Effect of biohumus and growth regulators on the content of pigments and catalase, spike productivity and grain quality of spring wheat

I N Besaliev, A L Panfilov, N S Reger, J A Karavaytsev and R R Abdrashitov
Federal Scientific Center for Biological Systems and Agrotechnologies RAS, Orenburg, Russia
E-mail: orniish_tzk@mail.ru

Abstract. The article is devoted to studying the influence of various treatment options for spring wheat seeds with biohumus and growth regulators. The results of field experiments led to the establishment of the effect of various seed treatment options on the content of chlorophyll, carotenoids and antioxidant enzymes, spike productivity indicators, grain quality indicators (nature, vitreous, raw gluten content). A positive effect of the studied preparations on the increase in the content of photosynthetic pigments, catalase, as well as the number of grains in an ear and the weight of grain per ear was established.

1. Introduction
In recent years, the interest of agricultural researchers and practitioners in biofertilizers and growth regulators has increased. This interest is due, on the one hand, to the limited use of mineral and organic fertilizers, which leads to a decrease in soil fertility and productivity, and losses in the quality of the products obtained. On the other hand, the cost of mineral fertilizers is very high, which does not always make crop production profitable. The amount of organic fertilizer is limited due to a decrease in livestock numbers. Under these conditions, the development and implementation of alternative drugs that can make up for the lack of nutrients are relevant and requires careful study.

It has been established that pre-sowing treatment of wheat and barley seeds with growth regulators (pilafen, pectin, pirafen) helps to increase the sowing quality of seeds, the formation of optimal agrocenosis density with an improvement in the activity of amylolytic enzymes [1], enrichment of the soil with microflora, contributing to the reproduction and preservation of soil fertility [2, 3].

The improvement in sowing qualities of seeds under the action of growth regulators is explained by the accelerated appearance of gibberellin substances, which affect changes in the activity and direction of metabolic processes [4].

Under conditions of increased abiotic stresses, caused primarily by the increase in aridity with increasing air temperature against the background of a lack of precipitation, it is important to study the pigment complex, which is represented by chlorophylls A and B, as well as carotenoids.

In most plants, chlorophyll A predominates over chlorophyll B, due to the wider participation of the first form in the photosynthetic process. Chlorophyll B accounts for about 15–25 % of the total chlorophyll [5, 6].

According to O.S. Amunova (2018) [7], a lack of moisture during the tillering-flowering period reduces the content of chlorophyll A and carotenoids; at the same time, variety-specificity by the
number of pigments was established, and a reliable correlation was obtained between the pigment content and the elements of wheat plant productivity.

In the work of O.S. Captain, G.A. Pryadkina, O.O. Stasik (2018) [8] found that the increase in the content of photosynthetic pigments in the late stages of ontogenesis was facilitated by the treatment of plants with a microelement complex.

Changes in the ratio of pigment-protein complexes contribute to the adaptation of the photosynthetic apparatus depending on weather conditions [9].

Some studies have shown an increase in the content of carotenoids in chloroplasts already 5–10 minutes after the start of the studied stressors (the effect is high – up to 42 °C, an increase in exogenous 1 and 10 nM H₂O₂) [10].

A positive effect of the growth regulator of pilafen in interaction with phytohormones (IAA, 6-BAP) and the trace elements of copper, as well as the organosilicon growth regulator Energy M on the potato antioxidant system was established [11, 12].

The use of Epin-extra (E.E.), the growth regulator of the brassin-steroid nature under the action of heavy metals (H.M.), contributed to a decrease in Cu²⁺ content in the roots, an increase in the length of the root and aerial parts of spring wheat, a decrease in the rate of superoxide generation and LPO intensity in leaves, and a change in catalase activity [13].

Growth regulators can be considered as a means to reduce water loss during a drought, given the positive effect on the relative conductivity of cells, transpiration rate [14, 15].

