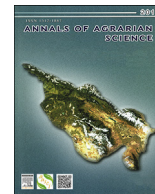




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## The effect of humic substances on winter wheat yield and fertility of ordinary chernozem

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### ABSTRACT

The effect of humic preparation BIO-Don on the productivity of winter wheat and soil properties has been studied in small plot and production experiments on ordinary carbonate chernozem. It was shown that pre-sowing seed treatment, the treatment of vegetative plants and soil with the preparation contributes to higher grain yields in comparison with the control variant. This effect is due to the presence of physiologically active substances - humic acids in the preparation. The positive effect on the high crop yield formation is also based on high availability of mobile forms of phosphorus on the variants treated with humic substances. This is due to the active control mechanism through root exudates and an increase in the number of microorganisms, which leads to mobilization of phosphorus by the plants that were treated with humic substances during the vegetation.

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### Introduction

The research summarized in the well-known monograph [1], has proved that humic substances have a stimulating and adaptogenic effect on the cellular and subcellular levels. Under the influence of humic substances the intensity of respiration, photosynthesis and water exchange are increased, the concentration of chlorophyll and ascorbic acid also grows, there is an impact on the processes of transcription and translation of the protein-synthesizing system, on the state of ribosomes, on the mitotic activity of meristematic tissue, the permeability of membranes is increased.

A broad spectrum of humic substances exist currently. Initially they were obtained from lignite and peat, but during the last twenty years, vermicomposts are increasingly used as a raw material for their production. The content of humic substances in that material is significantly lower than in lignite or peat, however, vermicompost is a renewable resource and contains a wide range of biologically active compounds: the waste products of microorganisms and earthworms.

### Materials and methods

Humic preparation BIO-Don is obtained by alkaline extraction of vermicompost. The preparation has an alkaline reaction (pH = 8,7), contains a relatively low amount of nutrients and is used in very low concentrations, so it cannot be regarded as an analogue of mineral fertilizers and the mechanism of its positive effect on the plants is different. The composition contains 2.24 g/l of humic acid. Numerous experiments have shown that humic substances are growth stimulators and adaptogens, which means that they relieve the stress after application of pesticides or minimize the negative effects of adverse weather conditions. Prior to the plants and soil treatment, the preparation is diluted to the optimum concentration of 0.001%.

The determination of several groups of microorganisms has been carried out: bacteria that use organic forms of nitrogen in the environment were enumerated by plate-counts on nutrient meat-peptone agar, the bacteria using mineral forms of nitrogen were enumerated on starch-ammonia agar, oligonitrophilic and nitrogen-fixing bacteria - on nitrogen free Ashby medium, coliform bacteria on Endo medium. The maximum number of colonies was detected on nutrient meat-peptone agar ( $2,75 \pm 0,17 \cdot 10^4$  CFU/ml of the preparation), and 78% of them were spore-forming bacteria belonging to genus *Bacillus*, which is due to the conditions of production of the biological product and the pH value. The presence of at least three different species of this genus was noted (presumably,

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based on colony morphology). These bacteria are non-pathogenic members of the normal soil microflora, they are also active hydrolitics involved in the decomposition of fresh organic material. Besides the spore-forming bacteria of the genus *Bacillus*, gram-positive cocci were found in low numbers, allegedly belonging to *Rhodococcus*, or *Micrococcus* genus (biochemical identification was not carried out). No nitrogen-fixing bacteria capable to grow actively on Ashby medium were found, only some oligonitrophilic microorganisms growing slowly on trace amounts of nitrogen. Coliform bacteria were also not detected, and no colonies at all were found growing on Endo medium, indicating a complete lack of Gram-negative bacteria in the preparation.

Thus, the preparation possess no microbiological threat, and even contains some valuable from an agronomic point of view bacterial cultures (*Bacillus* are included in the composition of many bacterial fertilizers). However, the contribution of microorganisms contained in the product in the mechanism of its biological activity is negligible in view of their low numbers, and the main role belongs to the humic substances.

To study the effectiveness of humic preparation for winter wheat several experiments have been performed since 2008.

On the territory of pilot production facility Nedvigovka the field experiment was performed. Soil type was ordinary carbonate chernozem Plot area - 50 m<sup>2</sup>. All experiments were performed in quintuplicate. The dose of the preparation for treatment of soil with humic preparation was 4 l/ha. Treatment with biologically active substances was held twice: in the autumn and topical treatment of shoots in spring in the phase of tillering. Winter wheat variety was "Zernogradsky-11". The effect of humic preparations was assessed by the dynamics of nutrients in the soil and by increase of the yield.

At the production facility FGBNU "DZNIISKH" a production experiment was held according to the scheme shown in Table 3. Soil type was ordinary carbonate chernozem, chosen crop was winter wheat variety "DonEco".

