



Plants Nutrition and Growth Stimulation with the Help of Nanotechnologies

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Abstract

The consistent use of some branches of agrochemical science in crop growing is in the concept of a comprehensive system approach we propose to the issue of plants nutrition and growth stimulation with the help of nanobiopreparations based on copper and cobalt nanopowders. We propose to optimize the technology of a number of agrochemical measures, and more precisely, to reduce them to a single pretreatment of seeds with a preparation containing necessary trace elements in the nano form.

This article presents toxic and stimulating effects of using nanoparticles in agro and biotechnology. The developed bio preparations, based on nanoparticles of metal-trace elements (iron, cobalt, copper), have a number of advantages over existing analogues and they are as it follows:

- multifunctional action;
- low consumption rates of preparations up to 1.0 g per hectare seeding rate, while pre-sowing treatment can be combined with dressing, which does not require additional energy costs;
- a comparative assessment of the toxicity of nanopreparations compared with salts of trace elements (iron and copper sulphates, cobalt chloride) proves that salts are 8-10 times more toxic than nanopreparations;
- low cost of nanopreparations (NP) compared with the cost of an equivalent dose of traditional mineral micro fertilizers and drugs to prevent plant diseases;
- ecological safety.

The uniqueness of the proposed technology lies in the fact that its use in agriculture does not require any special equipment or an increase in the staff of the enterprise. In the process of applying the proposed preparations, the natural processes of agrobiocenosis are not disturbed and soil fertility is preserved.

The effect of nanobiopreparations on living organisms and the effect of "low doses" compared to substances in the usual physical-chemical state, make possible to obtain scientifically based and objective results of the effect of nanoparticles on plants nutrition and growth stimulation.

Keywords: nanotechnologies, biotechnologies, preparations, plants growth stimulation, bio-accumulation, ecology.

1. Introduction

To apply nanotechnology in agriculture, we used biologically active nanopowders of metals. One of the determining factors of the biological effects of these nanoparticles is their physical chemical characteristics: chemical and phase compositions, average particle size, particle size distribution, specific surface area and degree of agglomeration of nanoparticles. The research that have taken place since 2011 at National Research Technological University "MISiS" together with Ryazan State Agrotechnological University, has shown that biological effects of nanoparticles are largely determined by the methods of their production. Much research work has been done on the biological

activity of copper, cobalt, and iron nanopowders obtained by low-temperature hydrogen reduction of metal hydroxides.

The possibility of effective use of nanopowders biogenic elements sized 20-45 nm in crop growing is shown in researches [1-10]. Within their framework the influence of nanomaterials on biological objects was studied in detail: degradation of nanoparticles when interacting with the biological material; their influence on metabolic processes; methods for obtaining this information; methods for standardizing nanoparticles as biologically active components; ways of homogenization and separation of the unification of the nanoparticles surface (disaggregation, ultrasonic treatment and centrifugation) have been developed.

Information about the properties of nanomaterials includes data on physical, physical-chemical, molecular-biological, cytological,

physiological, toxicological and environmental characteristics of nanomaterials. These results were obtained using adequate research methods, confirmed in accordance with the validity criteria adopted in the relevant areas of the study. Within the framework of these studies, we researched to determine the optimum parameters for the size, composition and concentration of nanoparticles for the following plant parameters:

- Biological activity.
- Accumulation of biopolymers.
- Changes in the structure and composition of biopolymers.
- Synergy of microelements in the process of vegetation.
- The state of antioxidant protection.
- Change in the activity of enzymes and phytohormones.

As a result of the research

- ecological and biological safety of metal nanopowders was proved, and the toxicity of metals nanopowders was 8-12 times less than that of their mineral salts currently used in agriculture;
 - with the help of modern physical-chemical methods of analysis, probe and electron microscopy, the absence of their accumulation in plants, soil, and animal products was proved;
 - the veterinary and toxicological characteristic was prepared for full development and safe introduction of bio preparations based on NPs of metals and their oxides (25-45 nm);
 - it was established that NPs of iron, cobalt and copper do not possess cumulative properties, but a prolonged action [11-15].
- The tested nanobiopreparations can be used to increase the yield and stress resistance of agricultural plants to adverse environmental factors, etc.

