p \leq 0.5$
* średnie wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy ($p \leq 0,05$)

After the harvest, the size and quality of the obtained seed yield were established (tab. 4). The highest yield – 422.7 g – was obtained from plants growing in the combinations where chitosan was applied three times, i.e. for seed dressing + seedling spraying + plant spraying at the beginning of anthesis. On the other hand, the lowest soybean seed yield was obtained from plants in the control combination. A good yield was also obtained from plants growing in the combination where chitosan was used only for seed dressing (on average, 353 g from a plot), and both for seed dressing and seedling spraying. Seeds with necrotic spots on the cover, i.e. diseased seeds, occurred in all experimental combinations. The greatest number of such seeds was obtained from plants gathered from control plots (on average, 5%), while the smallest from soybean plants from the combination where chitosan was used only for plant spraying at the beginning of anthesis (on average, 2.9%) (tab. 4).

Table 4. Yield and percentage infected seeds of soybean
Tabela 4. Plon oraz procent porażenia nasion soi

Experimental combination Kombinacja doświadczenia	Yield of pea seeds in g of field Plon nasion w g z poletka				Percentage of infected seeds Procent porażonych nasion			
	2002	2003	2004	mean średnia	2002	2003	2004	mean średnia
Seeds dressed with chitosan Nasiona zaprawiane chitozanem	242	425	392	353.0^{bc}*	3.2	3.6	3.2	3.3^{ab}*
Seeds dressed + seedlings sprayed with chitosan Nasiona zaprawiane + siewki opryskane chitozanem	240	345	404	329.7^{b}	4.0	2.0	3.5	3.2^{ab}
Seeds dressed + seedlings sprayed + plants at the beginning of anthesis sprayed with chitosan Nasiona zaprawiane + siewki opryskane + rośliny na początku kwitnienia opryskane chitozanem	357	455	456	422.7^{c}	2.0	5.0	1.7	2.9^{a}
Seedlings sprayed with chitosan Siewki opryskane chitozanem	190	275	367	277.3^{ab}	3.7	3.5	3.7	3.7^{ab}
Plants at the beginning of anthesis sprayed with chitosan Rośliny w fazie kwitnienia opryskane chitozanem	232	230	293	251.7^{a}	2.5	3.0	3.4	2.9^{a}
Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T	91	200	387	226.0^{a}	3.7	3.7	2.0	3.2^{ab}
Controll – Kontrola	80	368	224	224.0^{a}	4.7	5.0	5.5	5.0^{c}

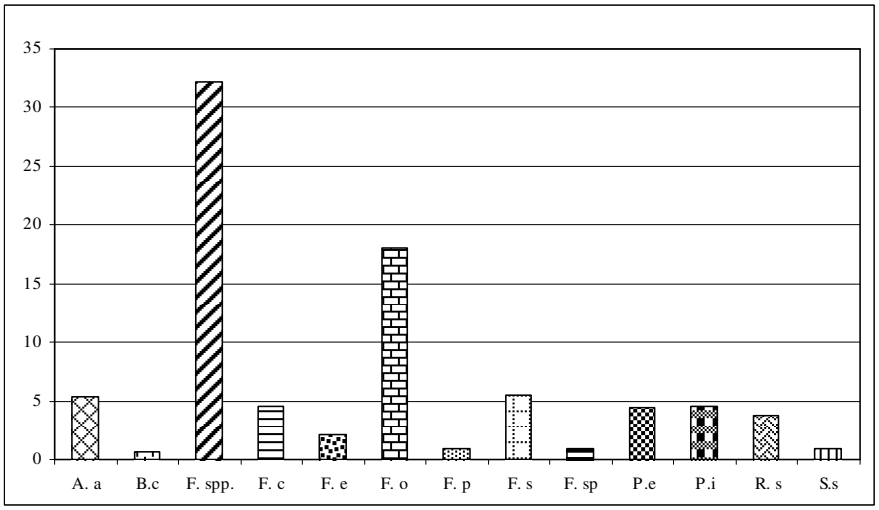
* mean in columns fellowed by the same letter do not differ significantly at $p \leq 0.5$
* średnie wartości w kolumnach oznaczone tą samą literą nie różnią się istotnie przy ($p \leq 0,05$)

The laboratory mycological analysis of the infected seedlings isolated 1784 fungi colonies belonging to 29 different species (tab. 5). *Fusarium oxysporum* f. sp. *glycines*, which constituted 18.1% of the obtained colonies, was the dominating species among all fungi colonies isolated from the infect soybean seedlings (tab. 5, fig. 1). This fungus was isolated in the greatest amount from the roots and the stem base of soybean seedlings from the combination with chemical seed dressing and from plants in the control combination.

