Enzymatic activity of spring wheat plants in the treatment of seeds with biostimulants and UFP of metals

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Abstract. The results of field experiments on the influence of different options of ultrafine metal particles, their oxides, their combinations, and biostimulants on the antioxidant activity of SOD, the content of malonovodialdehyde, and the amount of photosynthetic pigments in arid conditions are presented. The positive effect of the studied options of ultrafine metal particles and their oxides on the content of both types of chlorophyll is noted. There are phase of exit into the tube and the reduction of the type of chlorophyll b in the variants of the UFP of iron and silicon oxide and their combination. In the earing phase, an increase in the proportion of chlorophyll a over chlorophyll b was observed in most variants of the experiment. The amount of SOD enzyme under the action of metals UFP and their oxides increased, a positive effect among the biostimulant options was obtained by Agroverm and Siliplant. Biofertilizer Blago3 contributed to the reduction of the amount of malonovodialdehyde.

1. Introduction

The climate in the Orenburg region is characterized by an extremely continentality, as its territory is largely remote from the seas and oceans. The main feature of the region's climate is the frequent occurrence of droughts and dry winds. Drought, as a result of climate change, is the most powerful stress factor for agricultural crops [1].

Water-deficient plants produce an excessive amount of reactive oxygen species (ROI), which are highly reactive and cause damage to proteins, lipids, carbohydrates, and macromolecules [2].

Under the influence of adverse factors, the activity of antioxidant enzymes such as superoxide dismutase, catalase, and peroxidase increases [3]. At the same time, superoxide dismutase (SOD) acts as a primary barrier against ROI [4, 5].

The widespread use of metal nanoparticles in nature as products of industrial enterprises poses challenges for studying their effects on plants.

Nanoparticles (NPS) have a positive effect on morphological parameters, such as an increase in the seed germination rate, an improvement in the formation of roots and shoots and their correlation, as well as the accumulation of vegetative biomass of seedlings in many agricultural crops [6]. It was found that various antioxidant compounds are significantly increased in plants treated with Cu NPS, which indicates the activation of the protective mechanism by plants [7, 8].

The application of seed treatment with a colloidal solution of Cu, Zn nanoparticles caused an increase in SOD activity by 22% in drought conditions in wheat leaves [9]. ZnO nanoparticles with a concentration of 100 ppm contributed to an increase in the content of chlorophyll a in wheat seedlings by 55%,
chlorophyll \(b\) by 133\%, and iron NPS at a concentration of 20 ppm increased the content of chlorophyll \(a\) by 45\%, and chlorophyll \(b\) by 117 \% relative to the control. NPS ZnO and Fe significantly increased the activity of SOD in wheat leaves compared to the control by 47\% and 50\%, respectively [10].

Ag nanoparticles, due to their antibacterial and antifungal properties, are used to fight plant diseases [11].

In recent years, the need for agricultural preparations that would allow to preserve the crop or at least part of it under unfavorable abiotic factors has been increasing. Focused attention in this direction is paid to the effect of phytoregulators and biofertilizers on plants [12]. The use of amino acids, humates, phytohormones, nitrogen and sulfur can be effective for increasing plant resistance to various abiotic stresses [13].

In field experiments with spring wheat and spring barley, it was found that inoculation of seeds with biological preparations (rhizoagrin, flavobacterin) gives an increase in the grain yield of spring wheat - 23\%, barley – 12\% [14].

Treatment of wheat seedlings with riftal promoted the development of the root system, increased the level of endogenous auxins, and increased seed germination [15].

The use of melatonin on cotton in small doses (10-50 microns) increased the activity of SOD from 28.8\% to 39.2\% on average in relation to the control. High concentrations of melatonin (100-200 microns) increased the level of MDA content, which led to damage to cell membranes, and thus a negative effect on the germination of cotton seeds [16].

It was found that pre-sowing treatment with growth regulators reduces the content of heavy metals (HM) in the roots and in the aboveground part of wheat plants grown on solutions (HM) and weakens the severity of stress effects, mainly through changes in antioxidant activity [17].

It was found that the level of MDA content is an important indicator for evaluating the effectiveness of biological products as a criterion for the response to oxidative stress and/or the degree of resistance to this effect and the strength of cell membranes. The concentration of MDA in the treatment of humic-fulvate complex (HFC) and humic acid (HA) is reduced by 1.5-2 times compared to the control [18, 19].

Data on some effects of plant growth regulators and biofertilizers, namely, their effect on the accumulation of protective proteins, are very contradictory. These contradictions indicate the need for an in-depth study of the specific, and possibly varietal, features of the phyto regulators action on plants [20].

2. Problem statement

In the conditions of increasing aridity of weather factors during the growing season of spring wheat negatively affects the normal course of physiological and biochemical processes. Ultimately it reduces their productivity, and against the background of a wide market of natural drugs and chemical origin that can withstand the damaging effects of high temperatures and drought. So the questions of studying the mechanism of their impact on individual elements of the antioxidant system of plants are relevant.

