Effect of biopreparations on seed germination and fungal contamination of winter wheat

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^{1,2} Faculty of Forestry and Ecology, Faculty of Agronomy, Aleksandras Stulginskis University, Studentų St. 11, Akademija, LT-53361 Kaunas District The aim of the study was to investigate the influence of different biopreparations on the germination energy, germination and fungal infection of ecological winter wheat seeds.

Germination energy and germination of winter wheat seeds were determined after 24, 480 and 960 h, while fungal infection after 24 and 960 h of their treatment. In the studies biopreparations Biokal 1 (10 l t⁻¹), Biokal 2 (10 l t⁻¹), Biojodis (2 l t⁻¹) and Penergetic-p for roots (100 ml t⁻¹) were used. It was found that the values of germination energy and germination of winter wheat treated with the biopreparations after 480 h increased by 4.45 and 0.62%, as compared to treated seeds after 24 h. After 960 h of treatment the value of germination energy, as compared to seeds treated after 480 h, decreased insignificantly, while the germination of seeds has reliably decreased by 0.84%, as compared to treated seeds after 24 h, the value of germination energy was by 3.45% higher, but no significant decrease in germination was ascertained.

Biojodis after 24 h of the treatment was the most reliable in reducing wheat infection with the fungi of *Fusarium*, *Alternaria*, while Biokal 1 with *Penicillium* genera. The studied biopreparations after 960 h were insufficiently effective or had no influence at all on the development and spreading of fungi.

The diseases of coleoptiles and roots of winter wheat after 24 h and 960 h were most reliably prevented by the biopreparation Biokal 2.

Key words: ecological farming, wheat, biopreparations, germination energy, germination, fungi

INTRODUCTION

Pre-sowing preparation is one of the most actual problems in ecological farming, as the use of synthetic preparations for seed treatment is strictly forbidden in ecological farming. They have to be substituted by biopreparations, the choice of which is rather limited (Council Regulation (EB) No. 834/2007, 2007; Council Regulation (EB) No. 889/2008, 2008).

To become an important part in plant protection, biological preparations have to be efficient and reliable as much as chemical protection (Harman, 1991). Despite an ever increasing

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abundance of studies in the field, knowledge on natural antifungal preparations is scarce and barely applied in practice. Studies in Lithuania have revealed that biopreparations used for plant spraying during the vegetative period may be successfully applied also for presowing treatment of cereal seeds (Jankauskienė, Survilienė, 2009; Sliesaravičius et al., 2006). Biopreparations not only increase germination energy and germination of seeds, but also may reduce fungal infection of certain genera on the seeds (Gaurilčikienė et al., 2008; Jankauskienė, Survilienė, 2009; Pekarskas et al., 2007).

Soil is the main source of fungal infection (Lugauskas et al., 2004). High yield losses are caused by the fungi of Fusarium genus, which through infected grains are transfered to seedlings and damage them (Knudsen et al., 1995). A frequent cause of decline of the germinating seeds is the fungi of Penicillium genus, producing toxic metabolites in grain, which destroy the embryo and the most sensitive to infections - seedlings. Saprotrophic fungi (Mucor spp., Penicillium spp., Cladosporium spp.) reduce the germination power, while fungi of the Alternaria, Cocliobolus, Nigrospora, Aspergillus and Rhizopus genera reduce germination of seeds (Lacey, Magan, 1991; Ruza et al., 2004).

Literature analysis has shown that there are very few data on the effect of biopreparations on the germination energy and fungal infection of seeds. Besides, there are insufficient comparative studies on seed treatment against pathogens using different biopreparations, revealing their advantages and drawbacks.

The aim of the study was to investigate the influence of different biopreparations on the germination energy, germination and fungal infection of ecological winter wheat seeds.

MATERIALS AND METHODS

Studies on the influence of biopreparations on winter wheat 'Širvinta 1' seeds were conducted in the Laboratory of Biology and Plant Biotechnology Institute of the Faculty of Agronomy and at the Centre of Agroecology of Aleksandras Stulginskis University in 2008. During the studies stored seeds of winter wheat (yield of 2007) were treated with the certified biopreparations Biokal 1 (10 l t⁻¹), Biokal 2 (10 l t⁻¹), Biojodis (2 l t⁻¹) and Penergetic-p for roots (100 ml t⁻¹). Their effect was compared to that of the standard chemical seed treater Maxim Star 025 FS (fludioxonil 18.75 g l⁻¹ and ciproconazole 6,25 g l⁻¹), at 1.0 l t⁻¹ dose. The seed was treated using a precision laboratory seed treatment instrument Hege 11.