Among the genus of *Fusarium, F. culmorum, F. equiseti, F. poae, F. solani* and *F. sporotrichioides* were also obtained from the examined parts of soybean seedlings. Species of this genus totally constituted more than 32% of all isolated fungi colonies (fig. 1). Besides, *Altenaria alternata, Phoma exigua* var. *exigua, Pythium irregulare* and – sporadically – *Botrytis cinerea* and *Sclerotinia sclerotiorum* were isolated from the analyzed parts of soybean seedlings.

Saprophytic fungi were obtained as a result of the mycological analysis of the roots and the stem base of soybean seedlings. *Cladosporium cladosporioides* and *Penicillium nigricans*, whose isolates constituted 4.6% and 4.3% of all isolations, respectively, belonged to those isolated most frequently. The colonies of those saprophytes were isolated from the examined parts of soybean seedlings in each experimental combina-

tion. Besides, *Acremonium roseum, Aureobasidium pullulans, Epicoccum purpurascens, Humicola grisea, Papulaspora irregularis, Penicillium verrucosum* var. *cyclopium, Rhizopus nigricans* and *Talaromyces flavus* were isolated among the saprophytic fungi from the analyzed roots and stem base of soybean seedlings. Fungi from the genera of *Gliocladium* and *Trichoderma*, whose colonies were isolated much more frequently from the plant material of the combination after the application of chitosan as compared to chemical seed dressing with Zaprawa Oxafun T and the control, also belong to saprophytes (tab. 5).



A.a – Alternaria alternata; B.c – Botrytis cinerea; F. spp. – *Fusarium* spp.;
F.c – Fusarium culmorum; F.e – Fusarium equiseti; F.o – Fusarium oxysporum f. sp. glycines;
F.p – Fusarium poae solani; F.sp – Fusarium sporotrichioides; P.e – Phoma exigua;
P.i – Pythium irregulare; R.s – Rhizoctonia solani; S.s – Sclerotinia sclerotiorum

Fig. 1. Percentage of pathogenic fungi from infected soybean seedlings
Rys. 1. Udział grzybów patogenicznych w porażaniu siewek soi

2539 fungi isolates were obtained during the mycological analysis of the infected plants of soybean at anthesis (tab. 6). Over 25% of the obtained colonies were fungi from *Fusarium* genus (fig. 2). Among *Fusarium* spp., *F. oxysporum* f. sp. *glycines* was most frequently isolated and its colonies constitutes 14.3% of all isolations, totally (fig. 2). This pathogen mostly colonized infected soybean plants from the plots with chemical seed dressing with Zaprawa Oxafun T and the control, whereas the smallest number of isolates of this fungus was obtained from the studied plants of soybean taken from the combination after a triple and double application of chitosan.

Table 5. Fungi isolated from soybean seedlings (sums of isolates from the year 2002–2004)
Tabela. 5. Grzyby wyosobnione z siewek soi (sumy izolatów z lat 2002–2004)

