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Bioklad – an Effective Protectant of the Spring Wheat Seeds

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Abstract

Fractional extraction of the sum of lichens of *Cladonia* genus was carried out. The effect of presowing treatment of the spring wheat seeds with the preparation obtained from the ethanol extraction of lichens on the development of root rot agents was considered. Rather high efficiency in suppression of root rod was confirmed. The stimulating effect of the preparation on growth processes and increase in wheat productivity was revealed.

Key words: extraction, lichens of *Cladonia* genus, usnic acid, wheat, phytopathogens, pesticides

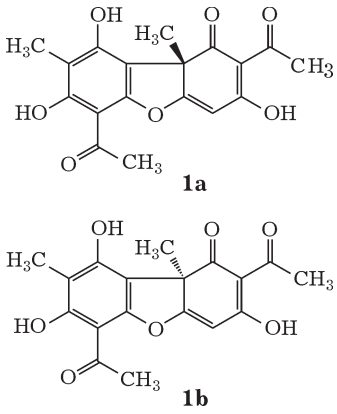
INTRODUCTION

Under conditions of West Siberia unfavourable for crop production (the spring-summer droughts alternate with over-moistening in the second half of the summer) a high level of fungal infections is marked almost every year. And a chemical method of protection arrangement system remains the main instrument for phytosanitary control [1]. However, the use of chemical pesticides leads to inevitable negative consequences such as bacterial diseases of crops, the emergence of resistant forms of phytopathogens, increased pesticide pressure resulting in significant changes of species composition of beneficial microorganisms, disturbance of biological equilibrium in agrocoenosises and general environmental deterioration [2, 3].

At present in view of necessity to use green variants of plant protection it is especially important to use new bioactive substances stimulating plant immunity, increasing their resistance to phytopathogens or unfavourable environmental conditions, and even acting as biofungicides reducing infection potential in crops. Given the fact that 75 % of fungal and 80 % of bacterial phytopathogens in Siberia pass through the seeds [4] the necessity of presowing treatment of seeds is self-evident.

The aim of our study was to investigate the effectiveness of new Bioklad preparation obtained from the bioactive compounds of extract of lichens of *Cladonia* genus for seed pretreatment in order to optimize the phytosanitary state of spring wheat crops, their resistance to diseases and for improving the grain productivity and quality. The use of *Cladonia* lichens extract as a natural fungicide is justified because the secondary metabolites of lichens, especially lichen acids [5] have a broad spectrum of fungicidal activity. The ability to suppress the development of various fungal colonies demonstrate first of all (+)-usnic acid (**1a**) and (−)-usnic acid (**1b**) – the main components of extracts from *Cladonia* genus lichens [6] (Scheme 1).

Various lichens of *Cladonia* genus contain both **1a** and **1b** compounds. Previously we obtained (−)-usnic acid **1b** $[\alpha]_D^{25}$ −450° (s 1.5, $CHCl_3$)) [7] from the air dry lichen *Cladonia stellaris*, and (+)-usnic acid **1a** ($[\alpha]_D^{25}$ +470° (s 1.6, $CHCl_3$)) from *Cladonia arbuscula* lichen. It is known that both optical isomers exhibit fungi-



Scheme 1.

cidal activity, but affect different types of fungal pathogens. [6] The use of ethanolic extract from a lichen mixture of *Cladonia* genus (*Cladonia stellaris, Cladonia rangiferina, Cladonia arbuscula, Cladonia uncialis*) for creating Bioklad agent allows to obtain a new natural wide spectrum pesticide.

RESULTS AND DISCUSSION

The investigated Bioklad agent was obtained from the extract of mixture of lichens (*Cladonia* genus). Extraction of raw materials was carried out by means of boiling with different solvents (hexane, ethyl acetate and ethyl alcohol) with successive increasing in their polarity. Analysis of the extracts by high performance liquid chromatography (HPLC) showed that the alcoholic extract highest by weight (1.7–1.8 % by weight of dry material), contains usnic acid **1** as a main component (peak 19 in the chromatogram, Fig. 1); its high fungicidal activity is noted in [6].

Bioklad preparation has been tested and registered in the State Register of Inventions of the Russian Federation in 2008 (Patent No. 2331194) in the list of agents against wheat diseases as a phytofungicide and plant growth regulator [8]. The chemical Raxil protectant (CS, 0.5 L/t) served as a reference for biological fungicide.

The improvement of seeds treated with the agents Bioklad and Raxil agents positively affected plant growth and development, increasing their resistance to unfavourable environmental conditions. This was revealed in the augmentation of planting density compared with the control by 20.7 and 19.8 %, respectively, at the 2–3 leaf stage and by 21.5 and 12.2 %, respectively, at the waxy ripeness stage. Viability of plants while using Bioklad also surpassed the chemical model by 6.3 %, and control one – by 2.0 % (Table 1).