2. Materials and methods

The studies took place at the Center for Nanotechnologies and Nanomaterials for the Agro-Industrial Complex at Federal State Budgetary Educational Institution of Higher Education Ryazan State Agrotechnological University (RSATU). The field tests took place at the agrotechnological station of RSATU (Stenki village, Ryazan region) and at Federal State Budgetary Research Institution "Ryazan Research Institute of Agriculture" (RRIA). The soil was light gray forest. Agricultural technology was carried out in accordance with regional recommendations. The experiment was small-scale and single-factor. The sown area of

the plot was 56 m², the harvesting area was 30 m². The replication was fourfold and the arrangement was systematic.

Nanomaterials produced in RTU MISiS and having the following characteristics were used in the experiment:

- nanopowders of iron (NP Fe), cobalt (NP Co) and copper (NP Cu) - fine homogeneous powders free of foreign matter, 99.98 % pure. The average particle size was 20-40 nm. To create a biologically active ultradispersed system, the metal suspension was subjected to ultrasonic treatment in aqueous medium.

The dispersion analysis [16] of experimental data was carried out according to B.A. Dospikhov [17].

Research Design:

- 1) Control - seeds without treatment with metal nanopowders.
- 2) Experiment - seeds treated with copper and cobalt nanopowders at a concentration of 0.1, 0.5 and 5 g per hectare seed rate (g / hsr).
- 3) Experiment - seeds treated with humic acids in ultrafine state.

The content of chemical elements at different phases of plant development was determined by atomic absorption spectrometry (KVANT – Z. ETA spectrometer). In the analyzer of this type, the transfer of the sample to the state of atomic vapor was carried out in a graphite tubular electro thermal furnace heated to the atomization temperature of the element being analyzed. A micropipette sample with a volume of 5 μl was injected into it. The control of the device and the processing of the analysis results were carried out with a personal computer with the software QUANT ZEEMAN 1.6.

To determine the activity of catalase and peroxidase in plant tissues, the following method was used: photometric kinetic test using a KFK-3-01- ZOMZ photoelectric colorimeter [18].

3. Results and discussion

3.1. The results of field tests of preparations based on nanoparticles.

The effect of NP on spring wheat was studied in field tests in 2013-2016. Ferrum NP at concentration of 0.5 g / hsr (2013) increased the yield of spring wheat cultivar "RIMA" by 17.6 % and gluten content by 4.5 %. The data of 2015 are presented in Table 1.

Table 1 Yield structure of spring wheat "RIMA" in 2015

Variant	Mass of 1000 seeds, g	The ratio to control, %	Grain ash, %	Mass fraction of crude gluten, %
Control	37.44	-	2.10	27.38±1.15
NP Fe	39.88	+6.53	2.12	28.95±1.18
NP Cu	41.02	+9.56	2.11	30.17±1.43
NP Co	41.59	+11.085	2.13	29.23±1.69

The increase of productive indexes with a one-time pretreatment of seeds with preparations containing 0.5 g of nanoparticles per hectare seed rate, ultimately led to some increase of spring wheat seeds yield: by 13.7 % when using ferrum NP, by 14.4 % when using cobalt NP and by 18.5 % when using copper NP. The mass fraction of crude gluten exceeded the control in all variants, with copper and cobalt NP at most, that is seen in Table 1. In terms of quality, the gluten of all variants was assigned to quality group II. In 2016, the yield of winter wheat "Moskovskaya-56" at Agrotechnological Station of Ryazan State Agrotechnological University was 43 dt / ha with the control yield of 22 dt / ha. The addition yield was 85 tons from 100 hectares.

It turned out that plants themselves are able to easily adsorb highly dispersed particles of biogenic metals and use the surface energy of the incoming nanoparticles, obtained by the seed during pretreatment. Nanoparticles affect biological objects at the cellular level, introducing their excess energy, increasing efficiency of processes occurring in plants, and also participating in processes of the microelement balance, i.e. they are bioactive.