Fungus species Gatunek grzyba	Experimental combination – Number of isolates – Kombinacja doświadczenia / Liczba izolatów																Overall Ogółem
	Seeds dressed with chitosan Nasiona zaprawiane chitozanem		Seeds dressed + seedlings sprayed with chitosan Nasiona zaprawiane + siewki opryskane chitozanem		Seeds dressed + seedlings sprayed + plants at the beginning of anthesis sprayed with chitosan Nasiona zaprawiane + siewki opryskane + rośliny na początku kwitnienia opryskane chitozanem		Seedlings sprayed with chitosan Siewki opryskane chitozanem		Plants at the beginning of anthesis sprayed with chitosan Rośliny na początku kwitnienia opryskane chitozanem		Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T		Controll Kontrola		Totally Razem	Totally Razem	
	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	
Acremonium roseum (Oud.) W. Gams	1	2	4	5	4	3	6	2	5	-	4	6	5	2	29	20	49
Alternaria alternata (Fr.) Keissler	5	10	4	2	3	4	4	8	8	3	6	10	16	14	46	51	97
Aureobasidium pullulans (de Bary) Arnaud.	-	6	-	-	4	3	2	3	-	-	1	4	2	4	9	20	29
Botrytis cinerea Pers.	-	-	1	-	-	-	-	-	1	-	1	5	1	4	4	9	13
Cladosporium cladosporioides (Fres.) de Vries	6	6	5	4	9	5	5	3	4	4	6	8	9	8	44	38	82
Epiccocum purpurascens Ehr. ex. Schl.	3	1	1	3	5	2	4	4	7	2	6	4	3	8	29	24	53
Fusarium culmorum (W. G. Sm.) Sacc.	4	13	2	1	3	-	8	8	8	6	3	9	9	9	37	46	83
Fusarium equiseti (Corda) Sacc.	-	3	-	1	-	1	1	-	1	1	5	6	7	11	14	23	37
Fusarium oxysporum Schl. f. sp. *glycines* Amst., Amst.	12	18	15	19	9	8	14	22	17	22	41	44	36	46	144	179	323
Fusarium poae (Peck.) Wollenw.	4	1	-	3	-	2	-	4	-	4	-	-	-	-	4	14	18
Fusarium solani (Mart.) Sacc.	1	2	5	8	5	6	9	9	10	8	11	7	10	8	51	48	99
Fusarium sporotrichioides Sherb.	1	2	-	1	1	-	4	4	1	1	-	-	-	-	7	8	15
Gliocladium catenulatum Gilman Abbott	11	12	9	12	12	9	5	4	6	5	4	5	4	5	51	52	103
Gliocladium fimbraiatum Gilman Abbott	5	4	1	2	4	8	-	1	2	1	-	-	-	-	12	16	28
Gliocladium roseum (Link) Bainier	1	1	3	4	6	5	3	-	5	3	3	1	2	1	23	15	38
Humicola grisea Domsch	5	4	-	-	-	2	2	4	5	4	7	10	12	17	31	41	72
Papulaspora irregularis Hotson	2	1	3	-	2	-	-	4	2	7	1	-	-	-	10	12	22
Penicillium nigricans (Bain.) Thom	9	6	2	3	2	2	4	4	6	7	6	10	4	12	33	44	77
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	2	3	3	1	3	2	3	4	3	-	8	5	8	6	30	21	51
Phoma exigua Desm. var. *exigua*	3	4	2	2	1	2	8	3	3	7	10	10	9	15	36	43	79
Pythium irregulare Buisman	9	-	5	-	3	-	8	3	9	3	14	6	20	3	68	15	83
Rhizoctonia solani Kühn	5	4	3	1	2	1	1	4	3	8	4	11	8	13	26	42	68
Rhizopus nigricans Ehrenberg	3	2	-	2	-	2	1	1	-	-	6	1	4	3	14	11	25
Sclerotinia sclerotiorum (Lib.) de Bary	1	-	-	-	-	-	-	-	2	-	1	4	5	3	9	7	16
Talaromyces flavus (Ben.) Stolk et Samson	1	2	1	3	6	-	5	8	6	9	-	-	-	-	19	22	41
Trichoderma aureoviride Rifai	7	9	-	1	1	3	2	1	3	2	3	1	2	1	18	18	36
Trichoderma harzianum Rifai	5	5	2	4	11	10	5	3	5	3	-	-	1	-	29	25	54
Trichoderma koningii Oud.	10	9	8	4	5	6	4	5	-	1	3	5	3	2	33	32	65
Trichoderma viride Pers. ex. S.F. Gray	4	2	4	5	3	1	-	-	2	6	1	-	-	-	14	14	28
Totally – Razem	120	132	83	91	104	87	108	116	124	117	155	172	180	195	874	910	1784

k – root – korzeń; pł – stem base – podstawa łodygi

Table 6. Fungi isolated from soybean plants at anthesis (sums of isolates from the year 2002–2004)
Tabela. 6. Grzyby wyosobnione z roślin soi w fazie kwitnienia (sumy izolatów z lat 2002–2004)

