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Biological Activity of Humic Preparations Containing Macro- and Microelements

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Abstract

The samples of humic preparations (HP) containing macro- and microelements of plant nutrition were obtained on the basis of humic acids (HA) isolated from brown coal of the Tisul deposit of the Kansk-Achinsk coal basin. The biological activity of humic preparations was tested under laboratory and field conditions on stony and loamy soils using the seeds of high-quality wheat varieties 'Iren' and 'Novosibirskaya 89'. A comparative assessment of the influence of the content of microelements cobalt, manganese, copper and zinc in humic preparations on the growth parameters of wheat was carried out. It is shown that the effectiveness of the use of humic preparations depends on the concentrations of all components, the method of their introduction, as well as on the edaphic properties of soil substrates.

Keywords: humic acids, biological activity, phytoactivity index

INTRODUCTION

Humic acids (HA) are the raw material for highly efficient humic preparations (HP) as plant growth stimulators with prolonged action. The ability of HA to stimulate plant growth was confirmed in the studies carried out using many plant species cultivated under different conditions [1–2]. The favourable effect of HA on plant development manifests itself both as the growth of roots or seedlings and as an increase in productivity. The use of HA in the form of soluble humates in agriculture causes an increase in the productivity of cereals, forage crops and vegetables by 10–30 % on average; seed germination and seedling emergence increase; plant metabolism and root formation improve. Humic acids contain aromatic and aliphatic domains representing O-, N- and S-containing functional groups with the high biological and chemical activity that may affect the activity of enzymes, stimulate root growth, cause an increase in the biomass. The fragments of HA with higher mobility may reach the root surface and interact with the cell membrane regulating the cell metabolism [3]. An increase in the permeability of the cell membrane simplifies the intake of nutrition substances and microelements inside the cell and accelerates plant respiration, synthesis of chlorophyll, saccharides, vitamins, amino acids [4]. After the application of HA, an increased content of amino acids (glutamate, aspartate, serine, glycine, methionine) was detected in the leaves [5]. The arrival of dissolved HA into a plant cell is accompanied by the enhancement of oxidation-reduction reactions according to the theory of Bach–Palladin–Szent-Gyorgyi: hydroquinone bianion $\leftrightarrow$ anion radical $\leftrightarrow$ benzoquinone. The presence of free radicals promotes an increase in energy reserve and the activity of the enzymatic system in the plant cell, enhancing its overall metabolism. It may be assumed that the effect of HA on plants is a result of many biological and chemical processes taking place simultaneously that depend first of all on the properties of humic substances (HS), chemical and biological properties of the soil and the plants.

The application of HP improves the resistance of plants against various diseases, allows not only a decrease in the norms of mineral fertilizers to be introduced into the soil but also minimization of the suppressing action of the high HP doses. In addition, HP weaken the negative effect of toxic substances in the soil, heavy metals and reduce soil toxicosis [6].

The biological activity of HP may be increased by introducing macro- and microelements for plant nutrition. The macroelements, which include the elements that are present in soil and in plants in substantial amounts, namely nitrogen, phosphorus, potassium, sulphur, magnesium, iron, are of special significance for plant growth and development at all the stages of the life cycle. Each element of the mineral plant nutrition has its own specific functions. The main role of microelements is the enhancement of the activity of various enzymes catalyzing many biochemical processes [7]. The microelements stimulate plant respiration and cause an increase in the rates of oxidation-reduction processes (Mn, Zn, Cu, Mo); they participate in the formation of organic acids and other compounds necessary for the synthesis of plant high-molecular compounds, such as proteins, fibres, lignin and so on (Mn, Cu, B); they affect the synthesis of chlorophyll in the leaves and enhance the intensity of photosynthesis (Mn, Zn, Cu, Mo, Co, B); they activate the enzymes of protein metabolism (Mn, Zn, Co, Fe).

The lack, as well as abundance of assimilable macro- and microelements in the soil disturb metabolic processes, cause various diseases, which leads to a decrease in the productivity.

The goal of the present work was to study the effect of the biological activity of complex humic preparations based on sodium humate (HumNa) and containing macro- and microelements on the growth parameters and the productivity of wheat under laboratory and field conditions.