Growth promoting effect of Bioklad is revealed in increasing of number of wheat stems. Thus, when it was applied a number of common and productive stems increased by 11.8 and 13.2 % respectively compared with control. If using Raxil these figures increased by 11.0 and 11.6 %, respectively.

Along with biometrics presowing treatment of seeds by Bioklad provided increase in accumulation of plants aboveground biomass at the flowering stage of wheat by 14.5 %, leaf area duration – by 24 %, and plants height by 23.9 % compared to the control (Table 2).

Investigations demonstrated that presowing seed treatment by Bioklad reduces the devel-

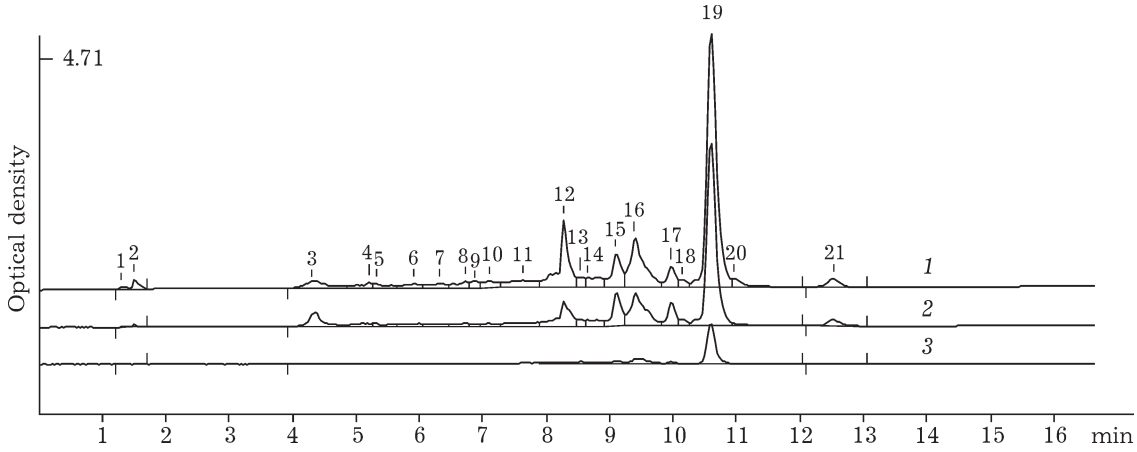


Fig. 1. HPLC chromatograms of alcoholic extract of *Cladonia* lichens, taken at a wavelength, nm: 230 (*1*), 280 (*2*), 360 (*3*).

TABLE 1

Effect of presowing treatment of wheat seeds with the agents on the growth processes (2003–2004)

Variants	Plant population, pcs/m^2		Survival,	Bushiness, pcs/plant.	
	2–3 leaf stage	Wax ripeness	%	Total	Productive
Control	420	385	89.4	1.27	1.21
Bioklad	507	468	91.4	1.42	1.37
Raxil	503	432	85.1	1.41	1.35

TABLE 2

Bioklad effect on the biometrics of wheat plants (2003–2004 years)

Variants	Biomass of plants, g/m^2		Leaf area duration, cm^2/plant	Plant height, cm
	Aboveground	Root		
Control	263.0	30.4	12.5	45.2
Bioklad	301.0	29.2	15.5	56.0

opment of root rot in spring wheat, increasing plant resistance to these pathogens. The degree of infestation of plants in the tillering phase being 4.3 %, biological efficiency of Bioklad is 53.5 %; at the milky stage the figures are 14.6 and 28.1 %, respectively. Raksil conceded in the tillering phase, when generative organs of wheat form (44.2%), but surpassed the test agent Bioklad at the milky stage of culture (37 %) (Fig. 2).

The improvement of the phytosanitary conditions of crops and stimulation of plant growth and development due to the seed treatment by biological agent Bioklad enhanced the increase in wheat productivity depending on the years by 0.4–3.2 hwt/ha (Fig. 3). Treatment with Raksil increased grain yield by 1.6–2.4 hwt/ha, depending on the year of research.

The observed positive effect of Bioklad agent led to the increase in thousand-kernel weight by 1–2 g (3.1–7.2 %), whereas the application of Raksil gave 0.3–1.9 g increase (0.9–6.8 %) (Fig. 4).

Thus, the use of Bioklad obtained from alcoholic extract of lichen of *Cladonia* genus for presowing treatment of the seeds results in essential improvement of the seeds and provides a significant increase in wheat yield and thousand kernel mass.

