The effect of biojodis on winter wheat and spring barley organic seed germination and contamination with fungi

Juozas Pekarskas¹, Aurimas Krasauskas², Jolanta Sinkevičienė³,

Daiva Šileikienė^{4*}

^{1,4} Studentų str. 11, Akademija 53361, Kaunas district This study analyses the effect of biological product biojodis and biojodis with sodium benzoate, which was used as a means to stop fermentation in biological product on germination energy and germination of organic winter wheat and spring barley seeds and contamination with fungi. It was found that biojodis and biojodis with sodium benzoate essentially increased germination energy and germination of organic winter wheat seeds compared to untreated seeds. No essential differences were observed on germination energy and germination of seeds treated with both products. Treating spring barley seeds with biojodis resulted in essential increase in value of their germination and productivity compared with seeds treated with biojodis with sodium benzoate. Treating seeds with a rate 3 l t⁻¹ increased germination value compared to treatment with a rate of 2 l he-1. Treating seeds with both products at a rate of 3 l he⁻¹ resulted in an essential increase in germination compared to untreated seeds and seeds treated with chemical seed treater. Treating organic winter wheat and spring barley seeds with biojodis and biojodis with sodium benzoate essentially reduced seed contamination with fungi compared to untreated seeds; however, it was markedly higher than contamination of seeds treated with chemical seed treatment maxim star. Seed contamination with fungi was more efficiently reduced by biojodis than biojodis with sodium benzoate. Greater rates of biological products were more effective as smaller rates.

Key words: organic seeds, treatment, biojodis, germination energy, contamination with fungi

INTRODUCTION

Preparation of seeds for sowing is one of the most pressing problems in organic farming, this method of farming system prevents environment from using synthetic chemical seed treatments. It is allowed to use solely certified chemical products in organic farming, extractions from medicinal plants and other materials that do not contradict the requirements of EU regulations (Council Regulation (EB) Nr. 834/2007, 2007; Council Regulation (EB) Nr. 889/2008, 2008).

Sowing seeds without treating with permitted means, can result in decreasing germination as

seed borne pathogens reduce their germination. The most frequent reason for seeds during storage is fungi of Penicillium genus. This pathogen produces and emits toxic metabolites which destroy seeds gem and shoots. Great damage for seeds is caused by fungi of Fusarium. They are able to penetrate into seed tissues and cells and produce strong toxins, in most cases fungal hyphae pass on to the shoots through contaminated seeds and injure them. In regions of colder climate conditions grain yield quality is mostly affected by fungi emitted by Fusarium, while in tropic and subtropic areas most problems are caused by fungi of genera Aspergillus, Penicillium and Alternaria (Schmidt, 1991; Knudsen et al., 1995; Ellner, 1997).

^{2,3} Aleksandras Stulginskis University, Faculties of Agronomy and Forest and Ecology

^{*} Corresponding author. E-mail: daiva.sileikiene@asu.lt

Research carried out in Lithuania showed that fungi of *Alternaria*, *Fusarium* and *Penicillium* prevail in seeds of winter wheat and spring barley. Their dissemination mostly depends on meteorological conditions of a certain year and seed humidity. The most important factors are relative air humidity and temperature during the period of seed and grain maturity and harvesting (Lugauskas et al., 2004). Fungi are more likely to damage broken, torn seeds or those polluted with outside materials. Mycelium first penetrates inside the seeds through natural gaps or those made by external damage (Ominski et al., 1994).

When yield is harvested and ecological factors change, seed's microbiota is also altered. Number of field fungi are usually decreased and storage fungi start to prevail. Grain and seed quality depends on fluctuations of pathogenic and saprotrophic fungi during the period of storage. This fluctuation results from interrelationship of relative substrate humidity, temperature and relative air humidity (Lacey, Magan, 1991; Ruza et al., 2004).

Research was carried out at Aleksandras Stulginskis University. Tested effects on different biological products of crop seeds – biological iodine products biojodis, biokal 1, biokal 2, penergeticp for roots were found to be the most effective because they caused increased seed germination energy and germination and reduced contamination with fungi (Sliesaravičius et al., 2006; Pekarskas et al., 2007; Pekarskas, Sinkevičienė, 2011).

