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Effects of Zinc Oxide Nanoparticle on Antioxidant System in Bean Leaves

Oguz Ayhan KIRECCI[1]✍, Serdar UZGEN[2], Tuba OKUTAN[3], Okkes YILMAZ[4]
[1]Bitlis Eren University, Hizan Vocational School, Bitlis, Turkey, [2]Bitlis Eren University, Department of Chemistry, Graduate Education Institute, Bitlis, Turkey, [3,4]Firat University, Faculty of Science, Department of Biology, Elazig, Turkey
[1]https://orcid.org/0000-0003-2205-4758,[2]https://orcid.org/0009-0002-7878-272X,[3]https://orcid.org/0000-0001-8745-0343,
[4]https://orcid.org/0000-0002-8276-4498
✉: oakirecci@beu.edu.tr

ABSTRACT
Nanotechnology can be most simply defined as technology at the nanoscale. Heavy metal stress often induces reactive oxygen species (ROS) and causes oxidative stress. Antioxidant enzymes, metabolites, flavonoids, carotenoids, polyols, cytosolic ascorbate, and peroxiredoxin play roles in ROS scavenging. Certain antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), and glutathione reductase (GR) defend against metal toxicity. In this study, the effects of zinc nanoparticles on certain biochemical parameters in the leaves of bean (Phaseolus vulgaris L.) were examined. For this purpose, ZnO nanoparticle concentrations of 0.1 mM, 0.01 mM, and 0.001 mM were applied. At the end of 120 hours, malondialdehyde, proline, glutathione, total soluble protein, and the activities of superoxide dismutase and catalase enzymes were determined. As a result, all findings from this study revealed that ZnO nanoparticle applications activated antioxidant defense mechanisms in the leaves of Phaseolus vulgaris L. It was determined that the mentioned ZnO nanoparticle exhibited more pronounced effects, especially at lower doses. Nano-sized metals were found to exert toxic effects on the leaves of *Phaseolus vulgaris* L."

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Çinko Oksit Nanopartikülünün Fasulye Bitkisi Yapraklarinda Antioksidan Sistem Üzerine Etkileri

ÖZET
Nanoteknolojinin en basit tanımı, nanoskalada teknoloji olarak ifade edilebilir. Ağır metal stresi genellikle reaktif oksijen türlerini (ROS) indükler ve oksidatif stres oluşturur. Antioksidan enzimler, metabolitler, flavonoidler, karotenoidler, polioller, sitozolik askorbat ve peroksiredoksin gibi maddeler ROS temizlenmesinde rol oynar. Katalaz (CAT), Askorbat peroksidaz (APX), Süperoksit dismutaz (SOD) ve Glutatyon redüktaz (GR) gibi bazı antioksidan enzimler metal toksisitesine karşı savunma yapar. Bu çalışmada, çinko nanopartikülünün fasulye (*Phaseolus vulgaris* L.) yapraklarındaki bazı biyokimyasal parametreler üzerindeki etkileri incelendi. Bu amaçla 0.1 mM, 0.01 mM ve 0.001 mM ZnO nanopartikül konsantrasyonları uygulandı. 120 saat sonunda malondialdehit, prolin, glutatyon, toplam çözünür protein ve süperoksit dismutaz ve katalaz enzim aktiviteleri belirlendi. Sonuç olarak, bu çalışmadan elde edilen tüm sonuçlar ZnO Nanopartikül uygulamalarının *Phaseolus vulgaris* L. yapraklarında antioksidan savunmayı aktive ettiğini ortaya koydu. Bahsi geçen ZnO nanoparçacığın, özellikle düşük doza bağlı olarak daha ciddi etkiler gösterdiği belirlendi. Nano boyuttaki metaller, *Phaseolus vulgaris* L. yapraklarında toksik bir etki oluşturdu.