The enzyme activity level of the antioxidant system also has variety-specificity, depending on the duration of the drought and the stage of plant development [16, 17].

2. Problem statement

Arid conditions of the steppe zone create stressful situations during the growing season of crops, expressed in the suppression of growth processes during the growing season and the restriction in the distribution of dry matter and nutrients in the second half of the growing season. Under these conditions, plant resistance to water stress acts as the leading indicator of adaptability to adverse factors. The actions of growth regulators and Biohumus from activation of the antioxidant system of the plant organism, the indicators of spike productivity are studied to substantiate their use.

The study aims to study the effect of biohumus and growth regulators on the content of chlorophyll, carotenoids and antioxidant enzymes, spike productivity and grain quality of spring soft wheat in a field experiment.

3. Methods and materials

In the experiment, plant growth regulators having different contents of organic and mineral substances are used.

Siliplant is a silicon-containing fertilizer, in which, in addition to silicon Si (7 %) and potassium K (1 %), microelements (mg/l) are readily available for plants in chelating form: Fe – 300; Mg – 100; Cu – 70–240; Zn – 80; Mn – 150; Co – 15; B – 90. ANO "NEST M" develops, registered and produces this fertilizer. The flow rate during the processing of seeds of grain crops 60 ml/t, working fluid 10 l/t.

Fertilizer "Blago 3⁺⁺" includes humic acids (up to 20 g/l), N (75–80), P₂O₅ (40–50), K₂O (100–110), MgO ≥ 9, Mn ≥ 0.9, Zn ≥ 0.7, Cu ≥ 0.5, Mo ≥ 0.4, Co ≥ 0.3. The content in the extract from sapropel of fulvic acids that break down organic and mineral compounds to ions is at least 6.9 g/l. The consumption of the drug during the processing of seeds of grain crops is 1 l/t, for processing by vegetation – 0.5 l/ha. Fertilizer is produced by Natural Resources LLC (Russia).

The growth regulator “Mival-Agro” is a bioorganic plant growth regulator based on silicon. It is produced by LLC AgroSil.

Biohumus is an organic fertilizer based on the products of the vital activity of earthworms as a result of processing compost of cattle or any biological waste susceptible to decay.

“Agroverm” is liquid humic biofertilizer, the basis for the production is Vermicompost, obtained with the help of red earthworms. This fertilizer contains amino acids, phytohormones, micro and
macro elements, humic substances. It is made by the LLC BioEraGrupp company (Russia). The consumption rate is 1 litre per ton of seeds of grain crops, and during processing by vegetation – 2 l/ha.

Composition: mass fraction of organic matter not less than 15 g/l, humic acids not less than 30 g/l, fulvic acids not less than 4 g/l, macrocell complex. It is used for the presowing treatment of seeds, planting material, root and foliar top dressing of plants.

In the experiment "Biohumus + Fe$_3$O$_4$ + Mo", the seeds were treated with ultrafine particles of iron and molybdenum. The studies used the UDC Mo (100–200 nm) produced by the Plazmotherm company (Russia, Moscow). Fe$_3$O$_4$ nanoparticles (80–100 nm, z-potential of 20 ± 0.14 mV) were purchased from Advanced Paw der Technologies (Russia, Tomsk). For preparing the UDM solutions, exact weighed samples were taken, placed in glass flasks with settled tap water, and they were intensively dispersed with ultrasound at a frequency of 35 kHz for 30 min. Pre-sowing seed treatment was carried out by pre-soaking for 10-15 minutes in solutions. The seeds served as a control without preliminary treatment.

The treated seeds were sown at the experimental site of the Federal State Budget Scientific Institution of the Federal Scientific Center of BST RAS. The soil of the plot is ordinary medium-humus chernozem (4.2–4.5 %), medium-thick (40–45 cm), medium loamy mechanical composition. The pH of the soil solution is neutral. Sowing of plots carried out by seeder SN-16.

