Original Research

The Effect of Flax Seed Dressing with Biopreparations, Chitosan, and its Derivatives on Fungal Communities in Soil

Katarzyna Wielgusz[1], Aleksandra Andruszewska[1], Elżbieta Pląskowska[2], Wojciech Szewczyk[3], Jan Bocianowski[4], Zbigniew Weber[5]*

[1]Department of Breeding and Agronomy of Fibrous Plants, Institute of Natural Fibres and Medicinal Plants, Wojska Polskiego 71B, 60-625 Poznań, Poland
[2]Department of Plant Protection, Wrocław University of Environmental and Life Sciences, Plac Grunwaldzki 24A, 50-363 Wrocław, Poland
[3]Department of Forest Phytopathology, Poznań University of Life Sciences, Wojska Polskiego 71C, 60-625 Poznań, Poland
[4]Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland
[5]Department of Phytopathology, Poznań University of Life Sciences, Dąbrowskiego 159, 60-594 Poznań, Poland

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Abstract

The effect of flax seed dressing with biopreparations, chitosan, and its derivatives on fungal communities in soil was evaluated. Biopreparation organisms consisted of: *Pseudomonas aureofaciens*, *P. fluorescens*, *Pythium oligandrum*, a mixture of photosynthetic and *Lactobacillus* bacteria, and unidentified yeasts and fungi. Chitosan and its derivative active ingredients were used: chitosan microcrystalline, chitosan acetate, and chitosan oligomers. Untreated flax seeds and seeds dressed with Zaprawa Oxafun T 75 DS fungicide containing carboxin and thiram active ingredients were used for controls. In general, *Pythium oligandrum* caused a greater decrease in soil fungal colony-forming units (cfu) than the Zaprawa Oxafun T 75 DS fungicide and all other preparations tested. *Pseudomonas aureofaciens* and *P. fluorescens* generally produced similar decreases in soil fungal cfu compared to fungicide controls. Chitosan and its derivatives almost always caused a decrease in fungal cfu, but these decreases were less pronounced than that in fungicide controls.

Keywords: *Pseudomonas aureofaciens*, *P. fluorescens*, *Pythium oligandrum*, chitosan microcrystalline, chitosan acetate, chitosan oligomers

Introduction

Disease suppression by biocontrol agents is the sustained manifestation of interactions among the plant, the pathogen, the biocontrol agent, the microbial community on and around the plant, and the physical environment. Biocontrol of soil-borne diseases is particularly complex because these diseases occur in a dynamic environment at the interface of root and soil known as the rhizosphere. The rhizosphere can change due to root growth, interactions with other soil biota, and weathering processes [1].

Biopreparations containing bacteria of the genus *Pseudomonas* [2-4] and other genera [5], *Pythium oligandrum* Drechsler species of the kingdom *Chromista* [6, 7], as

*e-mail: zweber@up.poznan.pl

well as chitosan and its derivatives [8-10], are characterized by high efficacy of plant protection against diseases caused by fungi and other groups of pathogens. Chitosan and its derivatives, as well as some biopreparations, affect the induction in plants of resistant responses related to synthesis of chitinase [11] and proteinase inhibitors [12], accumulation of callose [13], and permeability of cell membranes [14, 15]. Biopreparations [16], chitosan, and its derivatives [9, 17] also directly affect fungi by inhibiting their growth. The effect depends on the susceptibility of a particular species or even its isolate [18], the kind of biopreparation, or chitosan polymerization degree [8]. The direct effect of biopreparations, chitosan, and its derivatives on fungi is easy to assess in laboratory conditions. We tried to assess the effect of seed treatments on plots with flax in relation to soil fungi.

The aim of our work was to evaluate the effect of flax seed dressing with biopreparations, chitosan, and its derivatives on the population of fungi occurring in the rhizosphere.