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INTRODUCTION

The simplest definition of nanotechnology is nanoscale technology (Ramsden, 2016). Metallic nanoparticles increase seed germination, shoot and root growth, biomass production, and physiological activity (El-Gazzar et al., 2020). Plants produce reactive oxygen species in response to stress from various effects. Abiotic stresses such as UV rays, heavy metals, salinity, drought, herbicides, high temperature, and pollution are among these effects (Mehla et al., 2017). Oxidative stress occurs when the balance between antioxidants and reactive oxygen species (ROS) is disrupted. Nanotoxicity is also a factor that can cause oxidative stress (Cekic et al., 2017). Nanomaterials can induce excessive ROS production as a regulatory mechanism to protect stressed plant cells from further oxidative damage (Venkatachalam et al., 2017). Concentrations of nanoparticles (NPs) above the optimal ranges of metals such as Zn, Cu, Ag, Ce, and Ti produce stress and/or toxicity by generating ROS and disrupting cellular metabolism (Mehla et al., 2017). ZnO NPs affect the normal functionality of cells by causing changes in the structures of molecules such as proteins and DNA. Genotoxicity studies have shown that ZnO NPs affect plant DNA (Reddy et al., 2018). It has been stated that the application of ZnO and CuO NPs can lead to the oxidation and denaturation of proteins and the alteration of their structure and functions (Hidour et al., 2022). By controlling glucose metabolism and promoting the activity of antioxidant enzymes, ZnO nanoparticles improve the stress resistance of plants (Liu et al., 2022). Stress-responsive genes and transcription factors, which adapt to different stress situations, are distributed differently in response to stress signals, also causing the formation of ROS. Nanomaterials can boost photosynthesis, encourage plant development, and increase biomass and protein levels (Fazelian et al., 2020). Nevertheless, they have the potential to alter crop plant morphology and physiological processes when administered in high quantities. For example, the application of large concentrations of NPs in the root zone inhibits root growth, modifies the uptake of water and nutrients, and decreases leaf development and biomass output (Usman et al., 2020). Zinc oxide nanoparticles (ZnO NPs) have been shown to improve plant metabolism by increasing plant defense and stress tolerance (Hidour et al., 2022). However, some harmful effects of NPs on chlorophyll have resulted in oxidative stress, which slows down plant growth by affecting photosynthesis (Li et al., 2016). Nanotechnology is fundamentally a mindset—a way of thinking about the world based on precise perceptions at the atomic level. Zinc is an essential micronutrient for plants and plays a vital role in metabolic activities, including the synthesis and breakdown of macromolecules necessary for growth. It is crucial for chlorophyll synthesis and plays an important role in regulating plant growth hormones such as Indole-3-acetic acid (IAA) (Reddy et al., 2018). Many previous studies have reported the harmful effects of ZnO NPs on different plant species. For example, ZnO NPs have been shown to inhibit the growth of wheat and soybean plants (Salehi et al., 2021). In studies by Reddy-Pullagurala et al., ZnO NPs at 100 mg/L gradually delayed the germination period of Macrotyloma uniflorum. Under similar cultivation conditions, 1600 mg/L of ZnO NPs reduced germination by 40% in Medicago sativa and 20% in Solanum lycopersicum (Reddy et al., 2018). There are studies (Liu et al., 2022) indicating that ZnO NPs have increased the growth rate, enhanced biomass, and improved root growth of cluster bean (*Cyamopsis tetragonoloba* L.) and pearl millet (Pennisetum americanum) by promoting the increase of certain enzymes. Similar results have been observed in tomatoes (*Solanum lycopersicum*) and cabbage (*Brassica oleracea* L.), as well as in plants such as lettuce (*Lactuca sativa* L.). The main outcomes include positive changes in internal components such as chlorophyll, carbohydrates, and antioxidant enzymes (Liu et al., 2022). This study investigated the effects of ZnO nanoparticles at different concentrations on the antioxidant defense system of bean plant leaves at the 120th hour. Previous studies have highlighted both the positive and negative effects of ZnO nanoparticles (NPs) on plant growth; however, this study specifically investigates the impact of ZnO NPs on the antioxidant defense system of the bean plant.

MATERIAL and METHOD

Experimental Material

In our study, oilseed beans (*Phaseolus vulgaris* L.) seeds were used and obtained commercially. Germination tests were conducted, and plant cultivation and application procedures were carried out. Samples were frozen in liquid nitrogen and stored in a deep freezer at -40°C.

Germination of Plants and Formation of Experimental Groups

Following surface sterilization, seeds were soaked in water for 24 hours using an aquarium pump. Then, the planting process was applied to pots. The seeds were grown under controlled conditions at 25±2°C with 60-65% humidity. Plants irrigated with Hoagland culture solution (Hoagland and Arnon, 1938) were treated with ZnO nanoparticles prepared at concentrations of 0.1 mM, 0.01 mM, and 0.001 mM, added to the irrigation water after 12 weeks. The pH of the prepared nutrient solution was adjusted to be in the range of 5.6–5.8. As a result of the literature review, it was decided to use the current doses. Perlite was used as the cultivation medium. To observe the long-term effects, samples were collected at the 120th hour and analyzed. The experimental materials were grown in pots with a diameter of 21 cm and depth of 18 cm. The study was to include 12 pots for each concentration

and time, and one seed per pot. Due to the possibility of insufficient germination, 6 pots were prepared as replacements for each application and time. After applications, analyzes were performed with 6 randomly selected plants from concentration and time and 6 randomly collected leaves from each of these plants. Subsequently, samples were taken at 120 hours and transferred to the freezer. The results were analyzed with three replications.