In organic farming, extractions of medicinal plants were also found to be effective to be treated with crop seeds. Research has shown that certain medicinal plants have fungicidal substances and slow down the development of fungi (Pretorius et al., 2002; Fawzi et al., 2009).

Iodine is an indispensible microelement for people and animals; it is a composite part of thyroid hormones. The lack of it can cause varied deficiencies. Iodine is widely used in medicine to treat different diseases as well as in agriculture raising farm animals and plants, its lack in plants can cause different alterations, plant productivity and yield quality drops (Underwood, Suttle, 2001; Schöne et al., 2006; Sliesaravičius et al., 2006; Andersson et al., 2007; Flachowsky, 2007; Kotsilkov et al., 2009; Schöne et al., 2009; Leiterer et al., 2009).

The aim of this study is to determine the effect of biological iodine products biojodis and biojodis with sodium benzoate on germination and vigour of organic winter wheat and spring barley seeds and their contamination with fungi.

MATERIALS AND METHODS

For the research organic winter wheat and spring barley seeds were treated with biojodis products in 2005 at the Centre of Agroecology and Laboratory of Microbiology, Biology and Plant Biotechnology Institute, Agronomy Faculty, ASU.

The research was performed with winter wheat 'Širvinta 1' and spring barley 'Ūla' and treated with chemical seed treatment maxim star (i. e. fludijoksonil 18.75 g l⁻¹ and ciprokonazol 6.25 g l⁻¹) at a dose of l.0 l t⁻¹ for winter wheat and 1.5 l t⁻¹ for spring barley and biological products biojodis (Table 1) and biojodis with sodium benzoate.

Table 1. Chemical composition and parameters of biological product biojodis

Elements	Measurement units	Quantity	
Organic materials	%	5-8	
N	%	0.85-1.5	
P	%	0.90-1.5	
K	%	0.82-1.5	
Water soluble humates	%	0.15-0.7	
CaO	%	0.40-2.0	
Mg O	%	0.25-2.0	
Fe	%	0.08-0.2	
Mn	%	0.002-0.05	
Cu	%	0.008-0.01	
Zn	%	0.002-0.01	
Со	%	0.0005-0.002	
Mo	%	0.0005-0.002	
В	%	0.008-0.02	
J	mg l ⁻¹	0.10-0.75	
Saccharose	mg l ⁻¹	0.25-10.0	
Bacterial microbiota	colonies in a gramme	$10^7 - 10^{10}$	
Pathogenic microbiota	colonies in a gramme	none	
рН		7.1-7.8	

Biojodis is a liquid biological product made on the basis of aqueous extract of biohumus, enriched with biologically active iodine, biotransformators, characterized by resistance to temperature fluctuations (from 0° to +35 °C), very soluble in water. Biologically active iodine acts as a kind of sterilizer, characterized by microbicidal qualities and for this reason it may partially replace fungicides. Biojodis is composed of three parts: aqueous extract of biohumus, biotransformator and biologically active iodine. Prior to application of biojodis, those three parts are put together in certain proportions into one tankage and kept for 24 hours. Then the mixture is sprayed on plants or seeds are treated with it manually.

It is rather complicated to prepare biojodis product in such a way, especially to farmers in conditions of production. Thus prepared product should be used up quickly because if it is kept for a longer period of time, its efficiency decreases. Russian scientists recommended using 1% solution of sodium benzoate to stop the fermentation processes in the preparation. Test samples of biojodis with sodium benzoate were prepared and these tests were performed on them.

Seeds of winter wheat and summer barley were germinated in Petri plates covered with filter paper in the darkness for 3–4 days at 8–12 °C, later at 20 °C. Germination energy (%) was determined after 3 days, while germination (%) was calculated after 7 days. To determine germination energy, four samples were prepared with 100 seeds each. Seeds were put on filter and covered with a glass plate. In the course of seed germination, filter paper was kept damp.

