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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

INVESTIGATION OF THE EFFECT OF METAL OXIDE NANOPARTICLES ON ANTIOXIDANT ENZYMES IN THE LEAVES OF WHEAT GENOTYPES

Specialty: 2406.02 – Biochemistry

Field of science: Biology

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Baku – 2025

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GENERAL CHARACTERISTICS OF THE RESEARCH

The relevance of the topic and the degree of completion. A large part of agricultural cultivated areas is occupied by cereal crops, which play an important role in meeting the food needs of the world population. One of the pressing issues of the modern era is the shortage of food products and the necessity of taking preventive measures in this field. Increasing the productivity of agricultural crops could be a solution to this problem.

In recent years, interest in nanotechnologies (NT) in crop production has increased, which plays a significant role in the application of innovative agronanotechnological tools in agriculture, particularly in the development of highly productive genotypes that are resistant to adverse environmental conditions, diseases, and pests[1].

In biological research, initial experiments involving nanoparticles (NPs) were conducted primarily on plants due to their relatively short growth cycle and the rapid obtaining of research results. Although the concept of NP toxicity for agricultural crops is still at an early stage, the increasing concentration of NPs in the environment could cause serious damage to agriculture in the future[2].

In addition, the development of new methods for assessing the effects of NPs on biological objects is extremely important, as NPs can either stimulate or, conversely, inhibit biochemical processes in plants depending on their concentration and properties.[3]

In this regard, the use of NPs with an already established safety profile for living systems is more appropriate in plants.

The points outlined above justify the relevance of the current research work and the necessity of its implementation.

Object and subject of the research. The objects of the research were two-week-old seedlings of Gyrmyzy bugda, Garagylchyg-2,

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[1] Федоренко, В.Ф. 2011. Нанотехнологии и наноматериалы в агропромышленном комплексе / В.Ф.Федоренко М.Н.Ерохин, В.И.Балабанов [и др.] – Москва: ФГБНУ "Росинформагротех", – 2011. – 312 с.

[2] Bhagat, Y. Nanotechnology in agriculture: a review / Y.Bhagat, K. Gangadhara, C.Rabinal [et al.] // Journal of Pure and Applied Microbiology, – 2015. vol. 9, – p. 737-747.

[3] Yatts, D., Ling, Y. Nanoparticles could have a negative effect on plant growth // Nanotechnology News, – 2007. № 3. – p. 86-92.

Yagut, and Karabakh varieties belonging to (*Triticum durum* Desf.), and Mirbashir-128, Gobustan, Sheki-1, and Dagdash wheat varieties belonging to (*Triticum aestivum* L.), obtained from the Research Institute of Crop Husbandry. The subject of the research was the study of the effects of molar solutions of iron (III) oxide (Fe_2O_3), aluminum oxide (Al_2O_3), zinc oxide (ZnO), and titanium (IV) oxide (TiO_2) NPs on the morphological, physiological, and biochemical characteristics of the plants.

The purpose and tasks of the research. The aim of the research was to study the effects of different concentrations of Fe_2O_3, Al_2O_3, ZnO, and TiO_2 NPs on the activity of antioxidant enzymes and isoenzyme spectra, the content of photosynthetic pigments, the morphological characteristics, and the productivity of various wheat genotypes.

To achieve this aim, the following tasks were set:

– To study the effects of Fe_2O_3, Al_2O_3, ZnO, and TiO_2 NPs at different concentrations on the activity of ascorbate peroxidase (APO), polyphenol oxidase (PPO), superoxide dismutase (SOD), and catalase (CAT) enzymes in the leaves of wheat genotypes;

– To study the isoenzyme spectra of antioxidant enzymes (APO, SOD, CAT) in two-week-old seedlings of different wheat genotypes;

– To evaluate the effects of molar solutions of Fe_2O_3, Al_2O_3, ZnO, and TiO_2 NPs on the morphophysiological characteristics and productivity of different bread and durum wheat genotypes under normal irrigation conditions;

– To perform cluster analysis based on the productivity indicators of wheat genotypes under the influence of Fe_2O_3, Al_2O_3, ZnO, and TiO_2 NPs and to select wheat samples with high productivity;

– To study the effects of solutions of Fe_2O_3, Al_2O_3, ZnO, and TiO_2 NPs on the content of photosynthetic pigments in wheat seedlings;

– To investigate the correlation dependencies between the activity of antioxidant enzymes, the content of photosynthetic pigments, morphological characteristics, and productivity indicators in a number of bread and durum wheat genotypes based on the obtained data.

Research methods. After wheat seeds were treated with various metal oxide NPs, the activity of antioxidant enzymes, their isoenzyme composition, the content of photosynthetic pigments, and productivity indicators in two-week-old seedlings were studied using appropriate methods.

The main points of the dissertation for defense:

– Effect of the applied metal oxide NPs on wheat seedlings is dose-dependent: at lower concentrations, Fe_2O_3, ZnO, and TiO_2 NPs stimulate physiological and biochemical processes, while at higher concentrations, they exhibit toxic effects;

– Al_2O_3 NPs have an inhibitory effect on the activity of antioxidant enzymes, the content of photosynthetic pigments, and the productivity of the studied bread and durum wheat genotypes;

– Under normal irrigation conditions, Fe_2O_3, ZnO, and TiO_2 NPs at a concentration of 0.01 mM have a positive effect on the content of photosynthetic pigments, morphological characteristics, and the structure of the yield in various bread and durum wheat genotypes;

– Durum and bread wheat genotypes respond to the effect of NPs by regulating the activity of antioxidant enzymes and their isoenzyme composition. Depending on the chemical nature of the NPs, their photocatalytic activity, interaction with intracellular biomolecules, and ability to penetrate the cell, the isoenzyme spectra of antioxidant enzymes may change;

– Under normal irrigation conditions, the optimal dose of NPs in terms of their effect on the activity of antioxidant enzymes in the studied durum and bread wheat genotypes is 0.1 mM for Fe_2O_3, and 0.01 mM for ZnO and TiO_2 NPs;

– Highly significant positive correlations between the activity of PPO, CAT, and SOD enzymes (r=0.723**; r=0.653**; r=0.874**) are detected in the seedlings of both bread and durum wheat genotypes, while negative correlations exist with the activity of APO (r=-0.632**; r=-0.627**; r=-0.516**);

– Among the studied wheat genotypes, the highest productivity indicators were observed in Garagylchyg-2 (*Triticum durum* Desf.) and Dagdash (*Triticum aestivum* L.) genotypes, with this effect recorded upon the application of TiO_2 and ZnO NPs at a concentration of 0.01 mM.

Scientific novelty of the research. For the first time, the effects of different concentrations of metal oxide NPs on the biochemical, physiological, and agronomic characteristics of various wheat genotypes were studied comprehensively. The optimal concentrations of different metal oxide NPs that positively affected the activity of certain antioxidant enzymes, the amount of photosynthetic pigments, and the productivity of the studied wheat genotypes under normal irrigation conditions were determined.

Furthermore, the isoenzyme spectra of SOD, CAT, and APO were studied, which opens new horizons for future biochemical research. Correlation relationships between the studied indicators were determined, and the possible mechanisms of action of NPs were also discussed. The obtained results expand the understanding of the role of NPs as growth stimulators in plants and their contribution to increasing resistance.

Thus, the analysis of the reactions of different wheat genotypes to the effects of NPs creates new opportunities for identifying sustainable genotypes that are crucial for the development of agriculture in Azerbaijan.

Theoretical and practical significance of the research. The study of the effects of NPs on the photosynthetic apparatus and the morphological characteristics of seedlings significantly enhances the management of productivity through external influences.

During the conducted research, the positive or negative effects of metal oxide NPs on the activity of antioxidant defense system enzymes, morphophysiological characteristics, and productivity indicators in two-week-old seedlings of different wheat genotypes were studied, which has great practical significance. In the experiments conducted, the increase in APO activity proves the protective function of plants by focusing on the regeneration of hydroxyl radicals. To minimize risks, prevent the negative effects of NPs, and ensure the safety of agricultural crops, the optimal doses of Fe_2O_3, ZnO, and TiO_2 NPs were recommended. These doses had a positive effect on the morphophysiological characteristics and productivity of the studied wheat genotypes under normal irrigation conditions. Additionally, the possible mechanisms of nanoparticle effects were discussed. The Dagdash genotype, which demonstrated resistance to the effects of high doses of Fe_2O_3, NPs, is recommended as a valuable starting material for developing resistant wheat genotypes in plant breeding.

The research results can be applied in the development of recommendations to optimize the use of NPs in agriculture for the cultivation of new wheat genotypes with improved stress tolerance and enhanced productivity indicators.

Approbation of the research. The results of the dissertation were discussed at the “XXIII Republican Scientific Conference of Doctoral Students and Young Researchers” organized by the Ministry of Education of the Republic of Azerbaijan (Baku, 2019), at the IX International Scientific Conference on "Innovative Approaches in Modern Biology" dedicated to the 100th anniversary of Baku State University (Baku, 2019), at the XIX International Scientific-Practical Conference on "Integration Processes in Modern Science: New Approaches and Current Problems" (Russian Federation, Anapa, 2024), at the LXXXI–LXXXIII International Scientific-Practical Conferences on "Chemistry, Physics, Biology, Mathematics: Theoretical and Applied Research" (Russian Federation, Moscow, 2024), at the Scientific-Practical Conference on "The Role of National Leader Heydar Aliyev in Environmental Improvement in Azerbaijan" (Baku, 2024), at the LXXVII International Scientific-Practical Conference on "Natural Sciences and Medicine: Theory and Practice" (Russian Federation, Novosibirsk, 2024), and at the II International Plant Protection Conference (Pakistan, Tandojam, 2025).