Sampling of Plant Samples

Random samples were taken from plants in sufficient quantities, covered with aluminum foil, and rapidly frozen in liquid nitrogen. They were stored for analyses to be conducted at a temperature of -40°C.

Antioxidant Enzyme Analyses

The Superoxide dismutase (SOD) enzyme activity was performed with reference to the method specified by Sairam et al. (2002). The SOD activity was measured by recording a decrease in optical density of nitroblue tetrazolium (NBT). 3 ml of assay mixture consisted of 13 mM methionine, 25 mM nitroblue tetrazolium chloride, 0.1mM EDTA, 50mM phosphate buffer (pH 7.8), 50 mM sodium carbonate and 0.1 ml enzyme. Reaction was initiated with the addition of riboflavin and kept under two 15W fluorescent lamps for 15 minutes. Reaction was stopped by closing the light and using assay mixture without enzyme, giving the maximum coloration, as control. Complete assay mixture, which was not illuminated, was used as blank. SOD activity of one unit was defined as the amount of enzyme needed to cause 50% inhibition of NBT at 560 nm and was expressed in Unit/g.
Catalase activity (CAT) determination was carried out according to Aebi (1984). To measure CAT activity, the extract was combined with phosphate buffer and hydrogen peroxide (H_2O_2), and the variation in absorbance was recorded at a wavelength of 240 nm.

Determination of Malondialdehyde Content

HPLC apparatus was used for the determination of malondialdehyde content (Karatas et al. 2002). After the tissues were homogenized with Tris buffer, 1 mL of the resulting supernatant was taken and 10% perchloric acid ($HClO_4$) was added. The mixture was centrifuged at 5000 rpm for 5 minutes. The supernatant was then analyzed using an HPLC device through vials. In the HPLC system, a mobile phase consisting of a mixture of 30 mmol KH_2PO_4 and methanol (%82.5-%17.5 with H_3PO_4 at pH = 4.0) was used, along with an ODS-3 HPLC column (150 mm x 4.6 mm, 5 µm). The mobile phase flow rate was set to 1 mL/min, and the wavelength of the PDA detector was set to 244 nm. The results were calculated using the calibration curve obtained from standard mixtures, and the analysis was performed with Class VP 6.26 software (Shimadzu, Kyoto, Japan).

Prolin

The proline content was determined based on its reaction with ninhydrin which forms a colored complex. After adding 2-propanol, the absorbance of the sample solution and a reference solution at 510 nm using a spectrophotometer was determined. Results were expressed in proline milligrams per kilogram of honey (Codex Alimentarius Commission 2001).

Total Soluble Protein

Total protein contents of yeast cells were determined as Lowry's method (Lowry et al. 1951).

Measurement of GSH Content with HPLC

The GSH content was determined using an HPLC apparatus (Klejdus et al. 2004; Yilmaz et al. 2009). 1 mL of homogenate was taken, 1 mL of 10% TCA was added and it was deproteinized. After centrifugation at 6000 rpm, 1 mL was taken into autosampler vials. In quantitative measurements, analysis was performed using Shimadzu brand fully automatic HPLC device at 214 nm, and LC-10 ADVP UV-visible pump, SPD-M10AVP, PDA detector, CTO-10ASVP column oven, SIL-10ADVP autosampler, DGU-14A degasser unit and Class VP 6.26 operating program (Shimadzu, Kyota Japan)

Statistical Analyses

All experimental data were replicated three times under the application conditions. The experimental groups were subjected to a comparative analysis of variance with their respective control groups. The statistical analyses of the data were performed using SPSS 25.0 software. Analysis of variance (ANOVA) and the least significant difference (LSD) test were used to compare the groups with the control group. The data are presented as mean ± SD (standard deviation) and statistical significance was determined at $p<0.05$. Additionally, regression curves were statistically determined.