Experimental Procedures

Plants, Forecrops, Soil, and Weather Conditions

Fibre flax cv. Alba and oil flax cv. Szafir were used; they are mean susceptible and susceptible to *Fusarium oxysporum*, respectively. The plots were in the Pętkowo Experimental Unit of the Natural Fibre Institute in Poznań on a field that was previously cultivated with flax over 3 to 4 years before. Soil used was sandy-clay. Prior to sowing, soil was fertilized with nitrogen, phosphorus, and potassium in dosages of 12, 50, and 60 kg/ha, respectively. The flax was sown on May 6, 2003, and April 30, 2004. The vegetation period of 2003 was dry in April, June, and August (rainfall was high only in May and July), and in 2004 was characterized by high rainfall from April to August. Mean temperatures in successive months varied from 5 to 20ºC in 2003 and from 9 to 20ºC in 2004.

Biopreparations, Chitosan, and its Derivatives Used for Dressing of Flax Seeds

The following biopreparation organisms were used in the experiment: *Pseudomonas aureofaciens* Kluyver (Cedomon®; BioAgri S.A., Uppsala, Sweden); *Pseudomonas fluorescens* Migula (PSR; Department of Microbiology, University of Agriculture, Wrocław, Poland); *Pythium oligandrum* Drechs. (Polyversum®; Biopreparations, Czech Republic); and a mixture of photosynthetic and *Lactobacillus* bacteria, yeasts, and fungi (EM-A®; Greenland, Japan).

The chitosan and its derivative active ingredients were used: β-1,4-D-glucosamine (chitosan microcrystalline, deacetylation degree – 80%, molecular weight – 100,000, Plant Protection, Institute Poznań, Poland); chitosan acetate (high molecular weight chitosan dissolved in acetic acid, Plant Protection Institute, Poznań, Poland); chitosan oligomers (chitosan acetate degraded with chitosanolytic enzymes, deacetylation degree – 80%, Plant Protection Institute, Poznań, Poland).

Table 1. Mean squares from four-way analysis of variance for number of fungi isolated from soil of plots with flax.

Source of variation	Degrees of freedom	Mean squares
Cultivars (C)	1	77.31***
Years (Y)	1	220.41***
Fungi (F)	25	4265.32***
Treatments (T)	13	4410.90***
C × Y	1	237.06***
C × F	25	82.49***
Y × F	25	1729.63***
C × T	13	64.22***
Y × T	13	211.86***
F × T	325	203.61***
C × Y × F	25	74.90***
C × Y × T	13	17.71**
C × F × T	325	25.70***
Y × F × T	325	90.58***
C × Y × F × T	325	18.85***
Residual	4,368	7.09

** significant at $P < 0.01$
*** significant at $P < 0.001$

Untreated flax seeds and seeds dressed with the fungicide active ingredients carboxin and thiram (Zaprawa Oxafun T 75 DS®; Azot, Jaworzno, Poland) were used for controls.

Isolation of Fungi from the Soil

Fungi isolation was done with the Warcup [19] method modified by Mańka [20], which is based on a mixture of soil sample and sterile fine quartz sand. Two (0.5 kg) samples of soil from 5 to 15 cm in depth were taken from each plot with flax plants in the growth stage of green capsule maturity (second half of June – term of the fibre flax harvest). These two samples were mixed and then 1 g of soil was put into each of four Erlenmayer flasks with 149 g of fine quartz sand. From these two components mixed for ten minutes 10 subsamples with 30 mm^3 (50 mg) of the mixture from each Erlenmayer flask were transferred to sterile Petri dishes with Martin-Johnson medium [21]. After 7-10 days of incubation growing colonies were transplanted on slants and appropriate media in Petri dishes. Descriptions of macro- and microscopic colonies grown on the media allowed the species (or at least genus) to be determined [22-29].

Table 2. Occurrence of fungi in soil of plots with flax plants grown from seeds treated in different ways.

