AGRONOMSKI GLASNIK 6/2008.
ISSN 0002-1954

Original scientific paper
Izvorni znanstveni članak

EFFECT OF STONE MEAL ON CONTROL OF SEED-BORNE DISEASES IN WHEAT

UČINAK KAMENOG BRAŠNA U KONTROLI BOLESTI NA SJEMENU PŠENICE

Vesna Samobor, Dijana Horvat, B. Kesteli, M. Jošt

ABSTRACT

In organic seed production more than a half of seed lots are often discarded due to heavy infection with seed borne pathogens. To prevent this, various techniques are used: seed heat treatment, ultrasound treatment, brush cleaning and seed dressing with plant extracts or natural chemicals. In 2008, the efficiency of wheat seed treatment with the stone meal was tested and the results of preliminary investigation are presented. Two tests were performed using the official procedure for seed health testing.

Test 1: Heavily infected wheat seed was treated with the stone meal EKORAST (particle size <0.08 mm). Dry and wet treatment procedures were applied: both with 1, 2, 10 and 20%. Heavy application (10 and 20 kg per 100 kg of seed) significantly increased germination and energy of wheat seed, and it was effective against bacterial pathogens and some fungi, but only partially effective against heavy infection of *Fusarium* spp. However, the amounts of stone meal (10 and 20%) are practically not applicable, and adhesion of stone meal to the seed should be improved.

Test 2: Wheat cleaned seed of seven genotypes was wet treated with Ekorast (1 %) and chemical fungicide Vitavax 200FF (250 ml/100 kg). The untreated seed was used as control. There were no significant differences between the three variants in germination and energy. The stone meal Eko-rast was equal or better than Vitavax 200FF in seed protection against seed borne pathogens, and could be recommended for seed treatment in organic seed production. Search for an additive with better adhesion to seed is under way.

Keywords: wheat, seed borne diseases, seed treatment, stone meal

Talk given at 2nd Mediterranean Conference on Organic Agriculture – *Contribution to Sustainable Ecosystem*, Dubrovnik, April 2-4, 2008

SAŽETAK

Više od 50 % ekološki proizvedenog sjemena ne zadovoljava zdravstvene norme i mora se odbaciti zbog zaraze patogenim mikroorganizmima. Da bi se to spriječilo koriste se različite tehnike: tretiranje toplinom, ultrazvukom, četkanje i tretiranje biljnim ekstraktima (prirodnim kemikalijama). U 2008. godini učinak kamenog brašna ispitivan je u dva pokusa. Korištena je službena procedura za testiranje zdravstvenog stanja sjemena (NN. 4/2005 i 92/2006.).

Test 1. Jako inficirano zrno pšenice tretirano je kamenim brašnom 'Eko-rast'. Primijenjeno je suho i vlažno nanošenje, svako u koncentraciji od 1, 2, 10 i 20%. Kod jako zaraženog zrna, kameno brašno je značajno povećalo klijavost i energiju klijanja. Bilo je učinkovito protiv svih bakterijskih patogena i nekih patogenih gljivica. Djelomično učinkovito bilo je i kod jake infekcije *Fusarium* spp., ali količine apliciranog kamenog brašna (10 i 20%) nisu praktički primjenjive.

Test 2. Na sedam genotipova kvalitetno dorađenog sjemena primijenjeno je vlažno tretiranje kamenim brašnom 'Eko-rast' (1 %) i kemijskim preparatom 'Vitavax 200FF' (250 ml/100 kg). Kao kontrola služilo je netretirano sjeme. U klijavosti i energiji klijanja nije bilo značajne razlike između tri ispitivane varijante. U zaštiti sjemena od patogenih bakterija i gljivica kameno brašno bilo je jednako učinkovito ili bolje od Vitavaxa 200FF, pa se tretman sjemena kamenim brašnom može preporučiti u proizvodnji ekološkog sjemena žitarica. Traži se pogodan aditiv za bolje prijanjanje uz zrno.

