

The productivity of spring barley when using cobalt nanoparticles and liquid-phase biological product

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Abstract. The purpose of this research is to study the effect of growth activators on the yield and quality of spring barley products. For the most complete disclosure of the potential yield of spring barley against the background of the application of minimal doses of organic fertilizers, cobalt nanoparticles were used, as well as a liquid-phase biological product (LPBP) in various combinations (NPCo, Compost, Compost + NPCo, Compost+ LPBP 1%, Compost+ LPBP 2%, Compost+ LPBP 1%+ NPCo, Compost+ LPBP 2%+ NPCo). The size of cobalt nanoparticles was 40–60 nm, the phase composition - Co - 100%. A suspension of nanoparticles was obtained by dispersing with ultrasound in an aqueous solution in accordance with the requirements of the technical conditions. LPBP is a dark brown liquid with a specific odor, pH = 6.5–7.5, contains N, C, P, K, Ca, Mg, tryptophan, microorganisms. Cobalt nanopowder in solution contained 0.01 g per hectare seeding rate (20 mg L⁻¹), LPBP concentration was 1% and 2%. Compost was used as an organic fertilizer; it consisted of 90% of cattle manure and 10% of poultry manure at a dose of 20 t ha⁻¹. The seeds were soaked 30 minutes before sowing in double distilled water, in a suspension of nanoparticles and LPBP in accordance with the experimental options. The analysis of the results of the research has shown that the studied preparations have a stimulating effect, contribute to an increase in resistance to suboptimal weather conditions, an increase in productivity and product quality. It was noted that the best indicators were obtained with joint pre-sowing treatment of seed material by cobalt nanoparticles with 1% solution of a liquid-phase biological product. Thus, there was an activation of growth processes, the yield increased by 35%, the content of crude protein - by 20.9%, digestible protein - by 10.9%, and crude fat - by 78.8%.

Key words: cobalt nanoparticles, liquid-phase biological product, barley, yield, nutritional value.

INTRODUCTION

Currently, spring barley is grown in almost all regions of Russia. Barley grain is used up to 70% for feed, as well as for food and technical purposes. The fertility of soil of modern agricultural landscapes for the expanded reproduction of their productivity in organic farming presupposes the rejection of the integrated use of various means of chemicalization. One of the promising areas for increasing yields and product quality in

agricultural science and production is the use of various physiologically active substances. Pre-sowing seed treatment by biological stimulants in order to protect them from pests, diseases and stimulate germination is considered by scientists as an effective means of eliminating environmental pollution.

Among the numerous methods of increasing the productivity of grain crops, pre-sowing treatment is becoming more and more popular, it contributes to the disinfection of seeds from fungal and bacterial diseases, and also provides an increase in immunity and vigor of plant germination (Polishchuk et al., 2015; Efremova, 2016; Tombuloglu et al., 2020b). There are various methods of seed treatment - chemical, biological, physical impact. All methods are well researched, quite effective and do not require large energy and material costs in comparison with the treatment of crops during the growing season, pre-sowing soil preparation, fertilization, therefore they have found wide application not only among researchers, but also among agricultural producers and in personal subsidiary plots.

One of the safest methods is biological seed treatment - biological products, hormonal preparations, various natural growth regulators, nanomaterials and other growth regulators of multifunctional value, which, along with an increase in crop productivity, increase their resistance to unfavorable environmental factors, disease resistance, and nutrient assimilation and at the same time they are environmentally friendly (Polischuk et al., 2018; Chernikova et al., 2019; Polischuk et al., 2019; Seregina et al., 2020).

The polyfunctional liquid-phase biological product LPBP was developed at the FSBSI All-Russian Research Institute of Reclaimed Lands, its distinctive features are agronomically useful microflora, as well as physiologically active substances and nutrients for plants. LPBP enhances biochemical processes of plant growth and development, photosynthetic reactions, additional assimilation of nutrients, predominant accumulation of nitrogen in generative organs, etc. The effect of LPBP on growth and development is associated with an increase in metabolic processes, in particular, with the transformation of hard-to-reach organic soil compounds (Rabinovich et al., 2009 and 2015).