Based on the dissertation materials, a total of 18 scientific papers have been published, including 10 articles in local and international journals and 8 conference proceedings.

Organization where the dissertation was carried out. The dissertation was carried out at the Department of Biophysics and Biochemistry of Baku State University.

Volume and structure of the dissertation.

The dissertation consists of an introduction (12473 characters), five chapters: Chapter I – 42718 characters; Chapter II – 17158 characters; Chapter III – 32455 characters; Chapter IV – 75533 characters; Chapter V – 9529 characters, conclusions (2747 characters), results (2184 characters), practical recommendations (512 characters), a list of 282 references, and a list of abbreviations (323 characters). It is presented on 178 computer pages and has a total volume of 195632 characters. The dissertation includes 20 figures and 8 tables.

CHAPTER I. LITERATURE REVIEW

This chapter provides information on the development history of nanoparticles (NPs) and presents a comparative analysis of bibliographic data regarding the effects of various metal oxide NPs on the morphological and physiological characteristics of plants, as well as on the activity of the antioxidant system (AOS). According to the literature sources, the application of NPs in plant science may, in the future, contribute to the development of high-yielding genotypes, particularly those tolerant to unfavorable environmental conditions.

CHAPTER II. MATERIALS AND METHODS

As research material, the following wheat varieties obtained from the Azerbaijan Republic's Research Institute of Crop Husbandry were selected: Gyrmyzy bugda, Garagylchyg-2, Yagut, and Karabakh (durum wheat varieties), as well as Mirbashir-128, Gobustan, Sheki-1, and Dagdash (bread wheat varieties). Wheat seeds were treated using nanopriming and sonication methods with molar solutions of metal oxide NPs at various concentrations[4]. Under normal irrigation conditions, the activities of the enzymes PPO[5], APO[12], SOD[6], and CAT [7] were determined in two-week-old seedlings using

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[4] Marthandan, V. J. Seed priming: a feasible strategy to enhance drought tolerance in crop plants. / V.J. Marthandan, R. Geetha, K. Kumutha [et al.] // Int. J. Mol. Sci., – 2020, vol. 21, – p. 1-23.

[5] Ермаков И.П. и др. Физиология растений: учебник для студ. вузов. Москва: Академия, – 2007. – 465 с.

[6] Никерова, К.М. Определение активности супероксиддисмутазы и полифенолоксидазы в древесине *Betula pendula* var. *carelica* (*Betulaceae*) при разной степени нарушения ксилогенеза / К.М.Никерова, Н.А.Галибина, Ю.Л.Мощенская [и др.] // Растительные ресурсы, – Санкт-Петербург – 2019, т. 55, №2, – с. 213-230

[7] Шапкарин, В.В. Биохимия: сборник лабораторных работ / В.В. Шапкарин, А.П.Королев, С.Б.Гридина [и др.] – Кемерово: изд-во Кемеровского технологич. ин-та пищевой промышленности, – 2005. – 84 с.

spectrophotometric and titrimetric analysis methods. The isoenzyme composition of antioxidant enzymes was studied using the electrophoresis method in polyacrylamide gels (PAGE) at 4°C for 3 hours under a constant electric current of 30 mA[8, 9, 10]. Gels with 7% concentration were used for CAT, and 10% gels were used for SOD and APO. The isoenzymes were detected by staining the gels with specific dye buffers appropriate for each enzyme. The amount of photosynthetic pigments was determined using spectral analysis[11]. The yield characteristics of the wheat genotypes were evaluated based on international descriptors.

Statistical analyses. All experiments in the dissertation were conducted in three biological replicates. The reliability indicators and errors of the obtained results were determined based on the Student's t-test, and differences at $p \leq 0.01$ and $p \leq 0.05$ were considered statistically significant. Correlation analysis among the studied parameters was performed using the Pearson method, while the clustering of wheat genotypes based on yield traits was conducted using the Ward method with evaluation of Euclidean genetic distance indices, employing the IBM SPSS Statistics 26.0 software.

CHAPTER III. THE EFFECT OF METAL OXIDE NANOPARTICLES ON THE ACTIVITY OF ANTIOXIDANT ENZYMES IN WHEAT LEAVES

This chapter investigates the effects of metal oxide NPs at different concentrations on the activities of the enzymes SOD, CAT,

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[8]Davis, B.J. Disc Electrophoresis-II method and application to human serum proteins // Annals of the New York Academy of Sciences, – 1964, vol. 121, – p. 404-427.

[9] Mittler, R., Zilinskas, B.A. Detection of ascorbate peroxidase activity in native gels by inhibition of the ascorbate-dependent reduction of nitroblue tetrazolium // Analytical Biochemistry, – 1993, vol. 212, – p. 540-546.

[10] Anderson M.D., T.K.Prasad, C.R.Steward. Changes in ısozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings // Plant Physiol, – 1995, 109 (4), – p. 1247-1257.

[11] Калинина, А.В., Лящева, С.В. Состав и содержание пигментов фотосинтеза в листьях проростков озимой мягкой пшеницы // – Самара: Известия Самарского научного центра Российской академии наук, – 2018, т. 20, №2 (2), – с. 286-290.

APO, and PPO, includes isoenzyme analysis, and presents a comparative evaluation of the obtained results with bibliographic sources.

3.1. Effect of different concentrations of ferric (III) oxide nanoparticles on the activity of antioxidant enzymes in wheat leaves

Plants are periodically or continuously exposed to unfavorable environmental factors in their life activities, which leads to an increase in the production of reactive oxygen species (ROS) in plant cells, regulated by the AOS.

During the study, Fe_2O_3, NPs (0.1 mM, 0.2 mM, 0.3 mM, and 0.6 mM) were used for the treatment of different wheat seeds.

3.1.1. Effect of different concentrations of ferric (III) oxide nanoparticles on the activity of ascorbate peroxidase in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The results of the study showed a difference in the activity of ascorbate peroxidase (APO) between the control and treated wheat genotypes (Figure 1).

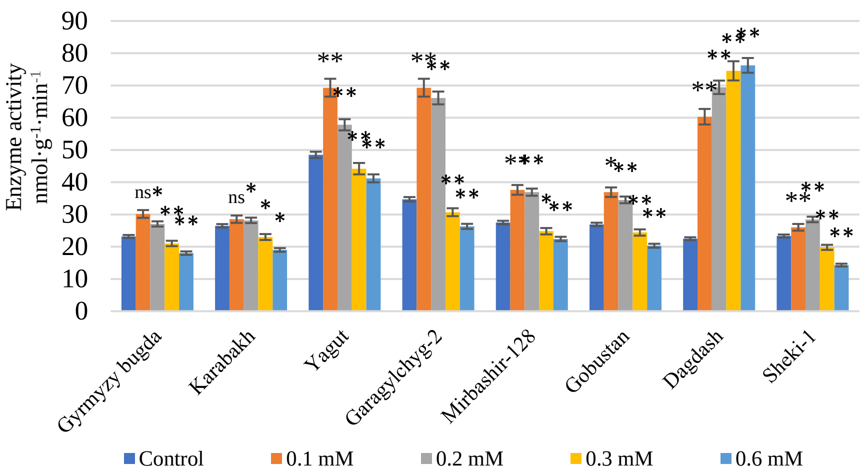


Figure 1. Effect of Fe_2O_3 nanoparticles on APO activity in wheat seedlings

At concentrations of 0.1 mM and 0.2 mM, the effect of NPs led to the formation of reactive oxygen species (ROS), which activated the cell's defense mechanisms and resulted in an increase in APO activity. In the Karabakh and Gyrmyzy bugda genotypes, the effect of NPs on enzyme activity was minimal, but in the Yagut and Garagylchyg-2 genotypes, APO activity increased by 42%, 19%, and 100%, 91%, respectively, compared to the control. In the case of bread wheat genotypes, the activity of the enzyme in Sheki-1 seedlings did not significantly change compared to the control. However, in Mirbashir-128 and Gobustan genotypes, this indicator increased by 36%, 34%, and 37%, 28%, respectively.

The highest activity of the APO enzyme was observed in Dagdash seedlings at a concentration of 0.6 mM of NPs, while the lowest activity was recorded in the Sheki-1 genotype at the same concentration. Increasing concentrations of NPs led to a decrease in APO activity in all other samples, except for the Dagdash genotype, which indicates the toxic effect of high doses of metal oxide NPs.

At Fe_2O_3 NPs concentrations of 0.3 mM and 0.6 mM, enzymatic activity in the Garagylchyg-2, Gyrmyzy bugda, and Karabakh samples decreased by 2%, 25%, 10%, 20%, and 13%, 28%, respectively, compared to the control. In Yagut genotype seedlings, activity decreased by 9% and 15%. In the Gobustan and Mirbashir-128 genotypes, activity also declined, amounting to 7% and 25%; 10% and 19%, respectively. A decrease in enzyme activity was also observed in Sheki -1 genotype seedlings compared to the control, amounting to 15% and 39%, respectively.