Ključne riječi: pšenica, bolesti na sjemenu, tretiranje sjemena, kameno brašno

INTRODUCTION

In conventional practice seed of cereal crops is treated prophylactically with agrochemical products regardless of the health status of the seed, and the risks that various levels of infection might pose. There are sound biological reasons for doing this, since the pathogens involved are highly adapted seed-borne fungi.

In organic production, the removal of derogation allowing the use of non-organic seed from January 2004 means that a minimum of two seed generations cannot be treated with conventional agrochemical products (Pearce et al. 2005).

Due to seed infections with seed borne pathogens, about 50% of all organically produced seed lots are discarded each year. Huge differences occur between years (in some years up to 90% discards) and crop, which makes planning of seed production impossible (Borgen, 2002, 2005).

Besides agro-technical measures (crop rotation, sowing time, crop density, harvest time, and crop mixtures), cereal seed infections with seed borne pathogens before sowing depend upon specific resistance to major systemic pathogens. Some diseases (*Pyrenophora graminea*, *P. teres*, *Ustilago nuda* and *Fusarium* spp.) could be controlled by seed size and seed density separation during seed cleaning. Brush cleaning and water-free heat treatment to control a range of seed pathogens could be useful. The efficiency of brush cleaning (removes up to 99.8% of the spores) to prevent seed borne transmission is comparable with the best chemical treatments known (Borgen, 2005), while heat treatment of the seed embryo will always have a negative side-effect on seed vigour. Aerated steam treatment can successfully control cereal seed-borne diseases when these are situated close to the seed surface (Forsberg G. 2004). Equipment for hot steam combined with ultrasound has been developed. It eliminates common bunt (*Tiletia tritici*) in wheat after four seconds, and in spelt after eight seconds without germination vigour decrease (Borgen et Al, 2005). A full-scale 'System for Thermal Seed Treatment' was developed for seed industry (STIIM, 2004). Full control of common bunt by coating the seeds with milk powder could be achieved in doses which reduce germination vigour of the seed, while mustard flour can be recommended as seed treatment in organic seed (Borgen and Lars, 2001). Skimmed milk powder and wheat flour in a concentration of 160g per kg of seeds reduced common bunt infection levels by 96%, and 62%, respectively. In most cases, the effectiveness of the skimmed milk powder is equal to the chemical seed-treatment (El-Naimi, 2000). Milk powder, wheat, maize and rye flour, mustard flour (*Sinapis alba*) and quinoa flour (*Chenopodium quinoa*) as alternative to chemical treatment of organic wheat and rye seed were examined, and some of them were efficient (Borgen and Lars, 2001). Seed treatment with acetic acid is also effective without negative impact on germination (Borgen and Nielsen, 2001; Lammerts van

Bueren, 2003; Nielsen et al., 2000). In India wheat seed treatment with plant extracts of *Canabis sativa*, *Eucalyptus globulus*, *Thuja sinensis* and *Datura stramonium* was fully effective against common bunt caused by the fungus *Tilletia tritici* (syn. *T. caries*). However, the experiment with the same seed treatments had no or very limited effect with extracts from the same species grown in Denmark, which has climate conditions very different from India. These methods had a significant but insufficient effect on disease suppression (Borgen, 2004a, 2004b). Lime milk and traditional mixture of $CuSO_4 \cdot 5H_2O$ with lime milk significantly decrease the number of plants infected with *T. caries*. Furthermore, $KMnO_4$ may show some efficacy, if it is accepted for use in organic farming (Łukanowski, 2006).

All these substitutes for chemical treatments offer a more or less effective and environmentally safe solution for organic seed. However, our earlier results with bactericidal effect of stone meal indicate to examine stone meal as an environmentally safe alternative to chemical seed treatment.

