UDC 547.99 + 632.934.1

New Combined Preparations Based on the Extracts of Larch and Lichen as Efficient Tool to Enhance the Productivity of Spring Wheat

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(Received April 24, 2012; revised May 13, 2012)

Abstract

A new efficient preparation with the broad range of action was developed on the basis of the aqueous solution of larch extract with the addition of a salt of 2,4-dichlorophenoxyacetic acid with bisamine and the extract of lichens of *Usnea* genus. The activity of this preparation is comparable with that of chemical fungicides. The preparation suppresses the development of mycosis and increases the productivity of spring wheat.

Key words: extract of larch (*Larix sibirica*), extract of lichens of *Usnea* genus, bisamine, 2,4-dichlorophenylacetic acid, spring wheat, phytopathogens, productivity

INTRODUCTION

Modern plant cultivation cannot do without plant protection agents. Annual potential losses of agricultural crops connected with plant pests, plant diseases and weeds in Russian Federation account for about 100 million t of conventional cereal units, or hundreds billion roubles in terms of money [1, 2].

The damage for spring wheat caused by hazardous organisms reaches 30 % of total croppage. In addition, because of common wheat affection by root rot, the protein content of grains decreases by 4.5–10 %, and gluten content decreases by 8–10 %; in the case of strong rust development, gluten content and grain glassiness decrease by 3–5 %; powdery mildew causes a decrease in gluten content by 3.5–8.6 % more [3].

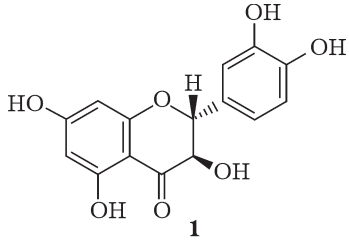
Under the conditions of ecological agriculture and plant protection, there is an increasing the demand for modern biologically active substances of plant origin that are able to stimulate plant immunity, to enhance plant stability against a number of diseases and to unfavourable environmental conditions [4]. The use of biologically active substances for treating the seeds and vegetating seedings provides a 1.5–3 times decrease in disease rate, an increase in productivity (by 1.9–8.0 centner/ha) and in grain quality, recovery of biological equilibrium in agrocoenoses and general ecological improvement [5].

The development of new preparations and improvement of those already registered ones requires their further studies under specific climatic and soil conditions with different cultivated plant species. Previously we carried out fractional extraction of the sum of lichens of *Cladonia* genus. The effect of pre-sowing treatment of the seeds of spring wheat with the preparation based on the alcohol extract of li-

chens on the development of root rot causative agents was considered. Rather high efficiency in their suppression was confirmed. The stimulating effect of the preparation on growing processes and an increase in grain productivity was revealed [6, 7]. In addition, we developed a preparation on the basis of the aqueous solution of the salts of abies extract with bisamine with the addition of a salt of 2,4-dichlorophenoxylacetic acid (2,4-D) with bisamine and the extract of lichen of the *Usnea* genus – a new efficient preparation of a broad action spectrum comparable in efficiency with chemical fungicides [8, 9]; it suppresses the development of fungal diseases and enhances the productivity of spring wheat and potatoes.

In the present paper we describe the results of the tests of two new preparations based on the extracts of Siberian larch (*Larix sibirica*). The extract of larch wood containing dihydroquercetin **1** as the major component is widely used in agriculture, promotes an increase in the productivity of agricultural crops and a decrease in the frequency of various diseases [10]. The major component of the extract of *Usnea* genus lichen is (+)-usnic acid **2** (Scheme 1).

The goal of the present study was to investigate the possibility of phytosanitary hygienics of spring wheat crops in the forest-steppe of West Siberia with respect to major diseases using the new preparations of plant origin.



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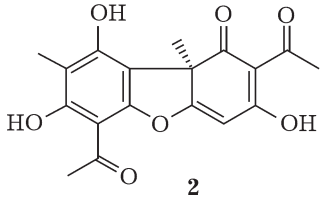

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EXPERIMENTAL

Preparation of larch extract. Air-dry larch chips (100 g) were extracted three times in a round-bottom flask 1 L in volume under heating to 75 °C in mixture of 300 mL of acetone and 300 mL of ethanol. After solvent removal, we obtained 12.2 g (12.2 %) of brown-coloured viscous substance containing dihydroquercetin (HPLC) as the major component.

Bisamine (*N*,*N*,*N*',*N*'-tetramethyldiaminomethane) is synthesized according to a procedure described in [11], from the aqueous solutions of formaldehyde and dimethylamine.