Soil samples were collected a month after the treatment of plants with humic substances, in the spring at the phase of tillering - booting, and in summer in the phase of grain ripening and harvesting. The samples were analyzed in the laboratory and the content of mobile forms of nitrogen - ammonium (GOST 26489-85), nitrate (GOST 26951-86), phosphorus and exchangeable potassium (GOST 26205-91), humus (GOST 26213-91), as well as the number of microorganisms by plate counting on solid nutrient media: copiotrophic bacteria (ammonifiers) on meat-peptone nutrient agar, copiotrophic (aminoautotrophic) on, oligotrophic microorganisms on water agar (non-filamentous bacteria and actinomycetes), actinomycetes on starch-ammonium agar, oligonitrophilic microorganisms on nitrogen-free Ashby medium, soil fungi on Czapek's sucrose-nitrate medium, cellulolytic actinomycetes and fungi - on Hutchinson medium supplemented with cellulose as sole carbon source [2]. The structural state of soil was monitored (dry and wet sieving method by Savvinov). The enzymatic activity of catalase and invertase was also accessed. The results were processed by methods of variation statistics [3].

At the time of planting the soil content of the mobile elements was estimated to be of medium enrichment with phosphorus and potassium, with middle humus content and medium activity of catalase and invertase. Soil moisture at planting was optimal, plowing and partition of the soil was good, there were no interruptions in sowing.

## Results and discussion

The results of the small plot experiment in pilot production facility "Nedvigovka" have shown that the use of humic preparation increases the availability of phosphorus to plants (Table 1). By the

phase of tillering the enrichment of soil with mobile phosphorus on the plot treated with humic substances can be already estimated as "high" - 5.53 mg/100 g soil. In the control options the enrichment with phosphorus was estimated as "medium", and the difference in the level of enrichment was statistically significant.

In the phase of booting the enrichment with mobile forms of phosphorus at the control variant moves into the category of "elevated" - 4.25 mg/100 g soil, while in the variant treated with humic preparation it remains high. In the phase of ripening grain all variants are characterized by high enrichment with mobile forms of phosphorus. However, the difference between the variants is not statistically significant, therefore, it is within the experimental error. Considering the dynamics of available phosphorus in the control variant, it can be seen that by the summer its content gradually increases, thus, the improvement of water and temperature conditions contributes to transfer of insoluble phosphorus into the mobile state, however, the use of humic preparation activates the mobilization of phosphorus.

The measuring of yield results has shown that the variant with humic substances obtained a significant increase of winter wheat yield of 7.2 hwt/ha of grain (the lowest average difference<sub>05</sub> = 2.02), which corresponds to the yield increase of 22.5%.

Thus, small plot experience has shown that the treatment with humic preparation contributes to obtaining higher yields of grain as compared with the control variant that is caused by the content of the physiologically active substances - humic acids.

The results of production experiment at the 48 ha field of DZNIISKH facility confirmed the high efficiency of humic preparation BIO-Don. The experiment has shown that the increase of winter wheat yield treated with humic preparation BIO-Don ranged from 6.9 to 12.8 hwt/ha, which is up to 35% compared to the background - mineral nutrition recommended for this area (Table 2).

The most effective way with the existing in 2014 weather conditions was a combination of preplant and two-time topical treatment of the leaves. In this variant, the yield increase reached 12.8 hwt/ha compared with the background.

Studying the dynamics of exchangeable potassium in the soil, and mobile forms of nitrogen has shown that when using humic preparations BIO-Don a tendency to increase in these elements can be traced. But there was a significant increase in the mobility of phosphorus, which was observed in all experimental variants throughout the experiment - at the tillering stage, in the phase of tillering - booting, in the phase of ripening grain - harvesting (Fig. 1).

The content of mobile forms of phosphorus in the soil under the influence of humic preparation was increased by 1,6-3 times. This is an important consequence of the application of the active substance as ordinary carbonate chernozem is characterized by low content of mobile phosphorus. In this experiment, the initial enrichment of soil with mobile phosphorus was average (for grain crops) a month later the phosphorus content at the control plots has not changed, and in variants treated with humic substances increased by 11,6–15,9 mg/kg and was evaluated as high. In the phase of booting provision of phosphorus in all variants it was comparable with the control and was high and stable. By the end of the vegetation the phosphorus content in the control variant decreased to an average level, while in variants treated with humic substances it remained high. Given the shortage of phosphorus, characteristic of calcareous chernozems, this was one of the factors for the plant nutrition optimization.

An interesting fact is that the increase in the content of mobile phosphorus occurred in the variants where the soil was treated with the preparation, and in the cases where the soil was not treated, and only topical leaf treatment of vegetating plants was

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