Effect of ultrafine metal particles and their oxides on antioxidant enzyme activity, spike productivity and grain quality of spring soft wheat

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Abstract. The results of field experiments in the scope of studying the effect of various options of ultrafine metal particles, their combinations, oxides on the content of antioxidant enzymes (catalase, malondialdehyde) as well as carotenoids in the tillering phases, stem elongation and spring wheat ear stage are presented. The influence of the above options on the formation of spike productivity (number of grains and grain weight per spike) and grain quality indicators (hardness, unit, gluten amount) are considered.

1. Introduction

The territory of the steppe zone of the Southern Urals is the zone of insufficient moisture. During the period of complex drought, plants suffer from various adverse environmental conditions [1].

Under the influence of drought, an excessive amount of reactive oxygen species (ROS) appears in plant cells that can damage the structure of membranes and macromolecules. At the same time, a defense system consisting of antioxidant enzymes (catalase, superoxide dismutase, peroxidase, etc.) that utilize the surplus of ROS is activated [2–4].

Under the influence of drought, an excessive amount of reactive oxygen species (ROS) appears in plant cells that can damage the structure of membranes and macromolecules. At the same time, a defense system consisting of antioxidant enzymes (catalase, superoxide dismutase, peroxidase, etc.) that utilize the surplus of ROS is activated [2–4].

Plant resistance to stress is determined by the increase and activity level of antioxidant enzymes [5–7].

Under drought conditions, the activity of such enzymes increases [8–11]. At low temperatures, the activity of superoxide dismutase, peroxidase, ascorbate peroxidase, and catalase depends on nitrogen deficiency as well as on a nitrate and ammonium option [12].

Studying the effect of different doses of Ni NPs on spring wheat seedlings showed that low concentrations (0.01 and 0.1 mg/l) did not change or stimulate growth processes, and higher doses (1 and 10 mg/l) significantly inhibited root growth and aboveground parts. The content of chlorophylls a and b significantly decreased only at the concentration of 10 mg/l. The number of carotenoids gradually decreased with the increasing concentration of Ni0 NPs [13].

A comprehensive study of the effects of NPs of iron oxide FeO, magnetite Fe3O4, copper Cu0, copper oxide CuO, nickel Ni0, nickel oxide NiO in various concentrations on seedlings of soft wheat
showed that they selectively affect the content of chlorophyll a and b, pigments, malondialdehyde (MDA). Metal nanoparticles and their oxides exhibit different biological activity depending on the applied composition and concentration of metals [14, 15].

The use of metal NPs in growing crops increases productivity by 18–25 % and increases the quality of agricultural products (the content of polysaccharides, protein, vitamins, microelements) up to 25 % [16, 17].

Processing seed material is one of the most effective methods of increasing the productivity of agricultural plants. Seed treatment has not only an economic advantage, but also a positive effect on plants, starting from the first stages of their development [18].

In field experiments with spring soft wheat, the treatment of seed material of Ag NPs ensured a yield increase of 12.1-38.7%. At the same time, the increase in yield was largely associated with an increase in the mass of grain per spike \(r=0.966\) [19].

The use of the NanoKremny preparation for seed treatment and spraying of spring wheat crops increased the number of grains in a spike by 26.9 %, the weight of grain from a spike by 39.5 % [20].

According to some researchers, iron nanoparticles have a positive effect on the content of chlorophyll and plant growth [21, 22].

2. Problem statement
With all the variety of data on the effect of nanoparticles of various elements and oxides on various metabolic processes in plants, most of them were obtained under invitro conditions, but it is equally important to evaluate the course and influence of these processes in the field.

The research objective was to study the effect of presowing treatment of spring soft wheat seeds (variety Uchitel) with nanoparticles of molybdenum, iron and silicon oxide, as well as their combinations on the antioxidant activity of enzymes, plant carotenoid content, spike productivity and grain quality in a field experiment.

3. Materials and methods
For research, we used SiO₂ NPs with the size of 30.7 ± 0.3 nm and a \(\zeta\) potential of 27 ± 0.12 mV and Mo NPs (size 100 ± 0.3 nm and a \(\zeta\) potential of 42 ± 0.52 mV) produced by the company Plazmoterm (Moscow, Russia, http://plasmotherm.ru). \(\text{Fe}_3\text{O}_4\) nanoparticles (80–100 nm, \(\zeta\)-potential 20 ± 0.14 mV) were purchased from AdvancedPowder Technologies (Tomsk, Russia, www.nanosized-powders.com).