2. Comparative characteristics of the effect of copper sulfate and copper NP on seeds of wheat cultivar "RIMA".

In the course of laboratory studies to find the optimum concentration of copper and copper sulfate NPs in order to stimulate the growth and development of spring wheat the concentration range of 0.1-5000 h / hsr was used. It was found out that the inhibition of growth and development of seedlings when treatment with copper NP begins at concentration of 500 g / hsr and copper sulphate NP at concentration of 100 g / hsr. Therefore, the use of copper sulphate at concentrations that are used at farms (up to 500 g) is dangerous. Copper excess in the surface layer of the soil inhibits the development of plants, in particular, slows the germination of grains and the development of the root system. The distribution of copper in plants is very variable. Copper in roots is bound mainly to the cell walls and extremely inactive. The highest concentrations of copper in shoots are always found in the phase of intensive growth at the optimum level of its intake. The manifestation of Cu toxicity is in dark green leaves; thick, short or

similar to barbed wire roots and oppressed shoot formation [19-22].

The root system of plants begins to develop at the earliest stages of germination, that's why it is the primary indicator of plant

development. The number of roots for a normally developed grain of wheat must be four.

Table 2 Roots number per one plant, pcs

Variant	Number	Variant	Number
NP Cu 0.1 g / hsr	4.04±1.32	CuSO ₄ 0.1 g / hsr	3.51±1.32
NP Cu 20 g / hsr	4.84±1.25	CuSO ₄ 20 g / hsr	4.49±1.11
NP Cu 100 g / hsr	4.58±1.16	CuSO ₄ 100 g / hsr	3.55±1.01
NP Cu 500 g / hsr	3.80±1.12	CuSO ₄ 500 g / hsr	0.31±0.12
NP Cu 2000 g / hsr	1.46±1.21	CuSO ₄ 2000 g / hsr	0.27±0.10
NP Cu 5000 g / hsr	0.99±0.23	CuSO ₄ 5000 g / hsr	0.15 ±0.10
Control - 4.09±1.1			

Seeds treated with copper NP at the concentration of the preparation from 0.1 to 500 g / hsr had, on average, 4 roots. This indicates the normal development of seedlings, hence, copper NP up to concentration of 500 g / hsr does not contribute to any change in the development of seedlings. However, at concentration of 2000 g / hsr, the number of roots has dropped to 1.4, which leads to dysfunction of absorption, and consequently to some shortage of minerals for the plant.

The toxic effect of sulphate is manifested, beginning at concentration of 100 g / hsr. There was a decrease in the development of the root system, which proves the ability of copper sulphate to inhibit the development of wheat seedlings

at given concentrations. With an increase in the concentration of copper sulfate, most seeds had practically no root system. Therefore, copper sulfate concentration of 100 g / hsr is critical for plants.

Laboratory tests are confirmed by field tests. For the control experiment, the seeds were treated with distilled water before planting. The effect of copper NP suspension and copper sulfate solutions at concentrations of 0.1 g / hsr, 20.0 g / hsr, 100.0 g / hsr and 500.0 g / hsr was studied.

During the growing season, the degree of spring wheat tillering was studied during the period of ear formation and the data are presented in Table 3.

Table 3 Spring wheat tillering degree

Variant	Tillering degree	Ratio to the control, %
Control	2.58±0.42	-
NP Cu 0.1 g / hsr	3.48±0.65	+34.88
NP Cu 20 g / hsr	2.85±0.72	+10.42
NP Cu 100 g / hsr	2.73±0.63	+5.81
NP Cu 500 g / hsr	1.47±0.51	- 43.02
CuSO ₄ 0.1 g / hsr	3.26±0.32	+26.35
CuSO ₄ 20 g / hsr	2.88±0.97	+11.62
CuSO ₄ 100 g / hsr	2.30±0.74	- 10.80
CuSO ₄ 500 g / hsr	1.75±0.54	- 32.1

Note: *-P ≤ 0.05

The characteristic degree of tillering for spring wheat is 2-3 plants from one seed. Based on the conducted studies, it was found that copper and copper sulfate NP at low concentrations stimulate the development and growth of spring wheat plants, which corresponds to the results of laboratory tests. However, when the specific quantity of the studied substances increases, different effects appear. So, the inhibition of tillering with the use of copper NP was observed at concentration of 500 g / hsr. The toxic effect of copper sulphate became noticeable even at concentration of 100 g / hsr. The tillering degree of spring wheat plants at this concentration was 10.8 % below the control. There were weak

shoots and the plants were scraggy and pale. At copper sulfate concentration of 500 g / hsr, the shoots were practically not detected.