The inhibitory effect of high concentrations of NPs can be explained by the overloading of the cell's defense mechanisms and the increased generation of ROS.

Analysis of the obtained results showed that under the influence of 0.1 mM Fe_2O_3 NPs, ROS were generated at moderate levels in the Garagylchyg-2 and Yagut genotypes, indicating that, compared to other variants, these genotypes are characterized by a more balanced regulation of free radical oxidation processes.

3.1.2. Effect of different concentrations of ferric (III) oxide nanoparticles on the activity of polyphenol oxidase in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The highest enzyme activity was observed in the Mirbashir-128 genotype at a nanoparticle concentration of 0.1 mM (Figure 2). The lowest PPO activity was recorded in the Yagut genotype at a concentration of 0.6 mM, during which phenol oxidation was almost entirely absent.

As seen in figure 2, PPO activity decreases with increasing concentrations of Fe_2O_3 NPs in all genotypes except for Dagdash. In Yagut seedlings, oxidation of polyphenols decreased by 87% under the influence of 0.6 mM NPs, which may be explained by the cytotoxic effect of the NPs. In Gyrmyzy bugda and Karabakh seedlings, an increase in enzyme activity was observed at the first two concentrations of NPs, whereas in the Yagut and Garagylchyg-2 genotypes, the experimental variants showed little difference from the control. These differences are likely related to the individual characteristics of each wheat genotype.

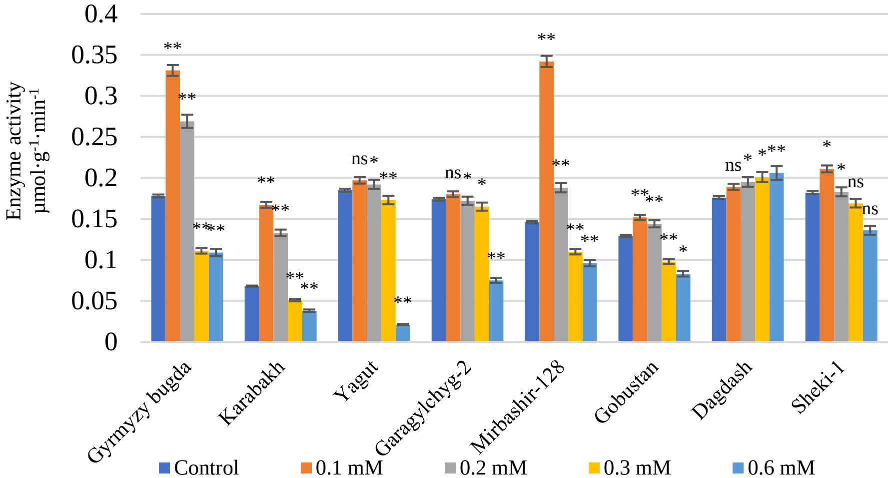


Figure 2. Effect of Fe_2O_3 nanoparticles on PPO activity in wheat seedlings

Regarding the bread wheat genotypes, treatment of the seeds of three out of four tested genotypes with 0.6 mM Fe_2O_3 NPs resulted in a decrease in the rate of polyphenol oxidation. This effect was most evident in the Gobustan genotype seedlings, where PPO activity was approximately 36% compared to the control. The highest enzyme activity was observed in the Mirbashir-128 genotype seedlings at a concentration of 0.1 mM. However, at 0.6 mM, the NPs had almost no effect on increasing PPO activity in this genotype. This is likely related to damage to cell membranes and proteins, as well as excessive accumulation of reactive oxygen species in response to high doses of Fe_2O_3.

In the Dagdash genotype, PPO activity increased with rising concentrations of NPs, which can be attributed to the genotype's genetic characteristics.

Thus, the conducted studies revealed that the rate of phenol metabolism in wheat seedlings varies depending on the individual characteristics of the plant and the dose of NPs. In this context, investigating the oxidation of polyphenols under the influence of metal oxide NPs in different wheat genotypes is of great importance.

3.1.3. Effect of different concentrations of ferric (III) oxide nanoparticles on catalase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The maximum activity of the catalase (CAT) enzyme was observed in the Mirbashir-128 genotype at a nanoparticle concentration of 0.1 mM, which was approximately 2.5 times higher than the control. The minimum enzyme activity was recorded in the Karabakh genotype at a concentration of 0.6 mM (Figure 3).

At lower concentrations (0.1-0.2 mM), NPs had a stimulatory effect on enzyme activity. However, at a concentration of 0.6 mM, CAT activity significantly decreased in all the wheat genotypes studied, except for the Dagdash genotype. This can be explained by the accumulation of numerous reactive oxygen species as a result of high doses of NPs, leading to a decrease in enzymatic activity and degradation of cell membranes. Moreover, it is essential to consider the individual characteristics of the wheat seedlings. Based on the research results, the optimal concentration of NPs was found to be 0.1 mM.

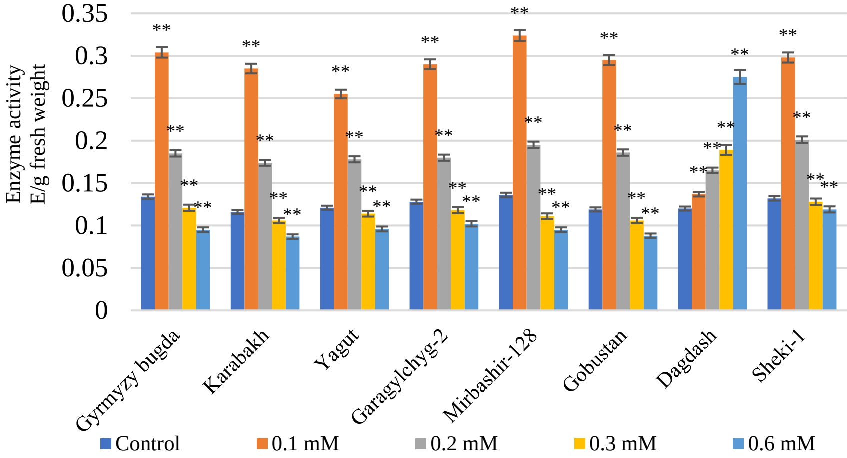


Figure 3. Effect of Fe_2O_3 nanoparticles on CAT activity in wheat seedlings

3.1.4. Effect of different concentrations of ferric (III) oxide nanoparticles on superoxide dismutase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

Fe_2O_3 NPs at concentrations of 0.1 mM and 0.2 mM led to an increase in SOD activity across all studied genotypes. However, at concentrations of 0.3 mM and 0.6 mM, a significant decrease in SOD activity was observed in all genotypes except for the Dagdash genotype (Figure 4).

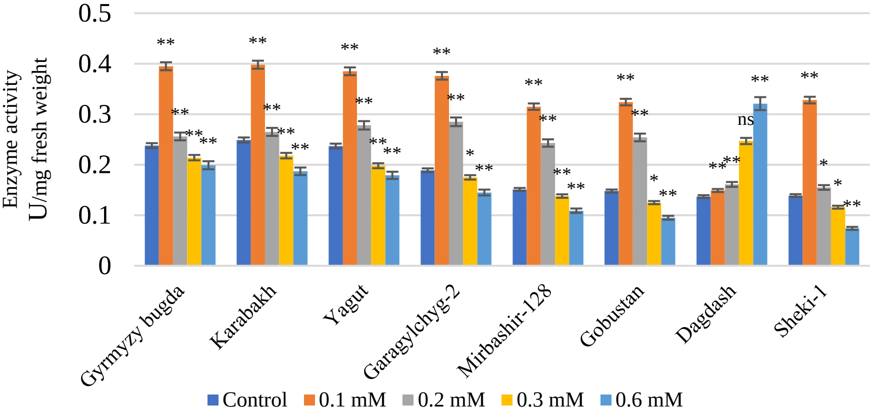


Figure 4. Effect of Fe_2O_3 nanoparticles on SOD activity in wheat seedlings

As is well known, low concentrations of NPs exert a stimulatory effect, whereas at higher doses, they can damage cellular structures through the generation of numerous ROS and thereby inhibit antioxidant system activity.

The highest SOD activity was observed in the Karabakh genotype at a nanoparticle concentration of 0.1 mM, showing an approximately 83% increase compared to the control. In contrast, the lowest activity was recorded in the Sheki-1 genotype at a concentration of 0.6 mM.

Thus, the increase in the activity of various antioxidant enzymes in the Dagdash genotype with rising concentrations of Fe_2O_3 NPs suggests that this genotype may be recommended for use in the breeding of stress-tolerant wheat varieties.

3.2. Effect of different concentrations of aluminum oxide nanoparticles on the activity of antioxidant enzymes in wheat leaves

In the experiments, the seeds of different wheat genotypes were treated with aluminum oxide nanoparticle solutions at concentrations of 0.001 mM, 0.01 mM, and 0.1 mM.

