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THE IMPACT OF THE BIOLOGICAL AGENT BIOJODIS ON THE INCIDENCE OF PATHOGENIC FUNGI IN WINTER WHEAT AND SPRING BARLEY

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Abstract

The research was designed to study the use of a biological agent Biojodis for winter wheat and spring barley seed and plant treatment against seed borne fungal stem base and leaf diseases as well as against toxic fungi occurring on harvested grain. Seed treatment with Biojodis did not exert any effect on seed contamination with *Fusarium* spp.; however an especially efficient control of seed borne stem base rots of winter wheat and spring barley was obtained. However, under the field conditions, plant damage by stem base rot of the crops sown with treated and untreated seed did not differ. The application of Biojodis did not exert any effect on winter wheat and spring barley foliar disease incidence. However, Biojodis reduced winter wheat grain infection with *Fusarium* genus fungi on harvested grain. A significant reduction in the content of these fungi was identified having used Biojodis for seed treatment; and three spray applications during the growing season. Biojodis reduced the content of *Fusarium sporotrichioides* in internal tissue of winter wheat grain, inappreciably affected *F. poae, F. avenaceum* and *F. culmorum*, and hardly had any impact on *F. langsethiae* and *F. tricinctum*. However, Biojodis did not show any significant effect on spring barley grain contamination with fungi.

Key words: wheat, barley, disease, biological agent

Introduction

Increased consumer awareness of food safety issues and environmental concerns has contributed to the growth in organic farming over the last few years. The organic farm sector in some of the countries of European Union is estimated to have grown around 30% a year since 1998. This form of farming is rapidly growing in Lithuania too. Over the last two years, the number of organic farms increased by 22% and certified area by 23% /Garliauskienė, 2008/. Restriction of the use of chemical products in the organic farming system prompts a constant search for new, more effective environment-friendly control methods and measures intended for the reduction of seed infection with pathogenic fungi. Pre-sowing seed preparation, plant protection against diseases, and the quality of the new harvest are one of the most relevant issues in organic farming. In the conditions favourable for the spread of fungi they can reduce seed germinating power, germination and seed weight /Abramson, 1998/, weaken plant root system, reduce plant green material and yield /Radisic et al., 1995/. Microscopic fungi that produce mycotoxins and release them into the environment reduce cereal grain sales value /Railienė et al., 2005/ and exert a negative effect on human and animal health /D'Mello et al., 1999; Mankevičienė, 2002/. The most common mycotoxins produced by *Fusarium* fungi identified on cereal grain are deoxynivalenol, zearalenone, T-2 toxin, nivalenol and others /Thrane et al., 2004/.

Biological agent Biojodis is a liquid organic fertiliser produced as an aqueous extract of bio humus supplemented by physiologically active iodine, bio transformers and microelements, which can be used on organic farms. However, the possibilities of its use have not been comprehensively studied yet. Iodine present in its composition has bactericidal properties, therefore from the phytosanitaric viewpoint it provides an effect similar to that of a seed treater. Cereal seed treatment and plant spray application during the growing season using the Biojodis agent of organic origin can enhance crop yield and improve grain quality. Moreover, fungicidal action of Biojodis has been noted /Sliesaravičius et al., 2006/. Research done at the Lithuanian University of Agriculture in 2005 showed that grain treatment of winter wheat cv. 'Širvinta 1' and spring barley cv. 'Ūla' with Biojodis resulted in an increase in grain vigour and germination and a reduction in the content of fungi on the surface of grain and fungi species composition /Pekarskas, 2005/. In field trials, Biojodis significantly increased spring barley grain yield /Pekarskas, Jonaitis, 2005/. In 2007 the study conducted at the Lithuanian Institute of Agriculture was designed to explore the use of the biological agent Biojodis for winter wheat and spring barley seed and plant treatment against seed borne fungal stem base and leaf diseases as well as against toxic fungi occurring on harvested grain.