Medium of Sabouraud dextrose agar with chloramphenicol (0.5 g l^{-1}) which stops development of bacteria were used for fungi isolation. Contaminated media were incubated in thermostat at 26 \pm 2 °C. Growing colonies of fungi were calculated on the 3rd, 5th and 7th day of the development. To determine prevalence of fungi, the researchers used detection frequency indicator A, calculated according to formula:

$$A = \frac{B}{C} \cdot 100\%$$
, (Лугаускас, 1988)

where B – number seed samples where the fungi of the same species were found; C – total number of the samples studied. Seed contamination with fungi propagules cfu g^{-1} (cfu – colony forming units) was determined by a dilusion method. Tests were replicated five times.

Fungi were isolated and identified according to cultural and morphological properties by a light microscopy method. (Domsch, 1980; Leslie et al., 2006; Ramirez, 1982; Lugauskas et al., 2002).

Acquired data were analysed and LSD (least significant difference) were determined by ANOVA for EXCEL (Tarakanovas & Raudonius, 2003).

RESULTS AND DISCUSSION

Treating seeds of organic winter wheat with biojodis and biojodis with sodium benzoate resulted in increased germination energy compared to seeds untreated with any biological product and treated with chemical seed treatment. All tested biological products essentially increased values of germination energy compared to untreated seeds, while compared to seeds treated with chemical seed treatment, only biojodis rates of 2 and 3 l t-1 and biojodis and sodium benzoate rate of 3 l t⁻¹ resulted in an essential increase. No essential differences in germination energy between seeds treated with both products were detected. Chemical seed treatment decreased germination energy of winter wheat seeds compared to untreated ones, but the decrease was insignificant. Biojodis and biojodis with sodium benzoate actually increased germination energy both compared to untreated seeds and seeds treated with chemical seed treatment. No essential differences were observed between biojodis and biojodis with sodium benzoate in their effect on germination; however, there was a tendency for greater increase in the case of treatment with biojodis than biojodis with sodium benzoate at rates of 3 l t⁻¹ compared with 2 l t⁻¹ (Table 2).

Treating spring barley seeds with biojodis and biojodis with sodium benzoate increased seeds germination energy, however, different products and their rates had different effect. Seeds treated with biojodis with sodium benzoate at a rate of 2 l t⁻¹ had the same germination energy as untreated seeds. Treatment with biojodis resulted in a marked increase in germination energy compared to seeds treated with biojodis with sodium benzoate. Biojodis products also increased germination of seeds. Treating seeds with biojodis and biojodis

Table 2. The effect of products biojodis and biojodis with sodium benzoate on germination energy and germination of winter wheat 'Sirvinta 1' seeds

Treatment	Germination energy, %	Seed germi- nation, %
untreated seeds	91.75	94.50
maxim star 11 t ⁻¹	92.25	94.12
biojodis 2 l t ⁻¹	93.50	96.50
biojodis 3 l t ⁻¹	93.50	96.63
biojodis with sodium benzoate 2 l t ⁻¹	93.13	96.25
biojodis with sodium benzoate 3 l t ⁻¹	93.50	96.50
LSD ₀₅	1.18	1.15

with sodium benzoate at a rate of 3 l he⁻¹ resulted in an essential increase of seeds germination compared to untreated seeds and seeds treated with chemical seed treatment. Treating seeds with equal rates of both biojodis and biojodis products resulted in greater increase in germination in case of treatment with biojodis rather than biojodis with sodium benzoate (Table 3).

Table 3. The effect of products biojodis and biojodis with sodium benzoate on germination energy and germination of spring barley 'Ūla' seeds

Treatment	Germination energy, %	Seed germi- nation, %	
untreated seeds	88.0	90.8	
maxim star 1.5 1 t ⁻¹	86.0	90.0	
biojodis 2 l t ⁻¹	90.0	93.0	
biojodis 3 1 t ⁻¹	92.0	93.5	
biojodis with sodium benzoate 2 l t ⁻¹	88.0	91.0	
biojodis with sodium benzoate 3 l t ⁻¹	91.0	92.0	
LSD ₀₅	0.75	1.14	

Biological product biojodis was created by Ukrainian scientists. After this preparation had been certified to be used on farms of organic production in Lithuania, research showed that is was very efficient in organical growing of most agricultural plants. Its application not only increased productivity of agricultural plants and improved their quality, but also decreased their vulnerability to disease (Sliesaravičius et al., 2006; Gaurilčikienė, 2008; Pekarskas, 2008a, b). Rese-

arch on treating organic winter rye, barley, wheat and spring barley seeds has shown that biojodis increased germination energy and germination and reduced their vulnerability to fungi (Pekarskas et al., 2009; Pekarskas, Sinkevičienė, 2011).