During vegetation, namely, at the stage of ear formation, we studied such parameters as plant height and chlorophyll content in leaves (Table 4). Chlorophyll is a pigment, the content of which in the leaves of plants determines photosynthetic activity [23, 24]. Copper, in turn, is an integral component of enzymes and proteins involved in the process of photosynthesis and its surplus leads to scorching of leaves and their subsequent dieback.

Table 4: Chlorophyll in leaves of spring wheat cultivar "Lada"

Variant	Chlorophyll, mg / g	Ratio to the control, %
Control	2.53±0.46	-
NP Cu 0.1 g / hsr	2.74±0.52	+8.30
NP Cu 20 g / hsr	2.66±0.49	+5.10
NP Cu 100 g / hsr	2.55±0.24	+0.80
NP Cu 500 g / hsr	2.07±0.18	-18.18
CuSO ₄ 0.1 g / hsr	2.65±0.21	+4.74
CuSO ₄ 20 g / hsr	2.34±0.31	-8.06
CuSO ₄ 100 g / hsr	2.16±0.17	-14.63
CuSO ₄ 500 g / hsr	1.34±0.18	-47.04

Note: *-P ≤ 0.05

The use of copper and copper sulfate NP affected the amount of chlorophyll in leaves of experimental plants. The greatest amount of pigment 2.74 mg / g was in leaves at copper NP concentration of 0.1 g / hsr, which exceeds the control by 8.3 %. In variants with NP, some increase in the amount of chlorophyll was observed up to concentration of 100 g / hsr. Oppression was observed only

from concentration of 500 g / hsr, and the difference with the control was 18.2 %.

Copper sulphate caused some negative result even at an active substance concentration of 20 g / hsr and the difference with the control was 8.06 % lower. The maximum concentration of copper sulphate (500 g / hsr) caused some decrease in the chlorophyll content by 47.04 % compared to the control.

The height of the plants was examined at the stage of ear formation. Copper NPs proved their high biological activity. The plant height exceeded the control by 7.4 % at nanoparticle concentration of 0.1 g / hsr. More chlorophyll in leaves intensified the process of photosynthesis. In this connection, the processes of growth, development and accumulation of biologically active substances in plants treated with copper NP were more active. The relative decrease in the height of the experimental groups of plants in comparison with the control was observed only at concentration of higher than 100 g / hsr. At nanoparticles concentration of 500 g / hsr, the height of spring wheat plants was 2.14 % lower than the control.

The mass of one thousand seeds is one of the main technological indicators, determined when checking the quality of crop production. The use of copper NP at concentrations of 0.1 g / hsr, 20 g / hsr and 100 g / hsr contributed to increase in the mass of one thousand seeds by 11.28 %, 13.33 % and 2.05 %, correspondingly. Seeds obtained when pretreatment with high concentrations of copper sulfate were less developed and weaker. At sulfate concentration of 500 g / hsr, the mass of one thousand seeds was 14.11 % lower than that of the control. Such a diverse nature of the action of copper and copper sulphate NP on plants suggested different yields of spring wheat cultivar "Lada".

Copper NPs prove their biological activity, in consequence of which, the yield of seeds exceeded the control practically in all variants. The highest yield was observed at copper NP concentration of 0.1 g / hsr and exceeded the control by 18.74 %. The yield of seeds in variants with copper nanoparticles was higher than the control one up to concentration of 100 g / hsr and only at concentration of 500 g / hsr the yield was 21.25 % lower than the control.

The pretreatment of wheat seeds with copper sulfate at concentration of 20 g / hsr caused yield increase by 6.2 %, which is the maximum value among the considered variants for treatment with sulfate. With some increase of copper sulfate concentration, the yield of spring wheat seeds was below the control by 18.75 %, and at concentration of 500 g / hsr, the difference with the control was 27.5 %, which proves the toxic effect of using copper sulfate as a micro-fertilizer.