Variety of spring soft wheat "Uchitel" is mid-season. The seeding rate is 4.5 million germinating seeds per hectare.

Plant sampling was carried out in the main phases of the vegetative development of plants – tillering, exit to the tube and heading.

The content of photosynthetic pigments (chlorophylls A and B, carotenoids) was determined by the standard method with modifications of Smashevsky (2011) [18]. After harvesting, plant productivity indicators were determined: the number of grains per ear, grain weight per ear, grain quality indicators: nature (GOST R 54895-2012) [19], vitreous (GOST 10987-76) [20], the content of crude gluten (GOST R 54478-2011) [21].

Differences were considered statistically significant at $p <0.05$.

**4. Discussion**

The effect of the studied growth regulators and Vermicompost on the content and ratio of pigments in spring wheat plants varies depending on the options and phase of the growing season (Table 1).

| Experience Options | The content of chlorophyll in the phase of exit into the tube, mg/g dry weight | The ratio of chlorophyll A/B | The content of chlorophyll in the earring phase, mg/g dry weight | The ratio of chlorophyll A/B |
|--------------------|-------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Control- water     | 5.600                         | 1.325                       | 4.22                        | 2.995                       | 1.25                        |
| Biohumus           | 9.299                         | 7.158                       | 1.30                        | 6.631                       | 1.416                       |
| Biohumus + Fe$_3$O$_4$ + Mo | 4.082                   | 10.050                      | 3.89                        | 15.732                      | 3.056                       |
| Agroverm           | 8.791                         | 1.806                       | 4.87                        | 14.483                      | 3.722                       |
| Mival Agro         | 5.543                         | 2.573                       | 2.15                        | 12.636                      | 2.967                       |
| Blago 3*           | 9.817                         | 4.505                       | 2.18                        | 5.522                       | 1.140                       |
| Siliplant          | 12.630                        | 3.587                       | 3.52                        | 3.319                       | 1.022                       |

An increase in the amount of chlorophyll A in the exit phase into the tube was obtained according to the following options: Siliplant (126 %), biohumus (66 %), Blago 3* (75 %) and Agroverm (57 %). The content of chlorophyll b in this phase in the experimental variants exceeded the control, except for the combination (Biohumus + Fe$_3$O$_4$ + Mo), but most significantly when treating seeds with Biohumus (440 %), Blago 3* (240 %) and Siliplant (171 %). The ratio of chlorophyll A and B was increased in variants with a lower content of chlorophyll B, including the control.
In the earing phase, the chlorophyll content in the variants with seed treatment with preparations was relatively higher relative to the control, except for the Siliplant variant with the highest values for the Biohumus + Fe₃O₄ + Mo (321 %), Agroverm (288 %) and Mival-Agro (238 %) variants.

According to the same variants, a positive dynamics was obtained for chlorophyll, and for the period of entry into the tube, it was heading, as well as the highest values for the amount of chlorophyll B. In the heading phase, all experimental variants provided a higher proportion of chlorophyll species in comparison with the control.

Thus, the content and ratio of chlorophyll A and B varied experimentally depending on the vegetation phase and the type of preparation. It should be noted that during the period from the exit to the tube to the heading, there was an increase in aridity. Under these conditions, according to the experimental variants using Biohumus + Fe₃O₄ + Mo, Agroverm and Mival-Agro, an increase for chlorophyll of both species was obtained. In contrast, in other variants, including the control, their content decreased. However, all experimental variants showed high values in the ratio of chlorophyll A and B in comparison with the control. This process may indicate a decisive role of growth regulators and vermicompost in stressful situations.

The activity of antioxidant enzymes is a response to negative biotic and abiotic factors, including drought [22, 23]. In our experiment, the aridity of the growing season was the main factor causing stress in plants.