Biokal 1 is a liquid biopreparation of natural origin, consisting of 57% herbs, 38% biohumus extracts and 5% etheric oils, healing water, as well as microelements, mineral and biologically active substances of natural origin. The extract of herbs consists of the great nettle (Urtica dioica L.), common horsetail (Equisetum arvense L.) and tetterwort (Chelidonium majus). Biokal 2 is a liquid biological preparation of natural origin, consisting of 45% herbs, 40% biohumus extract, 10% wood ash extract and 5% etheric oils. It also includes microelements and mineral substances of natural origin. Biojodis consists of a watery biohumus extract, a biotransformator and a biologically active iodine solution. Penergetic-p for roots is a growth stimulator produced from minerals and molasses of natural origin.

Germination energy and germination of winter wheat seeds was determined after 24, 480 and 960 h of the treatment of seeds, while fungal infection after 24 and 960 h. The treated seeds were kept in paper bags at 19 ± 1 °C temperature.

The winter wheat was germinated in Petri dishes with 25 seeds, with eight replications, on a filter paper in a thermostat at 20 ± 2 °C. Germination energy was determined after 3, germination after 7 days.

For the seed health test, 200 untreated and treated seeds from each variant, without surface sterilization, were incubated on Potato Dextrose Agar (PDA). The grains were kept in a thermostat at 26 ± 2 °C temperature. The infection level of seed was evaluated in % (0 – all seed healthy, 100% – all grain infected). The

raised colonies of fungi were calculated on the seventh day. Morphological characteristics of the fungi were studied using a light microscope and various descriptors (Satton et al., 2001; Leslie et al., 2006).

The method of blotter rolls was used for the determination of seed-borne root rot infection of seedlings. Analysis of each variant was done with 4 replications of 50 seeds. The seeds were incubated in wet blotter rolls in a thermostat for 7 days at 20–22 °C temperature and for 10–13 days in light at 15–18 °C temperature. Root and crown rot incidence on seedlings was estimated in points (0 points – healthy, 1 point – weakly, 2 points – moderately, 3 points – heavily affected) (Tinline et al., 1975) and the disease severity index (%) was calculated (Dabkevičius, Gaurilčikienė, 2002).

ANOVA was applied for the statistical processing of data (Tarakanovas, Raudonius, 2003).

RESULTS AND DISCUSSION

It was found that after 24 h of the treatment of ecological winter wheat seeds with biopreparations, their germination energy has increased (Table 1).

Having kept the treated seeds for 480 h, the germination energy increased essentially by 4.45%, as compared to the treated seeds after 24 h. Assessing the changes of germination energy after 960 h of treatment, in comparison to treated seeds after 480 h, germination energy decreased by 1.0%, but this reduction was not essential as compared to treated seeds

after 24 h, when the value of germination energy was by 3.45% higher. Having assessed the influence of different biopreparations on the germination energy of seeds, no essential differences were ascertained. Under the influence of chemical agent Maxim Star, as compared to that of biopreparations, the value of germination energy of winter wheat has decreased, but not essentially.

After 24 h of treatment of the ecological winter wheat seeds by biopreparations, the germination of seeds also augmented (Table 2). Seed germination was induced mostly by biopreparations Biokal 1 and Biokal 2, while the influence of Maxim Star has reduced seed germination in comparison to the untreated seeds. After 480 h the value of germination of the treated seeds increased by 0.62%, as compared to that of the treated seeds after 24 h. Assessing seed germination after 960 h, its value in comparison to that of the seeds treated after 480 h decreased by 0.84%, while in comparison to the treated seeds after 24 h, the value of germination was by 0.22% lower, but no significant decrease was ascertained. Comparing the influence of biopreparations on seed germination of ecological winter wheat among themselves and with untreated seeds, no essential differences in seed germination were ascertained, but comparing with Maxim Star, treatment of seeds with Biokal 1, Biojodis and Penergetic-p for roots resulted in by 0.67% higher germination of seeds.