3.3. The process of plants adaptation to stressful situations exemplified by biogenic metals nanoparticles in comparison with natural stimulators with humic acids in the ultradispersed state.

It is known that plants respond to mechanical effects and damage by changing the morphology or growth rate [25]. The enzymes of the antioxidant system take part in a number of biological processes, such as photosynthesis, respiration and protein metabolism. These enzymes have increased sensitivity to external factors, which makes it possible to use their activity as a test characteristic for determining the state of the plant. Fluctuations of

enzyme activity are possible when growing, but they should not vary by more than 30 % from activity when non-stress effect of external factors. In most cases, some increase of the activity of the antioxidant system enzymes indicates the mechanism of the plant's nonspecific response to stress [26, 27]. The increased activity of the antioxidant system enzymes can lead to deactivation of growth hormones. It is also possible to mutually inactivate the stimulus and molecules of enzymes due to sorption or other chemical interactions. This may serve as an explanation for the decrease in the activity of antioxidant enzymes noticed in a number of variants [28-31].

When investigating, optimal concentrations of copper and cobalt NP, 0.1, 0.5, 1.0, 5.0 g / hectare seeding rate (g / hsr) were chosen. For comparison, the effect of natural growth stimulants of humic acids in the ultradispersed state on seeds of spring wheat has been studied. During the growing season, the following parameters were determined: field germination, height and weight of plants, leaf surface area, net photosynthesis productivity, activity of redox enzymes, yield and chemical composition of the crop.

When the concentration of cobalt and copper nanoparticles 0.5 g / hsr in the phase of the third leaf, the length of the above-ground part of the wheat seedlings exceeded the control by 16 % and 18 %, respectively, and in a case with humic acid concentration of 1.0 g / hsr the length exceeded the control by 10 %. The underground part of seedlings developed more intensively than the aboveground part. The root length when treating seeds with cobalt nanoparticles at concentration of 0.5 g / hsr exceeded the control by 26.8 %, with copper nanoparticles by 18.5 %, and with humic acids at concentration of 1.0 g / hsr by 15 %. Laboratory studies have confirmed the effect of high biological activity of nanoparticles of cobalt, copper and humic acids in the ultradispersed state on processes associated with the growth and development of plants. The effect of nanoparticles was similar in all crops, but 8-12 % higher than when using humic acids at the same concentrations. Therefore, it can be concluded that nanoparticles act by their energy potential to activate metabolic cellular processes, and their action differs from that of micro fertilizers.

Field studies have shown that plants, when seeds were pretreated with copper nanoparticles, cobalt and humic acids in an ultradispersed state, had higher physiological parameters than the control ones during the growing season. One-time pretreatment of spring wheat seeds with cobalt or copper nanoparticles, as well as humic acids in the ultradispersed state contributed to an increase in the leaf surface area. At cobalt nanoparticle concentration of 1.0 g / hsr, the excess to the control was 13.1 %, in a case with humic acids by 8.6 % and copper nanoparticles by 14.0 %. The increase in the assimilation area of leaves resulted in some increase in the intensity of photosynthesis of spring wheat compared to the control by 12.7 % when using cobalt nanoparticles at concentration of 1.0 g / hsr, by 6.3 % when using humic acids and by 14.9 % when using copper nanoparticles.

Table 5: The leaf-area duration and the net productivity of plant photosynthesis

Spring wheat					
Variant	Leaf-area duration, thousand m ² / ha	Variant	Leaf-area duration, thousand m ² / ha	Variant	Leaf-area duration, thousand m ² / ha
Control	24.3±0.14	Control	24.3±0.14	Control	24.3±0.14
NP Co 0.5	26.8±1.31	HA 0.5	26.2±0.19	NP Cu0.5	25.8±0.09
NP Co 1.0	27.5±0.25	HA 1.0	26.4±0.07	NP Cu1.0	27.7±0.21
NP Co 5.0	25.9±0.15	HA 5.0	25.4±0.13	NP Cu5.0	24.9±0.16
Variant	Net productivity of photosynthesis, g / m ² · day	Variant	Net productivity of photosynthesis, g / m ² · day	Variant	Net productivity of photosynthesis, g / m ² · day
Control	4.7±0.02	Control	4.7±0.02	Control	4.7±0.02
NP Co 0.5	5.1±0.04	HA 0.5	4.9±0.01	NP Cu0.5	5.2±0.12
NP Co 1.0	5.3±0.03	HA 1.0	4.7±0.04	NP Cu 1.0	5.4±0.08
NP Co 5.0	4.8±0.01	HA 5.0	4.7±0.03	NP Cu 5.0	5.1±0.10

