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EFFECT OF THERMOTHERAPY, GRAIN TREATMENT AND LEAF SPRAYING WITH BIOLOGICAL CONTROL AGENTS ON SPRING BARLEY (*HORDEUM VULGARE*) HEALTH IN ORGANIC SYSTEM

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Abstract

Effect of grain treatment with Biochikol 020 PC, Biosept 33 SL, *Trichoderma viride* and thermotherapy on seedling health was tested. The aim was to determine their inhibitory effect on spring barley pathogens: *Bipolaris sorokiniana*, *Fusarium* spp. and *Drechslera teres*. Some advantageous effect of Biosept 33 SL and thermotherapy on plant health and pathogen control was noted. The development of *B. sorokiniana* was also significantly limited by Biochikol 020 PC but to a lower degree. Thermotherapy was favourable for *T. viride* development, but advantageous effect of that measure on *B. sorokiniana* control was observed only in the case of stem bases and leaves of young plants, where *D. teres* was noted in lower intensity. Leaf spraying with Biochikol 020 PC and Biosept 33 SL caused decreased infection with *B. sorokiniana* and *D. teres*. Alternative methods of disease control could be effective tools in barley protection. It is especially important in organic system where pathogenic fungi are a serious threat and pesticide application is prohibited.

Key words: barley, fungi, biocontrol, organic farming, *Bipolaris sorokiniana*, *Fusarium*, *Drechslera teres*

Introduction

There is a common opinion that organic agriculture favours maintaining biological balance in environment that can result in a better plant health. A basis of that theory is the belief that organic system conditions allow to rebuild and maintain proper interactions among microorganisms which can induce natural mechanisms of plant disease control. The opinion does not find confirmation in wide practice. Organic farming, without pesticides, herbicides or mineral fertilisers can

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lead to the risk of obtaining grain contaminated with harmful biological factors (Baturó 2002, Baturó and Sadowski 2005). It could create a problem because only propagation material produced in organic system is allowed for use there and chemical treatment is prohibited. Mycological analyses of spring barley grain carried out previously showed that *Bipolaris sorokiniana* is a serious problem in organic system. Besides *B. sorokiniana* also pathogenic species *Fusarium avenaceum*, *F. culmorum*, *F. graminearum* and *F. poae* were isolated, though in lower intensity in comparison to integrated and conventional systems. Also leaf infection with *Drechslera teres* and *B. sorokiniana* consist a serious problem in organic system (Baturó and Łukanowski 2001).

The health status of sowing material is of crucial importance, as it can be the source of pathogenic fungi that result in plant infection. It is possible to limit development of fungi transmitted with grain by applying both physical and biological methods which can be very useful in organic agriculture. Some of them were analyzed in the study reported in this paper. Due to revealed high organic grain infection, mainly by *B. sorokiniana*, the studies aimed at finding the possibility of minimizing the source of primary infection with tools approved for organic farming.

Materials and methods

The effect of cv. 'Damazy' grain treatment with Biochikol 020 PC (chitosan) 2.0%, Biosept 33 SL (grapefruit extract) 0.1%, antagonistic strain of *Trichoderma viride* (obtained from University of Agriculture, Wrocław) and thermotherapy with hot water (4 h – 30°C, 10' – 52°C) on seedlings' health in comparison with control was tested in 2002–2006. Plot experiment was carried out in four replications in organic farm in Kiełpin near Tuchola in Kujawy and Pomerania voivodeship, north-western Poland. The organic farm has belonged to the EKOLAND association (Polish national organic organisation which issues certificates to the farms meeting organic farming standards) for over 15 years.

For health status evaluation 200 plants from each field were sampled randomly (50 in each of four replications) at the beginning of tillering. The root and stem base infestation was evaluated according to a scale from 0 (healthy roots or stem bases) to 5 (roots with > 70% area damaged or stem bases completely rotted) and leaf infestation by *D. teres* according to a scale from 0 (healthy leaves) to 4 (leaves with > 50% area with spots). The idea was to take every year up to 30 plants with disease symptoms for mycological analysis from the sample of 200 plants. Yet, there were cases, with less than 30 plants with disease symptoms among the lot of 200, so isolation of fungi was performed from all the diseased plants. Details concerning plant numbers taken for mycological analysis from each joint sample in every year are shown in Tables 2 and 4. Fungi were isolated from 0.5-cm fragments of roots and stem bases on PDA medium, pH 5.5. Before placing them onto PDA medium the fragments were washed 10 min under tap running water, disinfected for 1.5 min in 1% of NaOCl and then washed three times with sterile water. After ade-

quate time of incubation at 23°C and after transferring on proper mediums fungi were identified according to mycological keys.