RESULTS and DISCUSSION

Prolin Content

The table provides the analysis of proline content in samples taken from *P. vulgaris* L. leaves at 120-hour intervals with NP applications. In the samples taken at 120 hours, a noticeable increase was recorded compared to the

control. The application at 0.01 mM concentration at 120 hours showed a high increase compared to the control but measured lower than other concentrations. The values at all of concentrations ($p<0.05$) are statistically significant. There was a significant increase in proline values at 120 hours compared to the control. In conclusion, ZnO NP significantly increased proline values at 120 hours.

Table 1. Effects of application group on Proline content in leaves of *P. vulgaris* L. plant
Çizelge 1. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Prolin miktarına etkileri

Application groups (120th hour)	Proline content (mg/g)
Control	**52,92±1.68**
0.1 mM ZnO	70.97±1.2*
0.01 mM ZnO	82.00±1.1*
0.001 mM ZnO	83.90±1.2*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: $p<0.05$

Proline plays a significant role in the physiological characteristics and growth of plants, assisting them in overcoming various stresses (Hayat et al., 2012). The accumulation of proline is a common response to various stresses (Verbruggen and Hermans, 2008) and is also an important indicator of lipid peroxidation (Dai et al., 2019). It is evident that as the concentration of stress decreases, the amount of proline increases in this study. This indicates that the plant is under stress. Additionally, it can be concluded that nanomaterials facilitate greater absorption, leading to an increase in proline content through accumulation.

It should be noted that the study reported by Amooaghaie et al. (2016), suggesting that the application of nanoparticles at low concentrations is suitable for plant growth, contradicts our findings. In their study, high proline content was determined at low concentrations, indicating that the plant was under stress when evaluated in conjunction with the antioxidant system. Due to differences in the material used, concentration, and method, results may vary.

In the case of Zn nanoparticles applied to Faisal and Shiralee varieties of canola at different concentrations (5 mg/L, 15 mg/L, and 25 mg/L), it was reported that they caused an increase in proline content. For mustard plants (*B. juncea*) treated with ZnO NPs, a gradual increase in proline content up to a concentration of 1000 mg/L in leaves and a decrease at 1500 mg/L were observed (Rao & Shekhawat, 2013). These studies align with our results, where an overall increase in proline content was determined.

Our research indicates that, at the 120th hour of application, the higher proline content compared to the control suggests that the applied nanoparticle molecules create stress conditions in the plant, leading to an increase in proline content as a part of the defense mechanism.

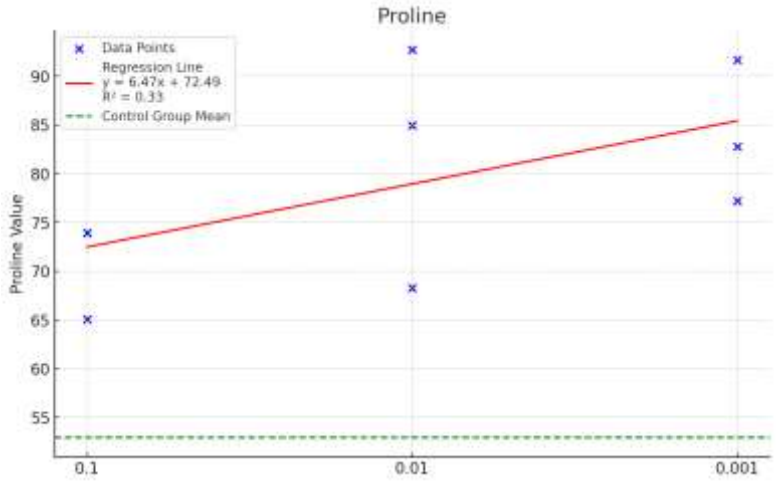

Figure 1. Regression curve of Proline content of application concentrations at 120th hour
Şekil 1. *Uygulama konsantrasyonlarının 120. saatteki Prolin içeriğine ait regresyon eğrisi*

Malondialdehyde Content

The table presents the effects of application groups on Malondialdehyde (MDA) content in leaves of *Phaseolus vulgaris* L. plants, as observed in Table 2. At 120 hours of application, it was observed that ZnO applications at 120 hours caused the increased MDA content increased as the concentrations decreased and all ZnO aplications higher than control.