2,4-D salt with bisamine is obtained by mixing the acid and amine at a molar ratio of 1 : 1 in aqueous solution. The white powder of 2,4-D in the amount of 0.85 g in 3.5 mL of water is charged into a cone flask; 0.4 g of bisamine is added to the resulting suspension under mixing with a magnetic stirrer. The white powder 2,4-D is dissolved completely, it is kept under mixing for 3 h. A yellowish solution (5.16 g) with 1.25 g of the amine salt is obtained. It is diluted with water to 10 g for convenience of subsequent usage.

Air-dry mixture of the lichens of *Usnea* genus (110 g) is crushed and extracted three times under boiling (4 h each time) by isopropanol. The solvent is removed from the joint extracts by simple distillation; the traces of the solvent are removed in a rotary evaporator in a bath for 30 min. The product is 7.6 g of the solid extract coloured brown. The basic component of the extract is usnic acid (HPLC). One drop of liquid soap (Liquid soap for technical purposes, TU 9144-080-00279611–2001, manufactured by Selenga Cellulose Card-Board Combine) is added to 0.03 g of the extract of the lichen of *Usnea* genus. Then the mixture is ground thoroughly with a spatula to obtain a homogeneous mass of yellowish-brown colour, diluted with hot water (50–60 °C) to the mass of 1.6 g.

Obtaining preparation No. 1. Larch extract in the amount of 0.2 g is placed in a bottle, then 0.008 g of the solution of the 2,4-D salt with bisamine is added (0.001 g of the salt, concentration 0.5 %), and 0.108 g of the solution of extracts of lichen of *Usnea* genus (0.002 g of the extract, 1.0 %). Then 0.1 g of liquid soap is added to the mixture, it is thoroughly ground

with a spatula to obtain a homogeneous mass of brown colour. Water is added to it to reach the mass of 10 g. The resulting preparation is then tested with wheat.

Obtaining preparation No. 2. Larch extract in the amount of 0.2 g is placed in a bottle, then 0.004 g of the solution of 2,4-D salt with bisamine is added (0.0005 g of the salt, concentration 0.5 %). Liquid soap (0.1 g) is then added to the mixture. It is thoroughly ground with a spatula to obtain a homogeneous mass of brown colour which is then brought to the mass of 10 g by adding water. The resulting preparation is further on tests with wheat.

Investigations were carried out in 2010 and in 2011 at the experimental field of Siberian Research Institute of Farming and Agriculture Chemization, Siberian Branch of the Russian Academy of Agricultural Sciences situated in the central forest-steppe Priob'ye agricultural landscape region of the Novosibirsk Region, with the crops of spring wheat. New preparations were used for seed treatment before seeding (May 25 and 18, in 2010 and 2011, respectively) and for spraying the wheat at the phases of stooling (June 21 and 24, respectively) and heading (July 13 and 5, respectively). To prepare the working liquid, the preparation was diluted with water at a ratio of 1 : 1000. For seed treatment, the consumption of the working liquid was 10 L/t, for the treatment of the crops during vegetation it is 200 L/ha. Chemical preparations Raksil, KS (0.5 L/t) and Falkon, KE (0.6 L/ha) were chosen as references. The experiment on seed treatment also included the treatment with Falkon (0.6 L/ha) in the heading stage to decrease the hazardous effect of leaf and stem infections. The experiments were carried out with four time repetition, the arrangement of the versions with respect ot seed treatment was systematic, with respect for crops treatment it was randomized; the area of plots in the first experiment was 50.4 and 43.2 m^2, in the second one – 16.8 and 13.2, corresponding to the years of investigation. Wheat was grown on fallow precursor according to the accepted technology.

All accounts and observations, as well the statistical treatment of th6e data obtained were carried out using generally accepted procedures and recommendations [12–15].

RESULTS AND DISCUSSION

The use of the new preparations based on larch extracts (Nos. 1 and 2) for the treatment of seeds had a positive effect on the growth and development of wheat increasing its stability against unfavourable environmental conditions.

