Bulletin of Biological and Allied Sciences Research
***ISSN:* 2521-0092**
www.bbasr.org
DOI: https://doi.org/10.54112/bbasr.v2024i1.90
*Bull. Biol. All. Sci. Res., Volume, **9**: 90*
Original Research Article ***Open Access***

INVESTIGATE THE IMPACT OF ZINC OXIDE NANOPARTICLES UNDER LEAD TOXICITY ON CHILLI (*CAPSICUM ANNUUM L*)

MUSHTAQ R[1*], SHAFIQ M[1], BATOOL A[2], DIN MI[3], SAMI A[4]

[1]*Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab P.O BOX. 54590, Lahore, Pakistan*
[2]*Department of Plant Breeding and Genetics, Faculty of Agricultural Sciences, University of the Punjab P.O BOX. 54590, Lahore, Pakistan*
[3]*Department of Chemistry, University of the Punjab P.O BOX. 54590, Lahore, Pakistan*
[4]*Agricultural Biotechnology Research Center, National Cheng Hsing University, Academia Sinica, Taipei, Taiwan*
**Correspondence Author Email Address: rabiamushtaq023@gmail.com*
(Received, 27th February 2024, Revised 8th December 2024, Published 11th December 2024)

Abstract *Capsicum annuum L is a commercially significant and valuable crop throughout the world. Weather variations and other stresses can significantly affect the growth and productivity of plants and limit crop productivity. One of the biggest stresses is lead poisoning since it hinders agricultural output and growth. Plants undergo biochemical, physiological, and morphological alterations in response to lead toxicity. As a result, the use of nanoparticles as an emerging method can significantly increase crop productivity. In this study, Kiar plants were employed to synthesize zinc oxide nanoparticles. Seed priming was performed using various applications of ZnO-NPs solution. In a field experiment, chilli plants were cultivated with various concentrations of lead acetate. Two different concentrations (250mg L-1 and 500mg L-1) were administered into the root zone. The following measurements were made after the ZnO nanoparticle supplementation: total chlorophyll content, carotenoids, peroxidase, catalase, flavonoids, and total phenolics content. Root and shoot length, fresh root weight and shoot weight, and dry root weight and shoot weight were all included in the morphological study. Nonetheless, the most noteworthy outcomes, proving that the concentration of ZnO-NPs affected chilli plants, was obtained upon applying the particles at a 150 ppm concentration. In comparison to untreated plants, the outcomes demonstrated that all plants treated with ZnO nanoparticles performed better when under stress.*

[Citation: Mushtaq, R., Shafiq, M., Batool, A., Din, M.I., Sami, A. (2024). Investigate the impact of zinc oxide nanoparticles under lead toxicity on chilli (*capsicum annuum L*). *Bull. Biol. All. Sci. Res.* **9***: 90. doi:* https://doi.org/10.54112/bbasr.v2024i1.90]
Keywords: *Chili; ZnO nanoparticles; Seed Priming; Lead toxicity; Morphological and Biochemical analysis*

Introduction

Capsicum annuum L is a native of the Americas and a member of the Solanaceae family. It was initially grown in Peru circa 7500 BC. There are almost 400 distinct types of chilies grown worldwide, making them the fourth most important crop. The five domesticated species of chili are *Capsicum annuum*, *Capsicum baccatum*, *Capsicum chinense*, *Capsicum pubescens*, and *Capsicum frustescens.* C. annuum is the most widely grown and economically significant of these species (Bal et al., 2022). Chilli pepper has been used for centuries for its medicinal properties. Farmers grow chili peppers in both tropical and temperate climates, with weather, soil conditions, and agricultural practices all influencing productivity. According to the FAO, Pakistan came in third place globally among nations that cultivate chilies in 2011, accounting for 5.87% of global production, behind China, Taiwan, and India (FAO 2013). 3,457,533 tons of red chilies we produced worldwide in 2011 (FAO Stats). 40,414 tons of red were produced in Pakistan. With 33% of the global production, India is the leading producer and exporter of chilies. The cultivation of chili covers an area of 1.43 lakh ha and yields 6.66 lakh tons (Deepa et al., 2022).