EXPERIMENTAL

Materials

HA from the humic brown coal of the Tisul deposit of the Kansk-Achinsk basin were chosen for investigation. The biological activity of HA was studied in the form of soluble sodium humates (HumNa) prepared through the alkaline extraction with a 1 % NaOH solution according to the standard procedure [8].

To carry out the tests, we prepared initial aqueous solutions of HP containing macro- and microelements: complex (HP 1 and HP 2), cobalt (HP 3), manganese (HP 4), copper (HP 5), and zinc (HP 6) according to Table 1. The preparations were obtained using carbamide, potassium sulphate, calcium nitrate, sodium metaborate, ammonium molybdate, manganese sulphate, copper nitrate, cobalt nitrate, and zinc nitrate. The weighed portions of the substances to be added to the hu-

TABLE 1

Humic preparations containing macro- and microelements

Component content, %	HP 1 (complex)	HP 2 (complex)	HP 3 (Co)	HP 4 (Mn)	HP 5 (Cu)	HP 6 (Zn)
HumNa	2.320	2.420	2.74	2.74	2.74	2.74
N	3.375	3.690	–	–	–	–
P_2O_5	0.380	–	–	–	–	–
K_2O	3.570	1.690	–	–	–	–
CaO	0.380	–	–	–	–	–
S	–	0.640	–	–	–	–
B	0.025	0.025	–	–	–	–
Mo	0.046	0.044	–	–	–	–
Mn	0.037	0.037	–	0.037	–	–
Co	0.021	0.021	0.021	–	–	–
Zn	0.006	0.006	–	–	–	0.006
Cu	0.006	0.006	–	–	0.006	–

mate solution were calculated from the total percentage of the active components taking into account the forms and doses of fertilizers containing macro- and microelements taking into account the values proposed in [7] (see Table 1).

Investigation procedures

Experiments were carried out according to the procedures described in [9, 10] in agreement with the State Standards GOST 12038–84 and GOST R 54221–2010 [11, 12]. Biological activity was evaluated by means of phytotesting, for which we chose the plants of a widespread annual gramineous crop – wheat varieties 'Novosibirskaya 89' and 'Iren'. The use of wheat seeds in experiments allows obtaining reliable quantitative and qualitative data both under laboratory and field conditions. In addition, it is the gramineous plants that are used for ramping soil surface as a measure against desertification.

Biological activity was measured from the value of phytoactivity index (PI), taking into account test functions: seed germinating power (GP), root length (RL) – the parameter depicting the response of a plant with respect to the content of biogenic elements, and seedling height (SH) – the parameter allowing us to evaluate the phytoenzymatic action of the preparation. The experiments were carried out in three replications.

Phytoactivtiy index is a generalizing parameter characterizing the deviations of test functions from the reference values; it is calculated in the fractions of a unity:

$$PI = \frac{(GP + RL + SH)}{300}$$

where GP, RL and SH are average values, % with respect to the reference [9].

At the same time, as the reference, wheat seeds were wetted in distilled water under the same conditions. In some experiments, we additionally measured the amount of roots (AR). All lots of seeds were preliminarily tested for germination capacity. The lots with seed germination capacity not lower than 90 % were chosen for experiments.

Concentrated humate solutions may have a negative effect on the growth and development of plants, so the solutions were diluted to 0.02 %. The concentrations of all macro- and microelements changed proportionally to the dilution. The solution pH was within the range of 6.5–8.2 and within the admissible limits for the crop involved in the experiments.

Laboratory tests

Under laboratory conditions, 20 wheat seeds were placed in Petri dishes in which the discs of filter paper were put on the bottom preliminarily, and the test solutions (HP1 – HP4) or water (reference test) were poured in. The solutions of the preparations were poured into the dishes so that the seeds were completely immersed in the liquid, but its height above the seeds was not more than 3 mm. After that, the dishes were placed into a thermostat (26 °C), where they were kept for 72 h in the dark.

Vegetation experiment

The next stage of HP tests was the vegetation experiment. In this experiment, wheat seeds were planted in the vessels filled with the substrate of the fertile soil layer of the chestnut soil of Southern Siberia, similar in its properties to the soil of Mongolia. The vegetation experiment was arranged in a greenhouse with controlled air temperature and the amount of watering. The introduction of HP was carried out in two versions: during pre-planting wetting of the seeds (a day before planting) and through watering of the emerging seedlings.