The growth stimulating effect of the preparations under study was observed as an increase in the thickness of plant stand in comparison with the reference during both years of investigation. In the phase of the 2nd and 3rd leaf, the number of plants per 1 m^2 increased by 13.2 and 4.2 %, respectively, while at the stage of waxy ripeness of the crops the difference from the reference was 17.9 and 2.2 %, respectively. The chemical reference (Raksil preparation) was behind the plant preparation No. 1 but exceeded the plant preparation No. 2 promoting an increase in the thickness of plant stand by 11.9 and 15.9 % in the corresponding phases of ontogenesis (Table 1). With the account of stooling, new biological preparations Nos. 1 and 2 exceeded not only the reference version in the number of steps per

TABLE 1

Effect of pre-sowing treatment of wheat seeds with the preparations on growth processes (2010–2011)

Versions	Thickness of plant stand, sp./m^2		Survival probability, %	Number of stems, sp./plant	Number of stems, sp./m^2	
	Phase of 2–3rd leaf	Waxy ripening of grains			Total	Productive
Reference	547	458	83.7	1.36	503	472
Preparation No. 1	619	540	85.8	1.63	582	547
Preparation No. 2	570	468	82.2	1.56	496	476
Raksil	612	531	86.6	1.48	600	553

TABLE 2

Effect of seed treatment with preparations on biometric parameters of spring wheat (2010–2011)

Versions	Plant height, cm	Biomass of plants			
		Stooling phase, g/100 plants		Flowering phase, g/m^2	
		Top	Root	Top	Root
Reference	55.5	13.2	2.0	364.0	45.7
Preparation No. 1	62.0	20.8	2.2	494.7	59.5
Preparation No. 2	62.6	22.7	2.4	499.2	57.3
Raksil	61.8	20.0	2.4	483.9	55.6

plant in this phase (by 19.8 and 14.7 %, respectively) but they also exceeded Raksil (by 10.1 and 5.4 %, respectively). Plant preparation No. 1 had a positive effect on the probability of survival of wheat plants (by 2.1 % higher with respect to the reference) and on bushiness of plants: the number of total and productive stems per 1 m^2 increased by a factor of 15.7 and 15.9 %, respectively, in comparison with the reference. With Raksil, the probability of survival, total and productive bushiness exceeded the reference by 2.9, 19.3 and 17.9 %, respectively.

The treatment of the seeds with new preparations Nos. 1 and 2 provided an increase in the accumulation of top and root biomass in the phase of wheat stooling (top – by 57.6 and 72.0 %, respectively; root – by 10.0 and 22.5 %, respectively) and in flowering phase (top – by 35.9 and 37.1 %, respectively, root – by 30.2 and 25.4 %, respectively), and the height of plants at the flowering stage (by 11.7 and 12.8 %, respectively) with respect to the reference (Table 2). In the version with Raksil these parameters were somewhat lower).

As a result of the studies, it was established that pre-sowing treatment of the seeds with the preparations under study had a positive effect on the phytosanitary situation in wheat crops with respect to the agents of root rot by increasing the stability of plants against these agents.

As an average for two years, using preparations Nos. 1 and 2 we succeeded in suppressing the development of the disease at the wheat stooling phase by 68.3 and 70.0 %, respectively (the index of disease development in the reference was 7.28 %). The occurrence of the disease decreased by 13.0 %, and by the phase of waxy ripeness of grains the development of root rot decreased under the action of these preparations by 40.6 and 30.1 %, respectively (the index of disease development in the reference was 27.6 %). The biological efficiency of Raksil turned out to be higher and equal to 81.6 % in the stooling phase and 50.4 % in the phase of the waxy ripeness of grains (Fig. 1).

As a result of these changes under the action of pre-sowing treatment of seeds with preparations Nos. 1 and 2, the average productivity of wheat with respect to the reference increased during the years of investigation by 4.7 and 4.5 %, while the mass of 1000 grains increased by 5.8 and 6.0 %, respectively. The application of Raksil provided the maximal increase in crops – by 13.1 % with respect to the reference (Table 3). In this situation, an increase in the mass of 1000 grains as a result of seed treatment with Raksil is comparable with the results of treatment with plant preparations (6.0 %). Sanitation of the seeds with new preparations (Nos. 1 and 2) caused some increase in the quality of the final product: with respect to the reference, the amount of gluten in grains increased by 0.8 and 0.4 %, respectively. The treatment of seeds with Raksil did not affect

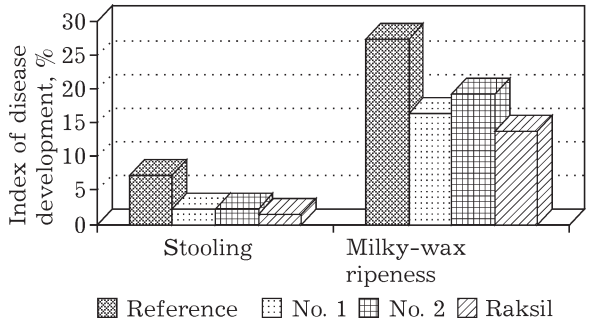

Fig. 1. Effect of pre-sowing treatment of seeds with preparations on affection of wheat plants by root rot (2010–2011).

