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BIUS – an Effective Agent for Increasing the Yield of Spring Wheat and Potatoes

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Abstract

A new efficient broad spectrum agent comparable in efficiency with chemical fungicides was developed on the basis of the aqueous solution of the salts of fir extract with bisamine, with the addition of the salt of 2,4-dichlorophenoxyacetic acid with bisamine and the *Usnea* lichen extract. The agent suppresses the development of fungal diseases and increases the yield of spring wheat and potato.

Key words: fir extract, bisamine, 2,4-dichlorophenoxyacetic acid, *Usnea* lichens, spring wheat, potato, phytopathogens, yield capacity

INTRODUCTION

Highly efficient grain production under present-day conditions is impossible without resolving the problem of plant diseases. The most dangerous diseases of wheat are brown rust, powdery mildew, Septoria spot, smut, root rot. Thus, the wheat yield losses caused by brown rust infection only are 12–40 % [1]. Crop shortfall from epiphytotics of root rot regularly observed from 1950s in affluent countries is 10–15 % annually, and sometimes is 50 % or more. Technological properties and sowing qualities of grain decrease as well [2]. Progressive diseases of winter and spring wheat in Russia are root rot and Septoria leaf spot taking 25 and 50 %, respectively in the structure of the pathogenic complex [3].

Loss of potato yield from Rhizoctonia root rot may vary from 5 to 45 % depending on growing conditions. In Novosibirsk region the value amounts up to 25–30 %. This is related with dying and growth retardation of plants during the growing season. Yield depression is also accompanied by a deterioration of food quality because of damage and changes in biochemical composition of potato tubers [4].

The use of modern biological preparations due to their low cost as well as prolonged and safe protective effect is one of the most important methods to increase the efficiency of grain cultivation, reduce chemical pressure on the plants and improve the ecological situation in agrocoenoses. In recent years both natural and synthetic organic compounds became of more and more great importance; in low doses they actively influence the metabolism of plants: stimulate their own immune system and thus allow the plants to induce a high level of resistance to pathogens and other adverse environmental factors [5].

The mechanism of action of these preparations is based on the elicitor effect of their components. After getting into the plants in certain amounts immune regulators cause increase in their immune status and provide a positive impact on growth activity, quality indices and pest infestation resistance [6].

In this connection the purpose of investigations was to create a new agent Bius based on extract of fir needles and to determine the effectiveness of its use for the treatment of seed potatoes and wheat grain, as well as vegetative winter wheat in order to improve phytosanitary condition of agrocoenoses in respect of diseases and to raise crop capacity and product quality.

RESULTS AND DISCUSSIONS

A newly developed agent BIUS is closest by composition to Biosil, Silk, Novosil preparations on the basis of triterpene acids from Siberian fir (*Abies sibirica*) needles. At the same time, BIUS is different from those preparations, because its triterpene acids of fir extract are transformed to water-soluble state as salts with bisamine. Biologically active lichen substances were used previously to create Bioklad preparation [7]. To prepare BIUS an extraction of air-dried fir greenery (needles and green branches) during methyl tertiary butyl ether boiling was used. Then the resulting extract was entered into reaction with N,N,N',N'-tetramethyldiaminomethane (bisamine); as a result, the acidic components of the fir extract were transformed into water-soluble amine salts. The salts of 2,4-dichlorophenoxyacetic acid (2,4-D) with bisamine and *Usnea* lichen extract were added to the obtained amine salt of fir extract.

Bisamine is a liquid with a boiling point of 85 °C; it is miscible with water in all proportions and little toxic, large-tonnage petrochemical product, easily synthesized from aqueous solutions of formaldehyde and diethyl amine. It is known to be used as a plant growth promoter [8]. Bisamine salts with carboxylic acids (formic and oxalic) demonstrate fungicidal properties [9]. Reaction of the fir extract with bisamine results in amine salts of triterpene acids, thus increasing their solubility in water, improving their fungicidal properties and bioavailability.

2,4-dichlorophenoxyacetic acid, a substance with a high biological activity, is a synthetic plant growth regulator [10]. Amine salt of 2,4-D is widely used in agriculture as a herbicide, which does not act on the cereals including wheat [11].

The major component of *Usnea* lichen extract obtained by thrice repeated boiling of air-dried mixture of lichens in isopropyl alcohol is (+)-usnic acid – a substance with potent fungicidal properties. Using extract of *Usnea* genus lichens is more efficient than that of *Cladonia* genus, because *Usnea* lichens can be obtained as forest harvesting waste [12].