Evaluation of the effect of HP was based on the quantitative and qualitative parameters. The SH was measured every week with an assignment to the phases of wheat development. At the end of vegetation, the following parameters were determined: the amount of above-ground phytomass, the amount and mass of seeds. All the versions of the experiment, both with the HP and with the methods of their introduction, were performed in three replications. Distilled water was used in the reference tests, which were carried out in three replications, too.

Field tests

The field tests of HP were carried out in two versions: during pre-planting seed treatment and during watering of the emerging seedlings (0.02 % with respect to HumNa). The investigation was carried out at the sites of technogenic landscapes, the dumps of the Zarechniy coal open pit of the JSC SUEK-Kuzbass. The sites for field experiments were determined relying on the properties corresponding to the arid extracontinental regions of Mongolia. Because of this, for the arrangement of experimental grounds, we chose the substrates represented by the eluvium of dense coal-embedding rocks similar to the stony soils of Mongolia, as well as loess-like carbonate loam – loose sedimentary rocks used as a model of loamy and clayey soil of arid regions. The use of these substrates allows a more reliable evaluation of the effect of HP because of the insignificant content of organic carbon (not higher than 3 %) in this soil.

In the versions with seed treatment, the seeds were wetted in the solutions of preparations for 24 h, and then planted. In the versions with the treatment of the emerging seedlings, soil watering was carried out 2 weeks after planting. The experiment was arranged in five replications at the sites 2 m^2 in the area. In the reference versions, distilled water was used instead of humate solutions in the same volumes as in the versions with the preparations. In all versions, the resulting effect of HP was evaluated from the effect of the preparations on the productivity of the cereals, which was determined at the end of vegetation season from the amount of dry phytomass. The summer wheat of the 'Novosibirskaya' variety was chosen as the crop for the experiment.

RESULTS AND DISCUSSION

Laboratory tests

Results of laboratory tests showed that all the preparations under test have a positive effect on the GP of wheat seeds (Table 2). The maximal biological activity with respect to this parameter was exhibited by the HP with the addition of cobalt (HP 3) and manganese (HP 4). These preparations also demonstrated the maximal positive effect for RL. A negative effect on RL was obtained from the application of the complex preparation HP 2. The sequence of HP efficiency is ranged as follows: HP 3 > HP 4 > HP 2 > HP 1. The maximal biological activity is exhibited by the preparations, the solutions of which contain either cobalt or manganese. The minimal values of PI are characteristic of HP 1 and HP 2, which are enriched with both macro- and microelements. The availability of biogenic elements may, on the one hand, fail to stimulate the development of the root systems of plants; on the other hand, calcium present in HP 1 promotes neutralization of the effect of HA [13].

TABLE 2

The values of test functions of the phytoactivity of humic preparations, % with respect to the reference (HumNa concentration 0.02 %)

Preparation	RL	SH	GP	PI
HP 1 (complex)	104.4	93.6	105.1	1.01
HP 2 (complex)	98.9	104.5	120.6	1.08
HP 3 (Co)	132.2	110.0	141.5	1.28
HP 4 (Mn)	132.0	97.1	127.5	1.19

To determine the dependence of PI on concentration, the solutions of HP with different concentrations were prepared from the initial solutions: 0.0005, 0.005 and 0.01 % (for HumNa). In this experiment, the seeds of 'Iren' wheat were wetted in the growing trays, 50 seeds per tray. The data obtained in phytotests are presented in Table 3.

Comparing the data shown in Tables 2 and 3, we may conclude that the effects from the use of HP 1 in the concentrations of 0.02 and 0.01 % practically do not differ from each other and are close to the reference. In addition, in both cases, the preparations suppress SH, and in the concentration of 0.01 % they also suppress RL. A maximally positive effect on seed germination is caused by HP 1 preparation in the minimal concentration (0.0005 %). The increments of RL and SH are 17 and 11 % against the reference, respectively. The PI values exceed the reference value by 12 %. It is possible that a decrease in CaO content causes a decrease in the degree of HA neutralization. The maximal positive effect of HP 3 and HP 4 preparations was exhibited for the concentration equal to 0.005 % with respect to HumNa (PI = 1.22 and 1.24, respectively). Seedling height after the use of these preparations exceeded the reference by 52 and 53 %.