Materials and Methods

The field and laboratory research was carried out at the Lithuanian Institute of Agriculture during 2006–2007. In this study the biological agent Biojodis was used for winter wheat 'Ada' (2006–2007) and spring barley 'Luokė' (2007) seed and plant treatments against seed borne fungal stem base and leaf diseases as well as against toxic fungi occurring on harvested grain. In field treatments, prior to sowing, wheat and barley seeds were treated with an aqueous solution of Biojodis (2 l t^{-1}; 3 l t^{-1}) and as a control treatment with Maxim star (fludioxonil + ciproconazole 18.75 + 6.25 g l^{-1}) 1.0 l t^{-1} in winter wheat and Kinto (triticonazole + prochloraz 20 + 60 g l^{-1}) 2.0 l t^{-1} in spring barley. The seed was treated using a precision laboratory seed treatment instrument Hege 11. Each plot's seed was weighed and treated separately. The field trial was replicated four times and was sown with a special drill SSFK-7 (winter wheat) and Hege 80 (spring barely). The plots were 20 m^2 (6 rows of plants, spaced at 15 cm) for winter wheat and 15 m^2 (12 rows spaced at 12.5 cm) for spring barely. During the season, winter wheat plants were sprayed with Biojodis from one to three times: 3 l ha^{-1} at the tillering stage (T-0); at the tillering and 1.5 l ha^{-1} at stem elongation (T-0; T-1); and at the tillering, stem elongation and 1.5 l ha^{-1} at heading (T-0; T-1; T-2). Spring barley plants were sprayed three times: 3 l ha^{-1} at the tillering stage, 1.5 l ha^{-1} at stem elongation and 1.5 l ha^{-1} at heading (T-0; T-1; T-2).

For phytotoxicity test, the seed germination power, germination (laboratory and field test) and leaflet and rootlet length of three week – old sprouts were quantified. The seed germination power was estimated after 3 days and germination laboratory test – after 7 days in plastic plates on wet sand (100 seeds in 4 replicates for each sample). For field germination test, we calculated the final emergence of 100 seeds in four rows for each treatment.

For seed health test, untreated and Biojodis also Maxim star and Kinto treated seeds (100 for each sample) without surface sterilization were incubated on Potato Dextrose Agar (PDA) for 7 days at 24^o C. To control fungus colony growth, the medium was supplemented with Triton 100 (0.8 ml l^{-1}), and to prevent bacteria growth, the medium was supplemented with citric acid (3 ml l^{-1}). The infection level of seed was evaluated in percent (0 – all seed healthy, 100% – all grain infected). The method of blotter rolls was used for the determination of seed-borne root rot infection of seedlings. The seeds were incubated in wet blotter rolls for 21 days at 20^o C 16 h daylight and 8 h darkness regime. Root and crown rot incidence on seedlings was estimated in points (0 – healthy, 1 – weakly, 2 – moderately, 3 – heavily affected), and disease severity index % was calculated. Similarly, crown rot was estimated in field conditions at plant tillering stage (on winter wheat after wintering). The grain yield was harvested at complete maturity by a plot harvester Wintersteiger Classic (winter wheat) and Wintersteiger Delta (spring barley).

Internal grain infection with fungi of harvested grain was determined by plating the surface-sterilized grains (200 for each sample) on PDA /Mathur, Kongsdal, 2003/. Microscopic studies of fungi were carried out after 7–8 days incubation at 26±2^o C. The infection level of grain was evaluated in percent. The purified single spore cultures of *Fusarium* species were identified on the basis of their cultural and morphological characteristics according to Nelson et al. (1983) and Leslie et al. (2006).

ANOVA was applied for the statistical processing of data. For data significance Fisher test was used. Averages for the other data were calculated.

In 2006, the autumn was prolonged, warm and wet. Winter wheat tillered out before winter very well. December and January were mild and wet. The vegetation of winter wheat continued until the middle of January. The weather conditions for winter wheat over wintering were favourable until the last ten-day period of January. At the end of January and in February severe frosts prevailed. In 2007, the spring was early. Warm and dry weather prevailed in March and April. In 2007, rainy and moderately warm weather conditions prevailed during the entire growing season. The weather conditions in 2007 were conducive to the spread and development of fungal diseases in cereals.

Results and Discussion

Assessment of Biojodis phytotoxicity for winter wheat plants. Laboratory analyses suggest that the biological agent Biojodis had no phytotoxic effect on winter wheat seed viability and seedlings. The seed germinating power of the Biojodis-treated winter wheat seed was slightly better than that of untreated seed; however, the difference was not significant. Having treated the seed with a mixture of chemical seed treater Maxim star and Biojodis, the retardant effect of the chemical seed treater on seedlings was lower compared with that of applied separately. The Biojodis had no significant effect on seed germination power and germination of winter wheat and spring barley, although other scientists have indicated that using this agent at a concentration of 2 l t^{-1} the wheat seed germination increased in comparison with the control treatment /Sliesaravicius et al., 2006/. Field emergence of the treated and untreated seed was similar (Table 1). The spring barley seedlings that emerged from Biojodis 2 l t^{-1} treated seed were slightly shorter than those in the control treatment.