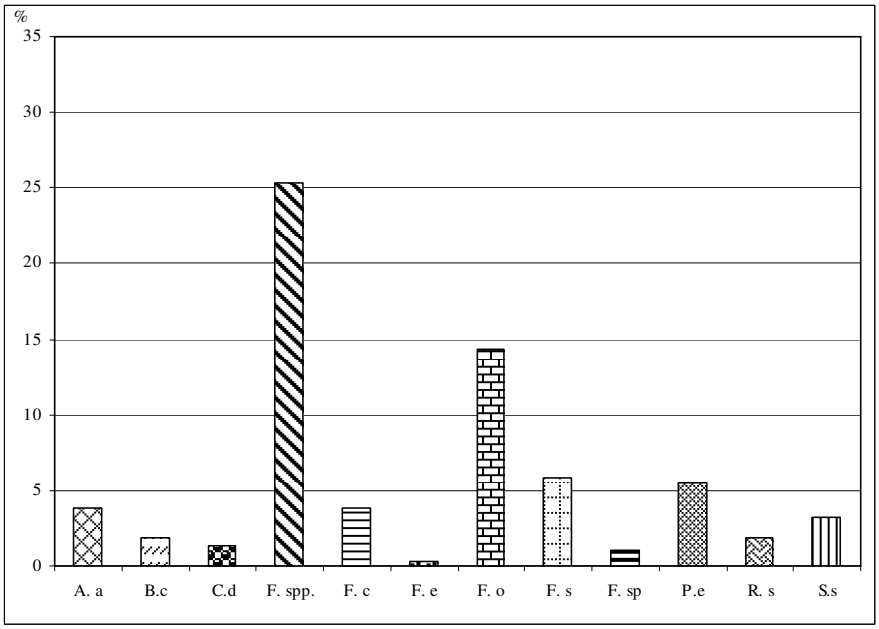
Fungus species Gatunek grzyba	Experimental combination / Number of isolates – Kombinacja doświadczenia / Liczba izolatów																
	Seeds dressed with chitosan Nasiona zaprawiane chitozanem		Seeds dressed + seedlings sprayed with chitosan Nasiona zaprawiane + siewki opryskane chitozanem		Seeds dressed + seedlings sprayed + plants at the beginning of anthesis sprayed with chitosan Nasiona zaprawiane + siewki opryskane + rośliny na początku kwitnienia opryskane chitozanem		Seedlings sprayed with chitosan Siewki opryskane chitozanem		Plants at the beginning of anthesis sprayed with chitosan Rośliny na początku kwitnienia opryskane chitozanem		Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T		Controll Kontrola		Totally Razem	Totally Razem	Overall Ogółem
	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	k	pł	
Acremonium roseum (Oud.) W. Gams	6	11	4	5	4	5	4	4	3	3	7	6	6	7	34	41	75
Alternaria alternata (Fr.) Keissler	3	4	2	4	4	4	4	3	6	8	9	16	13	16	41	55	96
Aspergillus niger van Tiegh	4	4	2	-	1	1	-	-	-	2	4	5	4	6	15	18	33
Aureobasidium pullulans (de Bary) Arnaud.	2	-	1	-	2	-	-	3	-	-	-	-	-	3	5	6	11
Botrytis cinerea Pers.	-	2	-	3	-	3	4	5	2	4	4	8	4	11	14	36	50
Cladosporium cladosporioides (Fres.) de Vries	5	9	3	1	4	5	4	7	7	5	7	7	8	13	38	47	85
Cylindrocarpon destructans (Zins.) Scholten	1	3	-	-	-	-	-	2	-	-	6	9	7	7	14	21	35
Epiccocum purpurascens Ehr. ex. Schl.	4	2	4	3	5	4	7	3	4	8	12	8	10	13	46	41	87
Fusarium culmorum (W. G. Sm.) Sacc.	6	10	5	1	2	2	5	6	7	4	9	16	10	15	44	54	98
Fusarium equiseti (Corda) Sacc.	-	-	1	-	-	-	-	3	2	1	-	-	-	-	3	4	7
Fusarium oxysporum Schl. f. sp. *glycines* Amst., Amst.	18	20	13	19	12	14	16	20	16	19	41	52	41	61	157	205	362