EXPERIMENTAL

High performance liquid chromatography (HPLC) was performed using a MiliChrom A-02 microcolumn chromatograph (EcoNova Co., Novosibirsk) with standard chromatography column (2 × 75 mm) filled with reversed phase sorbent (ProntoSIL 120-5-C18, with 5 μm particles, Bischoff, Germany). Gradient elution with simultaneous multiwavelength detection at

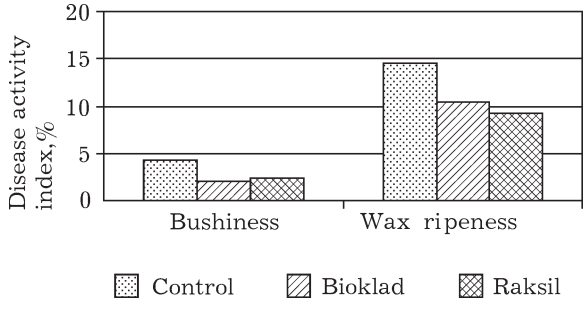


Fig. 2. Effect of presowing seed treatment by the agents on plants liability to root rots (2003–2004)

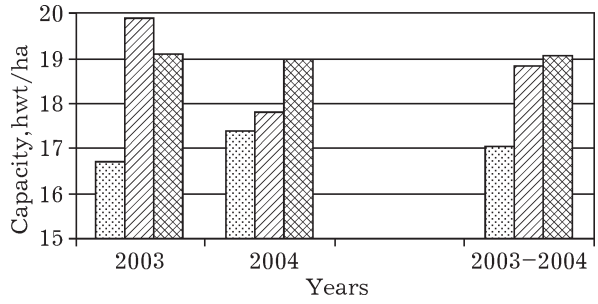


Fig. 3. Effect of presowing seed treatment by the agents on the yield of spring wheat. Design. see Fig. 2.

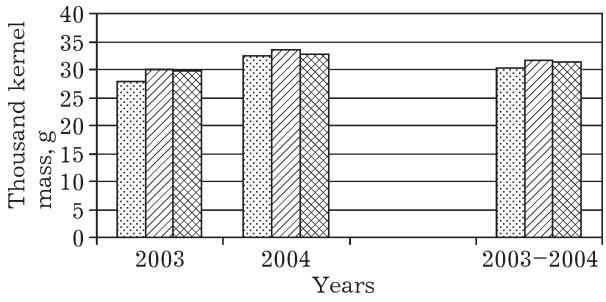


Fig. 4. Effect of presowing seed treatment by the agents on the thousand kernel mass. Design. see Fig. 2.

three wavelengths (230, 280, 360 nm) was used [9]. Methyl alcohol with 0.1% trifluoroacetic acid (TFA) was taken as eluent. Gradient was the following: 0–30 % methanol, 0.1 % TFA – 5 min, then 30–50 % methanol, 0.1% TFA – 5 min, then 50–70, 70 %, 0.1% TFA for 10 min, 70–90, 90 % , 0.1 % TFA for 10 min and 5 min to methanol. Temperature 35 °C, pressure 30–36 atm, the flow of 150 μL/min.

Bioklad was prepared by the following procedure. Air-dry mixture of lichens (292.6 g) of *Cladonia* genus (*Cladonia stellaris*, *Cladonia rangiferina*, *Cladonia arbuscula*, *Cladonia uncialis* gathered in the territory of the Republic of Altay) was crushed, loaded into a 2 L flask and extracted successively by 1.2 L of petroleum ether at 40–60 °C (three times for 4 h) and 1.2 L of ethyl acetate (three times for 4 h). The extracts obtained were removed and used for other purposes. Then the plant materials were extracted with 1.2 L of ethanol (three times for 5 h). Ethyl alcohol was removed and 7.5 g of the powder of light brown colour was obtained, the yield based on air-dry raw material was 1.73 %. Before use the powder it was ground into dust in a porcelain mortar.

Investigations were carried out in 2003–2004 years in the fields of the Siberian Research Institute of Farming and Agriculture Chemization in experimental production farm "Elitnoye" (Novosibirsk Region). Soil and environmental conditions were typical of the forest steppe zone of West Siberia. The agrotechnics of wheat spring growing ("Novosibirsk 15" released variety) was common for the region. Bioklad agent was used as disinfectant before sowing the wheat seeds (May 18 and 19, respectively according to the year of studies), consumption rate of 40 g/t in the powder form; the water-powder suspension was prepared before using. The chemical Raksil protectant (CS, 0.5 L/t) served as a model for biological fungicide. The rate of water consumption for each agent was 10 L/t of seed. The record plot area was 20.7 m^2, application was four replicated with regular arrangement. All calculations and observations during the experiment were carried out in accordance with current procedures and guidelines. The data were processed by variance analysis.

CONCLUSION

Plant protection ecologization, replacement of toxic chemical pesticides by less hazardous biological ones – is one of the most important tendencies of improvement of people's health, preservation of natural resources [3]. The Bioklad agent obtained from alcoholic extract of *Cladonia* lichens has a high efficiency. Its use provides a significant increase in plant population, the accumulation of aboveground biomass, the expansion of leaf area duration, plant height and bushiness, reducing infestation of root rot pathogens and, ultimately, increase the yield of grain without environmental hazard.

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