The aim of the work was to study the effect of pre-sowing treatment of spring wheat seeds (Teacher variety) with plant growth regulators, biofertilizers, ultrafine iron, molybdenum, silicon oxide, and their combinations on the antioxidant activity of the enzyme superoxide dismutase (SOD), the content of photosynthetic enzymes, and malondialdehyde in arid conditions of the Orenburg Cis-Ural region.

3. Materials and methods

For the research, we used SiO\(_2\) NPS with a size of 30.7 ± 0.3 nm and a \(\zeta\) potential of 27 ± 0.12 mV) and Mo NPS (with a size of 100 ± 0.3 nm and a \(\zeta\) potential of 42 ± 0.52 mV) manufactured by Plasmotherm company (Moscow, Russia). Fe\(_3\)O\(_4\) nanoparticles (80-100 nm, \(\zeta\)-potential 20 ± 0.14 mV) were purchased from Advanced Powder Technologies company (Tomsk, Russia).

The following concentrations were used for seed treatment: for Fe\(_3\)O\(_4\) 10\(^{-2}\), 10\(^{-3}\), and 10\(^{-4}\) mg / l; for SiO\(_2\) 10\(^2\), 10\(^3\), and 10\(^4\) mg / l; the mixed solution was obtained by mixing a suspension of Fe\(_3\)O\(_4\) (10\(^{-3}\) mg / l) and SiO\(_2\) (10\(^{-4}\) mg / l) in a ratio of 1: 1.
Next, the prepared solutions of NPS were used for pre-sowing treatment of seeds by pre-soaking for 10-15 minutes in solutions with different concentrations of nanoparticles (concentrations of $10^{-3}$ - $10^{-5}$ mg/l). The seeds was control without pretreatment.

In the experiment, plant growth regulators with different content of organic and mineral substances were used. Siliplant is a silicon-containing fertilizer, which, in addition to silicon Si (7%) and potassium (1%), contains trace elements (mg/l) in a chelated form that is easily accessible to plants: Fe – 300; Mg – 100; Cu – 70-240; Zn – 80; Mn – 150; Co – 15; B – 90. Consumption for grain crops is 60 ml / t, working fluid is 10 l/t.

Fertilizer Beno 3+ includes humic acids (up to 20 g/l), N (75-80), P$_2$O$_5$ (40-50), K$_2$O (100-110), MgO ≥ 9, Mn ≥ 0.9, Zn ≥ 0.7, Cu ≥ 0.5, Mo ≥ 0.4, Co ≥ 0.3. Consumption of the preparation for processing seeds of grain crops is 1 l / t, for vegetation processing is 0.5 ml/ha.

The growth regulator Mival-Agro is an Agro-bio-organic plant growth and development regulator based on silicon.

Biohumus is an organic fertilizer based on the waste products of earthworms as a result of processing the compost of cattle or any biological waste that is subject to rot.

Agroverm (TU 9819-001-6453141360-2015) is liquid humic biofertilizer. The basis for production is Vermicompost (GOST R 56004), obtained with the help of red earthworms.

Pre-sowing treatment of seeds was carried out by pre-soaking for 10-15 minutes. The control was seeds without pretreatment. The treated seeds were sown at the experimental site of the Federal Research Centre of Biological Systems and Agrotechnologies of the Russian Academy of Sciences. The soil of the site 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 was carried out with the SN-16 seeder. A variety of spring soft wheat Teacher is medium-ripened. The seeding rate is 4.5 million. germinating seeds per hectare.

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

The content of photosynthetic pigments (chlorophyll a and b, caratinoids) was determined by the standard method with modifications of Smashevsky [21].

The content of superoxide dismutase and malondialdehyde was determined by the method of Sibgatullina with modifications (2011) [22]. The differences were considered statistically significant at p<0.05.

4. Results discussion

The content of chlorophyll a in spring wheat plants in the tube release phase increased significantly under the influence of the combinations of UFP Fe+SiO$_2$ (by 73.3%), silicon oxide SiO$_2$ (by 72.6%) and especially significantly against the background of seed treatment with Fe * $10^4$ nanoparticles (by 108%) and Fe+Mo (by 167%).

Table 1. The content and ratio of chlorophyll a and b in spring wheat plants, depending on the pre-sowing treatment of seeds with ultrafine particles and their oxides.

| Experiment variant | Chlorophyll content in the tube exit phase, mg / g of dry weight | Chlorophyll a/b ratio | Chlorophyll content in the earing phase, mg / g of dry weight | Chlorophyll a/b ratio |
|--------------------|---------------------------------------------------------------|------------------------|---------------------------------------------------------------|------------------------|
|                    | a                                                                 | b                      | a                                                               | b                      |
| Control            | 5.600                                                           | 1.325                  | 4.22                                                           | 3.38                   | 2.995                   | 1.25                   |
| Fe+SiO$_2$         | 6.808                                                           | 0.686                  | 9.92                                                           | 7.639                  | 0.138                   | 55.35                  |
| Mo+SiO$_2$         | 9.710                                                           | 4.545                  | 2.14                                                           | 13.303                 | 4.040                   | 3.29                   |
The content of chlorophyll b also increased significantly in the variants, with the exception of the interaction of ultrafine iron particles and silicon oxide, where its amount decreased by half. The positive effect was manifested in an increase in its amount by 74.2% (Fe\(\cdot\)10\(^{-4}\)) and up to a fivefold increase in the variant with the use of Fe+Mo.