MATERIALS AND METHODS

The stone meal Eko-rast (particle size <0.08 mm) was used as an alternative for chemical treatment of seed. It is registered as natural mineral fertilizer for organic agriculture (Austria Bio Garantie, Bio Inspecta Swiss and Alicon Bio Certificate, Germany). Its mineral composition is shown in Tab. 1.

In 2008, the efficiency of wheat seed treatment with the stone meal was examined in two tests.

Test 1: Heavily infected wheat seed of unknown origin was treated (dry and wet treatment) with 1, 2, 10 and 20 kg of stone meal Eko-rast per 100 kg of seed.

Tab. 1. Mineral composition of stone meal Eko-rast

Mineral	Percentage
SiO_2	56.9 %
CaO	8.8 %
MgO	5.9 %
K_2O	3.0 %
P_2O_5	0.8 %
Fe_2O_3	1.3 %
Al_2O_3	8.7 %

Mineral	Percentage
Na	0.8 %
Mn	54.2 mg/kg
Zn	21.2 mg/kg
Cu	35.3 mg/kg
Pb	2.2 mg/kg
Cd	0.3 mg/kg

Test 2: To avoid uneconomic high concentration of the stone meal used in the first test, an improved way of application (Hege 11 equipment) was developed, and seven different cultivars/lines (Koleda, Divana, J801-1, J801-2, J801-10, J802-12, J803-37) of clean wheat were wet treated with stone meal (1kg/100 kg of seed), or with fungicide Vitavax 200FF (TMTD+Carbaxin) at 250 ml per 100 kg of seed. The third control variant was untreated. Fifty seeds of each cultivar were germinated in Petri dishes on wet filter paper in four replications. The official procedure for seed health testing was used (NG 4/2005 and 92/2006): in a germination chamber with NUV lamp the seed was exposed to the temperature of 20 ^oC for 24 hours, with 12 hours light and 12 hours dark period. After that, the Petri dishes were removed to low temperature (-25 ^oC) for 24 hours and returned to the germination chamber at temperature of 20 ^oC for 14 days. Energy and germination, as well as determination of fungi (under stereo microscope) and bacteria (under light microscope) were performed. After statistic analyses the results are presented in the tables.

RESULTS

Test 1: Only high doses of stone meal application slightly increased energy and germination. (Tab. 2) The effect was better with wet than with dry application. Fungicidal effect on *Fusarium* spp. was pronounced but did not satisfy regulations (It should be less than 10 %). At all other pathogen fungi and bacteria, the effect was complete.

During treatment and sample manipulation it was noticed that adhesion of stone meal to seed was not well enough. This was the reason why only high doses (10 and 20 kg/100kg of seed) were effective. Such high doses are not practically acceptable, so we decided to repeat the test.

Tab. 2. The effect of seed treatment with stone meal EKO-RAST (Test 1)

Seed treatment		Germination %	Energy %	Infected seeds - %				
				Fusarium spp.	*Aspergilus* spp.	*Acremoniela atra*	*Bacterium* spp.	*Trichoderma harzianum*
Untreated		94.0a	94.0 a	28 a	0.5	5	10	3
1 %	Dry	94.0a	94.0 a	23 ab	0	0	1	0
	Wet	95.0ab	95.0 ab	23 ab	0	0	1	0.5
2 %	Dry	94.5ab	94.8 ab	24 ab	0	0	0	0.5
	Wet	96.5 b	96.8 b	22 bc	0	2	0	0
10 %	Dry	95.5ab	95.8 ab	18 bc	0	0	0	0
	Wet	98.0 c	98.0 c	20 bc	0	0	0	0
20 %	Dry	96.0 b	96.0 b	17 c	0	0	0	0
	Wet	98.0 c	98.0 c	18 bc	0	0	0	0

Means followed by the same letter in a column are not significantly different at P= 0.05

Test 2: There were no significant differences in energy (95.5–96.2 %) and germination (96.1–96.8 %) percentage between untreated (control) and treated (Vitavax and Eko-rast) variants.