To carry out subsequent experiments, we prepared the solutions of HumNa (0.005 %) containing cobalt, manganese, copper and zinc cations in concentrations varied from 0.001 to 0.1 %. Results of the experiments listed in the Table 4 provide evidence of the positive effect of HumNa (0.005 %) on the growth parameters of wheat seeds (PI exceeds the reference by 39 %). The maximal biological activity is exhibited by HP 5 (copper content 0.001 %) and HP 6 (zinc content 0.001 %). Phytoactivity indices in these cases are equal to 1.91 and 1.95, respectively. These preparations have a positive effect on all test functions, especially on RL. The excess over the reference for RL in the case of the application of HP 5 is more than two times, and in the case of HP 6 three times. For HP 3 and HP 4 containing cobalt and manganese, in the concentration of 0.001 %, PI is equal to 1.51 and 1.49, respectively; all test func-

TABLE 3

Effect of the concentration of humic preparations on phytoactivity index

Preparation	Concentration for HumNa, %	SH	RL	GP	AR	PI
		% with respect to reference				
HP 1 (complex)	0.0005	111	117	107	106	1.12
>>	0.005	103	117	107	106	1.08
>>	0.01	99	99	104	103	1.01
HP 3 (Co)	0.0005	108	104	106	103	1.06
>>	0.005	153	114	98.7	104	1.22
>>	0.01	109	107	92.6	106	1.03
HP 4 (Mn)	0.0005	116	97	103	105	1.05
>>	0.005	152	118	103	103	1.24
>>	0.01	102	113	100	108	1.05

TABLE 4

Values of test functions of phytoactivity of humic preparations containing microelements (HumNa concentration 0.005 %)

Preparation	RL	SH	GP	AR	PI
	% with respect to reference				
HumNa	164.7	147.8	105.1	105.0	1.39
HumNa + Co^{2+} (0.001 %)	178.7	165.9	107.6	110.2	1.51
HumNa + Co^{2+} (0.01 %)	162.7	155.1	100.0	106.3	1.39
HumNa + Co^{2+} (0.05 %)	79.4	122.7	100.0	101.1	1.01
HumNa + Co^{2+} (0.1 %)	28.1	73.0	93.8	103.8	0.65
HumNa + Mn^{2+} (0.001 %)	186.3	149.2	112.1	105.8	1.49
HumNa + Mn^{2+} (0.01 %)	184.3	142.6	108.0	102.6	1.45
HumNa + Mn^{2+} (0.05 %)	151.3	162.5	103.2	102.4	1.39
HumNa + Mn^{2+} (0.1 %)	126.3	127.3	100.0	102.8	1.18
HumNa + Cu^{2+} (0.001 %)	235.5	198.6	138.1	103.9	1.91
HumNa + Cu^{2+} (0.01 %)	190.9	199.3	129.1	107.1	1.73
HumNa + Cu^{2+} (0.05 %)	82.5	116.9	127.0	111.0	1.10
HumNa + Cu^{2+} (0.1 %)	20.2	41.9	66.1	35.3	0.43
HumNa + Zn^{2+} (0.001 %)	358.2	118.8	107.3	110.2	1.95
HumNa + Zn^{2+} (0.01 %)	331.9	121.6	103.2	107.5	1.86
HumNa + Zn^{2+} (0.05 %)	243.9	112.5	108.0	112.6	1.55
HumNa + Zn^{2+} (0.1 %)	146.0	102.7	108.0	113.0	1.19

tions exceed the reference values. With an increase in the concentration of microelements in the preparations, a decrease in the biological activity and all test functions is observed. The lowest values are observed in the experiments involving the preparations with microelement content 0.1 %. Low efficiency was exhibited by the preparations with cobalt (0.1 %); all values were lower than in the reference experiments with water. The phytoactivity index was only 0.65. The lowest values were observed for the preparation with copper content 0.1 % (RL = 20.2, SH = 41.9, GP = 66.1, PI = 0.43). This preparation (HP 5), unlike others, exhibited a suppressing effect on the development of the root system (AR was more than twice lower than the reference). The excessive amount of microelements in the preparations, similarly to their lack, disturbs cell metabolism, the equilibrium of biochemical processes in plants, which leads to diseases (chlorosis, necrosis). In particular, copper in excess causes deceleration of plant development and further die-away.