In recent years, the application of nanotechnology in agriculture has increased and it is a vital instrument in achieving the global objective of sustainable food production. Nanoparticles are very small particles with dimensions ranging from one to 100 nanometers (nm). In plants, Nanoparticles have intriguing potential for increasing growth, improving stress tolerance, and precisely delivering vital components genes. Plants require a variety of nutrients for proper growth. Nanoparticles can transport critical minerals like iron and zinc, as well as macronutrients like nitrogen (Shang et al., 2019). Due to their large surface area, nanoparticles can encapsulate these nutrients and transfer them directly to plant cells, enhancing absorption efficiency and decreasing overall fertilizer use. Zinc (Zn) is an important micronutrient involved in plant growth and production, and a shortage will reduce agricultural output. Most enzymes, including carbonic anhydrase, carboxypeptidase, and superoxide dismutase, need zinc as a cofactor. Zinc deficiency can harm a plant by limiting its growth, and reducing the number of

tillers, resulting in chlorosis, smaller leaves, spikelet sterility, and lower-quality harvested products (Al Jabri et al., 2022). It is essential for crop nutrition because it participates in several enzymatic events, metabolic processes, and oxidation-reduction reactions.

Zinc oxide nanoparticles (ZnO-NPs) are white, odorless powders having an 81.38 g/mol molecular weight. Different techniques can be used to create ZnO-NPs in different sizes and forms. By changing reaction parameters including temperature, solvent, and time, ZnO-NPs can have different sizes, shapes, and surface chemistries. ZnO-NPs' usefulness and toxicity are determined by their size, shape, and surface chemistry (Sruthi et al., 2018). ZnO-NPs are developing as a viable tool in plant science, with huge implications for improved plant development and yield, one of the most important answers to the world's fast-rising population. Plants face a variety of environmental challenges, including toxic stressors, which are a significant threat to their growth, development, and survival. Lead (Pb) is the second most toxic heavy metal, following arsenic (As), which has no biological activity. Pb poisoning produces a variety of plant difficulties, including germination and yield development; however, its toxicity varies depending on time and concentration. Research has indicated that exposure to lead can impede the growth of plants in chilies, resulting in smaller leaves, stunted stems, and decreased biomass overall. While lead is absorbed by chili plants, research suggests that the fruits themselves often show lower lead contents than the roots and foliage (Ahmed et al., 2021).

Materials and methods

Study Area and Sample Collection

The pot experiment was performed at the Department of Horticulture, Lahore. Loamy Soil was collected from the Faculty of Agricultural Sciences. Different varieties of Chilli seeds were obtained from the Yuksel Seeds Company Lahore and washed with distilled water before sowing seeds were soaked overnight for germination.

Pre-Soil Analysis

The soil sample was air-dried and sieved to remove stones.

Soil Texture

To determine soil texture, dissolve 40g of soil in 40 ml of sodium hexametaphosphate solution. Keep this solution overnight. Next, use a hydrometer to assess soil texture. Take four readings at 40 seconds and 2 hour intervals. Loamy soil is characterized by a mix of sand, silt, and clay, resulting in excellent nutrient retention and drainage.

Electrical conductivity

For EC, take 1g of soil and make the suspension in 10 ml deionized water. After that note the reading on the EC meter.

0.03*10 = 0.3/1.33

Ec = 0.2255 ds/m

Potential of Hydrogen

For pH, take 200g of soil and make the saturated paste in distilled water. After that note the reading through pH meter. Then the pH reading was 8.17.