3.2.1. Effect of aluminum oxide NPs at different concentrations on ascorbate peroxidase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

Among the durum wheat genotypes, the lowest enzyme activity was observed in the Gyrmyzy bugda genotype at 0.1 mM NP concentration, which was 4–5 times lower than the control values. As for the bread wheat genotypes, the minimum enzyme activity at the same NP concentration was recorded in the Dagdash genotype. The highest enzyme activity was observed in the Yagut durum wheat genotype and the Mirbashir-128 bread wheat genotype belonging to the control group (Figure 5).

Thus, the analysis of the data showed that as the concentration of NPs increased, the activity of ascorbate peroxidase (APO) decreased. Consequently, it was revealed that increasing doses of NPs had a negative effect on enzyme activity and, accordingly, on the functioning of the antioxidant system in plants.

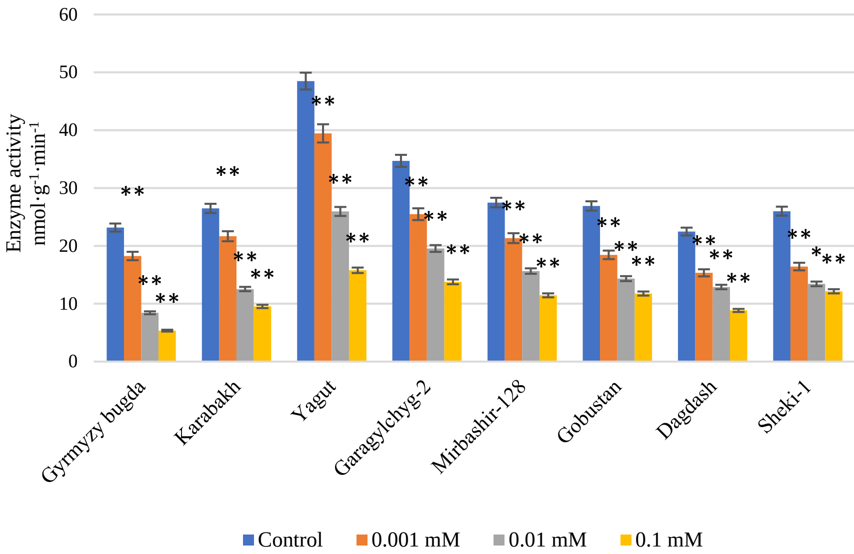


Figure 5. Effect of Al_2O_3 nanoparticles on ascorbate peroxidase activity in wheat seedlings

3.2.2. Effect of aluminum oxide NPs at different concentrations on polyphenol oxidase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

At all tested concentrations, the NPs exhibited an inhibitory effect on enzyme activity. The highest PPO activity was observed in the control variants of the Mirbashir-128 genotype, while the lowest activity was recorded in the Sheki-1 genotype at an NP concentration of 0.1 mM, which was 73% lower than the control (Figure 6).

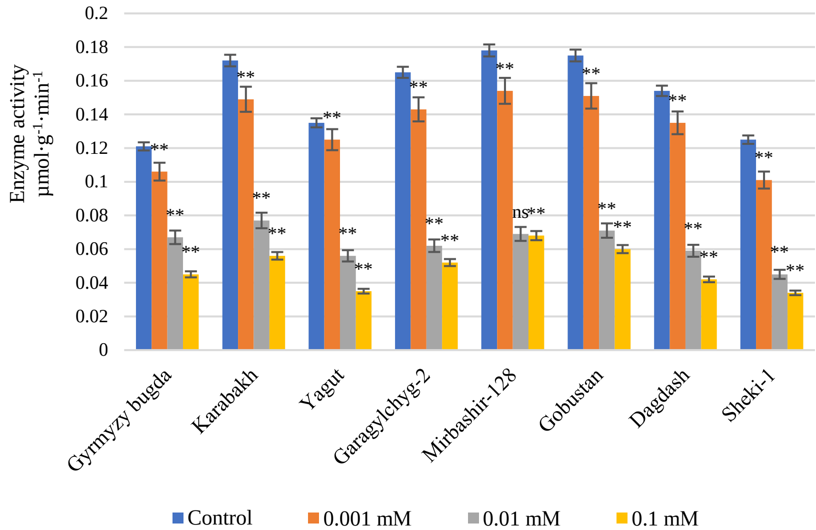


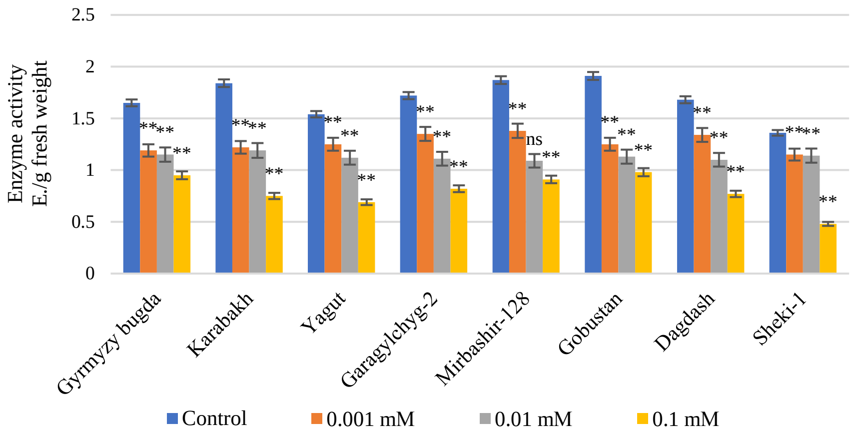
Figure 6. Effect of Al_2O_3 NPs on PPO activity in wheat seedlings

Analysis of the results revealed that the inhibitory effect of NPs increased with rising concentrations. Therefore, the use of Al_2O_3 NPs in agriculture is not advisable.

3.2.3 Effect of aluminum oxide NPs at different concentrations on catalase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The highest catalase (CAT) activity (1.91 E/g) was observed in the control samples of the Gobustan genotype. The lowest CAT activity was recorded in the seedlings of the Sheki-1 genotype at an NP concentration of 0.1 mM (Figure 7).

Analysis of the results revealed that increasing concentrations of NPs enhanced their inhibitory effects on the antioxidant system, thereby allowing the identification of the risks associated with the application of the mentioned NPs in agronomy.



Şəkil 7. Effect of Al_2O_3 nanoparticles on CAT activity in wheat seedlings

3.2.4. Effect of aluminum oxide NPs at different concentrations on superoxide dismutase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The effect of NPs was dose-dependent, with the highest superoxide dismutase (SOD) activity observed in the control samples of the Gobustan genotype, and the lowest activity recorded in the Sheki-1 seedlings at an NP concentration of 0.1 mM (figure 8).

At all tested concentrations, NPs exerted an inhibitory effect on enzyme activity. At 0.1 mM, NPs caused a significant reduction in SOD activity in wheat seedlings, indicating their harmful effects.

3.3 Effect of zinc oxide nanoparticles at different concentrations on the activity of antioxidant enzymes in wheat leaves

In the study, the effect of zinc oxide NP solutions at concentrations of 0.001 mM, 0.01 mM, and 0.1 mM on the activity of antioxidant enzymes in various wheat seedlings was investigated.

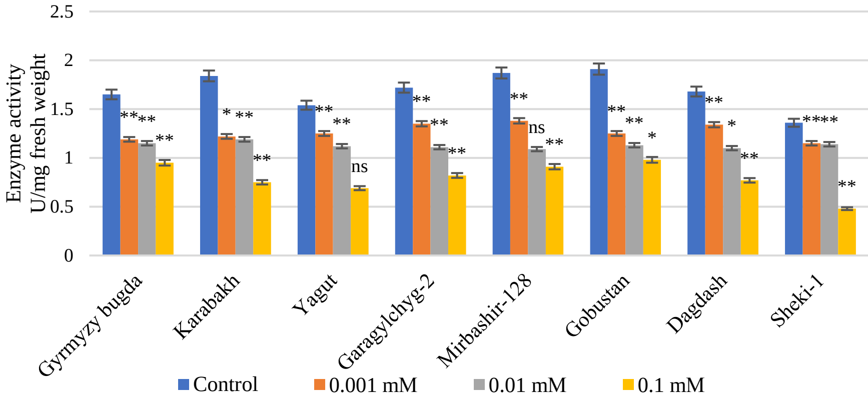


Figure 8. Effect of Al_2O_3 nanoparticles on SOD activity in wheat seedlings

3.3.1. Effect of zinc oxide nanoparticles at different concentrations on ascorbate peroxidase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

At all tested concentrations, the NPs had a positive effect on APO activity in wheat seedlings. According to figure 9, the concentration of 0.01 mM was identified as the optimal level for enhancing enzyme activity in most genotypes, with the exception of Sheki-1 and Mirbashir-128.

