

# Effect of Metal Oxide Nanoparticles on the Activity of Antioxidant Enzymes and Productivity of Wheat Genotypes

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## Abstract

The influence of different concentrations of NPs of biogenic metal oxides of iron, zinc, titanium and aluminum on the activity of antioxidant enzymes of different wheat genotypes was studied.. The objects of the study were different varieties of durum wheat (*Triticum durum* Desf.) Gyrmizi bugda, Garagylchyg-2, Yagut, Karabakh and soft wheat (*Triticum aestivum* L.) Mirbashir-128, Gobustan, Sheki-1 and Dagdash, purchased from the Research Institute (RI) of Agriculture of Azerbaijan. In order to establish the optimal concentrations of the presented nanoparticles when affecting the activity of antioxidant enzymes and the morphophysiological parameters of a number of durum and bread wheat varieties, we used solutions of different concentrations of 0.1, 0.01, 0.001, 0.0001 mMol nanoparticles. When acting on the activity of catalase, the optimal concentration was 0.01 mM for all studied durum wheat varieties, and 0.001 mM for bread wheat varieties. The activity of superoxide dismutase depended on the varietal characteristics. Titanium dioxide nanoparticles at a concentration of 0.01 mM had a stimulating effect on the activity of antioxidant enzymes, the content of photosynthetic pigments by 9-13% and the yield by 5-83%, taking into account the varietal characteristics of wheat varieties.

**Keywords:** nanoparticles, enzymes, wheat, catalase, superoxide dismutase, photosynthetic pigments, productivity

## **Introduction**

Nanoscience and nanotechnology are an expanding field of research that involves structures, devices, and systems with new properties and functions due to the arrangement of their atoms in the range of 1-100 nm. In the early 2000s, this field became the subject of growing public awareness and controversy, which in turn gave rise to the commercial application of nanotechnology. It contributes to almost all fields of science, including physics, materials science, chemistry, biology, computer science, and engineering [1]. Notably, in recent years, nanotechnology has found widespread application in medicine, especially in the field of cancer treatment. Thus, nanotechnology is a developing and promising area of interdisciplinary research, which opens up a wide range of opportunities in various sectors, including medicine, pharmaceuticals, electronics and agriculture [2]. In the agricultural sector, nanotechnology can be used to combat insect pests (pesticides and insecticides made from nanomaterials); to increase the productivity of agricultural crops using conjugated NPs and for the slow release of nutrients and water from plants.

Currently, the problems of studying the positive or negative impact of nanomaterials on biological objects are of particular interest. Such studies are becoming extremely relevant, since the range and number of nanoparticles (NPs) entering the environment is expanding, and it is necessary to develop methods for assessing the consequences of their impact on living organisms [3]. Biosafety of nanotechnology, the study of the behavior of NPs in the environment and living organisms is the subject of numerous studies.

In the first experimental studies on the biotesting of NPs, preference was given to plants, since they are diverse and are accessible objects with sensitivity to low-intensity environmental factors, sometimes exceeding the sensitivity of objects of animal origin by an order of magnitude. It is known that NPs smaller than 10 nm are capable of not only penetrating into plant cells, but also integrating into their membranes.

Plants cultivated *in vitro* are good model test objects for assessing the impact of NPs introduced into the nutrient medium. It is promising to study the features of morphogenesis, cytogenetic indicators and the interaction of NPs with intracellular structures.

The use of smart systems based on nanotechnology is growing day by day, the reason for which is the increased level of safety in the processing and transportation of food products [4].

Thus, developments in the sector of nanosensors, nanodevices and smart systems have led to easy and rapid detection of pathogens and environmental pollutants.

The aim of the study was to study the effect of various concentrations of NPs of biogenic metal oxides of iron, zinc, titanium and aluminum on the activity of antioxidant enzymes of various wheat genotypes.