Table 1. The effect of seed treatment with Biojodis on winter wheat and spring barley seed germinating power, germination and seedling development

Application	Germination power %	Germination %	Field germination %	Leaflet length cm	Rootlet length cm
		Winter wheat			
Untreated	84.5	95.2	88.2	25.6	24.8
Biojodis 2 l t^{-1}	87.5	95.5	88.8	25.6	23.4
Biojodis 3 l t^{-1}	88.2	95.2	86.0	24.8	22.3
Maxim star + Biojodis 1 + 2 l t^{-1}	86.0	95.0	83.0	22.9	25.2
Maxim star 1 l t^{-1}	85.2	92.2	87.8	21.1	27.3
LSD_{05}	6.14	3.48	8.11	1.72	2.81
		Spring barley			
Untreated	76.2	78.0	69.7	27.9	16.2
Biojodis l t^{-1}	74.7	77.5	70.0	25.1	16.3
Biojodis 2 l t^{-1}	74.2	74.5	69.0	26.1	15.6
Kinto 2 l t^{-1}	78.0	79.7	68.5	27.9	15.4
LSD_{05}	7.63	5.86	5.93	2.80	2.65

The effect of Biojodis on the winter wheat and spring barley seed contamination with pathogenic fungi. Analysis of seed contamination with fungi was done by sowing the seed treated with the test products on PDA as well as by the method of wet blotter rolls. On PDA winter wheat seed treated with Biojodis was heavily infested with *Alternaria* spp., *Penicillium* spp. and other fungi (Table 2). The seed treated with Biojodis was less contaminated with the fungi of *Penicillium* genus compared with untreated seed. The seed treated with Biojodis mixture with Maxim star and with only Maxim star was markedly less infested with fungi. Biojodis strengthened the effect of the chemical seed treater Maxim star against *Alternaria* spp. infection. The effect of Biojodis on the fungi of *Fusarium* genus on winter wheat seed was controversial. On PDA the content of these fungi in Biojodis treated grain was identified to be even higher than that on untreated grain, however, in the rolls of wet blotter both Biojodis and chemical seed treater gave a good control of *Fusarium* fungi. Having such controversial results, we can assume that both Biojodis and chemical seed treater are effective against only some of the fungi of *Fusarium* genera, the conditions for which were more favourable in the wet blotter rolls. A similar conclusion has been made by Sliesaravicius et al. suggesting that in the laboratory conditions Biojodis protected the germinating wheat spouts from diseases, because seed infection level was reduced by using this agent for seed treatment prior to sowing (2006).

Table 2. The effect of seed treatment with Biojodis on winter wheat seed infection

Application	Seed affected by fungi %					
	Fusarium spp.		*Alternaria* spp.		*Penicillium* spp.	Other
	PDA	Blotter	PDA	Blotter	PDA	PDA
Untreated	2.0	4.0	41.5	62.0	55.5	19.5
Biojodis 2 l t^{-1}	7.0	0	46.0	47.5	22.5	42.0
Biojodis 3 l t^{-1}	6.0	1.5	60.5	54.0	13.0	33.5
Maxim star + Biojodis 1 + 2 l t^{-1}	2.5	0	9.5	12.5	9.5	69.5
Maxim star 1 l t^{-1}	3.0	0	20.5	19.0	8.5	66.5

The spring barley seed used in the study was heavily infected with *Fusarium* spp. fungi. On PDA the seed treated both with Biojodis and chemical seed treater had a similar infection level of the *Fusarium* fungi compared with untreated seed (Table 3). However, in wet blotter rolls both Biojodis and Kinto effectively controlled *Fusarium* spp on seed. We can make the same assumption as that made when discussing winter wheat test results that Biojodis and Kinto were effective not against all *Fusarium* genus fungi, but gave an effective control of those for which conducive development conditions were created when seedlings were grown in wet blotter rolls. The infection with *Dreschlera* spp. propagules on barley seed was weak and the effect of Biojodis on these pathogens was one-to-many. The content of *Penicillium* genus fungi was slightly lower when a higher rate of Biojodis was used.