BIUS is an aqueous solution of a components mixture which is very easy to use. For treatment of seeds and vegetative plants the mixture is watered up to the desired concentration and applied in amounts required by the experiment.

The study has found that BIUS positively affects the phytosanitary condition of the wheat crops concerning root rot pathogens. On average, after two years' experience it suppressed the development of disease at the tillering stage of wheat by 44 %, the spread of the disease has decreased by 13 % (the value is comparable to the chemical disinfectant Raxil), and after applying BIUS at the milk wax stage of ripeness the development of root rot decreased by 39 %. Biological effectiveness Raxil was higher: 78 and 59 % according to the specified phases, respectively.

Seed treatment had a positive effect on wheat crop density: after BIUS applying at second and third leaf stage it increased by 19 plants/m^2 (501 plants/m^2 in control), and during the harvesting the difference with control (419 plants/m^2) was 40 plants/m^2. Seed sanitation with Raxil maximized increase in plant population up to 92 and 84 plants/m^2 in second and third leaf stage, and that of full ripeness, respectively.

BIUS has an evident growth-promoting effect on wheat (Table 1). Thus, the height of the plants at the flowering stage increases by 4 cm compared to control, while it is 3.6 cm with the use of Raxil.

In the tillering period accumulation of aboveground biomass of wheat was going more intensively with the use of Raxil, and in the flowering period with the use of BIUS. The growth of root biomass was equal during the first record (tillering) and increased by 15 % compared to the control. At the flowering stage accumulation of aboveground and root biomass was higher when applying BIUS (by 11.3

TABLE 1

Effect of seed treatment with preparations on biometrics of spring wheat (2009–2010)

Variants	Plant height, cm	Tillering phase, g/100 plants		Flowering phase, g/m^2	
		Biomas		Biomas	
		top	root	top	root
Control	82.2	16.7	2.0	617.3	62.9
BIUS	86.2	21.2	2.3	687.3	72.1
Raxil	85.8	22.5	2.3	664.0	69.0

and 14.6 % compared to the control). In the version with Raxil the values were 7.5 and 9.7 %, respectively.

As a result of seed treatment with BIUS the yield increased by 0.52 t/ha, or 13.8 % as compared to control (Table 2). When using a chemical fungicide the yield increased by 0.74 t/ha, or 19.6 %. Thousand kernel weight increased by 2.7 g after seed treatment with BIUS, and by 2.2 g after that with Raxil.

Improvement of seeds with the use of new perfects the quality of the final product, and grain protein content increased by 0.4 % when applying BIUS. However, seed treatment with Raxil had no effect on the protein content and it increased the gluten content of wheat by 1 %.

BIUS was applied also for spraying of vegetating wheat to reduce the air born infection of plants. The agent application in the tillering phase of wheat against powdery mildew, Septoria leaf spot and brown rust demonstrated biological effectiveness of 70, 25 and 35 %, respectively. The figures were higher for Septoria leaf spot (45 %) and lower for the other two diseases (57 and 8 %, respectively) in applying of wheat spraying in the heading phase. Treatment of wheat plantings in the heading phase with chemical fungicide Falcon ensured the maximum protection of plants against powdery mildew, Septoria leaf spot and brown rust (86.4, 80.0 and 100 %, respectively).

Growth-promoting effect of studied preparation was evident in the increase in wheat plant height during its flowering: the treatment of wheat in the tillering phase with BIUS provided the increase of 2.1 cm, in the heading stage it was 5.5 cm, and with Falcon – 4.9 cm (Table 3). Treatment of vegetating plants provided the increase in plants biomass in the flowering phase. Thus, the aboveground biomass increased exclusively after spraying of wheat plants with BIUS in the tillering stage (by 31 g/m^2 compared to control); and the root biomass – in applying the agent in the two phases of plant development (6.6 and 12.4 g/m^2, respectively). In applying Falcon the root biomass was more than control by 7.6 g/m^2.

Complex effect of wheat crops treatment with BIUS resulted in the yield increasing by 0.40 t/ha when applied at the tillering phase and 0.23 t/ha in the heading stage (Table 4). Spraying of crops with Falcon provided the maximum increase in grain yield by 0.82 t/ha.

Weight per 1000 kernels significantly increased only in applying Falcon. It is interesting, that there is an increase in the grain protein content when BIUS is applied.