## **Materials and methods**

The objects of the study were various varieties of durum wheat (*Triticum durum* Desf.) Gyrmzy bugda, Garagylchyg-2, Yagut, Karabakh and soft wheat (*Triticum aestivum* L.) Mirbashir-128, Gobustan, Sheki-1 and Dagdash, purchased from the Research Institute (RI) of Agriculture of Azerbaijan. In order to establish the optimal concentrations of NPs in their effect on the activity of AOS enzymes, the functioning of the FSA and the morphophysiological parameters of a number of hard and soft wheat varieties, we used solutions of NPs of various concentrations obtained as a result of ultrasonication of ferric oxide (15 mg/kg – series I; 30 mg/kg – series II; 50 mg/kg – series III; 100 mg/kg – series IV), zinc oxide (0.1 mM – series I; 0.01 mM – series II; and 0.001 mM – series III), aluminum oxide (0.1 mM – series I; 0.01 mM – series II; and 0.001 mM – series III) and titanium dioxide (0.1 mM – series I; 0.01 mM – series II; and 0.001 mM – series III).

### **Determination of CAT activity**

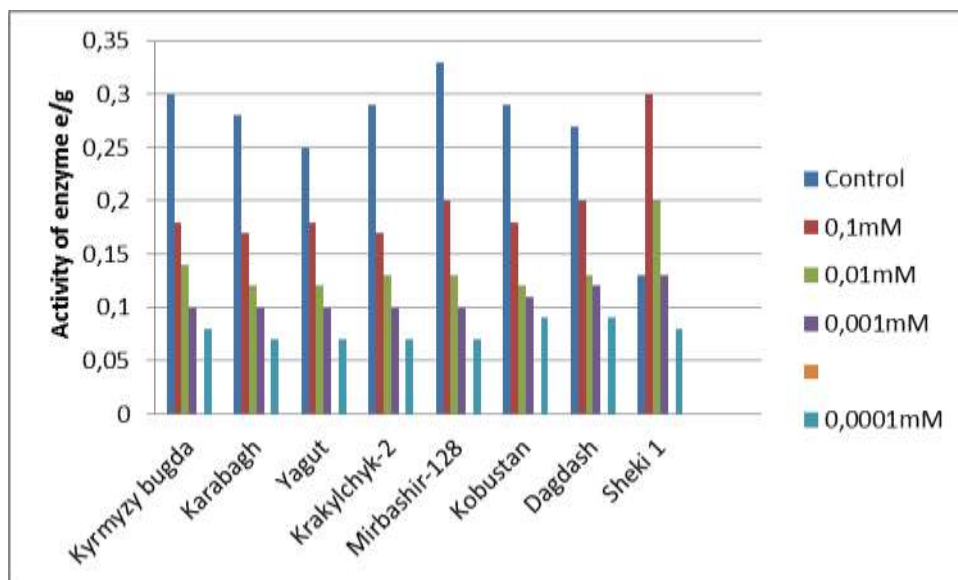
To determine the enzyme activity (CAT, KF 1.11.1.6), the method according to [9].

### **Determination of SOD activity**

The enzyme activity (SOD, EC 1.15.1.1) was assessed by inhibition of photoreduction of nitroblue tetrazolium (NBT) [11]. For this purpose, a plant sample weighing 1.5-2 g was ground with an extraction medium containing 50 mM Na,K-phosphate buffer (pH 7.8), 0.01 mM EDTA and 1% PVP solution, followed by filtration and centrifugation for 15 min at 8000 rpm.