Treatments of seeds before sowing			Colonies forming units of fungi ($10^5 \times g^{-1}$ of soil)*			
			Alba		Szafir	
preparations (substances)		ml(g)/1 kg of seeds	2003	2004	2003	2004
1.	Control	-	19.0[a]	17.2[a]	20.8[a]	17.1[a]
2.	Zaprawa Oxafun T 75DS	3	5.4[g]	6.6[e]	7.2[gh]	6.5[e]
3.	Cedomon	15	8.2[e]	7.9[e]	8.2[efg]	7.9[d]
4.	PSR	10	7.1[f]	7.5[e]	7.2[gh]	7.6[d]
5.	Polyversum	5	4.9[g]	4.6[f]	5.8[i]	5.2[f]
6.	EM-A	50	12.3[b]	13.2[b]	12.4[b]	12.0[b]
7.	0.01% chitosan acetate	120	8.6[e]	11.0[cd]	8.3[ef]	9.6[c]
8.	0.1% chitosan acetate	120	7.1[f]	8.0[e]	7.1[h]	7.1[de]
9.	0.5% chitosan acetate	120	8.3[e]	11.5[cd]	7.7[fgh]	9.9[c]
10.	0.01% chitosan microcrystalline	120	10.8[c]	10.2[d]	10.2[c]	9.8[c]
11.	0.1% chitosan microcrystalline	120	10.4[cd]	10.8[cd]	9.9[cd]	10.0[c]
12.	0.5% chitosan microcrystalline	120	10.9[c]	11.1[cd]	9.8[cd]	10.1[c]
13.	0.125% chitosan oligomers	120	9.7[d]	11.7[bcd]	9.1[de]	10.3[c]
14.	0.25% chitosan oligomers	120	9.6[d]	11.8[bc]	9.1[de]	10.5[c]

*in columns, means followed by the same letters are not significantly different.

Table 3. Mean squares (m.s.) from two-way analysis of variance for colonies forming units of fungi isolated from soil of plots with flax.

Source of variation	Alba				Szafir			
	2003		2004		2003		2004	
	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.	d.f.	m.s.
Species of fungi (F)	30	2,309***	25	507***	30	2,333***	27	592***
Treatments (T)	13	1,500***	13	1,024***	13	1,655***	13	911***
F × T	390	154***	325	62***	390	101***	351	46***
Residual	1,302	5	1,092	12	1,302	6	1,176	6

*** – significant at P<0.001
d.f. – degrees of freedom

Statistical Analysis

Four-way analysis of variance (flax cultivar, year, fungi species, seed treatments) was used to verify lack of particular factors' influence on isolated colony numbers. Fungal colonies from plots with different treatments were arranged in a completely randomized design. Homogeneous groups for the colony numbers were determined on the basis of least significant differences. A two-way analysis of variance was carried out to determine the effects of fungi (F), treatments (T), and the fungi × treatments (F × T) interaction. Contrast analysis was carried out for estimation of fungi occurrence in soil with flax [30]. Analysis of the data was performed using the statistical package GenStat v. 7.1 [31].

Results

Each of the four factors (flax cultivar, year, fungal species, and seed treatments) and all interactions significantly influenced the numbers of isolated fungal colonies (Table 1).

Mean numbers of fungal colony forming units (cfu) were highest in untreated flax seeds approximately in the following order: untreated > EM-A > CM = CO > CA > Cedomon > PSR > Zaprawa Oxafun T 75 DS > Polyvesum – Table 2 [CM=Chitosan microcrystalline, CO=Chitosan oligomers, CA=Chitosan acetate]. Mean squares from analysis of variance were significant at P < 0.001 for species of fungi (F), treatments (T) and F × T interaction (Table 3).

Table 4. Occurrence of fungi in soil of plots with flax plants grown from not treated seeds.

