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Biological protection of spring wheat from root rot in the forest-steppe zone of Eastern Siberia

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Abstract. We present the results of the trial of the biological drug BisolbiSan *(Bacillus subtillis strain H-13*, isolated by the All-Russian Research Institute of Agricultural Microbiology) for treatment of spring wheat seeds in comparison with the widely popular chemical fungicides Maxim and Maxim Plus in the forest-steppe zone of Eastern Siberia in 2016–2018. BisolbiSan contributed to a decrease in total seed contamination by 2.4 times compared to control, which was practically at the level of the chemical fungicide Maxim. Maxim and Maxim Plus oppressed the growth of the sprout and the main germ line, while BisolbiSan stimulated the growth and development of the root system, and did not inhibit the growth of the sprout. The prevalence of root rot in the variant with BisolbiSan was lower compared to control by 54 %, effectiveness of which was not significantly inferior to that of chemical protectants. In comparison with control variant, BisolbiSan increased vitreous content of grain by 16.9 %, the content of crude gluten by 3.9 %, contributed to obtaining a statistically reliable increase in the yield of 0.38 tons per hectare, which did not differ significantly from the increase in the variant with chemical protectants. In our experiment, the payback of 1 ruble of costs when treating seeds with BisolbiSan was 1.7, which is 0.5 and 0.2 rubles higher compared to Maxim and Maxim Plus, respectively. The profitability of the yield increase using BisolbiSan was 70.9 %, which is 54.5 % and 20.6 % more than when using Maxim and Maxim Plus, respectively.

1 Introduction

Modern farming methods have significantly increased yields, including through chemical control of plant pests. However, this has caused serious harm to human health and lead to a number of environmental problems, such as pollution of ground waters, soil, and a decrease in biodiversity [1-3]. Problems with the use of chemical pesticides have also affected the

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Irkutsk region, which is among the regions of Russia where the number of areas contaminated with pesticides is high (up to half of the surveyed areas).

The growing demand for stable and healthy diet requires effective control of pests and plant diseases through biological means, such as *Bacillus* [4], for example.

Bacteria from the *Bacillus* genus produce antagonistic compounds of different structure in relation to plants, pathogenic bacteria, fungi and viruses, as well as stimulate the systemic resistance of plants to pathogens [5].

Antagonistic bacteria of the *Bacillus* genus can control some plant diseases. Inparticular, the prospects of different strains of this bacterium for limiting the root rot of grains of various etiologies have been shown in [6-9]. In addition to the prospect of microbiological control of fungal pathogens of agricultural crops, the stimulating effect of the bacterium *Bacillus* and other groups of microorganisms on growth of shoots and roots of the plant, number of grains in the ear and the mass of 1000 grains, and increase in yield was reported in [10].

Root rot of various etiology is a harmful disease of cereals all over the world. The works of foreign researchers dedicated to studying the control of this disease most often declare fungi of genera *Rhizoctonia, Pythium, Fusarium* as pathogens of common root rot [11, 12]. In Eastern Siberia, this disease also causes serious damage to the spring wheat crop annually. Unlike in foreign studies, the main causative agents in the Irkutsk region of Russia are *Bipolaris sorokiniana (Sacc.) Shoemaker. Syn.:Helminthosporium sativum* Pammel, C.M. King et Bakke, *Helminthosporium sorokinianum Sacc., Drechslera sorokiniana (Sacc.)* Subram. Et P.C. Jain.; species of the genus *Fusarium* (*F. culmorum* (W.G.Sm.) Sacc. var. *culmorum, F. avenaceum* (Fr.) Sacc. var. *avenaceum, F. oxysporum* Schltdl. var. *oxysporum, F. graminearum* Schwab, etc.); species of the genus *Alternaria* (a complex of species *A. alternata* and others).

In Russia, the All-Russian Research Institute of Agricultural Microbiology created *Bacillus subtillis strain H-13* to fight a complex of plant diseases [13]. One of commercial names of the drug is BisolbiSan.