The content of chlorophyll a in the earing phase in the control and experimental versions with the UFP SiO\(_2\) and Fe\(\cdot\)10\(^{-4}\) relative to its amount in the exit phase into the tube decreased. An increase in the amount of this pigment was found in all experimental variants, except for the variant with silicon oxide (SiO\(_2\)).

The content of chlorophyll b in plants during the earing phase decreased for most of the experimental variants, with the exception of the Fe+Mo variant and the control, but the variants with ultrafine particles and oxides of Mo+SiO\(_2\), Mo\(\cdot\)10\(^{-4}\), and Fe+Mo exceeded the control in the amount of this type of chlorophyll.

A significant decrease in the amount of chlorophyll b in the tube release phase relative to the control was reflected in an increase in the chlorophyll a/b ratio in favor of the first type. The opposite pattern is typical for the reduction phase: a decrease in the proportion of chlorophyll a over chlorophyll b in comparison with the control and experimental version of Fe+SiO\(_2\).

One of the key elements of the antioxidant activity of plants is the enzyme superoxide dismutase (SOD) [23-25], which protects plant tissue cells from oxidative damage and the effects of adverse environmental factors.

According to our data (figure 1), pre-sowing treatment of seeds with UFP metals and their oxides contributes to the activation of the SOD enzyme in all the studied variants in the earing phase.

![Image of Figure 1](image-url)

**Figure 1.** The content of superoxide dismutase (SOD) in spring wheat plants, depending on the pre-sowing treatment of seeds with ultrafine particles and their oxides.

At the same time, the increase in the amount of superoxide dismutase when using molybdenum (Mo\(\cdot\)10\(^{-4}\)) and silica (SiO\(_2\)) as a variant was 2.847 – 3.114 units/g of protein (16.1 – 17.6%), and their combination (Mo+SiO\(_2\)) contributed to an increase in the amount of SOD by 11.506 units/g of protein.
or 64.9%. The most significant increase in the amount of this enzyme was obtained in the variants with the UF of iron (Fe \cdot 10^{-4}) – by 15.153 units/g of protein (85.4%) and the combination of iron and molybdenum (Fe+Mo) - by 15.287 units/g of protein or 86.2%. The effect of molybdenum is positive when it is used in combination with iron and silicon oxide and in comparison with the effect of the UF of iron. The combination of Fe+SiO₂ gave a positive effect on the growth of SOD by 4.774 units / g of protein, or 26.9%.

Depending on the seeds treatment with biostimulants, the content of this enzyme increased in the variants Mo+Fe+biohumus (by 4.2%), with Agroverm biofertilizer (by 72.2%) and with Siliplant growth regulator (by 145.9%), with a significant (by 62.0-76.0%) decrease in its amount in other variants of the experiment (figure 2).

![Figure 2. The content of superoxide dismutase (SOD) in spring wheat plants, depending on the pre-sowing treatment of seeds with growth regulators and biofertilizers.](image)

Malondialdehyde (MDA) is a marker of oxidative stress. It is formed in cells when fatty acids are destroyed by active oxygen forms [26]. Its increase indicates an increase in oxidative stress.

According to the data obtained in our studies (table 2), a decrease in the level of malondialdehyde in all three phases of accounting is detected in the fertilizer treatment Blago 3.

### Table 2. The content of malondialdehyde in spring wheat plants, depending on the treatment of seeds with growth regulators and biofertilizers Mmol/g of raw weight.

| №  | Experiment variant | The phase of plant development |
|----|--------------------|--------------------------------|
|    |                    | tillering | exit into the tube | earing |
| 1  | Control            | 0.0995    | 0.0066             | 0.0788 |
| 2  | Biohumus           | 0.1019    | 0.0761             | 0.1239 |
| 3  | Mo+Fe+biohumus     | 0.1404    | 0.0189             | 0.0768 |
| 4  | Agrovermiculitis   | 0.0592    | 0.0215             | 0.0852 |
| 5  | Mival-Agro         | 0.1716    | 0.0146             | 0.0430 |
| 6  | Blago 3+           | 0.0195    | 0.0016             | 0.0788 |
| 7  | Siliplant          | 0.0719    | 0.0405             | 0.1157 |

In the tillering phase, the effect was also obtained in variants with biofertilization of agricultural firms and Siliplant growth regulator. In the earring phase, a decrease in the MDA content was obtained.
when seeds were treated with the growth regulator Mival-Agro and when ultrafine particles of molybdenum and iron were combined with biohumus.

5. Conclusion
The most positive effect on the content of chlorophyll a and b and the content of enzymes is provided by the variants of seed treatment with ultrafine molybdenum particles (Mo · 10⁻⁴), Mo + SiO₂ and Fe + Mo compounds.

Agrofirm biofertilizer and Siliplant growth regulator increase the amount of superoxide dismutase in plants.

Reducing the amount of malondialdehyde (MDA) in spring wheat plants is promoted by biofertilizer Blago 3.

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