Experimental data on grain yield demonstrated a good response of plants to the treatment of spring wheat seeds in 1% of LPBP concentration - the increase in actual yield averaged 7% (Rabinovich et al., 2019).

Also, modern nanotechnology and nanomaterials are used to increase food production (Duhan et al., 2017; Ma et al., 2018).

Interest in the use of nanoparticles (NPs) in plant growing and agricultural practice is associated with their unique properties. Long-term studies of dispersed systems and NPs in particular have revealed the following features of the biological action of NPs. NPs have low toxicity, 7–50 times lower than the toxicity of metals in ionic form; have a prolonged and multifunctional effect; stimulate metabolic processes; easily penetrate into all organs and tissues; their biological activity is associated with the peculiarity of the structure of particles and their physicochemical characteristics; NPs exhibit a synergistic effect with natural polysaccharides (Rakhmetova et al., 2015; Olkhovskaya et al., 2019; Tombuloglu et al., 2020a).

A special place is occupied by research related to the pre-sowing treatment of seeds by nanoparticles, which makes it possible to increase the yield up to 30–40% (Churilov et al., 2019, 2020). The close attention of researchers to the use of NPs in the practice of

pre-sowing seed treatment is due to the fact that more intensive seed germination contributes to a lower consumption of reserve nutrients by the seed, its productive respiration and growth.

Cobalt occupies an important place among nanoscaled materials. Cobalt participates in the biological processes of plants, animals and humans, being an irreplaceable element, therefore, special attention should be paid to the analysis of the effect of nanosized cobalt on the main indicators of biological processes. The influence of cobalt on the intensity of photosynthesis, as well as an increase in the content of ascorbic acid in plants, was noted. The lack of cobalt can cause disturbances in nitrogen metabolism, a decrease in the amount of chlorophyll, carotenoids, vitamin E in the leaves. For a long time, cobalt was considered a trace element necessary only for animals. But at present, its usefulness or necessity for higher plants has also been proven (Minz et al., 2018).

The main function of cobalt is associated with its participation in fixing atmospheric nitrogen in the nodules of leguminous and non-leguminous plants. The participation of cobalt in the life of higher plants incapable of nitrogen fixation is specific or indirect. The metal stimulates cellular reproduction of leaves by increasing the thickness and volume of the mesophyll in the leaves, the size and number of cells in the columnar and spongy leaf parenchyma. The influence of cobalt on the formation and functioning of the photosynthetic apparatus of plants through the concentration of chloroplasts and pigments in the leaves was established. This is linked with an increase in the volume of the plastid apparatus due to the growth of organelles (Elizareva et al., 2018).

However, at present, a number of basic issues remain unresolved. These issues are related to the mechanisms on that how nanoparticles affect fluid translocation and photosynthesis, what chemical transformations bio-transformations of nanoparticles occur (or do not happen), how are effects electron transport chain, Calvin cycle and other photosynthetic compartments, how nanoparticles affect the soil organisms and plant performances (Rajput et al., 2019).

The purpose of our research was to study the effect of growth activators on the yield and quality of spring barley products. For the most complete disclosure of the potential yield of spring barley against the background of the application of minimal doses of organic fertilizers, cobalt nanoparticles were used, as well as a liquid-phase biological product (LPBP) in various combinations.

MATERIALS AND METHODS

The research was carried out in lysimeters designed by All-Russian Scientific Research Institute of Hydraulic Engineering and Land Reclamation with an undisturbed soil profile. The area of stationary field lysimeters is 1.13 m².

Lysimeters are charged with gray forest soil. Samples taken from a depth of 0–25 cm at the beginning of the experiment characterize the soil with a low content of organic matter from 3.8% to 5.4% (on average 4.6% ± 0.6).

The acid-base reaction of the soil is weakly acidic, pH from 5.3 to 6.4 (on average 5.7 ± 0.1). The provision of soil with mobile nutrients on average was as follows: potassium content - 833.5 mg kg⁻¹, total nitrogen - 0.12%, mobile phosphorus - 128 mg kg⁻¹, which characterizes this soil as averagely provided by these elements.