Table 2. Effects of application group on Malondialdehyde (MDA) content in leaves of *Phaseolus vulgaris* L. plant
Çizelge 2. P.vulgaris L. bitkisi yapraklarında uygulama gruplarının Malondialdehit (MDA) içeriğine etkileri

Application groups (120th hour)	MDA content (**nmol/g**)
Control	88±31,6
0.1 mM ZnO	147.92±28.71*
0.01 mM ZnO	157.82±10.56*
0.001 mM ZnO	179.66±23.31*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: $p<0.05$

Hydroperoxides accumulating in the environment disrupt membrane integrity. Large classes of biomolecules are affected by free radicals and their derivatives, with lipids being the most sensitive among them (Gulcin et al., 2004). Biological membranes are composed of a combination of polyunsaturated fatty acids, amphipathic lipids, and membrane proteins. Lipid peroxidation (LPO) is a process that begins with the oxidation of polyunsaturated fatty acids by radicals and extends in the form of autocatalytic chain reactions, culminating in the conversion of lipid peroxides into aldehyde derivatives, hydrocarbon radicals, and some volatile products (Unal, 1999; Kirecci, 2018). The membrane damage caused by lipid peroxidation (LPO) is irreversible.
At 120 hours, it was observed that as the concentration decreased in the ZnO application groups, the MDA content increased. This indicates that over time, superoxide radicals increase the MDA content. In a study conducted by Li et al. (2012) on tomato seedlings, it was noted that ZnO NPs applied to tomato seedlings (at concentrations of 10 and 50 mg/L) significantly increased the MDA content in the roots due to the increased concentration of H_2O_2. In the same study (Li et al., 2012), despite the increase in H_2O_2 content in wheat leaves, the unchanged MDA content and SOD activity were associated with POD, CAT, GR, and APX activities, as well as osmotic regulation. In cotton plants, it has been noted that exposure to high concentrations of nanoparticles leads to a decrease in the activities of SOD, CAT, and POX enzymes, resulting in lower lipid peroxidation (low MDA) (Venkatachalam et al., 2016). Faizan et al. (2021) reported a significant decrease in MDA by 31%, H_2O_2 by 28%, and O_2 by 31% concentrations in tomatoes upon the application of ZnO NPs. Separate applications of Zn and ZnO to tomato and wheat plants showed a significant increase in MDA content and H_2O_2 accumulation in tomato plants, but in wheat plants, although the H_2O_2 content slightly increased, the MDA content remained unchanged. However, 200 mg Zn/L and ZnO resulted in a significant increase in MDA content in wheat. Sunflower plants have been shown to exhibit effects related to water scarcity, such as electrolyte leakage, increased lipid peroxidation, H_2O_2 production, and proline accumulation, causing damage to the membrane (Ramadan et al., 2022). In our study, it was concluded that after a 120-hour nanoparticle application period, there was an increase in MDA content, and this increase was particularly higher at low concentrations. Although zinc is a molecule required for plants, in nano size and at low concentrations, it has caused increases in MDA content due to absorption.

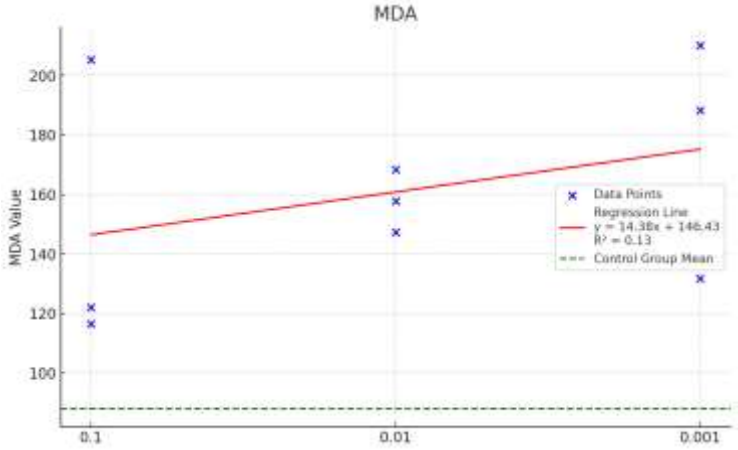

Figure 2. Regression curve of MDA content of application concentrations at 120th hour
Şekil 2. *Uygulama konsantrasyonlarının 120. saatteki MDA içeriğine ait regresyon eğrisi*

Glutathione Content

The table examines the effects of application groups on Glutathione (GSH) content in leaves of *Phaseolus vulgaris* L. plants, as observed in Table 3.Upon examining Table 3 regarding the effects on GSH content in the leaves of *Phaseolus vulgaris* L. plants, the lowest GSH content was obtained at 120 hours with the application of 0.001 mM ZnO nanoparticles (56.75±13.14 nmol/g), and this decrease was statistically significant ($p<0.05$).