TABLE 2

Effect of seed treatment on the productivity of wheat seed and grain quality (2009–2010)

Variants	Yield, t/ha		1000 kernel weight, g		Gluten, %	Protein, %
	Absolute	Increase	Absolute	Increase		
Control	3.77	–	39.8	–	28.6	13.8
Bius	4.29	0.52	42.5	2.7	26.2	14.2
Raxil		4.51	0.74	42.0	2.2	29.613.7

Note. Least significant difference (.05 level) is 0.52 for yield, and 2.5 – for 1000 kernel weight.

TABLE 3

Effect of plants spraying with preparations in the growing period on wheat biometrics (2009–2010)

Variants	Plant height, cm		Biomass, g/m^2	
	Mean	Difference	Top	Root
		Tillering		
Control	72.5	–	641	57.0
BIUS	74.6	2.1	672	63.6
		Heading		
BIUS	78.0	5.5	610	69.4
Falcon	77.4	4.9	619	64.6

The most harmful disease in potato plots within two years of research was Rhizoctonia root rot (*Rhizoctonia solani*), the major cause of reduced germination. After treatment with BIUS the number of dying plants reduced by 1.7 times compared to the control, with Maxim fungicide – by 5.5 times (the dying out of plants in control was 12 %). The data on shoot infection with Rhizoctonia root rot 4 weeks after planting indicate a high level of infestation – 30 %. Applying of Maxim and BIUS resulted in significant reduce of the numbers by 2.5 and 1.5 times, respectively. Ten weeks after planting (flowering phase) Rhizoctonia infection on stems continued to increase and reached 73 %. During this period Maxim suppressed the development of the disease most effectively – 3.7 times compared to the control, BIUS – 1.3 times. All studied preparations significantly reduced the number of stolons infected with Rhizoctonia: biological efficacy of Maxim was 75 %, and BIUS – 43 % (and in control 64 % of stolons were blasted and dead).

The total yield after use of the preparations was high; Maxim contributed to the yield increase by 14.1 t/ha, and BIUS – by 6.2 t/ha (Table 5).

Application of disinfectant conduced to significant increase in the yield of disease-free tubers (2-fold compared to control), and that of BIUS – by 1.5 times.

Thus, the study of the BIUS efficacy in yield increase and reducing infestation of spring wheat and potato showed the prospective viability of its applying in the system of pest protection of crops.

EXPERIMENTAL

Extraction of air-dried fir greenery (needles and young branches) was made by boiling with methyl *tert*-butyl ether (3 × 4 h), and then the extract reacted with N,N,N',N'-tetramethyldiaminomethane (bisamine). Air-dried fir greenery (320 g) were placed into 2 L capacity round bottom flask, then methyl *tert*-butyl ether was

TABLE 4

Effect of spraying crops during the growing season preparations wheat yield and grain quality (2009–2010)

Variants	Yield, t/ha		1000 kernel weight, g		Gluten, %	Protein, %
	Absolute	Increase	Absolute	Increase		
			Tillering			
Control	2.22	–	34.7	–	31.0	13.4
BIUS	2.62	0.40	36.3	1.6	30.2	14.2
			Heading			
BIUS	2.45	0.23	36.1	1.4	31.0	13.8
Falcon	3.04	0.82	37.4	2.7	31.0	13.6

Note. Least significant difference (.05 level) is 0.40 for yield, and 1.9 – for mass per 1000 kernels.

TABLE 5

Effect of spring treatment of potato tubers on the crop capacity and quality of the yield (2009–2010)

Variants	Yield, t/ha	Disease-free tubers, t/ha
Control	28.0	17.6
Maxim (0.4 L/t)	42.1	34.6
BIUS	34.0	26.0

Note. Least significant difference (.05 level) is 2.4 for yield, and 1.5 – for disease-free tubers.

put in and refluxed for 4 h. Then extract was filtered, and the solvent returned back into the flask with a meal by simple distillation. Extraction operation was repeated twice more. After removal of the solvent crude extract was placed for 30 min on a rotary evaporator at 50 °C and 24.75 g (7.73 %) of vitreous dark green mass was obtained.

Neutralization test for the extract was carried out by neutralization with alcoholic potassium hydroxide of free fatty acids in the weighed sample of the test extract (GOST 5476–80). 0.112 g of KOH per 1 g of extract was obtained (0.002 mol of KOH per 1 g of extract).

Bisamine (N,N,N',N'-tetramethyldiaminomethane) was synthesized by the method described in [13], from aqueous solutions of formaldehyde and dimethylamine.