Table 3. The effect of seed treatment with Biojodis on spring barley seed infection

Application	Seed affected by fungi %			
	Fusarium spp.		*Dreschlera* spp.	*Penicillium* spp.
	PDA	Blotter	PDA	PDA
Untreated	30.5	8.0	1.0	11.0
Biojodis 1 l t^{-1}	30.0	0	0.5	12.0
Biojodis 2 l t^{-1}	35.0	2.0	2.0	8.0
Kinto 2 l t^{-1}	39.5	2.0	0.5	8.0

The effect of Biojodis on seed-borne root rots of winter wheat and spring barley plants. The seedlings of winter wheat and spring barley were heavily infested with root rots caused by seed-borne pathogens. By blotter roll method it was determined that Biojodis was highly effective against seed-borne root rots of seedlings both in winter wheat and spring barley (Table 4). However, under field conditions, in spring during plant tillering stage the plants that emerged from treated and untreated seed did not differ in the root rot infection level. It is likely that these products did not any longer protect wheat and barley plants against the pathogens present in the soil.

Table 4. The efficacy of winter wheat and spring barley seed treatment with Biojodis against root rots

Application	Seed-borne root and crown rot Blotter roll test		Root and crown rot Field test at tillering stage	
	Incidence %	Severity index %	Incidence %	Severity index %
		Winter wheat		
Untreated	40.6	23.9	71.2	23.7
Biojodis 2 l t^{-1}	12.5	4.5	68.8	23.1
Biojodis 3 l t^{-1}	12.3	4.6	67.5	22.5
Maxim star + Biojodis 1 + 2 l t^{-1}	1.0	0.34	76.2	25.4
Maxim star 1 l t^{-1}	1.0	0.52	81.8	29.4
LSD_{05}			19.72	5.68
		Spring barley		
Untreated	44.8	20.3	100	16.7
Biojodis 1 t^{-1}	28.5	10.0	98.3	33.3
Biojodis 2 l t^{-1}	21.7	8.5	98.3	43.3
Kinto 2 l t^{-1}	12.2	6.5	100	30.0

The effect of Biojodis on the productivity of winter wheat and spring barley. In the winter wheat plots sown with Biojodis 3.0 l t^{-1} treated seed and with Biojodis 2.0 l t^{-1} treated seed and additionally sprayed T-0 with 3.0 l ha^{-1} and T-1 with 1.5 l ha^{-1}, a significant grain yield increase was obtained (Table 5). In other plots, only a yield increasing trend was observed. An increase in grain yield of different winter wheat varieties has also been demonstrated with Biojodis by Sliesaravicius et al. (2006). 1000 grain weight (TGW) increased significantly in the plots sown with the seed treated with Biojodis 3.0 l t^{-1}, Biojodis mixture with the chemical seed treater Maxim star and only with Maxim star. However, in the plots sown with Biojodis treated seed and additionally sprayed three times during the growing season, a significant 1000 grain weight reduction was obtained. Spring barley was additionally treated with Biojodis three times (at tillering, booting, and heading stages); however, no significant effect on grain yield and 1000 grain weight was identified. The chemical seed treater Kinto did not have any effect on the mentioned parameters either.

The impact of Biojodis on the harvested grain infection with toxigenic fungi. The analyses of harvested winter wheat grain showed that protection agents reduced internal grain infection with *Fusarium* fungi (Table 6), therefore a significant reduction was estimated using the Biojodis for seed treatment; Biojodis for seed treatment and 3-time spraying during the growing season; for seed treatment using Maxim star together with Biojodis. The protection agents did not exert any effects on harvested grain infection with *Alternaria, Aspergillus, Penicillium* fungi. Therefore, the application with Biojodis had no effect on infestation with pathogenic *Fusarium, Alternaria*, *Aspergillus*, *Penicillium* fungi in the barley grain (Table 6).

Table 5. The effect of winter wheat and spring barley seed and plant treatment with Biojodis on the productivity of crops and 1000 grain weight (TGM)