Fusarium solani (Mart.) Sacc.	5	6	4	7	4	6	10	11	9	7	18	22	16	24	66	83	149
Fusarium sporotrichioides Sherb.	3	2	1	-	-	-	-	1	2	1	3	4	4	6	13	14	27
Gliocladium catenulatum Gilman Abbott	12	14	11	12	14	17	10	10	10	9	5	9	5	7	67	78	145
Gliocladium fimbraiatum Gilman Abbott	5	1	1	3	4	4	-	5	-	2	-	-	-	-	10	15	25
Gliocladium roseum (Link) Bainier	8	9	9	15	10	13	8	7	10	12	7	5	6	3	58	64	122
Humicola grisea Domsch	5	3	3	1	5	-	6	5	6	5	9	12	10	14	44	40	84
Humicola fuscoatra Traaen	-	1	1	4	-	6	3	4	5	1	-	-	-	-	9	16	25
Mucor hiemalis Wehmer	5	2	-	-	4	1	5	4	3	5	5	6	6	6	28	24	52
Penicillium expansum Link ex S. F. Gray	7	2	-	-	-	3	3	6	5	6	5	8	5	9	25	34	59
Penicillium nigricans Bainier	4	2	4	3	2	-	3	-	1	1	8	6	9	15	31	27	58
Penicillium verrucosum Dierckx var. *cyclopium* (West.) Samson, Stolk et Hadlok	1	5	2	2	4	1	3	5	2	8	5	11	10	15	27	47	74
Penicillium verrucosum Dierckx var. *verrucosum* Samson, et al.	-	-	4	-	2	2	2	5	2	1	-	1	-	1	10	10	20
Phoma exigua Desm. var. *exigua*	4	9	2	8	3	5	7	6	4	8	16	20	24	25	60	81	141
Rhizoctonia solani Kühn	4	2	4	2	2	3	2	1	3	2	6	5	6	6	27	21	48
Rhizopus nigricans Ehrenberg	4	5	1	3	1	4	3	6	7	7	7	19	6	22	29	66	95
Sclerotinia sclerotiorum (Lib.) de Bary	2	2	1	4	2	1	6	3	4	5	9	17	10	15	34	47	81
Talaromyces flavus (Ben.) Stolk et Samson	1	-	-	2	-	-	1	1	4	3	1	3	2	4	9	13	22
Trichoderma aureoviride Rifai	5	6	-	6	2	1	-	1	-	2	1	-	-	-	8	16	24
Trichoderma harzianum Rifai	8	9	9	9	11	15	9	7	6	7	1	-	1	-	45	47	92
Trichoderma koningii Oud.	9	9	11	12	12	14	8	9	11	8	3	3	7	5	61	60	121
Trichoderma viride Pers. ex. S.F. Gray	16	14	10	12	12	17	8	10	12	12	2	7	2	2	62	74	136
Totally – Razem	157	168	113	141	128	151	141	163	153	166	210	285	232	331	1083	1405	2539