Note: *P ≥ 0.95

The productivity of plants depends directly on the intensity of photosynthesis, which correlates with the leaf-area duration. Thus, seeds pretreatment with the suspension of nanoparticles of cobalt,

copper and humic acids in the ultrafine state contributed to the activation processes associated with growth and development of

plants. The effect of humic acids is not so vivid as that of metal nanoparticles.

During the growing season, the activity of redox enzymes (peroxidase and superoxide dismutase) in spring wheat tissues in the phase of ear was determined. The change in the activity of enzymes is the response of the body to external influences, in particular the actions of NP.

Peroxidase activity in roots of spring wheat in all variants did not exceed the critical difference with a control ($\pm 30\%$), which is considered as the limit for normal plant development.

Therefore, the plant does not experience any stress disrupting the processes of electron transfer and neutralization of free radicals within the concentration range of nanoparticles from 0.1 to 5.0 g / hsr.

The activity of superoxide dismutase in roots and green mass of spring wheat significantly exceeded the control at all concentrations of cobalt and copper NP. Under the action of humic acids in the entire concentration range, the activity of superoxide dismutase remained below the control. Therefore, the effect of metal nanopowders and humic acids (to a lesser extent) has a beneficial effect on plants growth and development.

For comparison, the enzyme activities in sprouts of experimental crops in laboratory experiments on day 7 of their seed germination in a liquid nutrient medium with salts of copper and cobalt, at concentrations causing inhibition, were determined. The results are consistent with the findings that salt (copper sulfate and cobalt chloride) concentrations of 100 g / hsr are critical for plants. Changes in the activity of plant enzymes were 60-80 %, which confirms their danger at high concentrations.

The activity of catalase, an enzyme that in the process of metabolism destroys the formed hydrogen peroxide to water and molecular oxygen, in sunflower plants was within acceptable limits. The change in catalase activity in spring wheat plants with an increase in concentration reliably exceeds the control, both in roots and in green mass, but does not exceed 30 %.

Thus, it is assumed that the effect of metal NPs on plants is different from the action of salts and natural sources of biologically active compounds, humic acids. Metal NPs, unlike microelements and natural suppliers of biologically active compounds, humic acids, have some energy potential and are biological catalysts. They also have bactericidal properties and can complement and enhance traditional plant protection preparations. Their action is based on the fact that in soil conditions they gradually oxidize, creating conditions on the surface of the seeds that are unfavorable for the habitat of pathogenic microflora[32]. At the same time, the least energy-intensive cell membranes of bacteria, deprived of protective functions and access to oxygen, in particular, as a result of inhibition of respiratory chain enzymes, are affected (unlike plants and living beings).

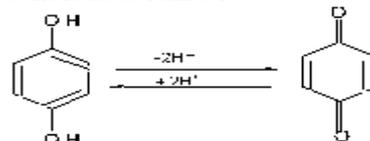
The lack of metals accumulation in the soil after pretreatment indicates a high penetrating ability of nanoparticles in plant cells. Unlike ions, nanoparticles do not possess a charge, so they do not form large complexes with carrier proteins, but they can freely pass through the pores of the plasmodesm in the bilipid layer of cell membranes whose size (50 nm) is commensurable with the size of nanoparticles (25-45 nm) and get into the cytoplasm. The redox potentials of copper and cobalt NPs in an aqueous medium are more negative than that of metals in the macro state and are -0.5V and -1.6V, respectively, approaching the potential of iron -0.44V, which is characterized by the reaction:



Metals in the nanostate are stronger reducing agents that allows them to realize the formation of protons in the aquatic environment by the reactions:



Indeed, the pH value after ultrasound treatment of nanoparticles has approximately the same pH values from 5.35 to 5.9 for 0.02 % aqueous solutions of cobalt and copper nanoparticles, therefore, the medium is acidic. Thus, once inside the cell NP, being the reducing agent and supplier of protons in the central vacuole of the seed cell, increases their number. Acidification increases the cells elasticity. Extensin, a protein that catalyzes the pH-dependent stretching of cell walls, plays an important role in this. The central vacuole transports protons and electrons to the mitochondria using known proton transfer vectors, ubiquinones (vitamin K1), having the corresponding transition potential from the oxidized to the reduced form 0.1 V.