Fungi	Colonies forming units of fungi ($10^4 \times g^{-1}$ of soil)*			
	Alba		Szafir	
	2003	2004	2003	2004
Acremonium falciforme (Carrion) W. Gams	7.2^{d}	6.1^{cdefg}	9.0^{c}	6.5^{defg}
Alternaria sp. Nees ex Fr.	14.5^{ab}	14.0^{a}	15.1^{a}	12.4^{a}
Chrysosporium pannorum (Link) Hughes	3.6^{fg}	3.0^{ghi}	3.2^{hij}	3.5^{ij}
Cladosporium sp. Fres.	7.2^{d}	8.5^{bc}	9.0^{c}	8.6^{bc}
Fusarium chlamydosporum Wollenw. et Reinking	14.2^{ab}	12.9^{a}	14.3^{ab}	9.3^{b}
F. gibbosum Appel et Wollenw.	7.2^{d}	6.1^{cdefg}	8.0^{cd}	4.2^{hij}
F. merismoides Corda	0.1^{j}	4.6^{defgh}	0.1^{k}	3.5^{ij}
F. oxysporum Schlecht.	15.4^{a}	10.9^{ab}	15.4^{a}	8.6^{bc}
F. solani Sacc.	13.0^{bc}	4.6^{defgh}	12.8^{b}	6.1^{efgh}
F. venenatum Schwabe	4.5^{ef}	5.7^{cdefgh}	4.8^{gh}	5.3^{fghi}
Gliocladium penicilloides Corda	6.2^{de}	6.1^{cdefg}	8.3^{cd}	4.6^{ghi}
Gonytrichum sp. (Grove) Höhnel	3.0^{fghi}	0.0^{i}	5.8^{ef}	0.0^{k}
Microdochium nivale Samuels et Hallett	0.2^{j}	7.0^{cdef}	1.5^{jk}	7.1^{cdef}
Mortierella polycephala Coemans	0.1^{j}	7.4^{cde}	5.5^{efg}	7.5^{bcde}
Phoma eupyrena Sacc.	3.3^{fgh}	7.0^{cdef}	5.1^{fg}	8.3^{bcd}
P. exigua Desm.	7.5^{d}	3.8^{fgh}	6.7^{def}	2.5^{j}
P. finetti Sialer, Ciancio, Gallitelli	7.2^{d}	4.6^{defgh}	8.0^{cd}	5.0^{ghi}
P. hedericola Boerema	3.6^{fg}	3.8^{fgh}	5.8^{ef}	3.9^{ij}
Penicillium adametzi Zaleski	7.8^{d}	7.0^{cdef}	7.1^{de}	7.5^{bcde}
P. citrinum Thom	3.6^{fg}	0.0^{i}	5.6^{efg}	6.5^{defg}
P. janczewski Zaleski	11.0^{c}	8.9^{bc}	9.6^{c}	7.1^{cdef}
P. lividum Westling	1.5^{ij}	2.7^{hi}	2.2^{ij}	6.1^{efgh}
P. nigricans (Bankier) Thom	2.7^{ghi}	4.2^{efgh}	2.9^{ij}	3.5^{ij}
P. purpurogenum Stoll	3.0^{fghi}	0.0^{i}	3.8^{ghi}	0.0^{k}
P. vinaceum Gilman & Abbott	8.1^{d}	6.6^{cdef}	9.0^{c}	3.5^{ij}
P. waksmani Zaleski	8.1^{d}	5.7^{cdefgh}	7.1^{de}	5.0^{ghi}
P. vermiculatum Dangeard	12.1^{c}	7.7^{bcd}	8.3^{cd}	7.1^{cdef}
Trichoderma koningii Oud.	3.6^{fg}	7.4^{cde}	3.8^{ghi}	7.1^{cdef}
T. viride Pers. ex Gray	1.8^{hi}	0.0^{i}	4.7^{ij}	6.1^{efgh}
Ulocladium botrytis Preuss	1.5^{ij}	5.7^{cdefgh}	2.6^{ij}	4.6^{ghi}
Umbelopsis vinacea (Dixon-Stewart) von Arx	7.2^{d}	0.0^{i}	9.9^{ij}	0.0^{k}
Total	190.0	172.0	208.0	171.0

* in columns, means followed by the same letters are not significantly different.