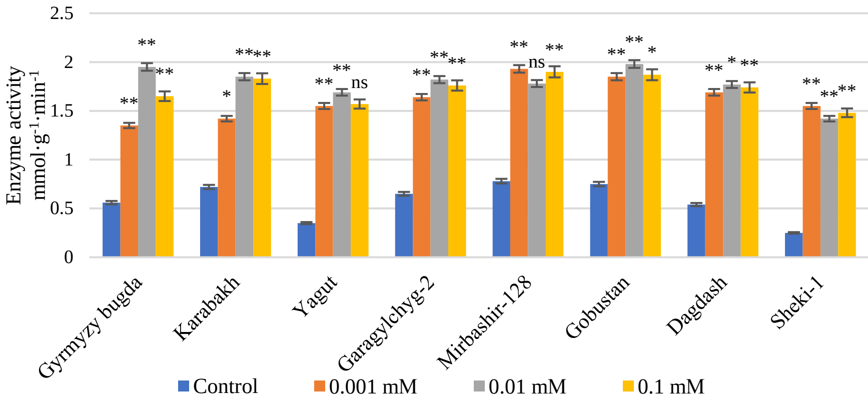


Figure 9. Effect of ZnO nanoparticles on APO activity in wheat seedlings

The highest APO activity was recorded in the Gobustan genotype at a concentration of 0.01 mM (164% higher than the control samples), while the lowest activity was observed in the seedlings of the Gyrmyzy bugda genotype at a concentration of 0.001 mM.

Although 0.01 mM was considered the optimal concentration for the studied wheat genotypes, both 0.1 mM and 0.001 mM concentrations also had a positive effect on enzyme activity. This suggests that the influence of NPs on APO activity may depend on the specific characteristics of the wheat genotypes.

3.3.2. Effect of zinc oxide nanoparticles at different concentrations on polyphenol oxidase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

According to the results of the experiments, NPs at the tested concentrations had a stimulatory effect on PPO activity in wheat seedlings, with 0.01 mM identified as the optimal concentration. The highest enzyme activity values were observed at 0.01 mM NP concentration, while the lowest values were generally recorded at 0.001 mM, except for the Mirbashir-128 and Sheki-1 genotypes (Figure 10).

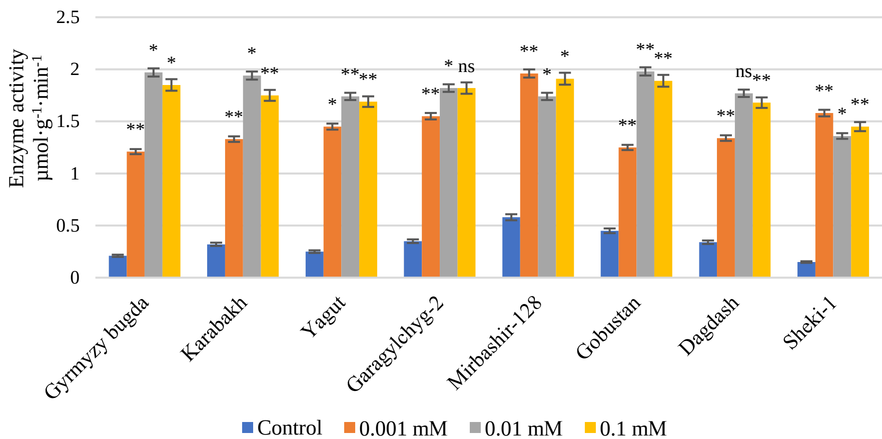


Figure 10. Effect of ZnO nanoparticles on PPO activity in wheat seedlings

The maximum PPO activity was observed in the Gobustan genotype under the influence of 0.01 mM NPs, which was 4–5 times higher than the control values. The minimum activity was recorded in the Gyrmyzy bugda genotype at an NP concentration of 0.001 mM.

3.3.3. Effect of zinc oxide nanoparticles at different concentrations on catalase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The highest CAT activity among the durum wheat genotypes was observed in the Gyrmyzy bugda seedlings at an NP concentration of 0.01 mM, which was five times higher than the control values for this genotype. Among the bread wheat genotypes, the maximum activity was recorded in the Gobustan seedlings under the same concentration.

The lowest enzyme activity under the influence of ZnO NPs at a concentration of 0.001 mM was observed in the durum wheat genotype Garagylchyg-2 and in the bread wheat genotype Dagdash. This suggests that the effect of NPs on PPO activity depends on the individual characteristics of the studied plant genotypes (Figure 11). Nevertheless, based on our results, the concentration of 0.01 mM was considered optimal for the effect of NPs on PPO activity.

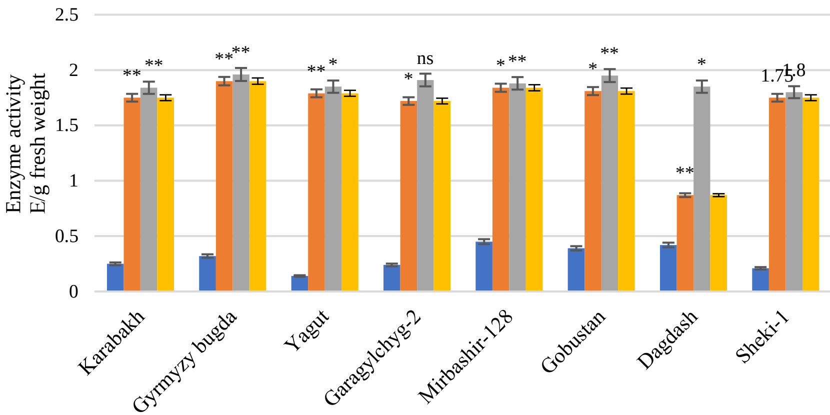


Figure 11. Effect of ZnO nanoparticles on CAT activity in wheat seedlings

3.3.4. Effect of zinc oxide nanoparticles at different concentrations on superoxide dismutase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

As shown in figure 12, the effects of NPs on durum and bread wheat seedlings differed. In both types of wheat seedlings, with the exception of the Mirbashir-128 and Sheki-1 genotypes, SOD activity increased under the influence of various concentrations of NPs compared to the control. Nevertheless, the concentration of 0.01 mM was identified as optimal for the effect of NPs.

Among the durum wheat genotypes, the highest SOD activity under the influence of ZnO NPs at a concentration of 0.01 mM was observed in the Gyrmyzy bugda seedlings, where it was five times higher than the control. Among the bread wheat genotypes, the highest SOD activity at the same concentration was recorded in the Gobustan seedlings.

The lowest SOD activity among the durum and bread wheat samples was observed in the Karabakh and Mirbashir-128 genotypes, respectively, at concentrations of 0.001 mM and 0.1 mM.

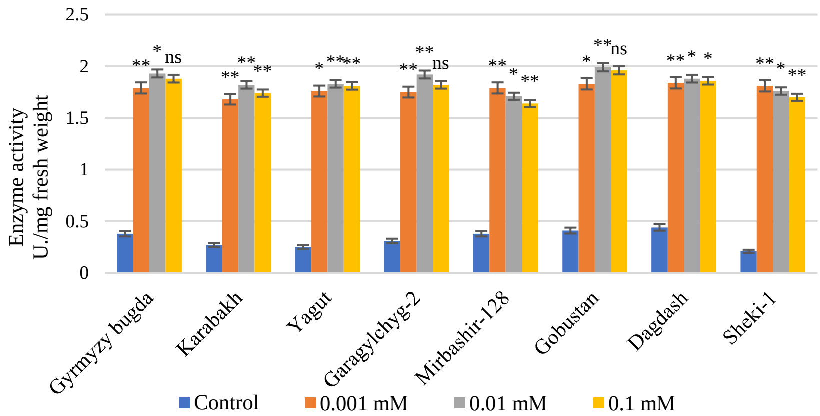


Figure 12. Effect of ZnO nanoparticles on SOD activity in wheat seedlings

Thus, the obtained results suggest that the effect of ZnO NPs on SOD activity in the studied wheat genotypes also depends on genotypic characteristics.

3.4. Effect of titanium dioxide nanoparticles at different concentrations on antioxidant enzyme activity in wheat leaves

We investigated the effect of TiO_2 NPs at concentrations of 0.001 mM, 0.01 mM, and 0.1 mM on the activity of antioxidant enzymes in the leaves of various wheat genotypes.

3.4.1. Effect of titanium dioxide nanoparticles at different concentrations on ascorbate peroxidase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

At all tested concentrations, TiO_2 NPs exhibited a stimulatory effect on APX activity in the wheat seedlings. The optimal concentration was found to be 0.01 mM (Figure 13). The highest enzyme activity was recorded in the Gobustan genotype at 0.01 mM concentration of NPs, while the lowest activity was observed in the Gyrmyzy bugda samples at 0.001 mM.

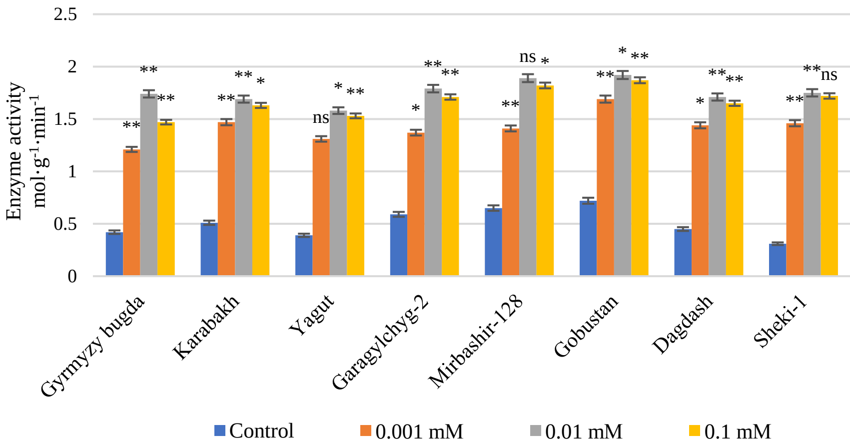


Figure 13. Effect of TiO_2 nanoparticles on APX activity in wheat seedlings

Although the 0.1 mM concentration of TiO_2 NPs had a positive effect on APX activity, its stimulatory effect was less pronounced. Furthermore, in order to minimize risks and prevent potential negative effects of the NPs, the 0.01 mM concentration was considered safer for the studied wheat genotypes.