In Croatia the most common and economically important seed borne pathogens are *Fusarium graminearum, Fusarium nivale, Alternaria alternata,* and together with *Bacterium* spp. the contamination of these pathogens is reported.

Contamination with *Alternaria alternata* was rather high 32-52 % (average 43.1), but was significantly reduced to 15.7 and 18.3 % respectively by both treatments - Vitavax 200FF and stone meal Eko-rast (Tab.3). The five new breed lines had 5-20 % lower infection than the two cultivars. (Cv. Divana is the parent cultivar of the five lines.) This could be connected with genotypic resistance of the lines, or it could be a side effect of selection to powdery

mildew (*Erysiphe graminis* D.C. f.sp. *tritici* E. Marchal) resistance, more precisely, the effect of the genes introduced from US wheat lines KS92WGRC21 and KS92WGRC22.

Tab. 3. The effect of seed treatment with stone meal Eko-rast (Test 2)

A) ***Alternaria alternata*** – The percentage of infected seeds

Cultivar/line	Seed treatment		
	Untreated	Vitavax 200FF	Stone meal*
Koleda	52 a	28	22
Divana	51 a	11	21
J 801-1	42 abc	13	15
J 801-2	42 abc	21	24
J 801-10	36 bc	10	20
J 802-12	32 c	15	7
J 803-37	47 ab	12	19
Average	**43.1 a**	**15.7 b**	**18.3 b**

B) ***Fusarium* spp.** – The percentage of infected seeds

Cultivar/line	Seed treatment		
	Untreated	Vitavax 200 FF	Stone meal*
Koleda	1	1	0
Divana	3	1	1
J 801-1	4	1.5	0
J 801-2	1	3	0
J 801-10	3	1	0
J 802-12	2	1	0
J 803-37	1.5	3	0
Average	**2.2 a**	**1.6 a**	**0.4 b**

C) ***Bacterium* spp.** – The percentage of infected seeds

Cultivar/line	Seed treatment		
	Untreated	Vitavax 200 FF	Stone meal*
Koleda	3	1	0
Divana	3	0	0
J 801-1	0	0	0
J 801-2	3	2	0
J 801-10	0	0	0
J 802-12	0	0	0
J 803-37	1	0	0
Average	**1.4 a**	**0.4 b**	**0 c**

Means followed by the same letter in a row are not significantly different at P= 0.05

As it was mentioned earlier (Borgen, 2005) proper seed cleaning reduces the number of infected seeds - discards of smaller, shrivelled and light seed. The good health status of cleaned untreated (control) variant of the seed samples could be seen in Tab. 3. Control variant with 1-4 % (average 2.2 %) infection with *Fusarium* spp. was far below the limit proposed by Croatian regulations. However, seed treatment was equally effective against *Fusarium* spp. The genotypic differences were small and not significant.

The seed contamination with *Bacteria* spp. was also rather low 1-3 % (average 1.4 %) and the repeated treatment with the stone meal, was very efficient. The genotypic differences in the level of infection are small and not significant.

CONCLUSION

The stone meal 'Eko-rast' was as the effective as chemical fungicide Vitavax 200FF in seed protection against seed borne pathogens. It could be

Untreated (control) *Vitavax 200FF* *Stone meal 'Eko-rast'*

Fig. 1 The seed infection with the seed borne pathogens after 14 days of germination

recommended as alternative in organic seed treatment at a dose of 1 % (1 kg per 100 kg of seed). However, further research is needed to obtain better adhesion of the stone meal to seed surface. Beside its fungicidal and bactericidal effects, the stone meal Eko-rast, has positive effect on the plant growth. The search for better adhesion and the examination of a stimulatory effect on the plant growth in field conditions is under way.