k – root, korzeń; pł – stem base, podstawa łodygi

Table 7. Fungi isolated from seeds of soybean (sums isolates from the years 2002–2004)
Tabela. 7. Grzyby wyosobnione z nasion soi (sumy izolatów z lat 2002–2004)

Fungus species Gatunek grzyba	Experimental combination / Number of isolates – Kombinacja doświadczenia / Liczba izolatów																
	Seeds dressed with chitosan Nasiona zaprawiane chitozanem		Seeds dressed + seedlings sprayed with chitosan Nasiona zaprawiane + siewki opryskane chitozanem		Seeds dressed + seedlings sprayed + plants at the beginning of anthesis sprayed with chitosan Nasiona zaprawiane + siewki opryskane + rośliny na początku kwitnienia opryskane chitozanem		Seedlings sprayed with chitosan Siewki opryskane chitozanem		Plants at the beginning of anthesis sprayed with chitosan Rośliny na początku kwitnienia opryskane chitozanem		Seeds dressed with Zaprawa Oxafun T Nasiona zaprawiane Zaprawą Oxafun T		Controll Kontrola		Totally Razem	Totally Razem	Overall Ogółem
	1	2	1	2	1	2	1	2	1	2	1	2	1	2	1	2	
Acremonium roseum (Oud.) W. Gams	6	2	3	-	-	-	5	2	4	2	-	-	6	7	24	13	37
Alternaria alternata (Fr.) Keissler	7	3	6	2	4	2	4	3	3	1	7	4	10	6	41	21	62
Aureobasidium pullulans (de Bary) Arnaud.	3	1	4	1	-	-	5	2	-	-	3	1	-	-	15	5	20
Botrytis cinerea Pers.	12	6	5	3	4	1	9	5	6	3	5	2	8	6	49	26	75
Cladosporium cladosporioides (Fres.) de Vries	7	4	4	2	3	1	3	1	3	1	6	3	3	4	29	16	45
Epiccocum purpurascens Ehr. ex. Schl.	4	1	3	-	-	-	4	2	-	-	1	1	-	-	12	4	16
Fusarium culmorum (W. G. Sm.) Sacc.	6	3	4	1	4	1	6	3	7	4	6	3	9	7	42	22	64
Fusarium oxysporum Schl.	7	2	5	3	3	1	11	7	9	5	8	4	11	6	54	28	82
Humicola grisea Domsch	3	1	2	1	2	1	4	2	3	1	3	1	-	-	17	7	24
Mucor hiemalis Wehmer	4	-	-	2	3	-	1	1	-	-	4	-	4	2	16	5	21
Penicillium expansum Link ex S. F. Gray	5	-	-	-	4	-	-	-	4	2	-	-	3	1	16	3	19
Penicillium verrucosum Dierckx var. *verrucosum* Samson, et al.	9	3	6	3	3	1	3	2	3	1	4	2	-	-	28	12	40
Phoma exigua Desm. var. *exigua*	6	1	3	1	2	1	5	3	4	2	6	3	8	4	34	15	49
Phomopsis sojae Lehman	12	4	4	2	3	2	6	2	5	3	4	1	3	5	37	19	56
Rhizoctonia solani Kühn	7	3	5	2	4	1	3	1	4	2	8	2	11	7	42	18	60
Rhizopus nigricans Ehrenberg	4	1	3	-	1	-	4	2	3	4	3	1	4	2	22	10	32
Sclerotinia sclerotiorum (Lib.) de Bary	9	2	4	1	-	-	5	3	6	3	6	4	10	3	40	16	56
Trichoderma aureoviride Rifai	5	2	4	4	6	5	4	1	1	1	-	1	3	2	23	16	39
Trichoderma harzianum Rifai	3	1	-	2	2	7	-	-	2	4	4	2	-	1	11	17	28
Trichoderma koningii Oud.	6	3	5	3	3	4	6	2	-	-	3	-	1	-	24	12	36
Totally – Razem	125	43	70	33	51	28	88	44	67	39	81	35	94	63	576	285	861

1 – seeds with spots, nasiona z plamami; 2 – seeds without spots, nasiona bez plam