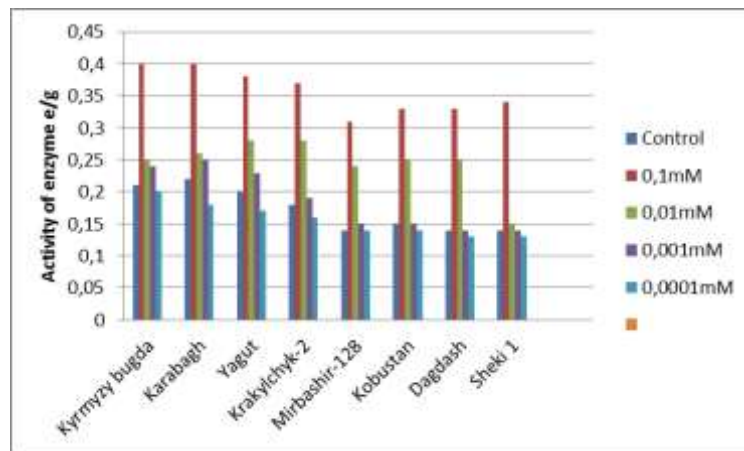
## **Results and discussion**

Catalase-.CAT (E.F. 1.11.1.6) is one of the enzymatic components of the antioxidant defense system of plants, which is a heme-containing enzyme with a molecular weight of about 250 kDa, catalyzing the decomposition of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into water and molecular oxygen. The enzyme is localized mainly in peroxisomes and glyoxysomes, its specific form has also been identified in mitochondria [6]. It is believed that, unlike peroxidases, it effectively acts at high concentrations of H<sub>2</sub>O<sub>2</sub>. As a result of the conducted studies, it was revealed that the activity of CAT in wheat sprouts under the influence of ferric oxide NPs depends on varietal characteristics (Fig. 1).



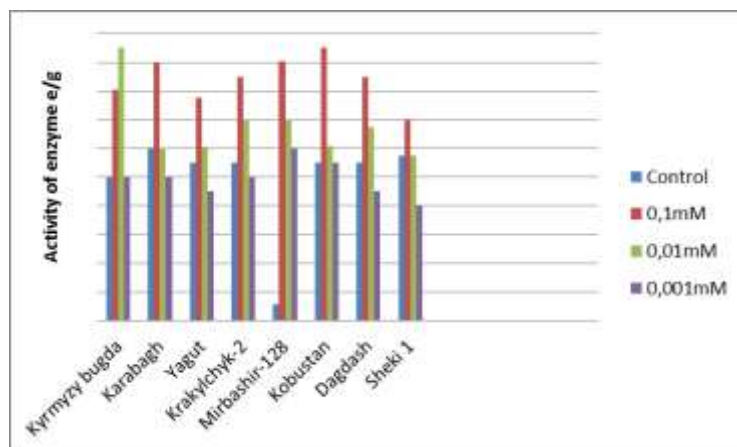
**Fig. 1** Effect of different concentrations of ferric oxide NPs on CAT activity in two-week-old sprouts of hard and soft wheat varieties

According to the presented data, the highest enzyme activity was observed in sprouts of the soft wheat variety Mirbashir-128 of the first series, which is approximately 2.5 times higher than the control values of this variety. The minimum enzyme activity was in sprouts of the hard wheat variety Karabakh of the fourth series. Thus, relatively low concentrations of ferric oxide NPs had a stimulating effect on CAT activity, whereas at a concentration of 100 mg / kg of soil, the enzyme activity significantly decreased in both hard and soft wheat varieties. Judging by the research results, the most optimal concentration of these NPs was 15 mg / kg of soil. SOD (E.F. 1.15.1.1) catalyzes the reaction of dismutation of two superoxide radicals to form H<sub>2</sub>O<sub>2</sub> and molecular oxygen [2]. The enzyme protects plant cells and tissues from oxidative damage and the effects of adverse environmental factors. H<sub>2</sub>O<sub>2</sub>, formed during the dismutation of the superoxide anion radical, can act as an SOD inhibitor, and therefore the effective operation of the enzyme depends on the functioning of other components of the AOS protection. In the course of experiments aimed at studying the effect of various concentrations of ferric oxide NPs on SOD activity, the following results were obtained in sprouts of hard and soft wheat varieties (Fig. 2).