3.4.2. Effect of titanium dioxide nanoparticles at different concentrations on polyphenol oxidase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

Based on the results of the experiments, TiO_2 NPs had a positive effect on PPO activity in all of the studied wheat genotypes (Figure 14).

The most optimal concentration for influencing PPO activity in wheat leaves was found to be 0.01 mM. Other tested concentrations of NPs also led to an increase in enzyme activity compared to the control. However, the highest level of activity was recorded in the Gobustan genotype at the 0.01 mM concentration, which was 156% higher than the control.

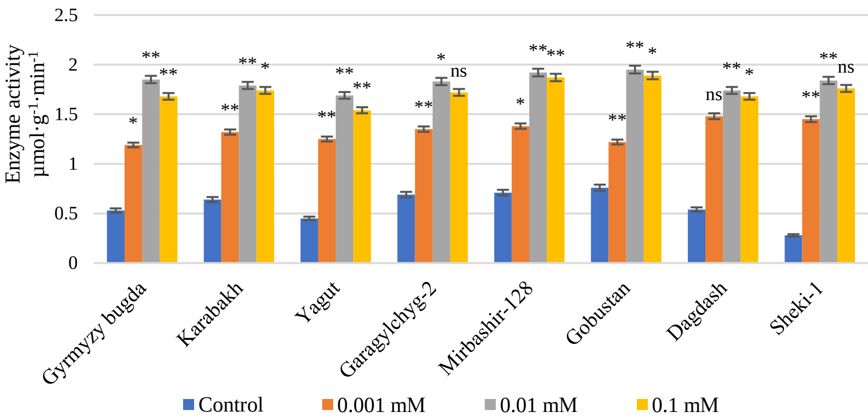


Figure 14. Effect of TiO_2 nanoparticles on PPO activity in wheat seedlings

The lowest PPO activity was observed in the Gyrmyzy bugda genotype at a TiO_2 NPs concentration of 0.001 mM**.**

3.4.3. Effect of titanium dioxide nanoparticles at different concentrations on catalase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

At all tested concentrations, TiO_2 NPs exhibited a stimulatory effect on CAT activity in the studied wheat genotypes.

The highest CAT activity was observed in the Gobustan genotype at a concentration of 0.01 mM, and among the durum wheat varieties, in the Garagylchyg-2 genotype. The lowest CAT activity, on the other hand, was recorded at a concentration of 0.1 mM in the Sheki-1 and Yagut genotypes (Figure 15).

3.4.4. Effect of titanium dioxide nanoparticles at different concentrations on superoxide dismutase activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

In all the studied wheat genotypes, TiO_2 NPs exhibited a stimulatory effect on SOD activity. The highest enzyme activity was observed at a concentration of 0.01 mM in the Garagylchyg-2 genotype among the durum wheat varieties, and in the Sheki-1 genotype among the bread wheat varieties (Figure 16).

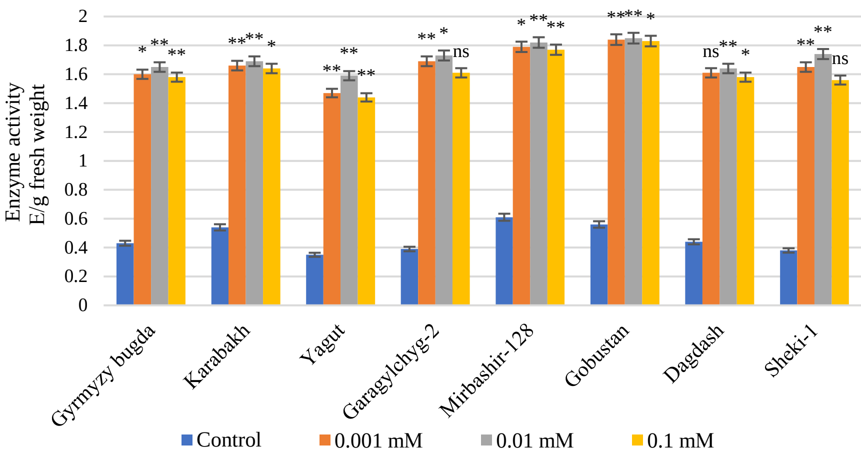


Figure 15. Effect of TiO_2 nanoparticles on CAT activity in wheat seedlings

Since the obtained values were significantly higher than those of the control, the 0.01 mM concentration of NPs can be considered optimal in terms of enhancing enzyme activity. The lowest CAT activity was observed at the 0.001 mM concentration in the Karabakh and Mirbashir-128 genotypes, respectively.

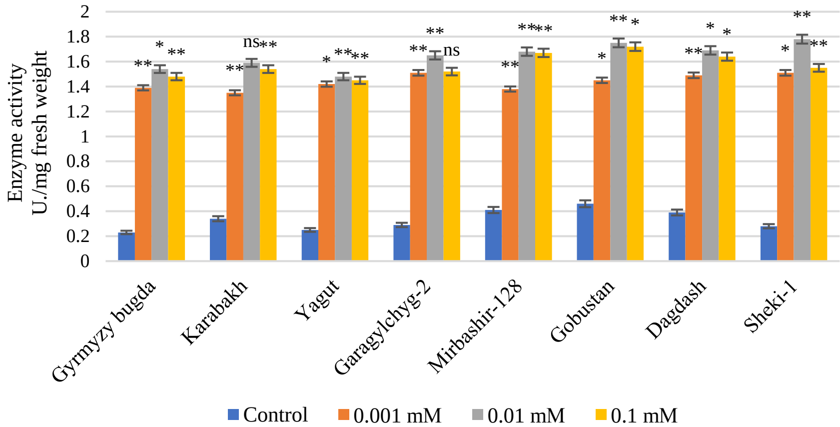


Figure 16. Effect of TiO_2 nanoparticles on SOD activity in wheat seedlings

Based on the information presented in this chapter, it can be asserted that various NPs influence the activity of antioxidant enzymes, and this effect is dose-dependent. The outcomes of nanoparticle application on plants depend both on the type of NPs and the concentration applied. Therefore, before using NPs, it is essential to select the types that positively affect metabolic processes, accelerate photosynthetic activity, increase the levels of chlorophyll a and b, and enhance antioxidant enzyme activity, as well as to determine their optimal dosages.

3.5. Isoenzyme analysis of antioxidant system enzymes

Under the influence of optimal concentrations of NPs, the isoenzyme composition of CAT, SOD, and APX enzymes was studied in two high-yielding varieties: Garagylchyg-2 (durum wheat) and Dagdash (bread wheat) genotypes (Figures 17, 18, and 19).

The results showed that TiO_2 and ZnO NPs significantly increased the expression of CAT and SOD enzymes, whereas the effect of Fe_2O_3 NPs was weaker. In the electropherograms of the APX enzyme, however, the spectral intensity was stronger under the influence of Fe_2O_3 NPs. This demonstrates that NPs play a regulatory role in the activity of antioxidant enzymes.

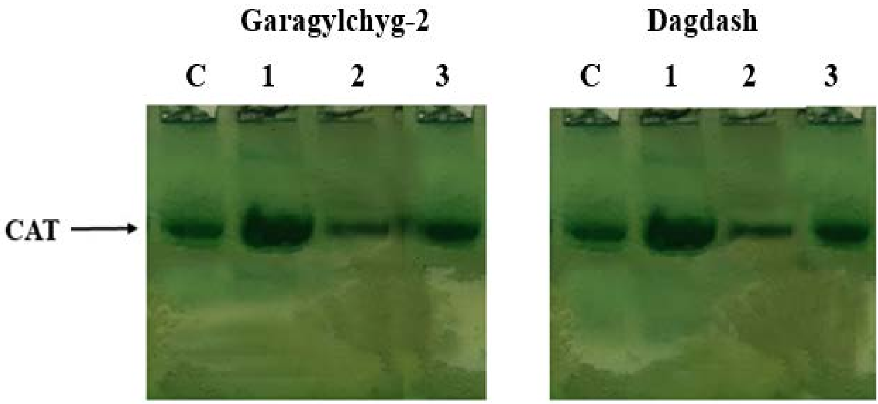


Figure 17. Isoenzyme composition of CAT in the leaves of Garagylchyg-2 and Dagdash genotypes: C-control; 1-sample treated with TiO_2 NPs; 2-sample treated with Fe_2O_3 nanoparticles; 3-sample treated with ZnO nanoparticles.