The efficacy of plant spraying with Biochikol 020 PC 2.0% and Biosept 33 SL 0.1% on leaf health was also determined in 2002 and 2004. Spraying was performed two and three times every two weeks, starting from shooting stage. At the beginning of dough maturity the flag leaf infestation by *D. teres* and *B. sorokiniana* was evaluated, as described above, with a 0–4° scale.

In all cases the levels of infestation were transformed to a disease index (DI) according to Townsend's and Heuberger's formula (Wenzel 1948) and data were analysed statistically by ANOVA and a Tukey test was used to compare means.

Results

Health status of roots, stem bases and leaves were different in every year (Tables 1, 2, 5) but some tendencies were noted. Some advantageous effect of Biosept 33 SL and thermotherapy was observed in the case of roots. Four-year studies revealed that roots of plants obtained from grain treated with Biosept 33 SL were significantly more healthy (DI 1.3%) in comparison to control. The highest DI (3.2%) was noted in treatment with *T. viride*, obviously due to great infestation of plants in that treatment in 2004 (7.0%). Further mycological analyses revealed that the infestation symptoms were not entirely caused by phytopathological agents. In 2003 infestation was relatively high, but in that year Biosept 33 SL showed the highest efficacy in plant protection.

Based on the mean disease index (Table 2) there was no significant difference in stem base health status, though plants were more healthy in the two last years of the studies.

Bipolaris sorokiniana was isolated in all treatments, both from roots and stem bases (Tables 3, 4), in lower isolate number as compared to control, except for roots of plants deriving from grain disinfected with hot water. Disease index in

Table 1

Root disease index at the beginning of tillering (%)

Grain treatment	Year of the study					2002–2006
	2002	2003	2004	2005	2006	
Control	1.5	8.9 a	0.2 c	1.0	1.2	2.6 bc
Thermotherapy	2.8	7.3 ab	0.0 c	0.0	0.8	2.2 c
Biosept 33 SL	3.3	1.8 b	0.6 bc	0.0	0.8	1.3 d
Biochikol 020 PC	1.0	6.6 ab	3.8 ab	2.6	1.2	3.0 ab
<i>T. viride</i>	1.6	2.9 ab	7.0 a	2.6	2.0	3.2 a
LSD _{$\alpha=0.05$}	n.s.	6.33	3.52	n.s.	n.s.	0.44

Values in the same line followed by different letters are significantly different.

n.s. – non-significant.

Table 2

Stem base disease index at the beginning of tillering (%)

Grain treatment	Year of the study					2002–2006
	2002	2003	2004	2005	2006	
Control	13.0 ab	11.6 ab	7.6 ab	2.4 bc	0.8	7.1
Thermotherapy	11.1 ab	15.8 a	6.4 b	1.4 c	0.0	6.9
Biosept 33 SL	14.4 a	10.7 ab	7.8 ab	2.0 bc	0.0	7.0
Biochikol 020 PC	10.6 ab	12.7 ab	7.6 ab	3.2 ab	0.4	6.9
<i>T. viride</i>	8.9 b	8.4 b	11.0 a	4.0 a	0.2	6.5
LSD _{α=0.05}	5.05	5.49	4.08	1.41	n.s.	n.s.

Values in the same line followed by different letters are significantly different.

n.s. – non-significant.