Application	Yield t ha^{-1}	Yield increase t ha^{-1}	TGW g	TGW increase g
Winter wheat				
Untreated	8.2	–	42.74	–
Biojodis 2 l t^{-1}	8.14	0.06	42.57	−0.18
Biojodis 3 l t^{-1}	8.52	0.32	44.3	1.55
Biojodis 2 l t^{-1}; T-0; T-1	8.57	0.37	43.05	0.3
Biojodis 2 l t^{-1}; T-0; T-1; T-2	8.29	0.09	40.83	−1.91
Maxim star + Biojodis 1 + 2 l t^{-1}	8.45	0.25	44.15	1.41
Maxim star 1 l t^{-1}	8.32	0.12	44.13	1.39
LSD_{05}	0.303		1.016	
Spring barley				
Untreated	6.74	0	54.75	–
Biojodis 1 l t^{-1}; T-0; T-1; T-2	6.75	0.01	55.37	0.62
Biojodis 2 l t^{-1}; T-0; T-1; T-2	6.72	−0.02	55.29	0.54
Kinto 2 l t^{-1}	6.74	0	55.16	0.42
LSD_{05}	0.24		1.190	

Table 6. Internal winter wheat and spring barley grain infection with pathogenic fungi

Application	Grain fungal infection level %			
	Fusarium	*Alternaria*	*Aspergillus*	*Penicillium*
Winter wheat				
Untreated	50.0	100	0.0	1.0
Biojodis 2 l t^{-1}	35.3	100	0.0	2.3
Biojodis 3 l t^{-1}	37.7	97	1.0	3.0
Biojodis 2 l t^{-1}; T-0; T-1	37.7	97	1.0	4.3
Biojodis 2 l t^{-1}; T-0; T-1; T-2	34.3	97	2.3	0.0
Maxim star + Biojodis 1 + 2 l t^{-1}	32.3	100	0.0	3.3
Maxim star 1 l t^{-1}	39.0	100	2.3	3.3
LSD_{05}	15.23	3.35	n. s.*	6.24
Spring barley				
Untreated	6.0	100.0	0.8	0.0
Biojodis 1 l t^{-1}; T-0; T-1; T-2	10.0	100.0	0.8	0.8
Biojodis 2 l t^{-1}; T-0; T-1; T-2	11.8	100.0	0.0	0.0
Kinto 2 l t^{-1}	7.5	100.0	0.8	1.8
LSD_{05}	9.58	n. s.*	1.99	n. s.*

*n. s. – not significant

The detailed study showed that Biojodis reduced the winter wheat grain infection with *Fusarium sporotrichioides*, but only inappreciably affected *F. poae, F. avenaceum* and *F. culmorum,* and hardly had any impact on *F. langsethiae* and *F. tricinctum* (Table 7).

Table 7. Internal winter wheat grain infection with *Fusarium* fungi

Application	Grain infection with *Fusarium* species %						
	F. poae	*F. langs-ethiae*	*F. sporo-trichioides*	*F. ticinc-tum*	*F. ave-naceum*	*F. culmo-rum*	*Fusarium* spp.
Untreated	8.0	0.0	6.7	7.7	19.3	4.3	4.3
Biojodis 2 l t^{-1}	1.0	2.0	6.3	4.3	24.3	0.0	0.0
Biojodis 3 l t^{-1}	0.0	3.3	1.0	13.3	18.0	2.0	2.0
Biojodis 2 l t^{-1}; T-0; T-1	2.0	1.0	2.0	14.3	12.0	5.7	1.0
Biojodis 2 l t^{-1}; T-0; T-1; T-2	8.7	0.0	3.3	6.7	16.7	1.0	0.0
Maxim star + Biojodis 1 + 2 l t^{-1}	1.0	0.0	0.0	21.0	11.0	0.0	1.0
Maxim star 1 l t^{-1}	7.0	2.3	4.3	12.0	11.3	2.0	1.0
LSD_{05}	5.41	n. s.*	5.44	10.14	13.04	4.99	3.28

*n. s. – not significant

Conclusions

1. Biojodis effectively cleaned both winter wheat and spring barley seedlings from seed-borne root rots (blotter roll test), however, under field conditions it did no longer have any effect on plant infestation with root rots.

2. In the plots of winter wheat sown with Biojodis 3.0 l t^{-1} treated seed and Biojodis 2.0 l t^{-1} treated seed and at plant tillering stage additionally sprayed with 3.0 l ha^{-1}, and at booting stage with 1.5 l ha^{-1}, a significant grain yield increase was obtained. Biojodis did not have any effect on spring barley productivity.

3. Biojodis treatment reduced internal grain infection with *Fusarium* fungi in harvested winter wheat grain. Biojodis was found to reduce *Fusarium sporotrichioides*, but it only inappreciably affected *F. poae*, *F. avenaceum* and *F. culmorum*, and hardly had any impact on *F. langsethiae* and *F. tricinctum*.

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