Species of fungal cfu occurring in soil depended on year and flax cultivar (Table 4). Total numbers of fungal cfu in soil of both flax cultivars with untreated seeds were higher in 2003 than 2004. The dominant fungal isolates in 2003 plots were *Fusarium oxysporum*, *Alternaria* sp., and *F. chlamydosporum*, whereas only *Alternaria* sp. was most numerous on plots with both flax cultivars in 2004.

Most preparations used for seed dressing decreased the number of fungal cfu isolated from soil (indicated by +, ++, or +++ in Tables 5-8). In 2003 on plots with fibre flax Alba cultivar numbers of cfu of *Fusarium merismoides*, *Mortierella polycephala*, and *Trichoderma viride* in all treatments were equal (indicated by 0 in Table 5) or greater (indicated by -, --, --- in Table 5) than in untreated controls.

Table 5. Contrast analysis for occurrence of fungi in soil with 'Alba' flax (Pętkowo 2003).

Fungi	Significant differences between treatments*						
	1-2	1-3,4	1-5	1-6	1-(7-9)	1-(10-12)	1-13,14
Acremonium falciforme	+	+++	+++	+++	+++	0	+
Alternaria sp.	+++	+++	+++	+++	+++	+++	+++
Chrysosporium pannorum	+++	+++	+++	+++	+++	+++	+++
Cladosporium sp.	+++	+++	+++	+	+++	+++	+++
Fusarium chlamydosporum	+++	+++	+++	+++	+++	+++	+++
F. gibbosum	+++	+++	+++	+++	+++	++	---
F. merismoides	0	0	0	---	0	---	0
F. oxysporum	+++	+++	+++	+++	+++	+++	+++
F. solani	+++	+++	+++	+++	+++	0	+++
F. venenatum	-	+++	+++	+++	+	+++	+++
Gliocladium penicilloides	+++	+++	+++	+++	+++	+++	+++
Gonytrichum sp.	+++	0	+++	+++	+++	---	+++
Microdochium nivale	+++	0	+++	+++	+++	++	---
Mortierella polycephala	0	0	0	---	0	0	0
Phoma eupyrena	+++	0	++	0	---	0	0
P. exigua	+++	+++	+++	+++	+++	+++	+++
P. finetti	+++	+++	+++	+++	+++	+++	+++
P. hedericola	++	++	+++	0	+	-	0
Penicillium adametzi	+++	+++	+++	+++	+++	+++	+++
P. citrinum	+++	+	+++	--	+++	+++	0
P. janczewski	+++	+++	+++	+++	+++	+++	+++
P. lividum	+++	-	0	0	+++	+++	---
P. nigricans	+++	0	--	++	0	0	0
P. purpurogenum	+++	+++	+++	---	+++	+++	+++
P. vinaceum	+++	+++	+++	+++	+++	+++	+++
P. waksmani	+++	+++	+++	+++	+++	+++	+++
P. vermiculatum	+++	+++	+++	+++	+++	+++	+++
Trichoderma koningii	0	0	-	0	0	+++	+++
T. viride	0	---	--	0	--	0	0
Ulocladium botrytis	0	0	0	+++	0	0	-
Umbelopsis vinacea	+++	+++	+++	+++	+++	+++	0

*Numbers of treatments are explained in Table 2.
+, ++, +++ (-, --, ---) means significant possitive (negative) contrast value at 0.05, 0.01, 0.001 level, respectively; 0 – means statistically insignificant contrast value.

Beside these three species of fungi only in some treatments (most often in treatments with chitosan oligomers) numbers of cfu equally or greater than in untreated controls appeared in 15 species of fungi.

In 2003 on plots with oil flax Szafir cultivar numbers of cfu of *F. merismoides* in all treatments were equal to or greater than in untreated controls (Table 6). Besides this species, only in some treatments (most often in treatment with EM-A) were numbers of cfu equal or greater than in untreated controls found in 13 species of fungi.