Table 3

Fungi isolated from roots of spring barley at the beginning of tillering in the years 2002–2006 (%)

Fungus	Control	Thermotherapy	Biosept 33 SL	Biochikol 020 PC	<i>T. viride</i>
<i>Aspergillus niger</i>	–	–	–	–	2.1
<i>Aspergillus</i> sp.	–	4.3	–	–	–
<i>Aureobasidium bolleyi</i>	–	–	2.9	–	2.1
<i>Bipolaris sorokiniana</i>	39.3	52.2	5.7	20.0	20.8
<i>Epicoccum nigrum</i>	3.6	–	–	–	–
<i>Fusarium avenaceum</i>	3.6	–	–	4.0	–
<i>Fusarium culmorum</i>	–	–	–	4.0	2.1
<i>Fusarium equiseti</i>	–	–	–	–	2.1
<i>Fusarium oxysporum</i>	–	–	–	–	4.2
<i>Fusarium sambucinum</i>	–	–	2.9	–	–
<i>Fusarium solani</i>	–	–	8.6	12.0	–
<i>Fusarium sporotrichioides</i>	–	–	–	–	2.1
<i>Gliocladium catenulatum</i>	25.0	–	–	–	–
<i>Gliocladium roseum</i>	–	–	40.0	8.0	6.3
<i>Mucor mucedo</i>	–	–	–	4.0	–
<i>Mucor</i> spp.	3.6	–	2.9	4.0	–
<i>Penicillium</i> spp.	3.6	–	–	16.0	20.8
<i>Periconia macrospinoso</i>	3.6	–	–	–	–
<i>Phoma</i> sp.	–	–	2.9	–	2.1
<i>Rhizoctonia</i> sp.	–	–	5.7	–	2.1
<i>Trichoderma viride</i>	14.3	39.1	28.6	28.0	31.3
Non-sporulating colonies	3.6	4.3	–	–	2.1
Total number of colonies	28	23	35	25	48
Number of plants with infested roots taken to analysis	24	22	31	30	52

that treatment was relatively low but in the case of roots *B. sorokiniana* accounted for 52.2% of all isolated colonies (with only 8% in the case of stem bases), despite of numerous isolates of *T. viride* both on roots and stem bases. Grain treatment with Biosept 33 SL resulted in the lowest plant infection with *B. sorokiniana*. It was isolated from 5.7% of roots and 11.1% of stem bases only. The pathogen was also significantly limited by Biochikol 020 PC but not so efficiently. Biosept 33 SL and Biochikol 020 PC and, to a smaller extent, antagonistic *T. viride* stimulated development of another antagonistic species, *Gliocladium roseum*.

Trichoderma viride was isolated frequently (both from roots and stem bases) in particular from symptomatic plants deriving from grain treated with antagonistic strain of *T. viride* or thermotherapy. It suggests that *T. viride* can develop on those symptomatic organs, from which pathogens were isolated in lower amounts. Thus, thermotherapy seems favourable for *T. viride* development which is a very advanta-

Table 4

Fungi isolated from stem bases of spring barley at the beginning of tillering in the years 2002–2006 (%)

Fungus	Control	Thermotherapy	Biosept 33 SL	Biochikol 020 PC	<i>T. viride</i>
<i>Acremonium</i> sp.	–	–	–	–	1.7
<i>Alternaria alternata</i>	–	–	–	–	0.8
<i>Aspergillus niger</i>	–	–	–	1.1	–
<i>Aureobasidium bolleyi</i>	–	–	–	1.1	–
<i>Bipolaris sorokiniana</i>	42.4	8.0	11.1	23.4	24.0
<i>Cladosporium herbarum</i>	–	–	1.1	4.3	–
<i>Fusarium avenaceum</i>	3.3	2.7	4.4	4.3	–
<i>Fusarium culmorum</i>	1.1	1.3	–	–	3.3
<i>Fusarium equiseti</i>	–	–	–	1.1	0.8
<i>Fusarium oxysporum</i>	3.3	–	–	–	–
<i>Fusarium poae</i>	–	–	–	1.1	–
<i>Gliocladium catenulatum</i>	–	12.0	–	1.1	–
<i>Gliocladium roseum</i>	7.6	–	17.8	19.1	17.4
<i>Mucor mucedo</i>	2.2	–	–	1.1	–
<i>Mucor</i> spp.	–	1.3	17.8	10.6	5.8
<i>Penicillium</i> spp.	5.4	8.0	20.0	21.3	14.9
<i>Phoma</i> sp.	–	–	–	1.1	–
<i>Rhizopus nigricans</i>	2.2	6.7	–	–	–
<i>Trichoderma koningii</i>	–	–	–	–	0.8
<i>Trichoderma viride</i>	31.5	57.3	23.3	7.4	29.8
Non-sporulating colonies	1.1	2.7	4.4	2.1	0.8
Total number of colonies	92	75	90	94	121
Number of plants with infested stem bases taken to analysis	45	67	70	76	81