TABLE 3

Effect of treatment of wheat seeds with preparations on the productivity and grain quality (2010–2011)

Versions	Productivity, centner/ha	Mass of 1000 grains, g	Gluten, %	Protein, %
Reference	23.30	35.10	35.4	16.3
Preparation No. 1	24.40	37.15	36.2	15.8
Preparation No. 2	24.36	37.20	35.8	15.6
Raksil	26.32	37.20	35.4	15.2

Note. NSR_{05} is 0.14 in productivity, 2.42 in the mass of 1000 grains.

TABLE 4

Effect of pre-sowing treatment of wheat seeds with preparations on the parameters of crop structure (2010–2011)

Versions	Ear length, cm	Number of spikelets per ear, sp.	Number of grains per ear, g	Mass of grains per ear, g
Reference	6.65	10.5	20.2	0.79
Preparation No. 1	7.25	11.8	26.4	1.07
Preparation No. 2	7.32	11.7	25.5	1.03
Raksil	7.62	12.3	27.4	1.13
NSR_{05}	0.32	0.4	1.8	0.1

the amount of gluten but caused a decrease in protein content of grains (see Table 3).

The new plant preparations provided the growth of all the elements of crop structure and only slightly gave way to chemical protectant Raksil (Table 4).

New biologically active substances were used also to spray vegetating plants fort he purpose of decreasing the affection of plants by aerogenic infections. During the years of investigation, wheat leaf blotch was the most widespread disease among all the leaf and stem diseases (the phase of grain ripening – 13.1 %), while powdery mildew and brown leaf rust were weakly developed in 2011 (4.4 and 3.5 %, respectively), while in 2010 wheat plants almost did not suffer from these diseases (0.12 and 0.72 %, respectively). In 2010 the preparations under study had a strong suppressing action on wheat leaf blotch agents, especially when applied in heading phase (61.8 and 65.8 %, respectively). The efficiency of their use in the phase of stooling was 17.1 and 18.7, respectively, but this effect was weaker than that of chemical reference Raksil: its application caused a decrease in the development of the disease by 83.4 % with respect to the reference.

In 2011, the biological efficiency of new preparations Nos. 1 and 2 applied at the stooling phase against powdery mildew was 60.2 and

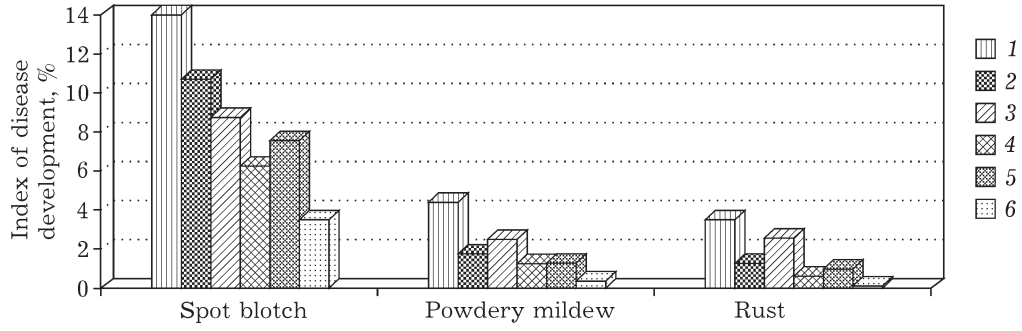

Fig. 2. Effect of crop treatment at wheat stooling and heading phases on the development of leaf and stem infections (2011): *1* – reference, *2* – preparation No. 1, stooling; *3* – preparation No. 2, stooling; *4* – preparation No. 1, heading; *5* – preparation No. 2, heading; *6* – Falkon.

TABLE 5

Effect of spraying of the plants with preparations during vegetation on biometric parameters of wheat (2010–2011)

Versions	Plant height, cm		Biomass of plants, g/m^2	
	Average	Difference	Top	Root
		Stooling		
Reference	54.7	–	397.3	39.8
Preparation No. 1	61.9	7.2	473.3	51.5
Preparation No. 2	64.2	9.5	464.6	54.3
		Heading		
Preparation No. 1	63.8	9.1	472.2	49.6
Preparation No. 2	62.5	7.8	440.1	59.1
Falkon	60.1	5.4	416.4	48.8

43.4 %, respectively, with respect to wheat leaf blotch 23.6 and 37.5 %, respectively, with respect to brown leaf rust – 64.0 and 26.8 %, respectively. Spraying at the heading phase caused an increase in this parameter with respect to all leaf diseases to 71.6 and 71.1 %, 55.3 and 46.1 %, 82.8 and 72.3 %, respectively. The treatment of wheat crops by chemical fungicide Falkon in the heading phase provided the maximal protection of the plants from powdery mildew, wheat leaf blotch and rust – 91.8, 75.0 and 100 %, respectively (Fig. 2).