An increase in the number of protons and electrons in the mitochondria causes more active synthesis of ATP, moreover, inorganic phosphate is usually present in sufficient quantity and is not a limiting factor. With an increase in the functional activity of cells ATP is expended on energy-dependent processes, as a result of which the concentration of ADP increases, and this in turn leads to an increase in the rate of electron transfer and the intensity of oxidative phosphorylation [33].

4. Discussion

Since nanoparticles consist of metal particles (99.9 %) with a large surface area, they have high reactivity, therefore, in the cell they can play the role of not trace elements, namely, energy suppliers. We believe that it is the starting processes of the transport of H⁺ ions into the matrix and an increase in the amount of ATP lead to an intensive growth of the plant root system, thereby increasing the amount of nutrients from the soil. Due to a larger amount of nutrients, the leaf surface increases and, as a result, a greater amount of solar energy is accumulated by the plant, which leads to an increase in crop yields.

It is necessary to take into account the dose-dependent biological dependence of nanoparticles. The objects of study, seeds germination energy and their morphological-physiological parameters, the results of which show that "small" doses are more effective, since the organisms, apparently, do not have a system of protection against them, and their signal stimulates the seed germination processes more. In the area of "small" doses per unit mass of grain, the effect is much higher than with "large" doses, but the effect of nanoparticles goes to a certain limit when increasing concentration.

5. Conclusion

- NP of copper, cobalt and humic acids in the ultradispersed state prove their biological activity, as a result of which, in almost all cases, the yield of seeds exceeded the control. The highest yield indicator was found at concentration of copper NP 0.1 g / hsr and exceeded the control by 18.74 %. The seed yield in the variants with copper nanoparticles was higher than the control up to concentration of 100 g / hsr and only at concentration of 500 g / hsr, the yield was lower than the control value by 21.25 %.

- The effect of using nanoparticles was similar in all crops, but higher by 8-12 % than when using humic acids at the same concentrations. The effect of metal NPs on plants differs from the action of salts and natural sources of biologically active compounds - humic acids. Metal NPs, unlike microelements and natural suppliers of biologically active compounds - humic acids, have energy potential and are biological catalysts.

- Wheat seeds pretreatment with copper sulphate at concentration of 20 g / hsr contributed to 6.2 % yield increase, which is the maximum value among the considered sulphate treatment options.

With an increase in the concentration of copper sulfate to 100 g / hsr the yield of spring wheat seeds was lower than the control by 18.75 %, which proves the toxic effect of copper sulfate as a micro fertilizer.

- The activity of antioxidant enzymes in sprouts of spring wheat in all variants did not exceed the critical difference with the control (± 30 %), which is considered as the limit for normal plant development. Therefore, the plant organism within the concentrations of nanoparticles from 0.1 to 5.0 g / hsr does not experience any stress disrupting the processes of electron transfer and neutralization of free radicals.

- NPs are consumed gradually, generating necessary hydrogen ions and electrons, quickly included in biochemical reactions at the time of formation. Thus, a prolonged effect of plant nutrition from a huge specific surface is achieved (hundreds of square meters per 1 gram of substance).

- NPs are introduced in micro doses and do not pollute the environment. Participating in the processes of electron transfer they enhance the action of enzymes that transfer nitrates to ammonium nitrogen, expand possibilities of affecting the cells respiration, photosynthesis, synthesis of enzymes and amino acids, carbohydrate and nitrogen metabolism, and directly to mineral nutrition of plants.

- They are environmentally safe. Working with them does not require any additional equipment. They are used in minimum quantities and have a prolonged and, especially important bactericidal action.

- Monitoring of the developed nano-technological processes and nanomaterials confirms that the application of NPs in crop production increases tolerance to unfavorable environmental factors and the yield.

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