**Fig. 2.** Effect of different concentrations of ferric oxide NPs on SOD activity in two-week-old sprouts of hard and soft wheat varieties

According to the results obtained during the experiments, the effect of ferric oxide NPs contributed to an increase in enzyme activity in all studied hard and soft wheat varieties of the first, second and third series, although in the sprouts of the fourth series, on the contrary, a significant decrease in SOD activity was observed. The highest enzyme activity was characteristic of the sprouts of the first series of the hard wheat variety Karabakh, which is approximately 83% higher than the control, the lowest for the sprouts of the fourth series of the soft wheat variety Sheki-1. According to the data presented in Fig. 34, it can be said that at relatively low concentrations, the effect of these NPs on SOD activity is stimulating in nature, which is of great practical importance. The data in Fig. 3 reflect the results obtained during the effect of a number of molar solutions (0.1 mM; 0.01 mM and 0.001 mM) of aluminum oxide NPs on catalase activity in the leaves of various wheat genotypes.

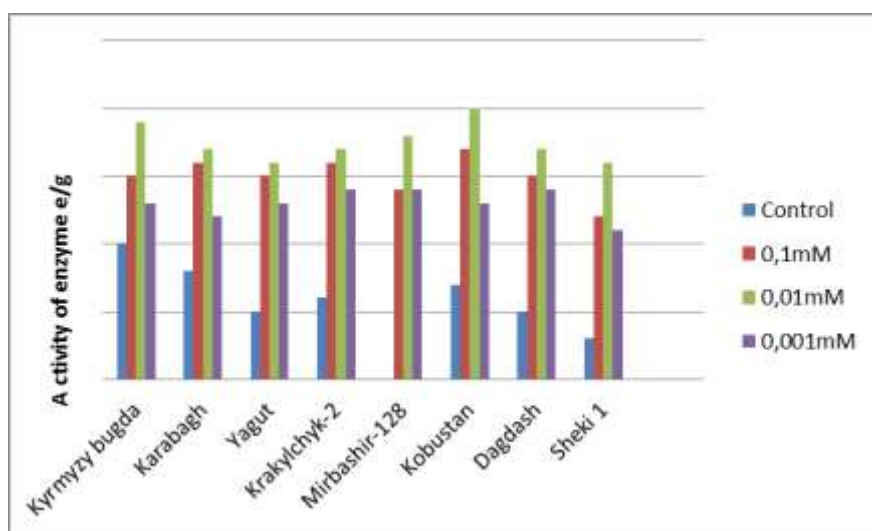


**Fig. 3** The influence of different concentrations of aluminum oxide NPs on CAT activity in two-week-old seedlings of durum and soft wheat varieties.

According to the data presented in Fig. 3 the maximum enzyme activity was observed in seedlings of the soft wheat variety Gobustan of the first series, which is 69% higher than the control values of the same variety. The lowest CAT activity was characteristic of seedlings of the soft wheat variety Sheki-1 of the third series.

Analyzing the data presented above, we can say that with an increase in the concentration of these NPs, the enzyme activity in the studied wheat varieties increased. Thus, at concentrations of 0.01 mM and 0.1 mM, a stimulating effect of these NPs was detected, although at a concentration of 0.001 mM, metal oxide NPs had an inhibitory effect on the activity of CAT in both durum and soft wheat varieties.