The influence of biopreparations on the germination energy and germination of agricultural crops has not been widely studied.

Transferrent (footon A)		Time after seeds treatment (factor B)							
Treatment (facto	Treatment (factor A)		after 480 h	after 960 h	means for factor A				
Untreated	_	94.00	99.67	99.00	97.56				
Maxim star	$1.0 l t^{-1}$	95.33	99.67	98.00	97.67				
Biokal 1	$10 l t^{-1}$	95.33	100	98.33	97.89				
Biokal 2	$10 l t^{-1}$	95.33	100	99.00	98.11				
Penergetic-p for roots	100 ml t ⁻¹	95.33	99.67	98.33	97.78				
Biojodis	$2 l t^{-1}$	96.67	99.67	100	98.78				
Means for facto	or B	95.33	99.78	98.78					
LSD ₀₅ : A – 1.503, B – 1.063, AB – 2.604									

Table 1. The effect of biological preparations on winter wheat laboratory germination energy, %

Treatment (factor A)		Time after seeds treatment (factor B)							
Treatment (facto	or A)	after 24 h	after 480 h	after 960 h	means for factor A				
Untreated	_	99.00	99.67	99.00	99.22				
Maxim star	$1.0 l t^{-1}$	98.67	100	98.00	98.89				
Biokal 1	$10 l t^{-1}$	99.67	100	98.33	99.33				
Biokal 2	$10 l t^{-1}$	99.67	100	99.00	99.56				
Penergetic-p for roots	100 ml t ⁻¹	99.33	99.67	99.67	99.56				
Biojodis	2 l t ⁻¹	99.00	99.67	100	99.56				
Means for facto	or B	99.22	99.84	99.00					
LSD ₀₅ : A – 0.654, B – 0.463, AB – 1.133									

Table 2. The effect of biological preparations on winter wheat laboratory germination, %

Studies conducted in 2008 with the seeds of grain crops have shown that after 24 h of the treatment of winter barley seeds with Biojodis germination energy and germination of seeds have essentially increased, while application of other biopreparations revealed only a tendency to increase germination energy and germination of seeds. Similar results were obtained also in the studies with winter rye seeds. In all studies the lowest values of germination energy and germination were obtained for seeds treated with chemical preparations (Pekarskas et al., 2007). Biojodis was efficient also soaking different vegetable seeds in its solution (Jankauskienė, Survilienė, 2009).

In the laboratory experiment the seeds of winter wheat were infected with the fungi of *Fusarium Alternaria* and *Penicillium* genera, which respectively comprised 5.5, 40.5 and 17.5% of all detected fungi (Table 3). The representatives of other genera comprised 56.0%. They belonged to *Aspergillus, Botrytis, Cla*-

dosporium, Dreschlera, Stemphylium, Mucor and Mycelia sterilia genera.

After 24 h of winter wheat treatment with different preparations, it was found that among the studied preparations the most efficient and reliable against fungal infection of winter wheat seeds was the chemical preparation Maxim Star, its biological efficiency against *Fusarium* genus comprised 100%, against *Alternaria* 87.7% and against *Penicillium* 34.3%.

Fusarium genus is widely distributed in the soil, its representatives – necrotrophs are plant pathogens, most of which cause a series of plant diseases (Webster et al., 2008). Among the studied preparations, Biojodis was the most efficient in reducing the amount of *Fusarium* genus fungi on seeds, its biological efficiency comprised 81.8%. Under the effect of Biokal 1, the amount of fungi from the genus *Fusarium* decreased by 1.5%, biological efficiency comprised 30.9%. Biokal 1 has an active