Table 3. Effects of application groups on Glutathione (GSH) content in leaves of *P. vulgaris* L. plant
Çizelge 3. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Glutatyon (GSH) miktarına etkileri

Application groups (120th hour)	GSH content (**nmol/g**)
Control	**335,91±33.6**
0.1 mM ZnO	119.92±33.6*
0.01 mM ZnO	77.92±7.58*
0.001 mM ZnO	56.75±13.14*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: $p<0.05$

Scientific observations on many plants have shown that glutathione is a significant player in determining their relative tolerance. Glutathione plays a role in detoxifying reactive oxygen species (ROS) through the ascorbate-glutathione cycle. Accumulated metal ions are detoxified by phytochelatins synthesized from glutathione in plants exposed to heavy metals (Yadav, 2010). Oxidative stress occurs when the balance between ROS and antioxidant defenses is disrupted (Tarrahi et al., 2018). Glutathione is a tripeptide found in various cellular organelles (Millar et al., 2003). Glutathione is a non-enzymatic antioxidant. ZnO NPs can induce oxidative stress by disrupting cellular metabolism or consuming cellular enzymatic and non-enzymatic antioxidants, thereby causing damage to cellular lipids, proteins, and nuclear DNA (Alkaladi, 2019).

At 0.001 mM concentration, ZnO was measured at the lowest level of 56.75 nmol/g after 120 hours. A generally decreasing level of GSH indicates an increase in oxidative stress. According to Alkaladi (2019), sub-lethal doses of ZnO NPs in Nile tilapia plants inhibit the activities of glutathione reductase (GR), glutathione peroxidase (GPx), and glutathione S-transferase (GST), alongside gene expression. Researchers reporting a decrease in GSH (glutathione) levels also noted an increase in lipid peroxidation (LPO) levels, indicating a significant increase in oxidative stress due to these results.

A study reports that Cu NP applied to tomato plants under salt stress resulted in a 13% increase in GSH levels compared to the control group. The study indicates that applying only salt resulted in an 81% increase in GSH, while Cu NP treatment led to a significantly high GSH content of 337%. According to the results, it was concluded that Cu NP has a positive effect on the GSH content of the plant (Pérez-Labrada et al., 2019). In the current study, however, the opposite result was obtained. It was observed that the application of ZnO NP led to a decrease in GSH content. This result may be due to differences in the experimental material used. Additionally, Cu NP and ZnO NP have different properties. Therefore, obtaining different results in living tissues, particularly in plants, can be inevitable. Overall, the impact of nanoparticles is influenced by various factors, including dose, treatment duration, application method, type of nanoparticle, and the plant species (Santás-Miguel et al., 2023).

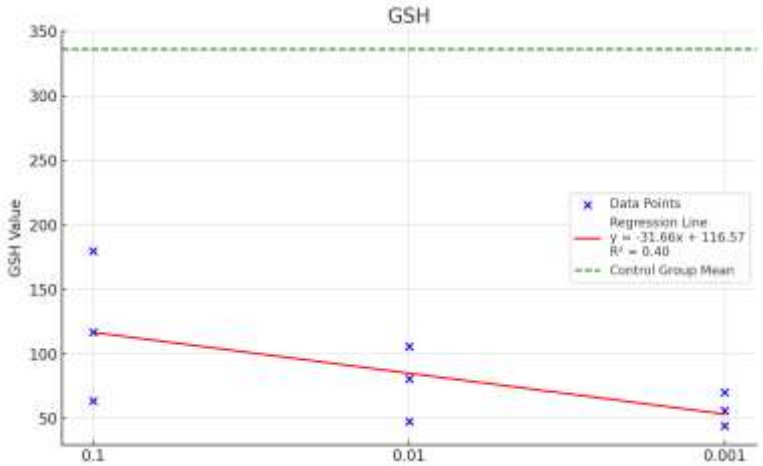

Figure 3. Regression curve of GSH content of application concentrations at 120th hour
Şekil 3. *Uygulama konsantrasyonlarının 120. saatteki GSH içeriğine ait regresyon eğrisi*

Total Soluble Protein Content

Changes in total soluble protein content are seen in Table 4. When examining the effects on the total soluble protein content in the leaves of *Phaseolus vulgaris* L. plants, it is observed that at 120 hours, as the concentrations of ZnO decrease, the soluble protein content also decreases.