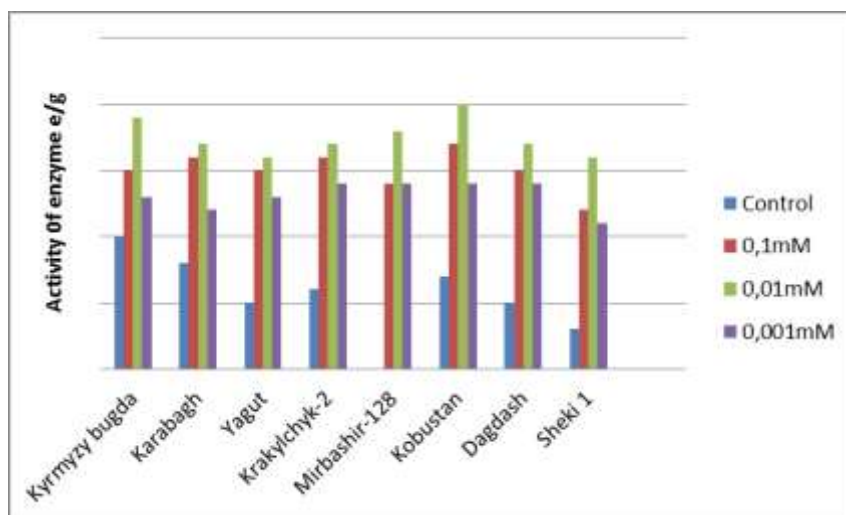
As part of the experiments, data were obtained on the effect of 0.1 mM; 0.01 mM and 0.001 mM concentrations of aluminum oxide NPs for 2-week-old seedlings of various durum and soft wheat varieties. The results are presented below (Fig. 4).



**Fig. 4** Effect of different concentrations of aluminum oxide NPs on SOD activity in two-week-old seedlings of durum and soft wheat varieties.

According to the data presented in Fig. 4, we can say that the effect of exposure to these NPs was dose-dependent. Thus, the highest enzyme activity was observed in seedlings of the soft wheat variety Gobustan at a concentration of 0.01 mM, which is 53% higher than the control values of this variety. Minimal SOD activity was characteristic of seedlings of the soft wheat variety Sheki-1 of the first series. A detailed examination of the data obtained shows that a 0.1 mM solution of metal oxide NPs had an inhibitory effect on the activity of the enzyme. Although a 0.001 mM concentration of aluminum oxide NPs had a stimulating effect on SOD activity, the maximum enzyme activity was characteristic of seedlings of the second series.

In the course of the studies, the effect of three concentrations of zinc oxide NPs (0.1 mM; 0.01 mM and 0.001 mM) on enzyme activity was studied. The results obtained are presented in (Figure 5.)

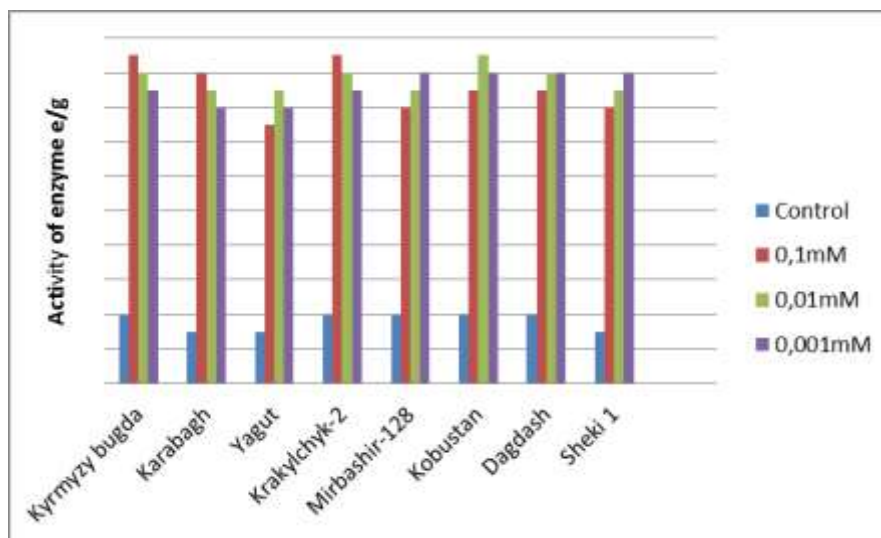


**Fig. 5** Effect of different concentrations of zinc oxide NPs on CAT activity in two-week-old seedlings of durum and soft wheat varieties.