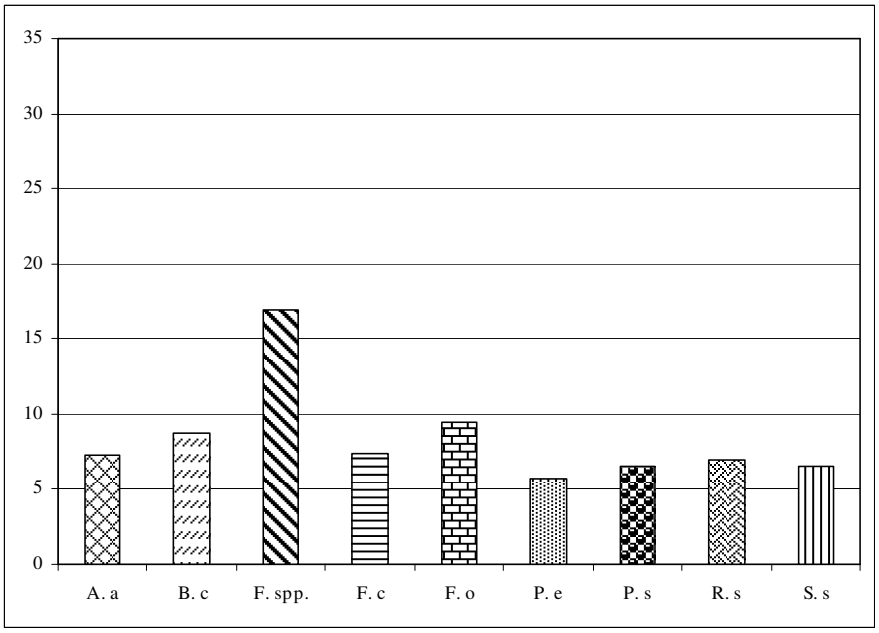


A.a – Alternaria alternata; B.c – Botrytis cinerea; C.d – Cylindrocarpon destructans;
F. spp. *– Fusarium* spp.; *F.c – Fusarium culmorum; F.e – Fusarium equiseti;*
F.o – Fusarium oxysporum f. sp. glycines; F.s – Fusarium solani; F.sp – Fusarium sporotrichioides;
P.e – Phoma exigua; R.s – Rhizoctonia solani; S.s – Sclerotinia sclerotiorum

Fig. 2. Percentage of pathogenic fungi from infected soybean plants at anthesis
Rys. 2. Udział grzybów patogenicznych w porażaniu roślin soi w fazie kwitnienia

Besides, the roots and the stem base of soybean were infected by *Altenaria alternata, Botrytis cinerea, Cylindocarpon destructans, Phoma exigua* var. *exigua, Rhizoctonia solani* and *Sclerotinia sclerotiorum.* The enumerated pathogenic fungi were most frequently isolated from soybean plants growing on control plants and on plots with chemical seed dressing with Zaprawa Oxafun T. Those species were isolated in smaller numbers from the examined parts of soybean plants treated with chitosan three times.

Among saprophytic fungi, the following were isolated from the roots and the stem base: *Acremonium roseum, Aspergillus niger, Cladosporium cladosporioides, Epicoccum purpurascens, Rhizopus nigricans* and *Talaromyces flavus* in addition to fungi from the genera of *Penicillium* and *Humicola* (tab. 6). Besides, *Gliocladium* spp. and *Trichoderma* spp. were obtained from the infected parts of soybean plants. The greatest number of colonies of those fungi were isolated from the parts of soybean growing on plots with triple application of chitosan, whereas the smallest number – from plants sampled from the control combination.



A.a – Alternaria alternata; B.c – Botrytis cinerea; F. spp. – Fusarium spp.;
F.c – Fusarium culmorum; F.o – Fusarium oxysporum; F.s – Fusarium solani;
P.e – Phoma exigua; P.s – Phomopsis sojae; R.s – Rhizoctonia solani; S.s – Sclerotinia sclerotiorum

Fig. 3. Percentage of pathogenic fungi from infected soybean seeds
Rys. 3. Udział grzybów patogenicznych w porażaniu nasion soi

After the harvest, a mycological analysis was also conducted on soybean seeds from which totally 861 isolates of different fungi species were obtained (tab. 7). Twice as many fungi isolates were obtained from spotted seeds as compared to those without any spots. Fungi from the genus of *Fusarium* (*F. culmorum, F. oxysporum*), which totally constituted 16.9% of all isolations, were frequently isolated from particular samples of soybean seeds (fig. 3). Besides, soybean seeds were colonized by such pathogenic fungi as *Altenaria alternata, Botrytis cinerea, Phoma exigua, Phomopsis sojae, Rhizoctonia solani* and *Sclerotinia sclerotiorum* (tab. 7). The following saprophytic species were also isolated from the analyzed seeds: *Acremonium roseum, Aureobasidium, Cladosporium cladosporioides, Epicoccum purpurascens, Humicola grisea, Mucor hiemalis, Rhizopus nigricans* and *Penicillium* spp. and *Trichoderma* spp.

DISCUSSION

Studies conducted by the author showed the best protective effect of chitosan in inhibiting soybean diseases in case of its triple application. It should be supposed that chitosan used for seed dressing protected the sprouting seeds from infection by soilborne fungi. The treatment of seedling spraying with chitosan extended the effect of this compound and, additionally, protected the leaves of young plants. On the other hand, the purpose of plant spraying at the beginning of anthesis was to protect the flowers and the sets as well as the seeds in pods from infection by plant pathogens. The application of chitosan in particular stages of soybean development had a positive effect on the density of plants and their healthiness as well as the size and quality of the yield.

The use of the discussed compound significantly decreased soybean infection, which was confirmed by a small proportion of plants with disease symptoms and the number of isolated plant pathogens. Such observations were made by Lee et al. [1999], who found out that soybean seeds submitted to soaking in a solution of chitosan showed a higher coefficient of sprouting, thicker hypocotyls and an increased weight of the seeds.