It should be noted that the preparations containing cobalt and zinc have a positive effect on the development of root system, that is, they promote an increase in the amount of roots, while the preparations containing manganese have a positive effect on the formation and development of side roots. The application of preparations based on HumNa containing cobalt, zinc and manganese

TABLE 5

Height of wheat plants over the development phases in comparison with reference versions, %

Preparation	Development phases				
	The third leaf	Tillering	Stem elongation	Earing stage	Complete maturity
			Seed wetting		
HP 1 (complex)	93.8	96.3	93.1	94.6	98.7
HP 2 (complex)	99.7	96.9	88.8	91.1	98.0
HP 3 (Co)	95.8	94.6	90.5	96.5	98.2
HP 4 (Mn)	100.4	100.6	93.6	95.9	98.0
			Seedling watering		
HP 1 (complex)	100.9	106.9	99.0	103.7	104.8
HP 2 (complex)	101.5	107.2	101.4	104.7	101.5
HP 3 (Co)	107.6	107.8	92.8	96.6	105.7
HP 4 (Mn)	90.1	97.7	96.6	98.8	95.9

TABLE 6

Parameters of wheat productivity from the results of vegetation experiment, % to the reference

Preparation	Parameter		
	Above-ground phytomass	Amount of seeds	Mass of seeds
	Seed wetting		
HP 1 (complex)	102.2	72.5	66.1
HP 2 (complex)	90.9	90.8	86.7
HP 3 (Co)	95.5	81.0	75.2
HP 4 (Mn)	108.8	97.2	97.6
	Seedling watering		
HP 1 (complex)	91.1	130.6	134.1
HP 2 (complex)	96.6	138.1	139.1
HP 3 (Co)	96.2	111.3	121.8
HP 4 (Mn)	95.5	107.5	112.8

cations promote the development of the root system of plants, which is important for soil structure strengthening.

Vegetation experiment

Results obtained in the observation of wheat development phases show that in the version with seed wetting in HP 1 – HP 4 preparations, the height of plants did not exhibit substantial differences (Table 5) and was close to the reference values. In the version with seedling watering, the differences in plant height were more evident. At the earliest stage, watering with the solution of the preparation with manganese (HP 4) revealed a trend to slower development of wheat. Higher values were observed after watering wheat seedlings with complex preparations HP 1 and HP 2 containing macro- and microelements.

In general, in the versions with different preparations, wheat height remained within the 5 per cent interval in comparison with the reference. For seed wetting, the plants were lower than in the reference versions, while for watering, they were higher. The negative effect of HP 1 detected in the laboratory experiment affects the subsequent stage of development, that is, plant height (see Table 5).

Evaluating the final results on wheat growth under the conditions of the vegetation experiment, we may also stress differently directed trends of the effect of HP used to wet the seeds and to water the plants (Table 6). For instance, the above-ground phytomass of wheat remained lower than the reference values almost in all versions, either after seed wetting or after plant watering.

The qualitative parameters of the productivity evaluated on the basis of the amount and mass of the obtained seeds turned out to be lower than the reference versions by 2.4–33.9 % for seed wetting and higher by 7.5–39.1 % for seedling watering. It is worth noting that the preparations modified with macroelements HP 1 and HP 2 (especially with the addition of Ca) were the most efficient ones in the case of watering.

Thus, the use of HP for seed wetting has a negative effect on the development of wheat and on its productivity. Quite contrary, the positive effect after seedling watering is observed both for seedling height and for productivity. The maximal effect was revealed for watering with HP 1 and HP 2 containing macro- and microelements (cobalt, manganese, copper, and zinc). The amount and mass of seeds exceed the reference values by 30–40 %.