In the studies, a rather high catalase content was obtained in the experimental variants in all the studied vegetation phases. There was a sharp decrease in this enzyme in the control of the earing phase (Table 2).

| Experience Options | Catalase content by vegetation phase, mg/g wet weight |
|--------------------|------------------------------------------------------|
|                    | tillering | exit to a tube | heading |
| Control- water     | 36.625    | 52.675         | 17.075  |
| Biohumus           | 98.425    | 72.050         | 37.925  |
| Biohumus + Fe₃O₄ + Mo | 91.075   | 51.275         | 64.525  |
| Agroverm           | 67.150    | 62.900         | 52.375  |
| Mival Agro         | 57.375    | 52.025         | 41.475  |
| Blago 3⁺           | 79.075    | 70.700         | 55.450  |
| Siliplant          | 62.550    | 62.925         | 43.675  |

In most experimental variants, the catalase content decreased from the tillering phase to the earing phase. But the effectiveness of the experimental options was different. In the first phase of accounting, the excess of control ranged from 56.7 % (Mival-Agro) to 168.7 % (Biohumus). In the phase of entry into the tube for variants that exceeded the variant without treatment, the increase in the amount of catalase was from 122.1 % (Biohumus) to 277.9 % (Biohumus + Fe₃O₄ + Mo).

Thus, the overall picture of the amount of this enzyme in spring common wheat plants during the vegetation phases had the form of an inverse parabola with growth towards the heading phase.

The decrease in efficiency from drugs in the exit phase into the tube is explained by an increase for catalase in control and the overall resistance of plants in this phase to adverse factors.

In general, the highest catalase content was facilitated by the experimental options with the presence of Biohumus, Agroverm, and Blago3 + growth regulator.

The influence of growth regulators and biofertilizers on the content of carotenoids is insignificant, especially in the phases of tillering and entering the tube. Only when treated with Biohumus and Siliplant, the amount of these pigments increased by 11 % in the tillering phase and by 7 % in the exit phase into the tube, respectively. In the heading phase in spring wheat plants, the carotenoid content increased with the use of Mival-Agro (by 14 %), Biohumus in combination with UDM of molybdenum and iron 9Ha 30 %) and Agroverm (by 68 %) (Table 3).
Table 3. The content of carotenoids in spring wheat plants, depending on the pre-sowing treatment of seeds with growth regulators and biofertilizers

| Experience Options | The content of carotenoids in the phases of vegetation, mg/g wet weight |  |
|--------------------|---|---|
|                    | tillering | exit to a tube | heading |
| Control- water     | 1.515 | 3.088 | 2.742 |
| Biohumus           | 1.678 | 2.691 | 1.945 |
| Biohumus + Fe$_3$O$_4$ + Mo | 0.684 | 1.150 | 3.569 |
| Agroverm           | 1.279 | 2.594 | 4.620 |
| Mival Agro         | 1.129 | 2.230 | 3.120 |
| Blago 3+           | 0.939 | 2.860 | 1.683 |
| Siliplant          | 0.782 | 3.297 | 0.969 |

The productivity indices of an ear of soft spring wheat in the variants using biohumus and growth regulators were significantly higher than in control. An exception was the variant of seed treatment by Agroverm, where the values of the number of grains in the ear and the mass of grain from the ear did not differ much from the control (Table 4). The greatest effect was obtained from the use of biohumus, biohumus in combination with the treatment of seeds with UDM of molybdenum and iron.