Salt of fir extract with bisamine was obtained as follows: 2.13 g of extract of fir greenery (conditionally 0.004 mol of triterpene acids by extract acid number) was dissolved by stirring in a mixture of 20 mL of ethanol and 10 mL of diethyl ether. 0.41 g (0.004 mol) of bisamine was added dropwise to the dark green solution, which was conditioned with stirring for 3 h at room temperature. Solvent was distilled off on a rotary evaporator and derived dark green mass (2.55 g) was triturated by spatula with addition of 1.00 g of emulsifier (liquid soap). The resulting substance was dissolved in warm water (40–50 °C, 9.2 g of water) at the rate of 0.2 g of extract per 1 mL of solution.

2,4-D salt with bisamine was obtained by acid and amine mixing (molar ratio 1 : 1) in aqueous solution. 0.85 g of white powder of 2,4-D in 3.5 mL of water was downloaded at the conical flask and 0.40 g of bisamine was added dropwise under magnetic stirring to the resulting suspension. 2,4-D as a white powder dissolved completely; the mixture was conditioned with stirring for 3 h. 5.16 g of a yellowish solution with 1.25 g of amine salt was received and then was diluted with water to 10 g for ease of use.

Air-dry mixture of *Usnea* lichen (110 g) was ground and extracted three times (4 h each) under reflux with isopropyl alcohol. From total extracts the solvent was removed by simple distillation, and its traces – by water bath for 30 min on a rotary evaporator. 7.6 g (6.9 %) of the solid brown extract was received. The base component of the extract is (+)-usnic acid (HPLC). To 0.03 g of *Usnea* lichen extract a drop of liquid soap was added, carefully triturated with a spatula until getting smooth yellow-brown substance and diluted with hot water (50–60 °C) to 1.60 g.

1 mL of a solution containing 0.2 g of a mixture of fir extract and bisamine was placed by volumetric pipette to a serum vial, 0.024 g of 2,4-D salt with bisamine (0.003 g of salt, 1.5 %) was added by weight as well as 0.108 g of solution of *Usnea* lichen extract (0.002 g of extract, 1.0 %). The resulting dark green solution was brought up to 10 g, and thus BIUS preparation was received, which was further used in accordance with the experiment.

Investigations were carried out in 2009–2010 on spring wheat and potato crops at the experimental field of Siberian Research Institute of Farming and Agriculture Chemization, Siberian Branch of the RAAS, located in the agrolandscape area of Ob central forest steppe (Novosibirsk Region).

Bius was used for pre-seeding treatment (15 and 25 May) and spraying at the wheat tillering stage (18 and 21 June) and heading stage (16 and 13 July, in 2009 and 2010, respectively). To prepare a working solution the agent was diluted with water in the 1 : 1000 ratio. Consumption rate for the seed treatment process was 10 L/t, and 200 L/ha – for spraying of crops in growing period. Chemicals Raxil (0.5 L/t) and Falcon (0.6 L/ha) were used in experiment as references. In the experiment with seed treatment with BIUS the plants were also treated with Falcon (0.6 L/ha) in the heading stage to reduce the harmfulness of leaf and stem infections. The experiments were performed in four repetitions, the arrangement of variants of seed treatment was systematic, that of crops was randomized; area of plots in the first case (seed treatment) was 33.0 and 50.4 m^2, in the second (crops treatment) – 14.8 and 21.6 m^2 according to the years of research. Wheat was grown after fallow in accordance with established procedure.

Field studies for revealing the influence of Bius on the phytosanitary condition and yield of potato were arranged as a single factor experiment with Lina variety, with potato as a forecrop and agrotechnics used in the region of research. The treatment of tubers by spraying with BIUS (dilution at a ratio of 1 : 1000) was carried out 1–3 days before planting. Fungicide Maxim, 0.25 FS was taken as a reference (400 mL/t). Consumption rate for working solution was 30 L/t. The experiment was repeated in three replication with plot size 16.3 m^2 and planting density 35.7 thousand plants/ha.

All records and monitoring, as well as statistical data manipulation was carried out by standard practice and recommendations.

CONCLUSION

Field studies have shown that a new biological product BIUS on the basis of aqueous solution of salts of fir extract with bisamine with addition of 2,4-D salt with bisamine and *Usnea* lichen extract is a promising growth promoter with fungicidal properties against fungal pathogens to be included in phytosanitary technology of spring wheat and potato cultivation.

The use of complex products, such as BIUS based on natural extracts allows to reduce the chemical load on the fields caused by application of synthetic fungicides and to increase the production of wholefood [14].

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