Table 5

Leaf infestation with *Drechslera teres* disease index at the beginning of tillering (%)

Grain treatment	Year of the study				2002–2006
	2002	2003	2005	2006	
Control	6.9 ab	3.9 b	4.5	23.2 a	9.6 ab
Thermotherapy	7.6 a	5.8 b	0.2	11.5 b	6.3 c
Biosept 33 SL	7.9 a	6.1 b	3.0	14.5 ab	7.9 bc
Biochikol 020 PC	6.4 ab	17.2 a	1.6	18.0 ab	10.8 a
<i>T. viride</i>	5.5 b	4.2 b	7.0	10.8 b	6.9 c
LSD _{$\alpha=0.05$}	2.01	6.05	n.s.	10.55	2.51

Values in the same line followed by different letters are significantly different.
n.s. – non-significant.

Table 6

Flag leaf disease index at the beginning of dough maturity (%)

Treatment	Biosept 33 SL			Biochikol 020 PC		
	2002	2004	mean	2002	2004	mean
Control	17.5	52.8 a	35.1 a	17.5	52.8 a	35.1 a
2 × spraying	18.3	41.8 b	30.0 b	17.5	40.0 b	28.8 b
3 × spraying	16.7	43.5 b	30.1 b	16.8	42.8 b	29.8 b
LSD _{$\alpha=0.05$}	n.s.	6.92	3.50	n.s.	9.16	4.64

Values in the same line followed by different letters are significantly different.
n.s. – non-significant.

geous phenomenon. It probably resulted in relatively low stem base infection of plants by *B. sorokiniana* in that treatment, and this was not the case in direct grain treatment with antagonistic strain of *T. viride*. Besides of *B. sorokiniana*, also pathogenic *Fusarium* spp. were isolated from roots and stem bases, though not frequently. *Trichoderma viride* grain treatment limited stem base infection with *F. avenaceum*. The pathogen was isolated in all the other treatments. Grain treatment with *T. viride*, beside thermotherapy, was advantageous for leaf health, as *D. teres* symptoms were significantly reduced in these treatments (Table 5).

Biosept 33 SL as Biochikol 020 PC sprayed onto the plants proved similarly, and not highly, efficient in protection of the flag leaf. Advantageous result occurred only in 2004 and influenced the mean value (Table 6). There was no significant difference in leaf infestation in the case of two or three sprays in the season.

Discussion

Results reported here showed that alternative methods of disease control could be effective tools in barley protection. Some tendencies to decrease the incidence

of *B. sorokiniana* on barley seedlings observed here are promising for organic system where pesticide application is prohibited and the pathogen, transmitted mainly with infected sowing material, is a serious problem due to root, stem base, leaf and head infection threat (Baturó and Łukanowski 2001, Baturó 2002, 2005, Baturó et al. 2002).

Good results were obtained with Biosept 33 SL grain treatment. Thermotherapy favoured the development of potentially antagonistic *T. viride*, but its advantageous effect of *B. sorokiniana* limitation occurred on stem bases only and on leaves of young plants, where *D. teres* was noted in lower intensity. Favourable effect of thermotherapy on *T. viride* development is a very advantageous phenomenon. The beneficial effect of *Trichoderma* spp. is due to direct parasitism on pathogenic fungi and to its effect on plants, such as enhancement of plant development and induced resistance (Harman 2000). The antagonistic process relies on production of antibiotics and hydrolytic enzymes and may be associated with competition for nutrients (Herrera-Estrella and Chet 1998). *Trichoderma viride* showed a good effect on health of pea (Łacicowa and Pięta 1996) and vegetables in organic farm (Sadowski et al. 2006). Gromovych et al. (1999) noted high or moderate antagonistic activity of *Trichoderma* strains toward *Fusarium* species, among them of *T. viride*. In the studies of Bharath et al. (2005) *T. viride* has proved efficient in reducing the incidence of many fungi. Cook and Maker (1983) found *Chaetomium*, *Penicillium*, and *Trichoderma* spp. the most effective fungal antagonists used for seed treatments. El-Meleigi and Hassan (2000) noted a successful, but not very high effect of *T. viride* in coating winter wheat seeds against *B. sorokiniana* and *F. graminearum*. The advantageous direct effect of grain treatment with antagonistic strain of *T. viride* was not observed here and *Fusarium* spp. were isolated after the treatment. It could have been due to direct grain treatment with *T. viride* conidia which were probably too weak without a special medium protecting them against non-favourable environment conditions.