Table 4. Productivity indices of an ear of spring soft wheat depending on biohumus and growth regulators

| Experience Options | The number of grains in the ear, pcs. | ± to control pieces | % | The mass of grain per ear, g | ± to control g | % |
|--------------------|--------------------------------------|---------------------|---|---------------------------|---------------|---|
| Control- water     | 11.7                                 | -                   | - | 0.31                      | -             | - |
| Biohumus           | 15.5                                 | +3.8                | 32.5 | 0.43                     | +0.12          | 38.7 |
| Biohumus + Fe$_3$O$_4$ + Mo | 14.8                                | +3.1                | 26.5 | 0.41                     | +0.10          | 32.3 |
| Agroverm           | 12.5                                 | +0.8                | 6.8 | 0.32                      | +0.01          | 3.2 |
| Mival Agro         | 13.9                                 | +2.2                | 18.8 | 0.40                      | +0.09          | 29.0 |
| Blago 3+           | 13.1                                 | +1.4                | 12.0 | 0.36                      | +0.05          | 16.1 |
| Siliplant          | 14.1                                 | +2.4                | 20.5 | 0.40                      | +0.09          | 29.0 |

Comment: the standard deviation did not exceed 5-7% of the average.

Changes in grain quality indicators were insignificant (Table 5).

Table 5. Grain quality indicators of soft spring wheat depending on vermicompost and growth regulators

| Experience Options | Nature, g/l | ± to control r/l | % | Vitreous, % | ± to control % |
|--------------------|-------------|-----------------|---|-------------|----------------|
| Control- water     | 756         | -               | - | 84          | -              |
| Biohumus           | 760         | +4              | 0.53 | 83          | -1             |
| Biohumus + Fe$_3$O$_4$ + Mo | 759      | +3              | 0.40 | 83          | -1             |
| Agroverm           | 753         | -3              | 0.40 | 80          | -4             |
| Mival Agro         | 758         | +2              | 0.26 | 85          | +1             |
| Blago 3+           | 761         | +5              | 0.66 | 80          | -4             |
| Siliplant          | 755         | -1              | 0.13 | 79          | -5             |

Comment: the standard deviation did not exceed 5–7 % of the average.

5. Conclusion
The study was carried out in the arid conditions of the Orenburg Urals. Presowing treatment of spring wheat seeds with biohumus preparations and growth regulators was carried out with increasing air temperature during the growing season. As a result of the study, it was revealed that the treatment of spring wheat seeds with humus preparations allows maintaining the stability of the pigment complex, the functioning of antioxidant enzymes, which contributes to the improvement of the spike productivity.
Acknowledgments
The studies were carried out in accordance with the research plan for 2019–2020 of the Federal Research Center for Biological Systems and Agrotechnology’s of the Russian Academy of Sciences (#0761-2019-0004).

Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical standards: All applicable international, national, and institutional guidelines have been followed.