Table 3. The effect of biological preparations on winter wheat seed infection, %

Treatment		After 24 h									
			Seeds affected by fungi %								
		Fusarium spp.		Alternaria spp.		Penicillium spp.		Other fungi			
		1	2	1	2	1	2	1	2		
Untreated	_	5.5	_	40.5	_	17.5	_	56.0	-		
Maxim star	$1.0 l t^{-1}$	0*	100	5.0*	87.7	11.5*	34.3	20.0*	64.3		
Biokal 1	$10 l t^{-1}$	4.0	27.2	38.0	6.2	9.5*	45.7	67.0	0		
Biokal 2	$10 l t^{-1}$	8.0	0	40.5	0	17.0	2.9	52.0	7.1		
Penergetic-p for roots	$100 \text{ ml } t^{-1}$	6.5	0	41.5	0	11.0*	37.1	54.5	2.7		
Biojodis	$2 l t^{-1}$	1.0*	81.8	28.0*	30.9	18.5	0	65.0	0		
LSD ₀₅		2.82		8.76		5.62		11.7			

1 - affected seeds, %; 2 - biological efficacy, %; *- significant at the 0.05 probability level

ingredient – *Chelidonium majus*, which hinders the spreading of *Fusarium culmorum*, *F. graminearum* and *F. oxysporum* fungi and has an antibacterial, antivirus and fungicidal effect (Pârvu et al., 2008; Saglam, Arar, 2003).

The fungi of Alternaria genus were the most abundantly widespread on the grain of winter wheat. Under the impact of Biojodis the number of fungi of the genus was reduced by 12.5%, while the efficiency of the preparation comprised 30.9%. Studies have shown that the development of the fungi of Alternaria genus is prevented by plants having pesticidic properties (Pretorius et al., 2002; El-Assiuty et al., 2006; Fawzi et al., 2009), however, in the carried out study the effect of Biokal 1 on the fungi of Alternaria genus was quite insignificant, the infection of seeds by fungi of the genus decreased only by 1.5%. Biokal 2 and Penergetic-p for roots had no effect preventing infection of seeds with the fungi of Alternaria genus. The most efficient reducing infection of wheat with the fungi of Penicillium genus among the studied biopreparations was Biokal 1, as infection with fungi of the genus in comparison to the control decreased by 8.0%, while the highest biological efficiency comprised 45.7%. The seed of winter wheat in the laboratory was reliably prevented also by Penergetic-p for roots, as in treated with it wheat the amount of Penicillium genus fungi decreased by 6.5%. The efficiency of Maxim Star against wheat seed fungi comprised 34.3%, however, it was lower than that

of biopreparations Biokal 1 and Penergetic-p for roots.

The efficiency of biopreparations against other detected seed fungi in the laboratory was not revealed.

Efficiency of biopreparations against disease causal agents after some time was studied with the treated grain additionally (Table 4). After 960 h, and compared with the study results obtained after 24 h, an increased infection of untreated seeds with the fungi of *Fusarium* (0.8%) and *Alternaria* (4.5%) genus was recorded, the fungi of *Penicillium* and other genera on the seeds of winter wheat were less widespread, respectively 7.5 and 12.8%.

Infection of winter wheat with the fungi of other genera (*Aspergillus*, *Botrytis*, *Cladosporium*, *Dreschlera*, *Stemphylium*, *Mucor*) after 960 h was reliably reduced under the influence of all studied biopreparations, as compared to the study after 24 h, this effect was not ascertained. The most efficient against fungal infection of wheat seeds was Penergetic-p for roots, which, as compared to the control, reduced infection of wheat by 13.2%, while its biological efficiency comprised 30.5%.

Reliable influence of biopreparations was ascertained also for separate genera of fungi. The most efficient among the studied biopreparations was Biokal 2, under the effect of which infection of seeds with the fungi of *Fusarium* and *Alternaria* decreased respectively by 1.3 and 7.0%. Biokal 2 consists of *Urtica dioica* L. extract. According to Hadizaseh et al.