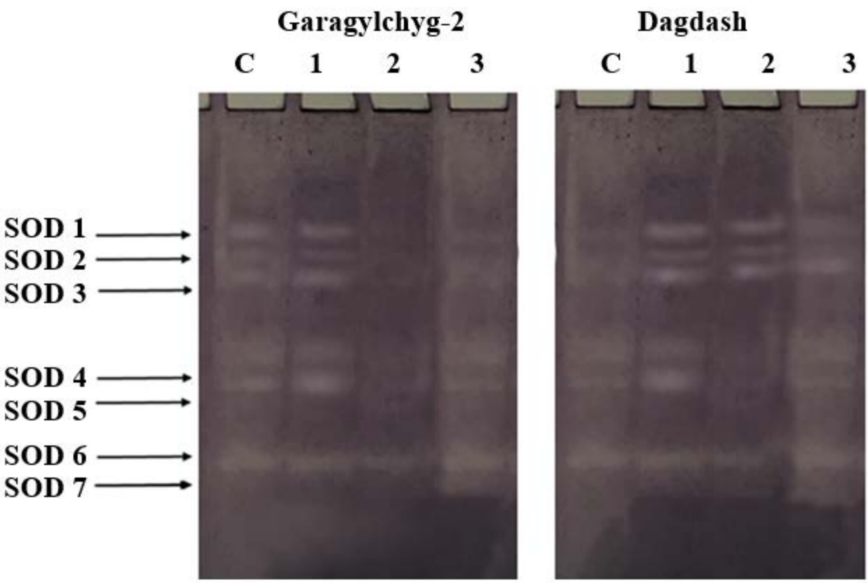


Figure 18. Isoenzyme composition of SOD in the leaves of Garagylchyg-2 and Dagdash genotypes: C-control; 1-sample treated with TiO_2 nanoparticles; 2-sample treated with Fe_2O_3 nanoparticles; 3-sample treated with ZnO nanoparticles.

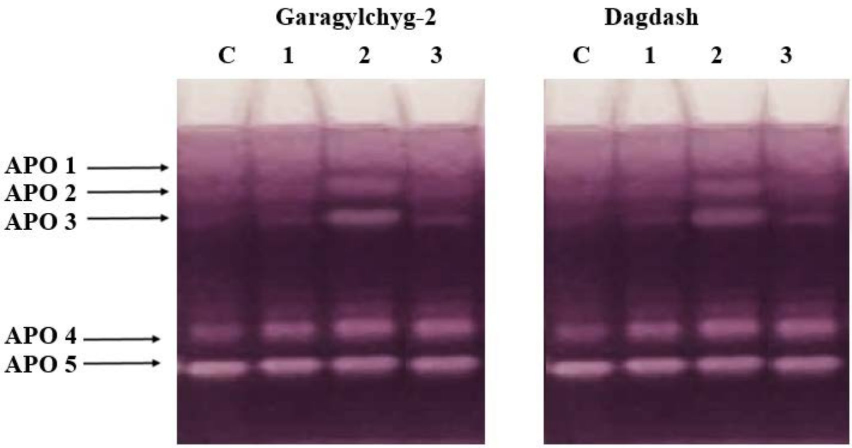


Figure 19. Isoenzyme composition of APO in the leaves of Garagylchyg-2 and Dagdash genotypes: C-control; 1-sample treated with TiO_2 nanoparticles; 2-sample treated with Fe_2O_3 nanoparticles; 3-sample treated with ZnO nanoparticles.

During the experiments, 7 isoforms of SOD and 5 isoforms of APX were identified, and their expression levels varied depending on the types of NPs used.

CHAPTER IV. EFFECT OF METAL OXIDE NANOPARTICLES ON MORPHOPHYSIOLOGICAL TRAITS AND PRODUCTIVITY IN DIFFERENT WHEAT GENOTYPES

There is a growing trend toward the progressive improvement of nanoparticle applications across all areas of agriculture, which has led to increased attention to the study of the biological safety of metals. It is important to note that certain metals and their oxides may possess toxic and pro-oxidant properties[12].

There is currently no comparative data on the effects of Fe_2O_3, TiO_2, and ZnO NPs on the morphological traits, productivity, and

[12] Короткова, А.М. Морфофизиологические изменения у пшеницы *(Triticum vulgare* L.) под влиянием наночастиц металлов (Fe, Cu, Ni) и их оксидов (Fe_3O_4, CuO, NiO) / А.М.Короткова, С.В.Лебедев, Ф.Г.Каюмов [и др.] // Сельскохозяйственная биология, – 2017, т. 52, №1, – с. 172-182.

content of photosynthetic pigments in the seedlings of the studied wheat genotypes.

For the first time, a comprehensive analysis has been conducted to examine the effects of these NPs on wheat genotypes.

Recent studies on the use of NPs in the cultivation of agricultural crops have demonstrated their dynamic impact on seedling growth and development. The functioning of the photosynthetic apparatus in plants largely depends on the type of NPs used and the activity of antioxidant enzymes. Therefore, investigating the influence of NPs on yield structure and photosynthetic performance holds significant theoretical and practical importance[13].

Under normal conditions, seed germination takes a long time; however, treatment with NPs allows for significantly improved germination rates, creating favorable conditions for the broader application of these NPs.

Given that a concentration of 0.01 mM has proven optimal for the activity of APX, SOD, CAT, and PPO enzymes, our research investigated the effects of 0.01 mM solutions of Fe_2O_3, TiO_2, ZnO, and Al_2O_3 NPs on photosynthetic pigment content and productivity in seedlings of various wheat genotypes under normal irrigation conditions.

Analysis of the presented data revealed that, with the exception of Al_2O_3 NPs, the others had a positive impact on the parameters mentioned.

Furthermore, the analysis showed that the most favorable results were obtained under the influence of Fe_2O_3 NPs, which provides a basis for their large-scale application in agriculture.

Thus, pre-sowing treatment of wheat seeds with NPs not only led to variable changes in morphophysiological parameters but also revealed genotype-specific sensitivity to different NPs.

Considering that the 0.01 mM concentration of NPs had minimal inhibitory effects on enzyme activity and the content of photosynthetic

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[13]Chen, YE. Comparison of photosynthetic characteristics and antioxidant systems in different wheat strains / Y. Su, CM. Zhang [et al.] //J. Plant Growth Regul. – 2018, vol. 37. – p. 347–359.

pigments in plants, this concentration can be considered the safest and most effective for use in future studies.

CHAPTER V. MULTIVARIATE STATISTICAL ANALYSIS OF MORPHOPHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN DIFFERENT WHEAT GENOTYPES

One of the main objectives facing agriculture today is the resolution of food shortages. Therefore, increasing the productivity of cereal crops is considered a key priority in modern times[14].

To assess the impact of antioxidant enzyme activity on productivity in wheat genotypes, it became necessary to conduct a correlation analysis to determine the relationships between photosynthetic pigment composition, biochemical parameters of the antioxidant system, and yield components. The correlations among these parameters will help identify potential mechanisms that ensure optimal plant growth and enhance their tolerance to stressful environmental conditions.

5.1. Correlation analysis of yield components in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

In all studied wheat genotypes, statistically significant positive correlations were identified among yield components at the 1% probability level. However, in bread wheat genotypes, a negative but statistically non-significant correlation ($r = –0.100$) was observed between the mass of grains per spike and yield (g/m^2). In contrast, in durum wheat genotypes, the correlation between these traits was statistically significant at the 5% probability level ($r = 0.306^*$).

Thus, to achieve maximum yield in the studied plants, it is essential to maintain the key yield indicators at optimal levels.

[14] Chaudhry, N. Bioinspired nanomaterials in agriculture and food industry: current state, predicted applications and problems / N.Chaudhry, S.Dwivedi, V.Chaudhry [et al.] // Microbe. Pathog., – 2018, vol. 123, – p. 196-200.

5.2. Correlation between photosynthetic pigment content and antioxidant enzyme activity in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

The presented analysis is of great importance for studying the tolerance mechanisms of plants against various stress factors. This interrelation reflects not only the physiological state of the plants but also their adaptive potential.

According to the obtained data, in durum wheat genotypes, positive correlations were found between the amount of photosynthetic pigments and PPO activity, whereas negative correlations were observed with the activities of SOD and CAT enzymes (Table 1). This indicates a protective response of the seedlings when pigment levels change. As for the bread wheat genotypes, a strong positive correlation was maintained between the pigments themselves, while either weak correlations were observed between pigment content and antioxidant enzyme activities, or no correlation was determined at all. This highlights differences in the mechanisms of their mutual interactions (Table 2).

Table 1.

Correlation relationships between photosynthetic pigment content and antioxidant enzyme activities in seedlings of durum wheat varieties (*Triticum durum* Desf.)

	Cl. a	Cl. b	Cl. a+b	Cl a/b	Car.	APO	PPO	CAT	SOD
Cl. a	1	0.970**	0.997**	-0.513**	0.975**	0.027	0.375**	-0.283*	-0.348**
Cl. b		1	0.986**	-0.666**	0.975**	-0.068	0.426**	-0.228	-0.298*
Cl. a+b			1	-0.553**	0.983**	0.006	0.387**	-0.274*	-0.340**
Cl a/b				1	-0.564**	0.492**	-0.558**	-0.177	-0.104
Car.					1	-0.040	0.399**	-0.253*	-0.320**

Note: **- Highly statistically significant, $p \leq 0.01$; * - statistically significant, $p \leq 0.05$ (based on Pearson's correlation coefficient).

Table 2.

Correlation relationships between photosynthetic pigment content and antioxidant enzyme activities in seedlings of bread wheat varieties (*Triticum aestivum* L.)