It was noted that the most efficient against all leaf and stem infections are preparations applied at the crops during the heading phase. In the majority of cases preparation No. 1 exhibited higher efficiency than preparation No. 2 but was behind Falkon in this parameter.

The growth stimulating effect of the preparations under study manifested itself as a substantial increase in the height of wheat plants during flowering: after treatment during stooling phase preparations Nos. 1 and 2 provided an increase in the parameter by 13.2 and 17.4 %, respectively, while applied at the heading phase they provided 16.6 and 14.2 %, respectively, while Falkon provided an increase by 9.9 % (Table 5). Treatment of wheat during vegetation has a positive effect also on the biomass of plants during flowering phase. For example, after spraying with plant preparations Nos. 1 and 2 in the stooling phase the top biomass increased with respect to the reference by 19.1 and 16.9 %, respectively, and the root mass increased by 29.4 and 36.4 %, respectively; in the heading phase the top biomass increased by 18.8

TABLE 6

Effect of spraying of crops with preparations during vegetation of wheat on the productivity and grain quality (2010–2011)

Versions	Productivity, centner/ha	Mass of 1000 grains, g	Gluten, %	Protein, %
		Stooling		
Reference	20.8	35.4	35.6	14.6
Preparation No. 1	23.8	36.7	36.0	16.6
Preparation No. 2	23.2	36.9	35.6	15.8
		Heading		
Preparation No. 1	24.5	36.6	36.4	14.4
Preparation No. 2	22.4	36.4	36.6	14.7
Falkon	24.1	36.6	36.2	16.0

Note. NSR_{05} is 0.26 for productivity, 0.40 for the mass of 1000 grains.

TABLE 7

Effect of spraying on wheat crops with preparations on the parameters of crop structure (2010–2011)

Versions	Ear length, cm	Number of spikelets per ear, sp.	Number of grains per ear, sp.	Mass of grains per ear, g
		Stooling		
Reference	6.42	10.0	18.8	0.72
Preparation No. 1	7.39	12.0	24.0	0.98
Preparation No. 2	7.48	11.9	24.3	0.99
		Heading		
Preparation No. 1	7.22	11.8	23.7	0.97
Preparation No. 2	7.33	11.7	24.3	0.98
Falkon	7.46	12.0	26.2	1.05
NSR_{05}	0.38	0.5	3.4	0.1

and 10.8 %, respectively, and the root mass by 24.6 and 48.5 %, respectively. In the version with Falkon the top and root biomass exceeded the reference by 4.8 and 22.6 %, respectively.

The integrated effect of the treatment of seeds with preparations Nos. 1 and 2 caused an increase in grain crops by 14.4 and 11.5 %, respectively, when applied at the wheat stooling phase and by 17.8 and 7.7 %, respectively, when applied at the heading phase. Spraying of the wheat crops with chemical fungicide Falkon promoted an increase in this parameter by 15.9 %. Similarly to the application of preparation No. 1 (in both phases), it provided reliable increase in crop capacity per hectare (see Table 5).

The treatment of wheat during the plant vegetation period had a positive effect on the mass of 1000 grains, which increased reliably with respect to the reference in all the versions of the experiment. It is necessary to stress the fact that protein content in what grains increased by 2.0 and 1.2 %, for treatment with preparations Nos. 1 and 2, respectively, and by 1.4 % for Falkon. After treatment of the crops at the heading phase, the amount of gluten increased by 0.8 and 1.0 % for preparations Nos. 1 and 2, respectively, and by 0.6 % for Falkon.

The treatment of wheat crops with new preparations Nos. 1 and 2 caused an increase in all the parameters of crop structure under study: the length of ears increased by 12.5 and 16.5 %, respectively, the number of spikelets per ear – by 17.5 and 20.5 %, respectively, the number of grains per ear 26.1 and 29.2, respectively; the mass of grains per ear – by 34.7 and 37.5 %, respectively (Table 7).

Similar results were obtained in the experiments with chemical reference (Falkon).

CONCLUSION

Thus, the field investigations showed that new preparations of plant origin, able to provide an increase in the phytosanitary situation in the crops of spring wheat and stimulation of the growth and development of plants are promising for inclusion into phytosanitary technologies for cultivation of spring wheat.

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