Nanoparticles produced at the Moscow Institute of Steel and Alloys (Dzidziguri et al., 2000). The size of cobalt nanoparticles was 40–60 nm, the phase composition Co - 100%. A suspension of nanoparticles was obtained by dispersing with ultrasound in an aqueous solution. LPBP is a dark brown liquid with a specific smell, pH = 6.5–7.5, contains N, C, P, K, Ca, Mg, trace elements: copper, zinc, manganese, iron, as well as tryptophan and microorganisms. The content of total nitrogen in the composition of LPBP is 0.2–0.5 g L⁻¹, mobile forms of potassium (K₂O) and phosphorus (P₂O₅) - 9.5 and 10 g L⁻¹, respectively. The concentration of the toxic elements: lead, mercury, nickel and arsenic is significantly lower than their maximum permissible concentrations.

Cobalt nanopowder in the solution contained 0.01 g per hectare of seeding rate, which corresponded to 20 mg L⁻¹, the concentration of LPBP was 1% and 2%. The compost was used as an organic fertilizer in all variants of the experiment, it consisted of 90% of cattle manure and 10% of poultry manure at a dose of 20 t ha⁻¹.

The seeds were soaked 30 minutes before sowing in double distilled water, in a suspension of nanoparticles and LPBP in accordance with the experimental variants (Table 1).

Table 1. The scheme of the experiment

No. of variants	Experiment variants	Abbreviations of names in variants in tables
1	Control	Control
2	Cobalt nanoparticles	NPCo
3	Compost (20 t ha ⁻¹)	Compost
4	Compost + Cobalt nanoparticles	C+ NPCo
5	Compost + Liquid-phase biological product 1%	C+ LPBP 1%
6	Compost + Liquid-phase biological product 2%	C+ LPBP 2%
7	Compost + Liquid-phase biological product 1%+ Cobalt nanoparticles	C+ LPBP 1%+ NPCo
8	Compost + Liquid-phase biological product 2% + Cobalt nanoparticles	C+ LPBP 2%+ NPCo

The spring barley variety ‘Kati’ was chosen as the object of the research. The mass of 1,000 grains is 46–56 g. It is grain fodder. The protein content is 10.9–14.5%. It is susceptible to root rot.

Sowing was carried out on May 2, 2020. The technology of growing spring barley in lysimeters mimicked the one generally accepted for gray forest soil in this region, but with some peculiarities of lysimetric research. On the plots, grooves were made 3–4 cm deep, at a distance of 12 cm, the seed rate was distributed at the rate of 55 grains per 1 running meter. In the phase, the pips was pulled in and 50 pieces were left per 1 running meter. Agrotechnology of growing crops is generally accepted for this zone. The growing season was 82 days. The barley was harvested in the full ripeness phase. Plants were cut at a height of 1–2 cm from the root collar, placed in bags indicating the variant number and repetition.

In the laboratory, the aboveground mass crop was dried to constant weight. After threshing the grain, the refined grain was weighed. Samples were taken from the crop by repetition, numbered, according to the registration journal, for sending to the laboratory. The mass of 1,000 seeds, grain moisture, nutritional value (crude protein, digestible protein, crude fat) were determined using standard determined by GOST methods.

Statistical processing of the results of the experiments was carried out by the method of variance analysis (Microsoft, Excel 2010). The shown significance is $p < 0.05$.

RESULTS AND DISCUSSION

Of greatest interest for the crop industry is the actual yield indicator - the volume of production per unit of sown area. In the research, the gross product yields for all variants of the experiment exceed the control value by 3.4–35.0%. The highest yield was obtained with pre-sowing treatment of barley seeds in a suspension of cobalt nanoparticles and a liquid-phase biological product at a concentration of 1% and the use of compost (variant 7) - 50.22 c ha^{-1} , which exceeds the indicator for the control variant by 35.0% (Table 2). Moreover, with the separate application of compost with a suspension of cobalt nanoparticles in the 4th variant and compost with a liquid-phase biological product in the same concentration (variant 5), the yield is significantly lower (41.25 c ha^{-1} and 41.42 c ha^{-1} , respectively). The improvement of plant growth and development could be facilitated by the formation of additional channels in seed cells under the influence of metal nanoparticles, which ensured the bioavailability of micro- and macroelements that make up LPBP.

It should be noted that with an increase in the doses of the liquid-phase biological product to 2%, a significant decrease in the yield of barley was observed, this is demonstrated by the 8th variant of the experiment, the result is directly related to the inhibition of plant growth and the formation of caryopsis.