	Cl. a	Cl. b	Cl. a+b	Cl. a/b	Car.	APO	PPO	CAT	SOD
Cl. a	1	0.993**	0.999**	0.463**	0.937**	0.144	0.366**	-0.212	-0.201
Cl. b		1	0.996**	0.378**	0.936**	0.112	0.403**	-0.186	-0.184
Cl. a+b			1	0.443**	0.937**	0.141	0.370**	-0.210	-0.201
Cl a/b				1	0.459**	0.215	-0.027	-0.252*	-0.220
Car.					1	0.047	0.387**	-0.190	-0.185

Note: **- Highly statistically significant, $p \leq 0.01$; * - statistically significant, $p \leq 0.05$ (based on Pearson's correlation coefficient).

5.3. Correlation relationships between photosynthetic pigment content and yield parameters in *Triticum durum* Desf. and *Triticum aestivum* L. seedlings

As a result of the calculations, positive correlations were observed between the photosynthetic pigment content and yield parameters in both durum and bread wheat genotypes (r = 0.379**, r = 0.351**, r = 0.407** and r = 0.523**, r = 0.492**, r = 0.411** respectively).

In all wheat genotypes, strong positive correlations were found between carotenoids and yield parameters, highlighting their significant role in protecting the photosynthetic apparatus and increasing cereal crop yield. The ratio between chlorophylls had a particularly positive effect on yield (r = 0.462**), which proves the different metabolic dynamics of pigments in bread genotypes. In durum wheat seedlings, a negative correlation (r = -0.470**) was observed between the chlorophyll ratio and grain weight, which can be explained by the adaptation of photosystems to light conditions.

In addition to the correlation analysis, cluster analysis of wheat genotypes' yield parameters under the influence of NPs was conducted, and a dendrogram was created (Figure 20). Based on the values of the Euclidean genetic distance index, the dendrogram indicates that cluster I effectively grouped the high-yielding genotypes (Gobustan, Karabakh, Dagdash, and Garagylchyg-2) under the influence of TiO_2, ZnO, and Fe_2O_3 NPs at a concentration of 0.01 mM, distinguishing them from other genotypes. Cluster II included medium-yielding genotypes, cluster III included low-yielding genotypes, and cluster IV represented the genotypes with the lowest yield (Gyrmyzy bugda, Sheki-1, Yagut). Thus, the applied clustering was effective in grouping the wheat genotypes into categories based on yield performance under the influence of NPs at a 0.01 mM concentration.

The results of the analyses provide a deeper understanding of the relationship between the biochemical status of plants and their productivity, laying the foundation for the development of effective methods to enhance the stress tolerance of agricultural crops.

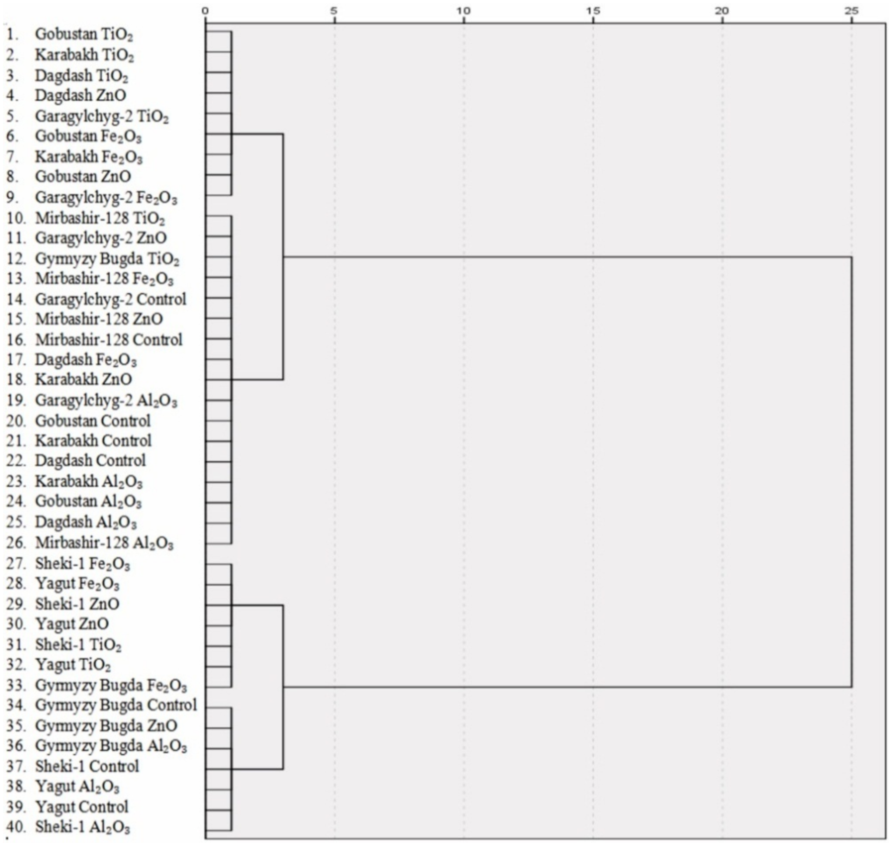

Figure 20. Cluster analysis of wheat genotypes based on yield indicators

CONCLUSIONS

1. The effects of Fe_2O_3, Al_2O_3, ZnO, and TiO_2 NPs on the activity of antioxidant enzymes, the content of photosynthetic pigments, and yield indicators vary depending on their concentrations and the characteristics of the studied genotypes, which are determined by heredity and their adaptive responses to metabolic changes [9, 13, 14].
2. In the studied durum and bread wheat genotypes, the optimal concentration of Fe_2O_3 NPs in terms of their effect on biochemical parameters was found to be 0.1 mM [3, 7].
3. Application of Fe_2O_3 NPs at a concentration of 0.01 mM to wheat genotypes resulted in a 5-19% increase in the content of photosynthetic pigments and an average 2-13% increase in yield compared to the control. The highest values were observed in the genotypes Garagylchyg-2 and Dagdash [5, 8].
4. In two-week-old seedlings of durum and bread wheat genotypes (excluding Mirbashir-128 and Sheki-1), the optimal concentration of ZnO NPs for influencing the activity of APO, PPO, CAT, and SOD enzymes was identified as 0.01 mM. Although a tenfold increase in concentrations of NPs also had a positive effect on enzyme activity in the studied seedlings, the stimulatory impact was less pronounced [10].
5. Under normal irrigation conditions, ZnO NPs at a concentration of 0.01 mM increased the content of pigments (chlorophylls a and b, and carotenoids) by 4-13% and enhanced yield by 2-16% compared to the control [8, 15].
6. TiO_2 nanoparticles at a concentration of 0.01 mM had a stimulatory effect on the activity of antioxidant enzymes and also led to a 9-13% increase in the content of photosynthetic pigments and a 5-16% increase in yield compared to the control. Notably, in the Gyrmyzy bugda genotype, yield increased by 83% relative to the control [9, 16].
7. Analysis of the isoenzyme composition of antioxidant enzymes in durum and bread wheat seedlings revealed five cytoplasmic isoforms of APO, seven of SOD, and one of CAT on the electrophoregrams. While TiO_2 and ZnO NPs significantly enhanced the expression of CAT and SOD enzymes, Fe_2O_3

nanoparticles had a weaker effect on these enzymes but increased the intensity of the APO enzyme spectrum [18].

8. In durum and bread wheat genotypes, there are statistically significant positive correlations between yield and the content of photosynthetic pigments, as well as between the content of photosynthetic pigments and PPO activity [17].

PRACTICAL RECOMMENDATIONS

1. Fe_2O_3, TiO_2, and ZnO NPs can be used as microfertilizers for seed treatment and plant cultivation in various wheat genotypes. In this case, the key requirement is to determine the optimal dose of NPs for the studied objects.
2. At all tested concentrations, Al_2O_3 NPs exhibited an inhibitory effect on the activity of antioxidant enzymes, the content of photosynthetic pigments, and the yield of various wheat genotypes, demonstrating the irrationality of their use in agriculture.

List of published scientific works on the topic of the dissertation

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3. Mammadli, G.H. Influence of ferric oxide nanoparticles on the activity of ascorbate peroxidase in wheat seedlings // The Scientific and Pedagogical News of Odlar Yurdu University, –2020, №58, – p. 35-43.
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9. Ismayılova, G.H., Azizov I.V. Effect of metal oxide nanoparticles on the activity of antioxidant enzymes and productivity of wheat genotypes // Advanced Studies in Biology, – 2024, vol. 16, №1, – p. 97-108.
10. Ismayilova, G.H., Azizov, I.V. The activity of catalase and superoxide dismutase under the influence of Fe and Zn oxide nanoparticles in wheat sprouts // Bulitin of Science and Practice (Бюллетень науки и практики), – 2025, т. 11, №2, – с. 59-66.
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The defense will be held on 27 June 2025 at 14:00 at the meeting of the Dissertation council FD 2.31 of Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at Baku State University.

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Electronic versions of the dissertation and its abstract are available on the official website of the Baku State University (www.bsu.edu.az).

Abstract was sent to the required addresses on 20 May 2025

Signed for print: 14.05.2025
Paper format: 60×90 1/16
Volume: 39266
Number of hard copies: 30