In 2004 on plots with fibre flax Alba cultivar numbers of cfu of *Penicillium vermiculatum* in all treatments were equal to or greater than untreated controls (Table 7). Besides this species only in some treatments (most often in treatment with EM-A) were numbers of cfu equal to or greater than in untreated controls occurring in 20 species of fungi.

Table 6. Contrast analysis for occurrence of fungi with 'Szafir' flax (Pętkowo 2003).

Fungi	Significant differences between treatments*						
	1-2	1-3,4	1-5	1-6	1-(7-9)	1-(10-12)	1-13,14
Acremonium falciforme	++	+++	+++	+++	+++	+++	+++
Alternaria sp.	+++	+++	+++	+++	+++	+++	+++
Chrysosporium pannorum	0	+++	++	0	+++	+++	+
Cladosporium sp.	+++	+++	+++	+++	+++	+++	+++
Fusarium chlamydosporum	+++	+++	+++	+++	+++	+++	+++
F. gibbosum	+++	+++	+++	+++	+++	+++	0
F. merismoides	0	0	0	---	0	---	0
F. oxysporum	+++	+++	+++	+++	+++	+++	+++
F. solani	+++	+++	+++	+++	+++	+++	+++
F. venenatum	0	++	+++	+++	0	0	++
Gliocladium penicilloides	+++	+++	+++	+++	+++	+++	+++
Gonytrichum sp.	+++	+++	+++	+++	+++	---	+++
Microdochium nivale	0	---	0	0	+++	+++	0
Mortierella polycephala	+++	+++	+++	-	+++	+++	+++
Phoma eupyrena	+++	+++	+++	+++	0	+++	+++
P. exigua	+++	+++	+++	+++	+++	+++	+++
P. finetti	+++	+++	+++	+++	+++	+++	+++
P. hedericola	+++	+++	+++	++	+++	+++	+++
Penicillium adametzi	+++	+++	+++	+++	+++	+++	+++
P. citrinum	+++	+++	+++	++	+++	+++	+++
P. janczewski	+++	+++	+++	+++	+++	+++	+++
P. lividum	+++	0	0	0	+++	+++	0
P. nigricans	+++	0	-	+	0	0	0
P. purpurogenum	+++	+++	+++	0	+++	+++	+++
P. vinaceum	+++	+++	+++	+++	+++	+++	+++
P. waksmani	+++	+++	+++	+++	+++	+++	+++
P. vermiculatum	+++	+++	+++	+++	+++	+++	+++
Trichoderma koningii	+++	0	---	0	0	+++	+++
T. viride	+	0	0	0	0	+	0
Ulocladium botrytis	+++	+++	+++	+++	+++	+++	+++
Umbelopsis vinacea	+++	+++	+++	0	+++	+++	+++

*Numbers of treatments are explained in Table 2.
+, ++, +++ (-, --, ---) means significant possitive (negative) contrast value at 0.05, 0.01, 0.001 level, respectively; 0 – means statistically insignificant contrast value.

In 2004 on plots with oil flax Szafir cultivar numbers of cfu of *Penicillium nigricans* in all treatments were equal to or greater than in untreated controls (Table 8). Besides this species, only in some treatments (most often in treatment with EM-A) were numbers of cfu equal to or greater than in untreated controls occurred in 13 specie of fungi.

Discussion of Results

Soil organisms respond sensitively to land management practices and climate [32]. The community of fungi depends on soil type [21], fertilization and watering [33], crop rotation [34, 35], and on many other factors.

Table 7. Contrast analysis for occurrence of fungi in soil with 'Alba' flax (Pętkowo 2004).