The main indicator of the quality of barley received for storage is its moisture content. The presence of liquid in the cereal is determined at the acceptance stage. Water makes changes in the grain, setting in motion a mechanism for activating the growth of microorganisms. The water content in the ‘body’ of the cereal suggests a lack of nutrients and a predisposition to shorten the shelf life expiration. An unacceptable level of humidity provokes the activation of chemical and physiological activity. The barley structure swells, the movement of enzymes turns on, the breakdown of biopolymer compounds, and the cereal germinates. The grain becomes friable, flowability and protection from machine impact are disturbed and the nature indicator falls. The level of moisture in the structure of the cereal negatively affects the biochemical and physical parameters that determine the food and market value of barley. Without violating the structural composition of carbohydrates, proteins and fats, it is impossible to remove chemically bound moisture. In this state, water is no longer a solvent, being molecularly linked to hydrophiles. Its removal will inevitably affect the change in the barley structure and its technological properties.

Table 2. The effect of cobalt nanoparticles and liquid-phase biological product on barley yield

No.	Experimental variants	Harvest of main products (c ha^{-1})		
		average	change %	c ha^{-1}
1	Control	37.20	-	
2	NPCo	40.76	9.6	3.6
3	Compost	41.25	10.9	4.1
4	C+ NPCo	41.25	10.9	4.1
5	C+ LPBP 1%	41.42	11.3	4.2
6	C+ LPBP 2%	43.87	17.9	6.7
7	C+ LPBP 1%+ NPCo	50.22	35.0	13.0
8	C+ LPBP 2%+ NPCo	38.48	3.4	1.3
	<i>LSD</i> _{0.95}	1.96		

The limit value of the moisture content of barley grain established by the standards is 15%. For the presented samples, the parameter is in the range of 10.46–10.78% and characterizes its condition as dry, which will reduce energy costs for processing and storage, however, excessively dried grain is devoid of elasticity, because of which an increase in the ash content of flour is possible (Table 3).

Table 3. Physical and energy indicators of barley grain

No.	Experimental variants	Moisture, %	Exchangeable E, MJ	Feed units, kg
1	Control	10.46	12.0	1.17
2	NPCo	10.68	12.2	1.20
3	Compost	10.74	12.2	1.21
4	C+ NPCo	10.48	12.2	1.19
5	C+ LPBP 1%	10.70	12.2	1.22
6	C+ LPBP 2%	10.57	12.1	1.18
7	C+ LPBP 1%+ NPCo	10.78	12.4	1.25
8	C+ LPBP 2%+ NPCo	10.77	12.1	1.21
	<i>LSD</i> _{0.95}	-	-	0.02

Considering that barley is cultivated mainly as a fodder crop, it is important to assess the energy potential of the obtained samples. In all variants of the experiment, the value of the exchange energy indicator was practically at the same level - 12.0–12.2 MJ, slightly higher - in the variant with the use of C + LPBP1% + NPCo. In all experimental samples, the content of feed units in the grain was optimal (with an average standard value of 1.13), the highest indicator was 1.25 kg and was also detected in the 7th variant of the experiment.

Table 4. Nutritional value of barley grain

No.	Experimental variants	Crude protein, %	Crude fat, %	Digestible protein, g
1	Control	11.57	0.80	10.1
2	NPCo	12.81	1.10	10.2
3	Compost	12.16	1.10	10.2
4	C+ NPCo	13.05	1.01	10.4
5	C+ LPBP 1%	13.61	1.34	10.9
6	C+ LPBP 2%	13.39	0.96	10.7
7	C+ LPBP 1%+ NPCo	13.99	1.43	11.2
8	C+ LPBP 2%+ NPCo	12.10	1.03	10.1
	<i>LSD</i> _{0.95}	0.52	0.06	0.19

Crude feed protein is the main source of nitrogen for animals, which is necessary for reproduction, milk formation, and deposition in the body. For cereal grains, its content varies from 10% to 15% in 1 kg of feed. A deficit of even 1% of fodder protein in the ration of livestock leads to an overconsumption of 2–3.5% of feed and, ultimately, to an increase in production costs. The grain products obtained as a result of the experiment correspond to the average parameters for the culture, the maximum obtained value of the fraction of crude protein calculated on dry matter is 13.99% and is presented in the experimental version when using C + LPBP 1% + NPCo (Table 4).