Biochikol 020 PC and antagonistic strain of *T. viride* limited *B. sorokiniana* to a smaller extent. Such factors as chitosan and antagonistic strains of *T. viride* were found active in pathogen control and reported in many papers. Chitosan is known as a potential elicitor of plant defence reactions and also an active inhibitor of fungal growth (Wojdyła 2004). Besides, many reports inform about the inhibiting impact of chitosan on growth and development of fungi and bacteria, although in some cases this impact was not observed (Stossel and Leuba 1984, Pospieszny et al. 1995). For instance: chitosan did not limit, nor *in vitro* neither after introduction into the soil, some forms of *F. oxysporum* (Skrzypczak and Orlikowski 1998). According to Pięta et al. (1998) for every fungus–chitosan or plant–chitosan model there can be a different biochemical mechanism and that is why it seems necessary to individually determine the effect of chitosan on growth and development of each phytopathogen. It is of great importance as fungicidal effect of chitosan depends, among others, on fungal species or even strains (Allan and Hadwiger 1979). This could perhaps explain not clear effect of Biochikol 020 PC on root and stem base health. Pięta et al. (2000) noted that treatment with chitosan satisfactorily protected germinated seeds of bean and next roots and stem bases against soil-borne

pathogens. In Petri dishes studies made by Pastucha (2001) liquid solution of chitosan added to medium was efficient in growth inhibition of pathogenic fungus colonies. Baturó (2003) showed that *B. sorokiniana* development on PDA medium could be inhibited by addition of Biochikol 020 PC. Studies reported here confirmed these effects in plot experiments. Biochikol 020 PC tested by Łukanowski (2003) also limited the growth of *Fusarium* spp.: it showed advantageous effect in the case of *B. sorokiniana*, while *Fusarium* spp. (e.g. *F. solani*) were not so efficiently limited. Roller and Covill (1999) observed antifungal properties of chitosan *in vitro* against a few filamentous fungi, but they also noted that some of fungal strains could be resistant to that chitin derivative. It shows that not all fungi are susceptible to chitosan. Biochikol 020 PC and Biosept 33 SL limited leaf infestation by *D. teres* and *B. sorokiniana*. It is very important because those pathogens are serious leaf pathogens of spring barley (Baturó and Łukanowski 2001, Almgren et al. 1999). Both preparations were tested by Wojdyła (2001, 2004) and spraying of ornamental plant leaves also resulted in their lower infection. Chitin, chitosan, and their derivatives are biologically active molecules regulating plant–phytopathogen interactions. Chitoooligosaccharides at high concentrations display fungal toxicity; inhibit fungal sporulation and growth of mycelium (Vasyukova et al. 2001). Also Biosept 33 SL, containing 33% grapefruit extract, directly effects pathogenic factors and induces in plants resistance to certain pathogens (Orlikowski et al. 2002). 7-geranoxicumarin, found in grapefruit juice, can have this effect (Orlikowski and Skrzypczak 2003). Advantageous effect of Biosept 33 SL and Biochikol 020 PC on health status of soybean was observed by Patkowska (2005). Patkowska (2006) also noted considerable protective effect of Biosept 33 SL against soil-borne pathogenic fungi, e.g. *Fusarium* spp., in germinating seeds, roots and stem bases of common bean, runner bean and pea.