The seeds treated with Biojodis 3 l t-1 and with chemical seed treatment maxim star l l t-1 were markedly less contaminated with fungi. However, these products were not effective against Aspergillus flavus. Reddy (1986) found that ultraviolet rays and some chemical mutagens treatment to A. flavus gave fungicides resistant mutants. Treating organic winter wheat seeds with both biojodis products essentially decreased seeds contamination with fungi compared with untreated seeds, however, contamination was markedly greater than in case of treating seeds with chemical seed treatment. Seeds contamination with fungi was more efficiently reduced by treating seeds with biojodis than biojodis with sodium benzoate. Greater rates of biological products were more efficient at reducing seeds contamination with fungi. Treating seeds with biojodis 3 l t⁻¹ essentially decreased seeds contamination with fungi compared to treatment with biojodis with sodium benzoate at a rate of 2 l t⁻¹ (Table 4).

Spring barley seeds were more contaminated with fungi than winter wheat seeds. Treating spring barley seeds with both biojodis products resulted in an essential reduction of seed contamination with fungi compared to untreated seeds, however, it remained markedly greater compared to seeds treated with chemical seed treatment maxim star. Seeds contamination with fungi were more successfully reduced by biojodis treatment than treatment with biojodis with sodium benzoate. Greater rates of biological products (3 1 t⁻¹) essentially decreased contamination of spring barley seeds compared with smaller 2 l t⁻¹ rate. Treating seeds with biojodis 2 l t-1 resulted in an essential reduction of contamination with fungi compared to treatment with biojodis with sodium benzoate at a rate of 2 l t-1, but no essential differences in numbers of fungi were detected between 3 l t⁻¹ rates (Table 5). The detailed study showed that Biojodis reduced the winter spring barley infection with fungi, but only insignificantly affected Fusarium equiseti (Table 5).

Table 4. The effect of product biojodis on number of fungi in winter wheat 'Širvinta 1' seeds

Treatment	Number of fungi colony forming units	Number of iso- lated fungi species	Dominating fungi species (%)
untreated seeds	6.3×10^{3}	6	Aspergillus flavus (56%) Fusarium semitectum (36%) Cladosporium herbarum (23%)
maxim star 1 l t ⁻¹	1.2×10^{3}	1	Aspergillus flavus (40%)
biojodis 2 l t ⁻¹	2.2×10^{3}	3	Aspergillus flavus (70%) Alternaria alternata (20%)
biojodis 3 l t ⁻¹	1.8×10^{3}	3	Aspergillus flavus (63%) Penicillium verrucosum (27%)
biojodis with sodium benzoate 21 t ⁻¹	3.0×10^{3}	3	Aspergillus flavus (40%) Penicillium verrucosum (40%)
biojodis with sodium benzoate $3 l t^{-1}$	2.2×10^3	4	Aspergillus flavus (36%) Penicillium verrucosum (27%) Fusarium semitectum (23%)
LSD ₀₅	0.89		

Table 5. The effect of biological product biojodis on number of fungi spring barley 'Ūla' seeds

Treatment	Number of fungi colony forming units	Number of isolated fungi species	Dominating fungi species (%)
untreated seeds	3.28×10^{4}	8	Fusarium poae Fusarium equiseti
maxim star 1.5 l t ⁻¹	0.56×10^4	5	Fusarium poae Fusarium culmorum Fusarium equiseti
biojodis 2 l t ⁻¹	1.88×10^4	7	Fusarium poae Fusarium sporotrichioides Fusarium culmorum
biojodis 3 l t ⁻¹	1.64×10^{4}	4	Fusarium poae Fusarium sporotrichioides Fusarium culmorum
biojodis with sodium benzoate $2 l t^{-1}$	2.52×10^4	6	Fusarium poae Fusarium equiseti Fusarium sporotrichioides
biojodis with sodium benzoate $3 l t^{-1}$	1.66×10^{4}	4	Fusarium poae Fusarium sporotrichioides Alternaria alternaria
LSD_{05}	0.23		

Usage of chemical seed treatments and biological products in compliance with requirements of EU Council regulations (EB) Nr. 834/2007, 2007 and (EB) Nr. 889/2008 in organic farming can be successful for seeds quality.