Of great importance in the composition of animal feed is the content of crude fat in it, which optimizes the normal functioning of the digestive glands, forms the structure of the protoplasm of all cells and accumulates energy reserves in the body. According to the experimental variants, its share in the dry matter composition was 0.8–1.43% (with an average value for cereal grains of about 2%), the highest indicator was also in the 7th variant of the experiment.

The share of digestible protein in the samples ranged from 10.1 to 11.2% with the highest value in the 7th variant. Probably facilitated by the action of cobalt nanoparticles access to the cells of the macronutrients of magnesium and potassium, which are involved in the synthesis of fats and proteins, activate the work of enzymes that promote the formation of proteins and fats in the grain of the experimental culture in the variant with the use of C + LPBP1% + NPCo proteins and fats are the largest.

CONCLUSIONS

From the obtained data, it can be concluded that the pre-sowing treatment of barley seeds with cobalt nanoparticles and liquid-phase biological product does not have a toxic effect and contributes to an increase in yield. Preliminary soaking of spring barley seeds in a suspension of cobalt nanoparticles at a dose of 0.01 g per hectare of seeding rate and 1% solution of a liquid-phase biological product against the background of organic matter showed the best result, which led to an increase in yield by 13 c ha⁻¹ (35%). At the same time, there was no decrease in the quality indicators: exchange energy and feed units. The amount of crude protein increased by 20.9%, digestible protein - by 10.9%, crude fat - by 78.8% compared to the control version of the experiment.

REFERENCES

- Chernikova, O., Mazhayskiy, Y. & Ampleeva, L. 2019. Selenium in nanosized form as an alternative to microfertilizers. *Agronomy Research* **17**(1), 974–981. doi: 10.15159/AR.19.010
- Churilov, D.G., Polischuk, S.D., Churilov, G.I., Churilova, V.V. & Byshova, D.N. 2020. Investigation of the long-term toxic effect of nanoparticles of different physical-chemical characteristics. *Agronomy Research* **18**(3), 1973 – 1991. doi: 10.15159/AR.20.186
- Churilov, D.G., Polischuk, S.D., Churilova, V.V., Churilov, G.I., Borychev, S.N. & Arapov, I.S. 2019. Biocompatibility conditions and biological activity of cobalt nanoparticles, depending on the size and concentration. *International Journal of Nanotechnology* **16**(6–10), 522–539. doi: 10.1504/IJNT.2019.106623
- Dzidziguri, E.L., Kuznetsov, D.V., Lyovina, V.V. & Sidorova, E.N. 2000. Perspective materials 6 Forestry 2019 IOP Conf. Series: *Earth and Environmental Science* **7** 92.
- Duhan, J.S., Kumar, R., Kumar, N., Kaur, P., Nehra, K. & Duhan, S. 2017. Nanotechnology: The new perspective in precision agriculture. *Biotechnol Rep (Amst)*. **15**, 11, doi: 10.1016/j.bTP.2017.03.002
- Efremova, Y.V. 2016. Growth stimulators as part of sustainable agriculture. *Russian Journal of Agricultural and Socio-Economic Sciences* **4**(52), 86–91. doi:10.18551/rjoas.2016-04.10
- Elizareva, E.N., Yanbaev, Y.A., Redkina, N.N., Kudashkina, N.V., Baykov, A.G. & Smirnova, A. P. 2017. Influence of some heavy metals compounds on the process of radish sprouts formation. *Modern problems of science and education*. **6**, 252.
- Ma, C., White, J.C., Zhao, J., Zhao, Q. & Xing, B. 2018. Uptake of Engineered Nanoparticles by Food Crops: Characterization, Mechanisms, and Implications. *Annu. Rev. Food. Sci. Technol.* **9**, 129–153. doi: 10.1146/annurev-food-030117-012657