Synthesis of Green ZnO Nanoparticles

Mix 50 milliliters of Kiar plant extract with 0.5 milliliters of zinc nitrate hexahydrate ($Zn(NO3)2•6H2O$) and stir at 90 degrees Celsius to make the mixture. A magnetic stirrer was used to mix in an aqueous solution of 1 M sodium hydroxide (NaOH). The churning continued for thirty minutes after the addition of NaOH. After five hours of drying the precipitates at 200 °C, a beige powder containing ZnO NPs was obtained. Next, the beige powder was calcined for four hours at 600 degrees Celsius in a muffle furnace. Zinc oxide nanoparticles were found in a white powder that was obtained (Aldalbahi et al., 2020).

Preparation of ZnO nanoparticle solution

For the preparation of nanoparticles solution, 0.01 g ZnO nanoparticles were added to 100 ml of distilled water to prepare 100 ppm solution, and 0.015g ZnO nanoparticles were added to 150 ml of distilled water to prepare 150 ppm solution.

For 100ppm Solution

100ppm = mass of solute (g)/volume of solution (ml)* 10^6

Mass of solute = 100*100/10^6 = 0.01g

For 150ppm Solution

150ppm = mass of solute (g)/volume of solution (ml)* 10^6

Mass of solute = 150*100/10^6 = 0.015g

Seed Priming

The Hybrid Seeds of different varieties of *capsicum annuum* (F1 19-14, 19-17, 19-19) were soaked in different concentrations of zinc oxide nanoparticles solution in Petri plates overnight.

Field Experiment

Seed Germination of Chilli

Different varieties of chilli seeds were purchased from the Yuksel Seeds Company Lahore and plastic bags were filled with the garden soil as it is good for keeping moist conditions and helps seeds to grow. After 5-6 days of sowing, seeds started germination.

Transplantation of chilli seedlings

After 15 days, seeds were transplanted to the pots under controlled conditions. The pots were filled with loamy soil and the sizes of pots were 6-10 inches wide and 7-9 inches of depth.

Lead Stress Induction

Chilli plants were subjected to lead stress through the spraying method. Two concentrations of lead solution were prepared. One is dissolving 250mg of Pb and the other is dissolving 500mg of Pb to the 1L of distilled water. After 7 days of transplanting, the plants were subjected to lead stress and were maintained throughout the experiment.

Collection of plant samples

After few days of application, plants attained maximum height then randomly collect the leaf

samples from the pots for experimental purpose. Fresh leaf samples were properly washed in tap and distilled water in the laboratory, dried at room temperature and tested for morphological (germination %, germination speed, germination mean time, plant height, root length, shoot length, fresh weight and dry weight) and biochemical analysis of chlorophylls (Ch-a and Ch-b), carotenoids, flavonoid, catalase, peroxidase and total phenolics.

MORPHOLOGICAL PARAMETERS

Germination percentage, Speed and mean time

The number of seeds-germinated was counted at six, twelve and eighteen days after seed treatment and the germination percentage, speed and mean time was determined.

Formulas

Germination percentage = number of days/total number of seeds*100

Speed of germination = number of days/germinated seeds

Germination mean time = number of days*germinated seeds/total number of seeds

Plant Height

When chilli plants attained maximum height, lengths of plants were measured by using RAYS Acrylic clear ruler of cm range.

Root Length

Roots were seperated from plants, washed with water and leave them for dry. After few minutes, they were measured by using Ray's ruler in cm range.

Shoot Length

Shoots were also treated with same above procedure and measured with ruler of cm range.

Fresh and Dry Root weight

Roots were seperated from plants and weigh them on weighing balance in grams. After that, they were dried in oven for 24 hours and again weigh them.

Fresh and Dry Shoot weight

Shoots were seperated from plants and treated with same above procedure.