A study reported an increase in protein levels in tomato, cauliflower, and cabbage plants as a result of the optimal use of ZnO NPs (Singh et al., 2013). Mehrian et al. (2015) investigated the effects of silver NPs on tomato plants. According to this study, significant decreases in protein content were observed as the concentration of Ag NPs

increased. In a study conducted with kidney beans, changes in Total Soluble Protein (TSP) content due to CeO2 NP applications were examined. The results indicated that protein content was not affected by the treatments conducted for 7 days, but significantly high protein contents were determined in the roots. The study also reported decreases in protein content in the leaves (Majumdar et al., 2014).

Table 4. Effects of application groups on Total Soluble Protein content in leaves of *P.vulgaris* L. plants
Çizelge 4. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Toplam Çözünebilir Protein miktarına etkileri

Application groups (120th hour)	Total Soluble protein Content (**mg/g**)
Control	1.61±0.19
0.1 mM ZnO	1.33±0.03*
0.01 mM ZnO	1.16±0.01*
0.001 mM ZnO	1.28±0.04*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: p<0.05

In a different study, the effects of ZnO NPs were investigated in *Leucaena leucocephala* plants under stress conditions induced by Pb and Cd applications. According to the research findings, the total soluble protein content did not increase in samples treated with ZnO NPs alone (Venkatachalam et al., 2017). A study reported that salinity stress and the application of different concentrations of Zn NPs did not significantly affect the total soluble protein values in spinach plants (Zafar et al., 2022). Additionally, a study conducted with corn plants suggests that the negative effects caused by arsenic can be mitigated with ZnO NPs (Khan et al., 2022).
Similarly, in our study, decreasing protein contents were observed in leaf tissues, which is generally consistent with the literature. Proteomic analyses are considered necessary for a better understanding of the mechanism.

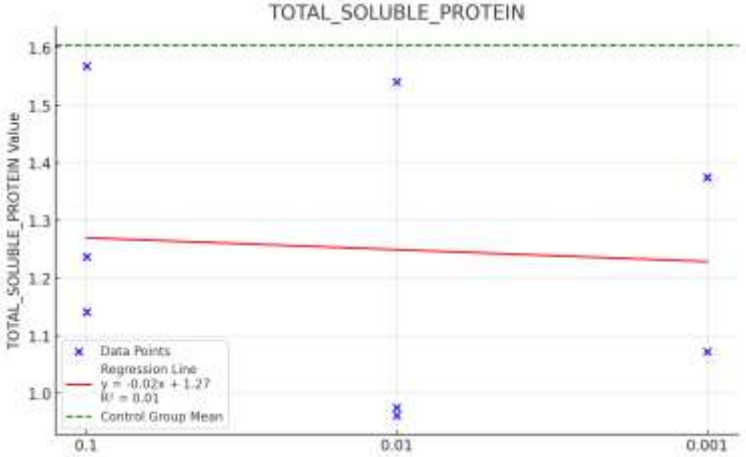

Figure 4. Regression curve of Total Soluble Protein content of application concentrations at 120th hour
Şekil 4. *Uygulama konsantrasyonlarının 120. saatteki Toplam Çözünebilir Protein içeriğine ait regresyon eğrisi*

Superoxide dismutase and Catalase enzyme activities

The superoxide dismutase (SOD) enzyme activity in leaves of *Phaseolus vulgaris* L. plants are presented in Table 5. The results obtained indicate that, in all concentrations of ZnO application, lower SOD enzyme activities were determined compared to the control. The effects of application group on catalase (CAT) enzyme activity in the leaves of *Phaseolus vulgaris* L. plants were examined based on the results in Table 5. It is observed that the effects of ZnO NP increase in all concentrations compared to the control. It is observed that CAT activity increases with decreasing concentrations of ZnO NP.

Table 5. The effects of application groups on Superoxide dismutase (SOD) and Catalase enzyme activities in leaves of *P. vulgaris* L. Plant
Çizelge 5. P. vulgaris L. bitkisi yapraklarında uygulama gruplarının Süperoksit dizmutaz (SOD) ve Katalaz (CAT) enzim aktivitelerine etkileri

Application groups (120th hour)	SOD Enzyme Activities (unit/g)	CAT Enzyme Activities (unit/g)
Control	**7.82±0.37**	**66.65±0.67**
0.1 mM ZnO	7.24±0.12*	121.09±4.44*
0.01 mM ZnO	7.20±0.05*	132.31±5.61*
0.001 mM ZnO	7.18±0.2*	206.64±8.45*

The evaluations in the tables were made between the control group and the other groups, and the statistical signs were observed between the control group and the other groups. *: p<0.05.