In earlier studies, Pięta et al. [1998], Pastucha [2001a] and Pięta and Pastucha [2002] found out that chitosan directly affects soybean pathogens, causing inhibition of their growth and development and morphological changes in the mycelium hyphas. The effect was inhibition of sporulation of pathogenic fungi as formation of their endospores, which probably contributed to the decrease of propagation units of the fungus in the soil environment, and next to plant infection. According to Allan and Hadwiger [1979] and Ryan [1988] and Ghaouth et al. [1991], chitosan enhances the immunological system in plants, causing the formation of anti-bacterial and anti-fungal compounds such as phenols, phytoalexins or propteinases in the cells. Besides, plants' cell walls treated with chitosan undergo lignification, which makes the development of the pathogenic fungus in the plant impossible.

The author found out in her studies that *Botrytis cinerea, Sclerotinia sclerotiorum* and *Rhizoctonia solani* as well as fungi from the genera of *Fusarium, Pythium, Phomopsis* constituted a considerable danger towards soybean cultivation. Chitosan applied a few times both in the form of a dressing and spraying remarkably inhibited plants' infection by those pathogens.

The protective effect of chitosan towards soybean through inhibition of the development of *Rhizoctonia solani* and fungi from the genera of *Fusarium* and *Pythium* was proved by Li BaoYing and MaShuMei [1997]. On the other hand, Wojdyła and Orlikowski [1997] found out that already a singular application of chitosan at the concentration of 0.1% inhibited the development of *Fusarium oxysporum* f. sp. *dianthi, Botrytis cinerea*, *Sphaerotheca pannosa* var. *rosae* and *Peronospora sparsa* on the plants of carnation and rose.

CONCLUSIONS

1. Chitosan proved to be effective prevention against fungi pathogenic towards soybean.

2. Multiple application of chitosan (i.e. for seed dressing and spraying the seedlings and then plants at anthesis) proved the most effective in inhibiting soybean infection by pathogenic fungi colonizing its parts.

3. Soybean plants in all stages of development and growth are threatened by *Alternaria alternata, Botrytis cinerea, Phoma exigua* var. e*xigua, Sclerotinia sclerotiorum, Rhizoctonia solani* and *Fusarium* spp.

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CHITOZAN JAKO ZWIĄZEK OGRANICZAJĄCY WYSTĘPOWANIE CHORÓB SOI

Streszczenie. W latach 2002–2004 na polu monokultury soi w miejscowości Czesławice k. Nałęczowa prowadzono badania, których przedmiotem były rośliny soi odm. Polan oraz chitozan. Do badań używano wodny roztwór mikrożelu chitozanu w stężeniu 0,1%. W prowadzonych badaniach testowano wpływ tego związku na ograniczenie chorób soi powodowanych przez grzyby chorobotwórcze przeżywające w glebie. Roztwór chitozanu zastosowano wielokrotnie, tj. w formie do zaprawiania nasion, oprysku siewek, oprysku roślin na początku kwitnienia oraz w kombinacjach mieszanych. W trakcie trwania doświadczenia w poszczególnych fazach rozwojowych soi prowadzono obserwacje polowe, podczas których określano liczebność i zdrowotność roślin. Porażone rośliny pobierano do analizy mikologicznej. Wyniki uzyskane z przeprowadzonych obserwacji polowych oraz wykonanych analiz mikologicznych wykazały, że chitozan stosowany w kombinacji mieszanej (do zaprawiania nasion + oprysku siewek + oprysku roślin na początku kwitnienia) był najskuteczniejszy w ochronie soi przed infekcją grzybów patogenicznych przeżywających w glebie. W kombinacjach tych uzyskano najlepszą obsadę siewek soi i roślin w fazie kwitnienia. Przeprowadzona analiza mikologiczna porażonych organów siewek oraz roślin w fazie kwitnienia soi wykazała, że chitozan okazał się skuteczny w ograniczaniu infekcji roślin soi przez grzyby z rodzaju *Fusarium, Pythium, Phomopsis* oraz *Botrytis cinerea, Sclerotinia sclerotiorum* i *Rhizoctonia solani*.

Słowa kluczowe: chitozan, soja, grzyby patogeniczne

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