References
[1] Karpova T A 2015 Effect of presowing treatment with growth regulators on physiological and biochemical processes during germination and sowing qualities of wheat and barley seeds Niva of the Volga Reg. 2(35) 32–8
[2] Rabinovich T Yu, Kovalev N T and Smirnova Yu D 2015 The use of new fertilizers and biological products in the cultivation of spring wheat (Triticum aestivum L) and potatoes (Solanum tuberosum) Agricult. Boil. 50(5) 665–72
[3] Buchanan B B, Cruissem R L and Jones (ed) 2006 Biochemistry and molecular biology of plants (Rockville, Maryland, USA: American Society of Plant Physiologist)
[4] Kostin V I., Dozorov A V and Isaichev V A 2018 On the issue of stimulation of agricultural plants under the influence of physical and chemical factors during seed treatment Bull. of the Ulyanovsk State Agricult. Acad. 2(42) 67–71
[5] Beale S I 1999 Enzymes of chlorophylli biosynthesis Photosynthesis Res. 60 43–73
[6] Nokagawara E., Sakuraba V., Vamasato F., Tanaka R., Tanaka A. 2007 Clp protease controls chlorophyll b synthesis by regulating the level of chlorophyllide a oxygenase Plant J. 49 800–9
[7] Korotkova A and Lebedev S Nov. 2015 Influence of iron of nanoparticles on induction of oxidative damage in Triticum vulgare Ecol. Environment and Conservat. 21(Suppl. iss) 101–11
[8] Lebedev S, Yausheva E, Galaktionova L and Sizova E September 2016 Impact of molybdenum nanoparticles on survival, activity of enzymes, and chemical elements in Eisenia fetida using test on artificial substra Environ Sci. Pollut. Res. 23(18) 18099–110 Retrieved from: https://doi.org/10.1007/s11356-016-6916-6
[9] Tyutereva E V and Voitsekhovskaya O V 2011 Reaction of a chlorophyll-deprived b barley mutant chlorine 3613 to a prolonged decrease in illumination. Dynam. of chlorophyll content, growth and productivity Plant Physiol. 58(1) 3–11
[10] Korotkova A M, Sizova E A and Lebedev S V 2015 Influence of NPs Ni on the induction of oxidative damage in Triticum vulgare Oriental J. of Chem. 31 137–45 Retrieved from: http://dx.doi.org/10.13005/ojc/31.Special-Issue1.17
[11] Kirillova I T and Sorokina T N 2012 The influence of growth regulators of pilafen and a trace element of copper on the components of the antioxidant system and productivity of potatoes (Solanum tuberosum L.) Sci. notes of Oryol State Univer. Ser. Natural, Techn. and Med. Sci. 3 103–106
[12] Galaktionova L, Gavrich I and Lebedev S 2019 Bioeffects of Zn and Cu Nanoparticles in Soil Systems Toxicol. Environ. Health Sci. 11 259–70 DOI: 10.1007/s13530-019-0413-5
[13] Lukatkin A S, Gruznova K A, Bashmakov D I and Lukatkin A A 2019 Effect of the growth regulator Epin-extra on wheat plants under the action of heavy metals Agrochem. 2 81–8
[14] Li L and Staden J 1998 Effects of plant growth regulators on drought resistance of two maize cultivars South Afric. J. of Botany 64(2) 116–20
[15] Kebede F, Kang M and Bekele E 2019 Chapter Five: Advances in mechanisms of drought tolerance in crops, with emphasis on barley Advan. in Agron. 156 265–314
[16] Srivalli B, Sharma G and Khanna-Chopra R 2003 Antioxidant defense system in an upland rice cultivar subjected to increasing intensity of water stress following by recovery Physiol. Plant 119 508–12
[17] Lebedev S, Gavrish I, Galaktionova L and Korotkova A M 2019 Assessment of the toxicity of silicon nanooxide in relation to various components of the agroecosystem under the conditions of the model experiment *Environmental geochem. and health* 41(2) 769–82
Retrieved from: https://doi.org/10.1007/s10653-018-0171-3

[18] Polishuk S D., Nazarova A A and Kutskir M V 2015 Ecologic-Biological Effects of Cjbalt, Cuprum, Copper Oxide Nano-Powders and Humic Acids on Wheat Seeds Modern Appl. Sci. 9(6) 354–64

[19] GOST R 54895-2012 *Grain. Method for determining the nature* 2013 (Moscow: Standartinform)

[20] GOST 10987-76 *Grain. Method for determining glassiness* 2001 (Moscow: IPK Standards Publ. House)

[21] GOST R 54478-2011 *Grain. Method for determining the quantity and quality of gluten* 2012 (Moscow: Standartinform)

[22] Galaktionova L V, Korotkova A M, Voskobulova N I et al 2019 Influence of pre-sowing priming on the parameters of Pisumsativum seedlings Conference on Innovations in Agricultural and Rural development *IOP Conf. Ser. Earth and Environmental Sci.* 341 012094 DOI: 10.1088/1755-1315/341/1/012094

[23] Lebedev S, Korotkova A and Osipova E 2014 Influence of Fe0 nanoparticles, magnetite Fe3O4 nanoparticles, and iron (II) sulfate (FeSO4) solutions on the content of photosynthetic pigments in Triticum vulgare *Russ. J. of Plant Physiol.* 61(4) 564–9 DOI: 10.1134/S1021443714040128