In this current study, in the ZnO NP application group, the SOD activity is lower compared to the control group. The lower SOD (Superoxide dismutase) activity at high concentrations may disrupt the antioxidant response involved in clearing reactive oxygen species from leaves, similar to the findings of Salehi et al. (2021). In another study with corn plants, high concentrations of ZnO NPs reduced SOD activity (Srivastav et al., 2021). On the other hand, zinc nanomaterials have been shown to increase SOD and POX enzyme activities, while also demonstrating a protective role against oxidative damage by reducing CAT activity (Venkatachalam et al., 2017).
In the results of this study, in all applications of ZnO NP, an increase in Catalase (CAT) activity compared to the control was observed. In the ZnO NP applied group, as the concentration decreased, CAT activity in all groups was significantly higher compared to the control.
In maize plants, SOD activity increased with the application of ZnO NPs at a concentration of 50 mg/L, but a decrease was observed with increasing concentrations (Srivastav et al., 2021). In light of the data, it can be said that superoxide radicals are dismutated by the SOD enzyme and then broken down and removed from the environment by CAT activity. Additionally, the high CAT activity can be explained by the excessive formation of H_2O_2 in the environment. Higher SOD activities have been identified in ZnO applications, possibly due to the Zn-SOD isoenzyme structure. When all the results and the literature are considered together, it can be seen that there is consistency in the relationships between the enzyme activities obtained and NP applications.

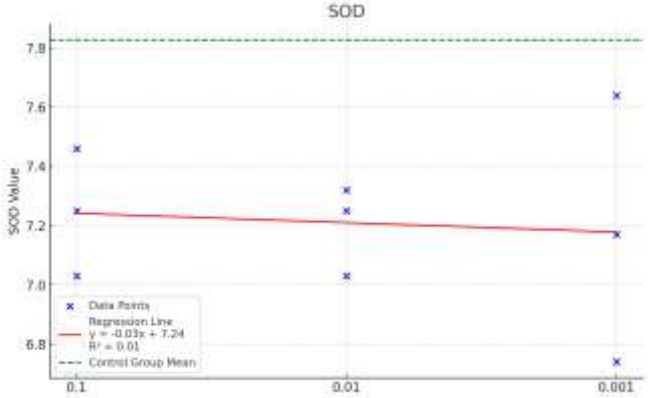

Figure 5. Regression curve of SOD and CAT enzyme activities of application concentrations at 120th hour
Şekil 5. *Uygulama konsantrasyonlarının 120. saatteki SOD enzim aktivitesine ait regresyon eğrisi*

Figure 6. Regression curve of CAT enzyme activity of application concentrations at 120th hour
Şekil 6. *Uygulama konsantrasyonlarının 120. saatteki CAT enzim aktivitesine ait regresyon eğrisi*

CONCLUSION

As a result, all findings from the present study revealed that ZnO NP application activates the antioxidant defense system in *Phaseolus vulgaris* L. leaves. It was determined that the metal nanoparticles in question caused negative effects, especially following exposure to low doses and for a duration of 120 hours. This situation may be attributed to their significantly small size. Nano-sized metals caused a toxic effect on the leaves of *Phaseolus vulgaris* L. Biological systems make it challenging to clearly elucidate the antioxidant mechanism. Obtaining consistent results in living cells is not possible due to factors such as the organism's genetics, developmental stage, applied molecules, and dosage, which play a primary role. However, according to the results of this study, nano-sized metals have induced a toxic effect on the leaves of *Phaseolus vulgaris* L. It should be noted that the duration of exposure to NPs also plays a role in the formation of this toxic effect. Numerous studies have reported that nanoparticles help cope with stress conditions in plants (You and Chan, 2015; González-García et al., 2021; Mushtaq et al., 2020). Specifically, it has been noted that nanoparticle applications lead to an increase in

antioxidant enzyme activities. The results suggest that nanoparticles act as agents that promote antioxidant defense. In the present study, similar results were obtained, and it was concluded that, in addition to stressing the plant, the rising antioxidant defense markers stimulate the plant's defense system. The increase in antioxidant activities will be an important defense response for the plant to overcome various stresses. Metal oxide nanoparticles are used in many fields. However, there is a potential danger that these substances can lead to serious adverse effects. The results obtained will contribute to the literature, emphasizing the need for more detailed research in this area. The results of this study emphasize the necessity of careful consideration in the use of nanomaterials. Particularly, in the face of global environmental disasters such as impending scarcity and drought, it is essential to establish sustainable systems and develop technologies.

Contribution Rate Statement Summary of Researchers

The authors declare no conflict of interest.

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