Field tests

The results of field tests showed that the effect of preparations on the phytomass of cereals is exhibited not in a similar manner and depends on edaphic factors (physical and chemical properties of the substrates at the sites). The productivity of dry phytomass at the sites of the field experiment was not high: 2 to 8 dt/ha. With respect to the reference versions in which distilled water was used, the effect of preparations was positive in a number of experiments (Fig. 1).

The most clearly pronounced HP effect was exhibited on the substrates represented by stony rocks. It should be stressed that under the conditions of insufficient moisture characteristic of these rocks, the most efficient preparations are HP 3 and HP 4, containing cobalt and manganese, respectively. The efficiency of HP 1 was approximately at the same level, which, in addition to the effect of Co and Mn, may be also due to the presence of macroelements with balanced composition (N, P, K and Ca). In the case of HP 2, also enriched with Co, Mn, N and K but without P and Ca, the effect of the preparation turned out to be minimal.

In general, the effect of HP on the phytomass of wheat grown on stony rocks was similar for the versions with seed wetting and for the sites where the preparations were introduced with watering.

At the experimental sites on loamy rocks, the effects of the preparations were not similar to each other. Under the conditions of more substantial humidification, a negative effect of the preparations was detected for HP 3 and HP 4 (see Fig. 1). A negative but less clearly expressed result of the application of HP 1 was detected in the versions with watering. Under the same conditions, the maximal positive effect on wheat phytomass was exerted by HP 2. It is evident that the observed effect is due to the presence of sulphur in this modification, and to the highest nitrogen content (see Table 1). The absence of phosphorus and potassium in HP 2 may have been compensated by their presence in the substrates of the sites. In the versions with seed wetting, the effect of HP 2 was lower than that of HP 1. Nevertheless, the productivity, in this case, was higher than the reference values by 13 % on average.

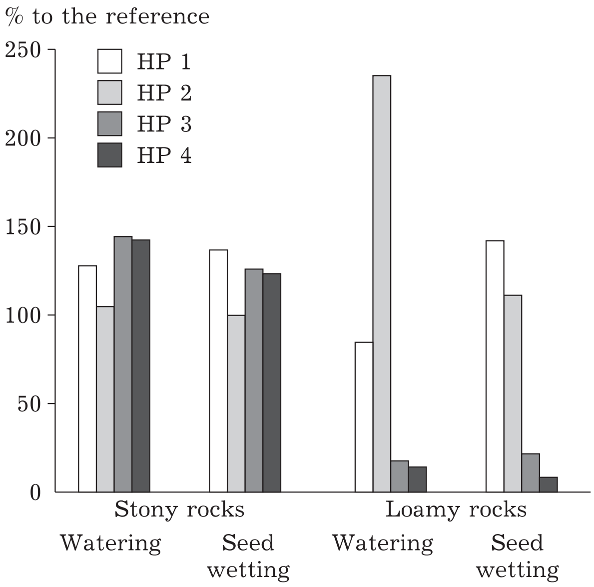

Fig. 1. Productivity of wheat phytomass at recultivation sites with respect to the reference, %.

CONCLUSION

The results of the studies allow us to conclude that the effect of preparations based on HumNa containing macro- and microelements on the development and productivity of wheat depends on several factors. It was revealed in laboratory tests that the biological activity (PI) depends on the concentration of the applied preparations and on the concentrations of macro- and microelements present in these preparations. The maximal biological activity is exhibited by the preparations (0.005 % with respect to humate) in which the content of microelements (cobalt, manganese, copper, zinc) does not exceed 0.001 %.

Experiments on vegetation showed that wheat seedling watering as the method to introduce the preparations for providing high growth characteristics and productivity turned out to be more efficient than seed wetting. High results were obtained for watering wheat seedlings with HP 1 and HP 2 preparations containing both macro- and microelements. The amount and mass of the seeds in the case of the application of these preparations exceed the reference values by 30–40 %.

Field tests showed that the choice of HP for use against desertification should be made, taking into account edaphic and climatic conditions over the territories of application. The largest effect of the application of HP solutions is observed at stony soil with an acute deficit of moisture, where almost all the preparations under study have a positive effect.

So, the balanced content of macro- and microelements in HP, consideration of the biological composition and structure of the soil, as well as the correctly chosen method of HP introduction will allow the most efficient application of the preparations.

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