To prepare NP solutions, the exact amounts of the preparations were placed in glass bulbs with deionized tap water and were intensively dispersed with ultrasound at the frequency of 35 kHz for 30 minutes. Solutions were prepared several times to reduce the amount of nanomaterials. The following concentrations were used for seed treatment: \(10^{-2}, 10^{-3}\) and \(10^{-4}\) mg/l for \(\text{Fe}_3\text{O}_4\); \(10^{-2}, 10^{-3}\) and \(10^{-4}\) mg/l for SiO₂; a mixed solution was obtained by mixing a suspension of \(\text{Fe}_3\text{O}_4\) (\(10^{-3}\) mg/l) and SiO₂ (\(10^{-4}\) mg/l) at the 1:1 ratio.

Next, the prepared NP solutions carried out 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 were selected). The seeds served as a control without preliminary treatment.

The treated seeds were sown at the experimental site of the FSSI FRC BST RAS. The soil of the site was ordinary medium-humus (4.2–4.5 %) and medium-thick (40–45 cm) chernozem of medium loamy mechanical composition. Ph of the soil solution is neutral (7.0). The site was sown by seeder SN-16 with mid-season spring soft wheat variety Uchitel. The seeding rate was 4.5 million germinating seeds per hectare.

Plants were sampled in the main phases of the vegetative development, specifically, tillering, stem elongation and earing.

The content of photosynthetic pigments (chlorophyll a and b, carotenoids) was determined by the standard method with modifications of Smashevsy, 2011 [17]. After the harvest, the following plant productivity indicators were determined: the amount of grain in a spike, the mass of grain per a spike, grain-unit (GOST R 54895-2012) [23], grain hardness (GOST 10987-76) [24], gluten content (GOST R 54478-2011) [25].

Differences were considered statistically significant at \(p <0.05\).
4. Results and discussion

According to the research results, the content of the catalase enzyme significantly changed both in the experimental options and in the vegetation phases.

In the tillering phase, the variants of ultrafine particles Fe$_3$O$_4$, Mo + SiO$_2$, SiO$_2$, Fe$_3$O$_4$, Mo, and Fe$_3$O$_4$ + Mo contributed to an increase in the content of this enzyme by 41–93 % and only with the option Mo its content was practically at the control level.

In the phase of stem elongation against the background of the last option (Mo), a more than twofold increase in the catalase content (131%) was obtained, slightly less in the Fe$_3$O$_4$ + Mo option (88 %). For other options, the catalase content did not exceed the control one (Fig. 1).

Figure 1. Catalase content in spring soft wheat plants by vegetation phases, depending on the options of ultrafine particles, Mmol/g of fresh weight

In the earing phase, the amount of this enzyme was significantly higher with all options of ultrafine particles with the highest excess values for the option Mo + SiO$_2$ (288 %), SiO$_2$ (181 %) and Fe$_3$O$_4$ + Mo (154 %).

Thus, the catalase content increased in all the main phases of the spring wheat vegetative period, especially in the experimental options containing the ultrafine particles of molybdenum, silicon oxide, and iron.

A significant role under the conditions of drought and an increase in air temperature is played by the content of malondialdehyde (MDA). Thus, according to I.A. Nilova, L.V. Topchieva, A.F. Titova [26] the content of MDA at a temperature of 43 °C was significantly higher than at 37 °C.

According to our data, in the early phases of vegetation a significant increase in malondialdehyde was promoted by the Mo + SiO$_2$, Fe$_3$O$_4$ + Mo, and SiO$_2$ ultrafine particles (Fig. 2).

In this case, the Mo + SiO$_2$ option with the highest MDA values both in the tillering phase and in the stem elongation phase should be noted.

By the earing phase, against the background of a general decrease in this enzyme in spring wheat plants, the excess of control had been obtained for the following options: Fe$_3$O$_4$ + SiO$_2$, Mo, Fe$_3$O$_4$.

The dynamics of MDA content in vegetation phases is associated with ultrafine particles options. According to the control option and combination of ultrafine particles Fe$_3$O$_4$ + Mo, the maximum MDA content accounts for the tillering phase, in other combinations (Mo + SiO$_2$, SiO$_2$) it accounts for the phase of stem elongation or earing (Fe$_3$O$_4$ + SiO$_2$, Fe$_3$O$_4$, Mo). However, in any case, the positive effect of the studied options on the content of malondialdehyde in spring wheat plants has been established.

The role of carotenoids is to dissipate excessive light energy with the objective to preserve the photosystem from damage [27].
Figure 2. Content of malondialdehyde in spring soft wheat plants according to the vegetation phases with regards to the options of ultrafine particles, Mmol/g of fresh weight

In our studies, the content of this pigment varied depending on its options of ultrafine particles (Fig. 3). In the tillering phase, it was greater in comparison with the control on the Mo + SiO₂ option (32 %) and a decrease in other options. In the yield phase, the same excess (30 %) was obtained according to the Fe₃O₄ + Mo option. During earing, an increase in the amount of this enzyme was ensured by the variants Mo + SiO₂ (6 %), Mo (10 %), and Fe₃O₄ + Mo (67 %).