Despite many benefits of this biological method for the environment and human health, its application in agricultural production is still very limited. Therefore, the purpose of our research was to test the biological drug BisolbiSan for treatment of spring wheat seeds in comparison with some of the most sought-after chemical fungicides, Maxim and Maxim Plus, in the Irkutsk region, which is located in the forest-steppe zone of Eastern Siberia and has a large spread of this disease on spring wheat. The goals of the research were to determine its effectiveness in suppressing root rot on seeds, to establish its effect on prevalence of root rot in the field, yield, quality of spring wheat grains, and to calculate economic indicators.

2 Materials and methods

BisolbiSan is a contact fungicide (bactericide) and a seed and planting material treatment for fighting a complex of diseases based on *Bacillus subtillis strain H-13* and metabolites obtained during the cultivation of the strain. It is produced by Bisolbi Inter, LLC. Maxim is a contact fungicide treatment, with 25 g/l fludioxonil active substance. Maxim Plus is a contact-system fungicide treatment, with 25 and 25 g/l diphenoconazole and fludioxonil active substances. Maxim and Maxim plus are manufactured by Syngenta, LLC. Application rates of the drugs are: for BisolbiSan 1 l/t; for Maxim 2.0 l/t; and for Maxim Plus 1.5 l/t; in the control group, seeds were treated with distilled water. The working fluid volume was 10 l/t of seeds; seeds were treated 5 days prior to sowing.

The experiment was carried out in the forest-steppe zone of Eastern Siberia in an experimental crop rotation at the Irkutsk Scientific Research Institute of Agriculture test field in 2016–2018. We have used zoned agricultural engineering; the precursor was peas. The area of the experimental field is 70.0 m^2. The experiment was repeated 3times with the *Buryatskaya ostistaya* spring wheat variety without application of fertilizers.

Contamination of wheat seeds by diseases was determined according to"RussianStandard12044-93".The prevalence of root rot was assessed according to the methodology of the All-Russian Research Institute for Plant Protection.

Statistical processing of the results of the experiments was carried out using a variance method [14].

Grain vitreousness was determined using "Russian Standard10987-76", the quantity and quality of gluten – according to "Russian Standard 54478-2011", the weight of one liter of grain in grams –with "Russian Standard54895-2012."

3 Results and discussion

The seeds used in the experiment had a total contamination rate of 72.9 % and were infected with a complex of fungi: *Fusarium sp.* (49.3 %), less *p. Bipolaris sp.* (10.3 %), *Alternaria sp.* (9.3 %), *Penicillium sp.* (4.0 %). Quite often, seeds were contaminated with fungi of multiple genera at the same time (Table 1).

Table 1. Results of phytopathological analysis of spring wheat, average for 2016–2018

Variant	Healthy sprouts, %	Pathogens, %				Total contamination, %
		Alternaria sp.	*Bipolarissp.*	*Fusarium sp.*	*Penicillium sp.*	
Control	27.1	9.3	10.3	49.3	4.0	72.9
Maxim	75.1	0.3	0.3	17.3	7.0	24.9
Maxim Plus	92.0	3.0	1.0	2.7	1.3	8.0
BisolbiSan	70.0	6.0	2.0	15.7	6.3	30.0

Our research has revealed the high effectiveness of BisolbiSan, which contributed to obtaining 70 % of healthy sprouts. The effect of BisolbiSan on a complex of seed pathogens was close to that of the Maxim fungicide based on fludioxonil. The effectiveness results of BisolbiSan and Maxim against fungi *Fusarium sp.* were rather close, and species *Penicillium sp.* were not affected by these drugs. We also found out that MaximPlus was more effective against all fungi in the experiment, as total seed contamination compared to control decreased by 9 times. Research of other scientists also demonstrated higher efficiency of chemical fungicides, based on diphenoconazolein particular, compared to biological ones based on *Bacillus* [15], and against the leaf spotting caused by *Bipolaris sorokiniana,* which also causes root rot in our region, the *Bacillus subtillis strain TE3* showed some promising signs of biological control [16].