The highest CAT activity was found among durum wheat varieties in seedlings of the second series of Gyrmyzy Bugda, which is 6 times higher than the control values of this variety, among soft wheat varieties in seedlings of the third series of Gobustan, which is 400% higher than the control. Minimal enzyme activity under the influence of zinc oxide NPs was characteristic of the third series of the durum wheat variety Karabakh, and among soft wheat varieties - for the first series of the Dagdash variety

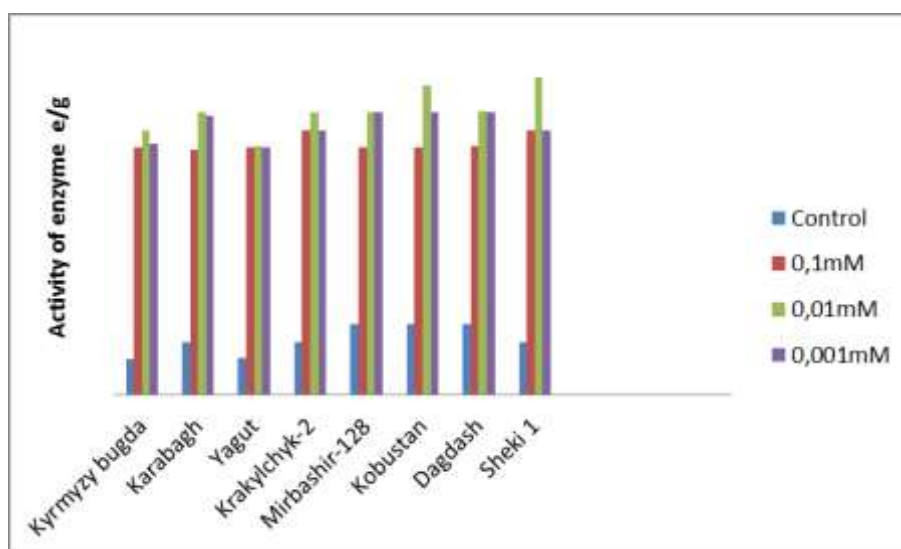
Upon closer examination of the data presented in Fig.5 one can see the dependence of the effect of zinc oxide NPs on the activity of CAT on the varietal characteristics of the crops under study. Despite the fact that increasing the concentration of NPs contributed to an increase in enzyme activity compared to the control in all durum wheat varieties studied, the optimal concentration was 0.01 mM. As for soft wheat varieties, with an increase in the concentration of metal oxide NPs, the activity of CAT decreased; the optimal concentration was 0.001 mM.

The indicators in Fig. 6 made it possible to establish the stimulating effect of metal oxide NPs on the activity of the SOD enzyme in all studied wheat varieties. The nature of the action of NPs was different in durum and soft wheat varieties. So, if in durum wheat varieties, with an increase in the concentration of these NPs, SOD activity increased, in soft wheat varieties (Mirbashir-128 and Sheki-1), on the contrary, a decrease in enzyme activity was observed. As for the soft wheat varieties Gobustan and Dagdash, the optimal concentration of zinc oxide NPs was 0.01 mM.



**Fig. 6.** Effect of different concentrations of titanium dioxide NPs on CAT activity in two-week-old seedlings of durum and soft wheat varieties

According to the data presented in Fig. 6, the maximum enzyme activity was characteristic of the seedlings of the second series of the soft wheat variety Gobustan, as for hard wheat varieties - for the seedlings of the second series of the Garagylchyg-2 variety. Minimal CAT activity under the influence of these NPs was observed in seedlings of the first series of the durum variety Karabakh and soft wheat variety Gobustan.



**Fig. 7.** Effect of different concentrations of titanium dioxide NPs on SOD activity in two-week-old seedlings of durum and soft wheat varieties



In accordance with the data in Fig 7., the stimulating effect of metal oxide NPs on enzyme activity was established in all studied wheat varieties.