Better development of *G. roseum*, in treatment with Biosept 33 SL, Biochikol 020 PC and *T. viride*, should be noted as advantageous phenomenon. *Gliocladium* spp. are known for their antagonistic abilities towards pathogens and their presence in environment is advantageous. Teperi et al. (1998) obtained satisfactory results in *F. culmorum* control in wheat by *Gliocladium* spp. Antagonism through antibiosis is considered a particularly promising strategy for biological protection of seeds (Baker 1990). *Gliocladium roseum* can effectively control root rots and seedling diseases caused by *Fusarium* spp. (CastejónMuñoz and Oyarzun 1995). Also according to Knudsen et al. (1995) and Jensen et al. (2000, 2002), *G. roseum* shows potential as a biological control agent against seed-borne diseases on cereals caused by *Fusarium* spp. and *B. sorokiniana*. Also Dal Bello et al. (2003) claim that *G. roseum* can be used to reduce seedling blight of wheat caused by *B. sorokiniana*. It suggests that high concentration of that fungus in treatment with Biosept 33 SL could have resulted in a relatively low infection with *B. sorokiniana*.

The results reported here are promising. Synthetic chemical fungicides have been long used for reducing the incidence of plant diseases. However, they are costly, can cause environmental pollution, and may induce pathogen resistance. The use of biocontrol agents or biopreparations for plant disease control may not be more effective than other control methods, but is commonly encouraged for en-

vironmental and other reasons (Cook 1993, Campbell 1994). According to Alabouvette et al. (2006) biological control practices need an integrative approach, and more knowledge than chemical control. Although biological control is a strategy element that was proposed half a century ago, it still needs development and studies reported in this paper should be continued and carefully performed.

Conclusions

1. Alternative methods of disease control used in the reported studies could be effective tool in barley protection.

2. Biosept 33 SL, hot water treatment, antagonistic strain of *T. viride* and Biochikol 020 PC may be considered for *B. sorokiniana* control, after further studies.

3. Severe threat of leaf infection by *D. teres* in organic system requires further studies on alternative methods of plant protection.

Streszczenie

WPŁYW TERMOTERAPII, ZAPRAWIANIA ZIARNA I OPRYSKIWANIA LIŚCI CZYNNIKAMI OCHRONY BIOLOGICZNEJ NA ZDROWOTNOŚĆ JĘCZMIENIA JAREGO (*HORDEUM VULGARE*) W SYSTEMIE EKOLOGICZNYM

Celem badań było poszukiwanie proekologicznych możliwości zminimalizowania pierwotnej infekcji roślin, której źródłem jest materiał siewny. Badano wpływ zaprawiania ziarna jęczmienia jarego preparatami Biochikol 020 PC i Biosept 33 SL, zarodnikami izolatu *Trichoderma viride* o silnych właściwościach antagonistycznych, a także odkażania termicznego na zdrowotność korzeni, podstaw źdźbła i liści. Szczególną uwagę zwrócono na ograniczenie *Bipolaris sorokiniana*, grzybów rodzaju *Fusarium* i *Drechslera teres*. Biosept 33 SL i termoterapia wykazały korzystny wpływ na zdrowotność roślin i ograniczyły rozwój patogenów na korzeniach i podstawach źdźbła. Biochikol 020 PC również ograniczał występowanie *B. sorokiniana*, ale nie tak znacząco. Odkażanie termiczne wpłynęło pozytywnie na rozwój *T. viride* i ograniczyło zasiedlenie podstaw źdźbła przez *B. sorokiniana*, a także było prawdopodobnie przyczyną słabszego porażenia liści młodych roślin przez *D. teres*. Opryskiwanie roślin Biochikolem 020 PC i Bioseptem 33 SL poprawiło ich zdrowotność. Opryskiwane liście były w mniejszym stopniu zainfekowane *B. sorokiniana* i *D. teres*. Przedstawione wyniki dowodzą, że alternatywne metody ochrony roślin mogą stanowić narzędzie ochrony jęczmienia jarego. Jest to szczególnie istotne w systemie ekologicznym, gdzie występowanie grzybów patogenicznych stanowi zagrożenie, a stosowanie pestycydów jest zabronione.

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