- Minz, A., Kumari, Sinha A., Kumar, R., Kumar, B., Prerna Deep, K. & Bhushan Kumar, S. 2018. A Review on Importance of Cobalt in Crop Growth and Production. *Int. J. Curr. Microbiol. App. Sci.* **7**, 2978–2984.
- Olkhovskaya, I., Bogoslovskaya, O., Jablokow, A. & Glushchenko, N. 2019. Yield indicators of spring barley after pre-sowing seed treatment by metal nanoparticles. *Russian nanotechnology* **14**. 55–62. doi: 10.21517/1992-7223-2019-1-2-55-62. (in Russian).
- Polischuk, S., Fadkin, G., Churilov, D., Churilova, V. & Churilov, G. 2019. The stimulating effect of nanoparticle suspensions on seeds and seedlings of Scotch pine (*Pinus sylvestris*). 2019. *IOP Conference Series: Earth and Environmental Science* **226**(2019), 012020. doi: 10.1088/1755-1315/226/1/012020
- Polischuk, S.D., Nazarova, A.A., Churilov, G.I., Borychev, S.N. & Byshov, N.V. 2018. Nanopowders of cuprum, cobalt and their oxides used in the intensive technology for growing cucumbers. *International Journal of Nanotechnology* **15**(4–5), 352–369. doi: 10.1504/IJNT.2018.094792
- Polishchuk, S.D., Nazarova, A.A., Kutskir, M.V., Churilov, D.G., Ivanycheva, Y.N., Kiryshin, V.A. & Churilov, G.I. 2015. Ecologic-biological effects of cobalt, cuprum, copper oxide nano-powders and humic acids on wheat seeds. *Modern Applied Science* **9**(6). 354–364. doi:10.5539/mas.v9n6p354.
- Rakhmetova, A., Bogoslovskaya, O., Olkhovskaya, I., Zhigach, A., Ilyina, A., Varlamov, V. & Glushchenko, N. 2015. Concomitant action of organic and inorganic nanoparticles in wound healing and antibacterial resistance: Chitosan and copper nanoparticles sin an ointment as an example. *Nanotechnologies in Russia* **10**, 149–157. doi:10.1134/S1995078015010164
- Rabinovich, G.Yu., Fomicheva, N.V. & Smirnova, Yu.D. 2009. A method of obtaining a liquid-phase biological agent for plant growing and agriculture. RF Patent No. 2365568 (in Russian).
- Rabinovich, G.Yu., Smirnova, Yu.D., Vasilyeva, E.A. & Fomicheva, N.V. 2015. Innovative technology for solving problems of agroecology. *Regional ecology* **6**(41), 7–15 (in Russian).
- Rabinovich, G.Yu., Smirnova, Yu.D. & Bulycheva, V.O. 2019. The effectiveness of the pre-sowing treatment of spring wheat seeds by the biological product LPBP. *Bulletin of Science and Practice* **5**(6), 137–144. doi: 10.33619 / 2414-2948 / 43/18 (in Russian).
- Rajput, V., Minkina, T., Sushkova, S., Chokheli, V. & Soldatov, M. 2019. Toxicity assessment of metal oxide nanoparticles on terrestrial plants. *Comprehensive Analytical Chemistry* **87**, 189–207. doi: 10.1016/bs.coac.2019.09.003
- Seregina, T., Chernikova, O., Mazhayskiy, Y. & Ampleeva, L. 2020. Features of the influence of copper nanoparticles and copper oxide on the formation of barley crop. *Agronomy Research* **18**(1), 1010–1017. doi: 10.15159/AR.20.025
- Tombuloglu, H., Ercan, I., Alshammari, T., Tombuloglu, G., Slimani, Y., Almessiere, M. & Baykal, A. 2020a. Incorporation of Micro-nutrients (Nickel, Copper, Zinc, and Iron) into Plant Body Through Nanoparticles. *Journal of Soil Science and Plant Nutrition* **20**, 1872–1881. doi: 10.1007/s42729-020-00258-2
- Tombuloglu, H., Slimani, Y., Tombuloglu, G., Alshammari, T., Almessiere, M., Korkmaz, A.D., Baykal, A. & Samia A.C. 2020b. Engineered magnetic nanoparticles enhance chlorophyll content and growth of barley through the induction of photosystem genes. *Environmental Science and Pollution Research* **27**, 1–11. doi: 10.1007/s11356-020-09693-1