Fungi	Significant differences between treatments*						
	1-2	1-3,4	1-5	1-6	1-(7-9)	1-(10-12)	1-13,14
Acremonium falciforme	0	+++	+++	0	+	0	+
Alternaria sp.	+++	+++	+++	+++	+++	+++	+++
Chrysosporium pannorum	+	0	0	0	0	0	0
Cladosporium sp.	+++	+++	+++	0	+++	+++	+++
Fusarium chlamydosporum	0	0	+	+	0	0	0
F. gibbosum	+++	+++	+++	+++	+++	+++	+++
F. merismoides	+++	++	+++	0	+++	0	0
F. oxysporum	+++	+++	+++	+++	+++	+++	+++
F. solani	+++	+++	+++	+++	+++	+++	+++
F. venenatum	+	0	+++	---	0	---	---
Gliocladium penicilloides	++	+++	+++	++	0	+	+++
Microdochium. nivale	++	+++	+++	0	+++	++	++
Mortierella polycephala	+++	+++	+++	0	+++	+++	+++
Phoma eupyrena	+++	+++	+++	0	++	++	0
P. exigua	+++	+	0	+++	+++	+++	0
P. finetti	+++	+	++	0	0	++	0
P. hedericola	0	+++	+++	-	+	++	0
Penicillium adametzi	+++	+	+++	0	+++	++	0
P. janczewski	+	+	+++	--	+	0	---
P. lividum	+++	+++	+++	0	++	+++	+++
P. nigricans	+++	+++	+++	0	+++	+++	+++
P. vinaceum	++	0	+	0	0	0	0
P. waksmani	+++	+++	+++	0	+++	0	+++
P. vermiculatum	0	--	0	---	---	---	---
Trichoderma koningii	+++	+++	+++	+++	+++	+++	+++
Ulocladium botrytis	+	++	+++	0	+	0	0

*Numbers of treatments are explained in Table 2.
+, ++, +++ (-, --, ---) means significant possitive (negative) contrast value at 0.05, 0.01, 0.001 level, respectively; 0 – means statistically insignificant contrast value.

Fungicides, biopreparations and some natural substances used for seed dressing against diseases can also lead to changes in the population of organisms. Soil microbial activity and biomass are decreased by fungicides and other pesticides [36-38]. Fungicides and other synthetic plant protection preparations used for seed dressing decreased the population of fungi occurring in the soil [39-41]. Dressing of seeds and treatment of aboveground parts of American ginseng with Polyversum decreased the population of soil fungi [42]. A similar effect was observed in our work with flax seeds treated with Polyversum. Dressing of seeds with Polyversum decreased the total number of fungi cfu equally (cv. Alba in 2003) or even greater than the fungicide Zaprawa Oxafun T 75 DS (cv. Alba in 2004 and cv. Szafir in 2003 and 2004). A similar effect to Zaprawa Oxafun T 75 DS was noted also in treatments with Cedomon and PSR (cv. Alba in 2004 and cv. Szafir in 2003). The smallest decrease of total cfu of fungi was found in treatment with EM-A. This may be connected with the large number of different species occurring in EM-A. We could not find any information in the literature on the effect of EM-A. Polyversum contains *Pythium oligandrum*, which is able to destroy a wide range of species of fungi [43]. In our work the range was larger in 2003 than in 2004, which might be influenced by weather or other factors.

Table 8. Contrast analysis for occurrence of fungi in soil with 'Szafir' flax (Pętkowo 2004).