Treatment		After 960 h									
		Seeds affected by fungi %									
		<i>Fusarium</i> spp. <i>Alternaria</i> spp.			Penicillium spp.		Other fungi				
		1	2	1	2	1	2	1	2		
Untreated	_	6.3	_	45.0	_	10.0	_	43.2	_		
Maxim star	$1.0 l t^{-1}$	0*	100	12.7*	71.8	2.0*	80.0	32.0*	25.9		
Biokal 1	$10 l t^{-1}$	7.5	0	42.0	6.7	12.0	0	41.0^{*}	5.1		
Biokal 2	$10 l t^{-1}$	5.0*	20.6	38.0*	15.6	14.5	0	35.5*	17.8		
Penergetic-p for roots	$100 \text{ ml } t^{-1}$	8.5	0	46.5	0	17.5	0	30.0*	30.5		
Biojodis	$2 l t^{-1}$	10.0	0	44.0	2.2	18.0	0	34.9*	19.2		
LSD ₀₅		0.91		3.19		1.00		1.71			

Table 4. The effect of biological preparations on winter wheat seed infection, %

1 - affected seeds, %; 2 - biological efficacy, %; *- significant at the 0.05 probability level

studies (2009), Urtica dioica L. efficiently hinders the spreading of Alternaria alternata. However, after 24 h of treatment this preparation had no effect on the spreading of the fungi of Alternaria genus. The efficiency of other studied biopreparations against the genus of *Fusarium* and Alternaria has not been revealed. The studied preparations failed to reduce the amount of fungi of *Penicillium* genus on wheat.

Growing plants in the laboratory by the method of blotter rolls, 36.0% of winter wheat coleoptiles and roots, and 39.0% of seeds were damaged by root rot (Table 5).

The most efficient to protect wheat coleoptiles and roots against fungi was Biokal 2, under the influence of which wheat coleoptiles and roots were damaged the least –17.0 and 10.0%, respectively, and this reduction in damage was statistically reliable. Biokal 2 had insignificant effect on the spreading of fungi on seeds.

In literature there are data pointing out that growth stimulators not only induce germination energy, but also help plants to acquire resistance against diseases (Halter et al., 2005). After the seed treatment with Penergetic-p for roots, it reliably protected the roots of winter wheat grown by this method against root rot fungi, and a reliable reduction by 26.0% of fungal infection was ascertained. Under the effect of this preparation, the amount of damage caused by disease agents on seedlings decreased by 15.0%, however, it had no essential impact on the fungal infection of seeds. All the studied preparations reliably reduced the intensity of root rot on the coleoptiles and roots of wheat. The most efficient among the studied preparations reducing disease intensity on the coleoptiles and roots of wheat was Biokal 2.

Seeds treated with Biojodis were the least damaged (26.0%) by fungi. Biojodis has reliably protected the roots (19.0%) and coleoptiles (27.0%) of wheat against fungi.

After 960 h period all the studied biopreparations reliably protected the coleoptiles of winter wheat (Table 6). Coleoptiles which sprouted from wheat grains treated with Biokal 2 and Penergetic-p for roots were the least damaged, respectively 30.0 and 25.5%. These preparations effectively protected the roots of wheat as well. As compared to the control, damages on the roots of seeds treated with Biokal 2 were reliably reduced by 18.0%, while treated with Penergetic-p for roots by 10.0%.

During the study it was found that after 24 h and 960 h both Biokal 2 and Penergetic-p for roots have essentially protected the coleoptiles and roots of wheat against fungi.

All the studied biopreparations reduced the intensity of spreading of root rot disease. The intensity of fungal infection of wheat seeds has essentially decreased after treatment with Biokal 1 (1.0%), that of roots with Biokal 1 (0.6%), Biokal 2 (1.2%) and Biojodis (0.8%).

The effect of biopreparations against damage of seeds by fungi has not been revealed.

	After 24 h							
Treatment		Coleoptiles		Ro	Seeds			
		1 2		1 2		1		
Untreated	_	36.0	1.14	36.0	1.12	39.0		
Maxim star	$1.0 \ l \ t^{-1}$	3.0*	0.01*	0*	0*	10.0*		
Biokal 1	$10 l t^{-1}$	30.0	0.82*	26.0	0.52*	34.0		
Biokal 2	$10 l t^{-1}$	17.0*	0.40*	10.0*	0.20*	32.0		
Penergetic-p for roots	100 ml t ⁻¹	21.0*	0.78*	10.0*	0.30*	31.0		
Biojodis	$2 l t^{-1}$	27.0*	0.74*	19.0*	0.38*	26.0		
LSD ₀₅		8.82	0.20	11.64	0.10	8.30		

Table 5. The efficacy of winter wheat seed treatment with biopreparations against root rots, %