Upon detailed examination of the numerical values, it was possible to establish that the highest indicators of SOD activity were characteristic of the second series of wheat seedlings; therefore, the optimal concentration of these NPs for their effect on enzyme activity was 0.01 mM

Judging by the data presented in Fig.78, the highest SOD activity was characteristic of the second series of wheat seedlings, from hard varieties - Garagylchyg-2, from soft varieties - Sheki-1. The obtained values were much higher than the control values of these varieties. As for the minimum enzyme activity, it was observed in wheat seedlings of the first series among durum varieties - Karabakh, among soft varieties - Mirbashir-128.

Based on the data obtained, presented in this chapter, we can draw a general conclusion that NPs of various metal oxides can affect the activity of antioxidant enzymes, the effect of which is dose-dependent.

Thus, the results of the action of NPs on plant processes depend both on the type of NPs and on their applied concentrations, therefore, before using them, it is necessary to establish the type and optimal dose of NPs that have a positive effect on metabolic processes and the activity of antioxidant enzymes.

Analysis of the data the Table 1 (below) showed that, with the exception of aluminum oxide NPs, all other NPs (ferric oxide, titanium dioxide, and zinc oxide) had a positive effect on such crop elements as (ear length, ear weight, number of grains in an ear, grain weight in an ear, grain).

## **Conclusions**

1. The effect of NPs of ferric iron, aluminum, zinc and titanium dioxide oxides on the activity of antioxidant enzymes, the content of photosynthetic pigments and the structure of the crop depended both on the applied concentrations of NPs and on the varietal characteristics of the crops under study.
2. Treatment of seeds of durum wheat varieties Gyrmyzy Bugda, Karabakh and soft wheat Mirbashir-128 with ferric iron oxide NPs at a concentration of 100 mg/kg made it possible to isolate them into a separate group to obtain high-quality flour products.
3. Durum wheat varieties Gyrmyzy Bugda, Garagylchyg-2 and soft wheat Dagdash, Gobustan were invulnerable to the action of increased doses of ferric iron oxide NPs, which was of great practical importance in breeding work for obtaining resistant wheat varieties.
4. When affecting the activity of CAT in all studied durum wheat varieties, the optimal concentration was 0.01 mM, in soft varieties – 0.001 mM. SOD activity depended on varietal characteristics.
5. Titanium dioxide NPs at a concentration of 0.01 mM had a stimulating effect on the activity of antioxidant enzymes, the content of photosynthetic pigments by 9-13% and yield by 5-83%, taking into account the varietal characteristics of wheat seedlings.

**Table 1.** The influence of NPs of trivalent iron, aluminum, zinc and titanium dioxide oxides on the yield structure of durum and bread wheat varieties under normal irrigation conditions