Fungi	Significant differences between treatments*						
	1-2	1-3,4	1-5	1-6	1-(7-9)	1-(10-12)	1-13,14
Acremonium falciforme	+++	+++	+++	+++	+++	++	+++
Alternaria sp.	+++	+++	+++	+++	+++	+++	+++
Chrysosporium pannorum	+++	++	0	0	++	0	0
Cladosporium sp.	+++	+++	+++	+++	+++	+++	+++
Fusarium chlamydosporum	+++	+++	+++	+++	+++	+++	+++
F. gibbosum	+++	+++	+++	+	+++	+++	0
F. merismoides	+++	+++	+++	0	+++	+++	+++
F. oxysporum	+++	+++	+++	+++	+++	+++	+++
F. solani	+++	0	+++	0	+++	0	--
F. venenatum	+++	+++	+++	+++	0	0	++
Gliocladium penicilloides	++	+++	+++	+	0	0	+++
Microdochium nivale	+++	+++	+++	+++	+++	+++	+++
Mortierella polycephala	+++	+++	+++	+++	+++	+++	+++
Phoma eupyrena	+++	+++	+++	+	+++	+++	+++
P. exigua	+++	+++	+++	---	+++	+++	+++
P. finetti	+++	++	+	++	+++	+++	+++
P. hedericola	+	0	+++	0	0	0	-
Penicillium adametzi	+++	+++	+++	++	+++	+++	+++
P. citrinum	+++	+++	+++	+++	+++	+++	+++
P. janczewski	+++	+++	+++	0	+++	+++	+
P. lividum	+++	++	+++	++	0	0	++
P. nigricans	0	0	0	0	0	0	0
P. vinaceum	+++	+	0	---	+	-	0
P. waksmani	+++	0	+++	0	+++	++	0
P. vermiculatum	++	+++	+++	0	+	+	0
Trichoderma koningii	+++	+++	+++	+	++	+++	++
T. viride	+++	+++	+++	+	+++	+++	+++
Ulocladium botrytis	++	+++	+++	--	+	0	0

*Numbers of treatments are explained in Table 2.
+, ++, +++ (-, --, ---) means significant possitive (negative) contrast value at 0.05, 0.01, 0.001 level, respectively; 0 – means statistically insignificant contrast value.

The functional properties of chitosan products should be carefully monitored to effectively utilize the products [44]. In our work three products – chitosan acetate, chitosan microcrystalline and chitosan oligomers – had a similar influence on decrease, sometimes on increase, or did not influence the number of cfu of particular species of fungi in 2003 and in 2004. More differences can be found between 2003 and 2004 than between flax cultivars Alba and Szafir in those years. Dressing of soybean seeds with chitosan increased the population of *Pseudomonas* bacteria, did not change the population of Bacillus genus bacteria, but decreased the population of fungi in the soil [45]. In our work all flax seed treatments also decreased the total number of fungal cfu in the soil. In Pastucha's [45] work only the population of antagonistic fungi of the genera *Gliocladium* and *Trichoderma* was increased. In our work in 2003 on plots with both flax cultivars, most of the seed treatments did not influence or increase the population of *T. koningii* and *T. viride*. However, in 2004 all seed treatments increased the population of *T. koningii* in comparison with controls. The differences in these two years were probably due to weather conditions. Precipitation in 2003 varied from 5 to 20 mm in successive months (apart from July, when it was 48 mm), and in 2004 varied from 20 to 52 in successive months. The air temperatures in spring of 2003 were lower than in 2004.

Conclusions

- In comparison to untreated control changes of fungal communities in soil, we observed not only in treatment with flax seeds dressed with Zaprawa Oxafun T 75 DS fungicide but also with all other preparations.
- The changes depended on preparation used for seed treatment, fungi species, and flax cultivar.
- Polyversum containing *P. oligandrum* often caused a greater decrease in the number of fungal cfu than Zaprawa Oxafun T 75 DS fungicide.
- Biopreparations containing *P. aureofaciens* or *P. fluorescens* often caused a similar decrease in soil fungal cfu to that observed with Zaprawa Oxafun T 75 DS.
- Chitosan and its derivatives almost always produced a lower decrease in soil fungal cfu than Zaprawa Oxafun T 75 DS.
- In all treatments numbers of cfu equal to or greater than in untreated controls were observed for *F. merismoides*, *M. polycephala*, and *T. viride* (cv. Alba, 2003), *F. merismoides* (cv. Szafir, 2003), *P. vermiculatum* (cv. Alba, 2004), and *P. nigricans* (cv. Szafir, 2004).
- In some treatments numbers of cfu equal to or greater than in untreated controls were also found for particular species of fungi in successive years, and flax cultivars.

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