All the drugs studied contributed to the formation of more roots in wheat sprouts. However, Maxim and Maxim Plus compared to control especially oppressed the growth of sprouts, by 4.7 and 2.7 cm respectively, and slightly oppressed the growth of the main germ root – by 0.9 and 0.8 cm respectively. BisolbiSan stimulated the growth and development of the root system. The growth of the above-ground part of the plant was not significantly influenced either positively or negatively (Table 2).

Table 2. Biometric indicators of wheat sprouts, average for 2016–2018

Variant	The length of the sprout, cm	The length of the main root, cm	Average number of roots, units.
Control	15.2	11.5	4.1
Maxim	10.5	10.6	4.5
Maxim Plus	12.5	10.7	4.4
BisolbiSan	15.0	12.4	4.5

In the field, the prevalence of root rot in the variant treated by BisolbiSan was lower compared to control by 54 % and almost at the level of chemical treatments (Table 3).

Table 3. Root rot of spring wheat in the sprouting phase, average for 2016–2018

Variant	Prevalence, %	Average plant damage rate, %	Development, score
Control	42.9	1.4	0.67
Maxim	16.9	1.5	0.2
Maxim Plus	21.9	1.2	0.1
BisolbiSan	19.7	1.2	0.4
Least Significant Difference$_{05}$	19.5	–	–

The drugs studied in our experiment positively affected the quality of grain of spring wheat (Table 4). Compared to control, BisolbiSan has increased grain vitreousness by 10.0 % and raw gluten content by 2.3 %.

Table 4. Quality of spring wheat grains, average for 2016–2018

Variant	Mass of one liter of grain, g/l	Vitreousness, %	Raw gluten content, %	Gluten quality group	The nature of gluten
Control	733	59	32.6	I	Good
Maxim	719	69	37.7	I	Good
Maxim Plus	732	70	34.7	I	Good
BisolbiSan	728	69	34.9	I	Good

Pre-sowing treatment of seeds contributed to the increase in grain amount of plants, the productivity of grain crops, and as a result, to obtaining a steady increase in the harvest yield (Table 5). Application of BisolbiSan allowed to obtain statistically reliable increase in the yield compared to control.

The difference in yield increase when using all treatments in the experiment is statistically insignificant and is within the least significant difference.

Researchers note the economic benefit of seed treatment compared to control, where seeds are not treated with fungicides [17]. In our experiment, the treatment of seeds with the biological drug BisolbiSan is economically more beneficial due to low costs per hectare: the payback of 1 ruble of costs was 1.7, which is 0.5 and 0.2 rubles higher compared to Maxim and Maxim Plus, respectively. The profitability of the yield increase using BisolbiSan was 70.9 %, which is 54.5 % and 20.6 % more than when using Maxim and Maxim Plus, respectively.

Table 5. Harvest structure and spring wheat yield, average for 2016-2018

Experimental variant	Length of the ear, cm	Number of spikes in the ear, units	Number of grains in the ear, units	Weight of grains of 25 ears, g	Mass of 1000 grains, g	Number of plants during harvesting, units/m^2	Productive number of stalks, units	Yield, t/ha	Yield increase, t/ha
Control	6.1	10.9	23.1	20.5	34.5	432	1.02	2.19	-
Maxim	6.7	11.8	27.5	27.6	36.9	419	1.1	2.58	0.39
Maxim Plus	6.3	11.0	22.9	21.2	36.9	485	1.03	2.64	0.45
BisolbiSan	6.7	12.2	28.7	27.0	37.0	423	1.1	2.58	0.38
Least Significant Difference$_{05}$, yield	0.26								

4 Conclusion

Thus, to reduce the chemical pressure on agrocenosis of spring wheat and for production of organic products, BisolbiSan may well be acceptable as an alternative to chemical treatment. With 100 % seed contamination, it effectively reduces seed infection, providing 70 % of healthy sprouts, significantly limits the prevalence of root rot at the level of chemical fungicides efficiency, while positively affecting the growth and development of plants and allowing them to form a reliably high harvest of good quality grains, and increases the profitability of spring wheat products.

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