REFERENCES

1. Borgen, A. and K. Lars. 2001. Effect of seed treatment with milk powder and mustard flour in control of common bunt (*Tilletia tritici*) in wheat and stem smut (*Urocystis occulta*) in rye. In: Biddle, A.J. (Ed.) Proc. BCPC Symp. No. 76: 'Seed treatment: Challenges & opportunities'. British Crop Protection Council, Farnham.
2. Borgen, A. and B. Nielsen. 2001 Effect of seed treatment with acetic acid in control of seed borne diseases. In: Bidle, A.J. (Ed.) Proc. BCPC Symp. No. 76: 'Seed treatment: Challenges & opportunities'. British Crop Protection Council, Farnham.
3. Borgen, A. 2002. Control of seed borne diseases in organic cereals and pulses. 4th ISTA - PDC Seed Health Symp.: Healthy seeds, the basis for sustainable farming. Wageningen, Netherlands, 29th April-1th May, p.18.
4. Borgen, A. 2004a. Organic seed treatment to control common bunt (*Tilletia tritici*) in wheat. *Seed Testing International* 128:p.8-9.
5. Borgen, A. 2004b. Control of seed born diseases in organic seed propagation. In: Osborn T., van Bueren, Edith Lammerts and Ranganathan Radha (Fds.), Proc. 1st World Conf. on Org. Seed. Challenges and opportunities for organic agriculture and the seed industry. IFOAM, pp. 170-171.
6. Borgen, A. 2005. Removal of bunt spores from wheat seed lots by brush cleaning. Seed Info, ICARDA 29.
7. Borgen, A., Krebs N. and Langkjær C. 2005. Novel development of heat treatment techniques for seed surface sterilisation. 5th SHC Seed Health Symp., Angers France, 10th -13th May Abstrac Booklet, p.28.
8. El-Naimi, M, H.Toubia-Rahme and O.F. Mamluk. 2000. Organic seed-treatment as a substitute for chemical seed-treatment to control common bunt of wheat. European Journal of Plant Pathology, 106(5):433-437.
9. Forsberg, G. 2004. Control of cereal seed-borne diseases by hot humid air seed treatment. Doctoral thesis Swedish University of Agricultural Sciences Uppsala, p.49.

10. Lammerts van Bueren, E.T., P.C. Struik and E. Jacobsen. 2003. Organic propagation of seed and planting material: an overview of problems and challenges for research. NJAS 51-3:263-277.
11. Łukanowski, A. 2006. Alternative methods of seed treatment against common bunt of wheat Phytopathol. Pol. 41:79–82.
12. Nielsen, B.J., A.Borgen and L.Kristensen. 2000: Control of seed borne diseases in production of organic cereals. In: The Brighton Conference – Pest and Diseases 2000 (1). British CropProtection Council, Great Britain: 171–176.
13. Pearce, B., J. Thomas, F. Lyon, F. Harris, G. Davies 2005. Cereal varieties for organic production: developing a participatory approach to seed production and varietal selection. Project Summary.
14. STIIM 2004 Project: System for thermal seed treatment, an integrated approach to implementation and management in the EU seed industry. Project leaders: Ringstad Lage and Enfält Patrik.
15. Wolfe, M.S., S. Clarke, D. Gibbons, F. Harris, K. Hinchsliffe, H. Jones, F. Lyon, B. Pearce and J. Thomas. 2005. A participatory approach to variety trials for organic systems. A poster for EFRC events. http://www.efrc.com/manage/authincludes/article_uploads/EFRCbulletinartcle NIABOct2004.pdf

Authors' address – Adresa autora:

Vesna Samobor
e-mail: vsamobor@vguk.hr

Dijana Horvat
Križevci College of Agriculture
1, M. Demerca, 48260 Križevci, Croatia

Branko Kesteli
Agropan d.o.o.
44, Koprivnička, 10000 Zagreb

Marijan Jošt
JOST Seed-Research
21, S. Radića, 48260 Križevci, Croatia

Received - Primljeno:

October 17, 2008