Figure 3. Content of carotenoids in spring soft wheat in the vegetation phases with regards to the options for ultrafine particles, Mmol/g of fresh weight

In general, it can be noted that the options with ultrafine particles of molybdenum were the most effective for increasing the content of carotenoids.

But the effect of ultrafine particles on the content of staining enzymes is lower than on the content of antioxidant enzymes.
The productivity indices of a spring soft wheat spike vary significantly depending on the options of ultrafine particles. Almost all options provided an increase in the number of grains in a spike and grain mass per a spike (Table 1). The most significant growth values were provided by the following ultrafine particles: Fe$_3$O$_4$ + SiO$_2$, SiO$_2$, and Mo.

### Table 1. Spike performance indices

| № | Experiment option       | Number of grains in a spike, pcs. | +,- to the control of pieces | Grain mass per a spike, g | +,- to the control of grams |
|---|------------------------|-----------------------------------|-----------------------------|--------------------------|-----------------------------|
| 1 | control                | 11.7                              | -                           | 0.31                     | -                           |
| 2 | Fe$_3$O$_4$+SiO$_2$    | 14.7                              | + 3.0                       | 25.6                     | 0.43                        | + 0.12                     | 38.7                        |
| 3 | Mo+SiO$_2$             | 13.7                              | + 2.0                       | 17.1                     | 0.37                        | + 0.06                     | 19.4                        |
| 4 | SiO$_2$                | 14.7                              | + 3.0                       | 25.6                     | 0.42                        | + 0.11                     | 35.5                        |
| 5 | Fe$_3$O$_4$            | 13.6                              | + 1.9                       | 16.2                     | 0.39                        | + 0.08                     | 25.8                        |
| 6 | Mo                     | 17.2                              | + 5.5                       | 47.0                     | 0.51                        | + 0.20                     | 64.5                        |
| 7 | Fe$_3$O$_4$+Mo         | 12.3                              | + 0.6                       | 5.1                      | 0.34                        | + 0.03                     | 9.7                         |

Note: the standard deviation did not exceed 5–7 % of the average one

The effect of the studied ultrafine particles options, their oxides and combinations on the grain quality indicators is less significant than on the spike productivity, but positive increases in grain nature and especially in vitreous should be noted. It should be noted that the Fe$_3$O$_4$ + SiO$_2$, SiO$_2$ ultrafine particles with higher values of unit and vitreousity are formed during their application (Table 2).

### Table 2. Grain unit and hardness

| № | Experiment option       | Grain unit, g/l | +,- to the control | Grain hardness, % | +,- to the control |
|---|------------------------|-----------------|-------------------|-------------------|-------------------|
| 1 | control                | 756             | -                 | 84                | -                 |
| 2 | Fe$_3$O$_4$+SiO$_2$    | 762             | + 6               | 87                | + 3               |
| 3 | Mo+SiO$_2$             | 758             | + 2               | 85                | + 1               |
| 4 | SiO$_2$                | 761             | + 5               | 87                | + 3               |
| 5 | Fe$_3$O$_4$            | 758             | + 2               | 88                | + 4               |
| 6 | Mo                     | 760             | + 4               | 80                | - 4               |
| 7 | Fe$_3$O$_4$+Mo         | 759             | + 3               | 86                | + 2               |

Note: the standard deviation did not exceed 5–7 % of the average one

The positive effect of the ultrafine particles options on the crude gluten content was obtained (Table 3).

### Table 3. Gluten content and quality in spring soft wheat grain

| № | Experiment option       | raw gluten content, % | +,- to the control | indicator IDK-1, pcs |
|---|------------------------|-----------------------|-------------------|---------------------|
| 1 | control                | 44                    | -                 | 98                  |
| 2 | Fe$_3$O$_4$+SiO$_2$    | 46                    | + 2               | 103                 |
| 3 | Mo+SiO$_2$             | 46                    | + 2               | 104                 |
| 4 | SiO$_2$                | 45                    | + 1               | 101                 |
| 5 | Fe$_3$O$_4$            | 44                    | -                 | 102                 |
| 6 | Mo                     | 42                    | - 2               | 100                 |
| 7 | Fe$_3$O$_4$+Mo         | 45                    | + 1               | 104                 |

Note: the standard deviation did not exceed 5–7 % of the average one

The improvement in the spike productivity and grain quality of spring soft wheat should be considered as a consequence of the positive influence of the treatment of ultrafine metal particles and their oxides on metabolic processes in plants during the growing season.
5. Conclusion
Thus, the study of the effectiveness of various options of ultrafine metal particles, their combinations and oxides showed a positive effect of molybdenum or its combination with silicon oxide, a combination of ultrafine particles of iron with silicon oxide. These options contributed to an increase in catalase, malondialdehyde, carotenoids, and spike productivity.