CONCLUSIONS

1. Biojodis and biojodis with sodium benzoate essentially increased germination energy and

germination of winter wheat seeds compared to untreated seeds. No differences in seeds germination energy and germination between biojodis and biojodis with sodium benzoate were found. Treating spring barley seeds with biojodis was more successful, results show essential increase in seeds germination energy and germination values compared to treatment with biojodis with sodium benzoate. Treatment with a rate of 3 l t⁻¹ resulted in essential increase in germination

energy value compared to treatment with a rate of 2 l t⁻¹ and in case of treatment with biojodis with sodium benzoate at a rate of 3 l he⁻¹, essential increase of germination values was observed compared with untreated seeds and seeds treated with chemical seed treatment. Treating seeds with equal rates, greater increase in germination was found in case of treatment with biojodis than biojodis with sodium benzoate.

2. Treating winter wheat and spring barley seeds with biojodis and biojodis with sodium benzoate resulted in reduced seeds contamination with fungi compared to untreated seeds, however, contamination with fungi remained markedly greater than in case of seed treatment with chemical seed treatment. Seed contamination with fungi was more successfully reduced by biojodis than biojodis with with sodium benzoate. Greater preparation rates were more efficient than smaller ones.

Received 04 January 2013 Accepted 21 August 2013

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Juozas Pekarskas, Aurimas Krasauskas, Jolanta Sinkevičienė, Daiva Šileikienė

BIOLOGINIO PREPARATO BIOJODŽIO POVEIKIS EKOLOGIŠKŲ KVIEČIŲ IR MIEŽIŲ SĖKLŲ DYGIMO ENERGIJAI, DAIGUMUI IR TARŠAI MIKROMICETAIS

Santrauka

Straipsnyje nagrinėjamas biologinių jodo preparatų biojodžio ir biojodžio su natrio benzoatu, kuris naudotas kaip rūgimo procesus biologiniame preparate stabdanti priemonė, poveikis ekologiškų žieminių kviečių ir vasarinių miežių sėklų dygimo energijai, daigumui ir sėklų taršai mikromicetais. Nustatyta, kad biojodis ir biojodis su natrio benzoatu ženkliai padidino žieminių kviečių sėklų dygimo energiją ir daigumą, palyginti su neapdorotomis sėklomis. Esminių žieminių kviečių sėklų dygimo energijos ir daigumo skirtumų tarp biojodžiu ir biojodžiu su natrio benzoatu apdorotų sėklų nenustatyta. Vasarinių miežių sėklų apdorojimas biojodžiu labai padidino dygimo energiją, palyginti su apdorojimu biojodžiu su natrio benzoatu. Dygimo energiją ženkliai padidino 3 l t⁻¹ preparato norma, palyginti su 2 l t⁻¹ norma, o apdorojus biojodžiu ir biojodžiu su natrio benzoatu 3 l ha-1 norma, labai padidėjo sėklų daigumas, palyginti su neapdorotomis ir sintetiniu beicu beicuotomis sėklomis. Sėklas apdorojus vienodomis normomis biojodis labiau didino sėklų daigumą nei biojodis su natrio benzoatu. Apdorojus ekologiškų žieminių kviečių ir vasrinių miežių sėklas biojodžiu bei biojodžiu su natrio benzoatu ženkliai sumažėjo sėklų tarša mikromicetais, palyginti su neapdorotomis sėklomis, bet ji buvo daug didesnė negu beicuotų sėklų. Sėklų taršą mikromicetais labiau mažino biojodis nei biojodis su natrio benzoatu. Didesnės biologinių preparatų normos buvo efektyvesnės už mažesnes.

Raktažodžiai: sėklos, apdorojimas, biojodis, daigumas, dygimo energija, tarša mikromicetais