1 - incidence, %; 2 - severity index, %; *- significant at the 0.05 probability level

Treatment		After 960 h							
		Coleoptiles		Ro	Seeds				
		1	2	1	2	1			
Untreated	_	44.0	1.50	30.0	1.30	25.0			
Maxim star	$1.0 l t^{-1}$	2.0*	0.01*	0*	0*	5.0*			
Biokal 1	$10lt^{-1}$	25.0*	0.50*	30.0	0.70*	24.0			
Biokal 2	$10lt^{-1}$	14.0*	0.60	12.0*	0.10*	18.0			
Penergetic-p for roots	$100 \text{ ml } t^{-1}$	18.5*	1.10	20.0*	0.90	22.0			
Biojodis	$2 l t^{-1}$	26.5*	0.90	29.0	0.50*	19.0			
LSD ₀₅		4.16	0.97	8.92	0.41	7.41			

Table 6. The efficacy of winter wheat seed treatment with biopreparations against root rots, %

1 - incidence, %; 2 - severity index, %; *- significant at the 0.05 probability level

CONCLUSIONS

1. The values of germination energy and germination of the winter wheat seeds treated with biopreparations after 480 h of treatment have essentially increased in comparison to the treated seeds after 24 h. After 960 h, the value of germination energy in comparison to the treated seeds after 480 h decreased insignificantly, while the germination of seeds decreased essentially in comparison to the treated seeds after 24 h, germination energy was significantly higher.

2. Biopreparations had no essential influence on the germination energy of seeds, while seed treatment with a synthetic preparation has reliably reduced seed germination, as compared to Biokal 1, Biojodis and Penergetic-p for roots.

3. Biojodis after 24 h of the treatment was the most efficient in reducing infection of wheat with the fungi of *Fusarium*, *Alternaria*, while Biokal 1 with *Penicillium* genera. The studied biopreparations after 960 h were insufficiently effective or had no influence at all on the development and distribution of fungi.

4. The diseases of coleoptiles and roots of winter wheat after 24 and 960 h were most efficiently prevented by Biokal 2.

Received 5 February 2015 Accepted 25 March 2015

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BIOLOGINIŲ PREPARATŲ POVEIKIS ŽIEMINIŲ KVIEČIŲ SĖKLOS DAIGUMUI IR UŽTERŠTUMUI MIKROSKOPINIAIS GRYBAIS

Santrauka

Straipsnyje nagrinėjama skirtingų biologinių preparatų Biokal 1 (10 l t⁻¹), Biokal 2 (10 l t⁻¹), biojodžio (2 l t⁻¹) ir Penergetic-p šaknims (100 ml t⁻¹) poveikis ekologiškos žieminių kviečių sėklos dygimo energijai ir daigumui bei užterštumui mikroskopiniais grybais. Žieminių kviečių sėklos dygimo energija ir daigumas nustatyti ją apdorojus biologiniais preparatais praėjus 24, 480 ir 960 val., o užterštumas mikroskopiniais grybais – po 24 ir 960 val. Nustatyta, kad praėjus 960 val. po apdorojimo, sėklos dygimo energija, palyginti su sėklos po 480 val. dygimo energija, sumažėjo nežymiai; sėklos daigumas sumažėjo 0,84 %, palyginti su sėklos, apdorotos po 24 val., jos dygimo energija buvo 3,45 kartus didesnė. Biojodis (2 l t⁻¹), praėjus 24 val. po kviečių sėklos apdorojimo, efektyviai sumažino kviečių taršą *Fusarium*, *Alternaria*, o Biokal 1 (10 l t⁻¹) – Penicillium genčių mikroskopiniais grybais. Tirti biologiniai preparatai po 960 val. kviečius apsaugojo nepakankamai arba neturėjo teigiamo poveikio stabdant mikroskopinių grybų plitimą. Efektyviausiai iš tirtų preparatų pašaknio ligų plitimą žieminių kviečių daigų koleoptilėse ir šaknyse po 24 ir 960 val. apribojo biologinis preparatas Biokal 2 (10 l t⁻¹).

Raktažodžiai: ekologinis ūkininkavimas, kviečiai, biologiniai preparatai, dygimo energija, daigumas, mikroskopiniai grybai