Acknowledgment
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 for animal care and use have been followed.

References
[1] Tikhonov V E 2005 Drought in the steppe zone of the Southern Urals (Orenburg)
[2] Bolwell G P 1997 Mechanisms for the Generation of Reactive Oxygen Species in Plant Defense – a Broad Perspective Physiol. Mol. Plant Pathol. 51 347–66
[3] Rizhsky L, Hallak-Herr E and Breusegem F V et al. 2002 Double antisence plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase Plant J. 32 329–42
[4] Polovnikova M G and Voskresenskaya O L 2008 The activity of antioxidant protection components and polyphenol oxidase of lawn plants in ontogenesis in urban environments Plant physiol. 55(5) 777–85
[5] Sairam R K, Deshmukh P S and Saxena D C 1998 Role of Antioxidant Systems in Wheat Genotypes Tolerance to Water Stress Biol. Plant. 41 387–94
[6] Shalata A, Mittova V, Vohkita M, Guy M, and Tal M 2001 Response of the Cultivated Tomato and Its Wild Salt-Tolerant Relative Lycopersicon pennellii to Salt-Dependent Oxidative Stress: The Root Antioxidative System Physiol. Plant. 112 487–94
[7] Mitteler R 2002 Oxidative Stress, Antioxidants, and Stress Tolerance Trends Plant Sci. 7 405–9
[8] Nikolaeva M K, Mayevskaya S N, Shugaev A T and Bukhov N G. 2010 Effect of drought on the content of chlorophyll and the activity of enzymes of the antioxidant system in the leaves of three varieties of wheat, differing in productivity Plant physiol. 57(1) 94–102
[9] Zhang J and Kirkham M B 1994 Drought-Stress Induced Changes in Activities of Superoxide Dismutaze, Catalase and Peroxidase in Wheat Species Plant Cell. Physiol. 35 785–791
[10] Zhang J and Kirkham M B 1996 Enzymatic Responses of the Ascorbate-Glutathione Cycle to Drought in Sorghum and Sunflower Plants Plant Sci. 113 39–47
[11] Sairam R K, Deshmukh P S and Saxena D C 1998 Role of Antioxidant Systems in Wheat Genotypes Tolerance to Water Stress Biol. Plant. 41 387–94
[12] Polesskaya O G, Kashirina E I and Alekchina N D 2004 Changes in the activity of antioxidant enzymes in leaves and roots of wheat depending on the form and dose of nitrogen in the environment Plant physiol. 51(5) 686–91
[13] Zotikova A P, Astafurova T P and Bureinina A A et al. 2018 Morphophysiological features of wheat germ (Triticumaestivum L.) when exposed to nickel nanoparticles Agricult. Biol. 53(3) 578–86
[14] Korotkova A, Sizova E 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
[15] Korotkova A M, Lebedev S V and Gavrish I A 2017 The study of mechanisms of biological activity of copper oxide nanoparticle CuO in the test for seedling roots of Triticum vulgare Environ Sci. Pollut. Res. Int. 24(11) 10220–33 DOI: 10.1007/s11356-017-8549-9
[16] Korotkova, Gavrish I, Lebedev S and Halikov B July 2019 Comparative analysis of cell viability of Triticum vulgare after exposure to copper nanoparticles Open Bio. 9(S1) pp 303 DOI: 10.1002/2211-5463.12675

[17] 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

[18] Lozhnikova V N and Slasty I V 2010 Growth of spring barley plants and the activity of endogenous hormones under the influence of silicon Agricult. Biol. 3 102–7

[19] Usanova Z I, Vasiliev A S and Ivanyutina N N 2015 Efficiency of using nanosilver technologies for the cultivation of spring crops Basic res. 2-22 4934–9

[20] Semina S A and Ostroborodova N I 2018 The influence of a silicon-containing preparation on the formation of the yield of spring soft wheat Niva of the Volga Reg. 2(47) 29–34

[21] Voica C, Polescu L and Lazar D A 2003 The influence of the magnetic fluids on some physiological processes in Phascolus vulgaris Rv. Roum. Biol 48 9–15

[22] Galaktionova L, Gavrish I and Lebedev S 2019 Bioeffects of Zn and Cu Nanoparticles in Soil Systems Toxicol. Environ. Health Sci. 11 259–270 DOI: 10.1007/s10653-019-0413-5

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

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

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

[26] Lebedev S, Gavrish I, Galaktionova L and Korotkova A M 2019 Assessment of the toxicity of silicon nanoxide 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

[27] 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) 564–69 DOI: 10.1134/S1021443714040128