Options	Ear length, cm	Ear weight, g	Number of grains in an ear, pcs.	Grain weight in an ear, g	Grain yield, g/m <sup>2</sup>
KYRMYZY BUGDA					
Control	8,5±0,2	1,5±0,3	25	1,1±0,02	224±1,2
Fe <sub>2</sub> O <sub>3</sub>	9,6±0,3	1,8±0,1	32	1,5±0,01	315±1,5
TiO <sub>2</sub>	10,5±0,1	2,1±0,2	35	1,7±0,03	410±1,3
Al <sub>2</sub> O <sub>3</sub>	8,2±0,4	1,4±0,3	23	1,0±0,01	222±0,2
ZnO	8,9±0,2	1,6±0,1	26	1,3±0,02	225±0,1
YAGUT					
Control	8,8±0,2	1,8±0,3	19	1,8±0,03	278±0,3
Fe <sub>2</sub> O <sub>3</sub>	9,8±0,3	2,1±0,4	20	1,9±0,4	310,5±0,4
TiO <sub>2</sub>	10,5±0,4	2,7±0,5	29	2,7±0,5	318,7±0,5
Al <sub>2</sub> O <sub>3</sub>	8,7±0,5	1,5±0,1	17	1,5±0,1	274,5±0,1
ZnO	9,5±0,1	2,8±0,3	30	2,8±0,3	319,8±0,3
KARABAGH					
Control	9,6±0,1	1,9±0,4	29	1,3 ±0,04	384 ±3,7
Fe <sub>2</sub> O <sub>3</sub>	10,5±0,2	2,1±0,3	35	1,8±0,05	421±4,1
TiO <sub>2</sub>	11,2±0,4	2,5±0,1	38	2,0±0,04	435±3,2
Al <sub>2</sub> O <sub>3</sub>	9,1±0,1	1,8±0,2	27	1,1±0,03	379±4,4
ZnO	10,2±0,3	2,2±0,3	32	1,9±0,02	391±2,5
KARAKYLCHYK-2					
Control	10,5±0,3	2,1±0,1	38	1,5±0,04	395±4,5
Fe <sub>2</sub> O <sub>3</sub>	12,6±0,4	3,2±0,3	43	1,9±0,05	425±5,4
TiO <sub>2</sub>	14,3±0,5	4,3±0,5	48	2,5±0,03	438±3,6
Al <sub>2</sub> O <sub>3</sub>	9,6±0,2	1,8±0,2	36	1,1±0,02	386±2,5
ZnO	13,4±0,1	2,8±0,4	42	1,8±0,01	405±5,2
MIRBASHIR -128					
Control	10,2±0,2	2,2±0,3	35	1,7±0,05	391±3,2
Fe <sub>2</sub> O <sub>3</sub>	10,6±0,3	2,5±0,2	36	1,9±0,01	395±4,1
TiO <sub>2</sub>	11,3±0,1	2,8±0,1	38	2,1±0,02	405±5,1
Al <sub>2</sub> O <sub>3</sub>	10,0±0,4	2,1±0,4	34	1,5±0,03	350±4,7
ZnO	12,4±0,3	2,9±0,5	38	2,2±0,04	399±0,1
KOBUSTAN					
Control	9,6±0,1	1,9 ± 0,4	29	1,3 ± 0,04	384,8 ±3,7
Fe <sub>2</sub> O <sub>3</sub>	10,5±0,2	2,1±0,3	35	1,8±0,05	421±4,1
TiO <sub>2</sub>	11,2±0,4	2,5±0,1	38	2,0±0,04	435±3,2
Al <sub>2</sub> O <sub>3</sub>	9,3±0,1	1,6±0,2	24	1,1±0,03	378±4,4
ZnO	10,8±0,3	2,4±0,3	37	1,9±0,01	425±1,7
DAGDASH					
Control	9,7±0,2	2,1±0,1	28	1,2±0,01	381±4,3
Fe <sub>2</sub> O <sub>3</sub>	11,5±0,5	2,4±0,2	34	1,7±0,02	389±3,2
TiO <sub>2</sub>	12,4±0,3	2,8±0,2	35	1,8±0,02	435±4,6
Al <sub>2</sub> O <sub>3</sub>	9,5±0,4	1,9±0,1	26	1,0±0,01	376±3,5
ZnO	11,6±0,1	2,7±0,3	30	1,9±0,02	438±5,4
SHEKI-1					
Control	8,5±0,1	1,8±0,3	20	1,8±0,03	275±0,3
Fe <sub>2</sub> O <sub>3</sub>	9,8±0,3	2,1±0,4	21	1,9±0,4	311,5±0,4
TiO <sub>2</sub>	10,5±0,4	2,7±0,5	29	2,7±0,5	319,7±0,5
Al <sub>2</sub> O <sub>3</sub>	8,1±0,5	1,5±0,1	18	1,5±0,1	271,5±0,1
ZnO	9,5±0,1	2,8±0,3	30	2,8±0,3	318,8±0,3

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