Biochemical Parameters

Estimation of Total Chlorophyll content and Carotenoids

Twenty grams of fresh chilli plant leaves were rinsed with tap water to get rid of any debris. to create a solution with 80% acetone. For ten to fifteen minutes, leaf samples were kept at room temperature after being homogenized in an 80% acetone solution. Samples that had been finely powdered were put in centrifuge vials and centrifuged. Centrifugation was run at 12,000 rpm for five minutes. Each sample's supernatant was gathered into test tubes, and the absorbance was assessed using a control solution of 80% acetone. To determine the quantity of carotenoids, chlorophyll a, and chlorophyll b (del Pilar Sánchez-Camargo et al., 2019) in each sample, absorbance measurements were made at 663 nm, 645 nm and 470nm and then further computed using the formula published by Litchtanthaler and Welburn (1983) (Singh and Kumar, 2020). By using Palta (1990) formula, total chlorophyll content was estimated (Ashenafi et al., 2023).

Chlorophyll a= 12.7 *OD663-2.69*OD645

Chlorophyll b=22.9*OD645-4.68*OD663

Total chlorophyll content=20.2*OD645 +8.02*OD663

Carotenoids= 1000*470nm-3.27*Chl a-10.4*Chl b/229

Determination of Flavonoid Content

The Park et al. (2008) procedure was used to determine the total flavonoid content (Lim et al., 2020). 0.1 g of the generated enzyme extract dissolved in 3 mL of deionized water. 500 microliters of diluted plant extract and 0.1 milliliter of 10% aluminum chloride hexahydrate are combined to create the solution. Next, add 2.8 ml of deionized water, 100 microliters of 1 M potassium acetate, and 1.5 ml of 95% alcohol. After carefully blending the mixture, it was allowed to sit at for forty minutes at room temperature. Forty minutes were spent measuring the reaction mixture's absorbance at 415 nm.

Estimation of Peroxidase Activity

Fresh leaf samples from cucumber plants were collected to determine peroxidase activity. In a pestle and mortar, 1 gram of leaves were homogenized with 2 ml of 0.05 mol L-1 sodium phosphate buffer. A 0.05 mol L-1 sodium buffer solution was created from a previously prepared 0.2 moll-L solution with a pH of 6.5. For fifteen minutes, the homogeneous mixture was centrifuged at 13,000 rpm. The supernatant from the plant sample was then isolated for future use. To make the 0.3% H2O2 solution, combine 3 milliliters of H2O2 with 97 milliliters of pure water. The 0.2% guaiacol solution was prepared by dissolving 0.2 g guaiacol in distilled water. A 3 mL solution was prepared to test the activity of peroxidase. Using a micropipette, 50 microlitres of enzyme extract was mixed with 1000 microlitres of 0.05 mol L-1 sodium phosphate buffer solution and 1000 microlitres of 0.3% H2O2 solution. The absorbance of samples was measured at 470 nm for 1 minute against a blank using a UV-1600 spectrophotometer (Cao et al., 2019).

Estimation of Catalase Activity

Fresh plant samples were gathered in order to determine catalase activity. The 0.2 mol-1 L solution was used to prepare a 0.05 mol-1 L sodium phosphate buffer solution for crushing. 1 gram of leaves were homogenized in 2 milliliters of 0.05 mol-1 L sodium phosphate buffer solution. The enzyme extract samples were centrifuged at 13000 rpm for 10 minutes, and the supernatant was collected to determine catalase activity. To make a 0.3% H2O2 solution, 3 ml of H2O2 was mixed with 97 ml of distilled water. The volume of 3 ml solution was created by combining 1000 microliters of H2O2 solution and 1900 microliters of distilled water with 100 microliters enzyme extract. After preparing the solution, each sample is transferred to the cuvette's absorbance was measured against a blank. Using a

UV-1600 spectrophotometer, the absorbance of each sample for CAT activity was determined at 240 nm (Gan et al., 2022).

Estimation of Total Phenolic Content

Using the Folin Ciocalteu technique, the total phenolic content of the enzyme extract was determined. Fresh leaves in 500 microliters were mixed with an 80% acetone solution and centrifuged for 15 minutes at 4 °C at 10,000 rpm. Following collection, the supernatant was diluted with two milliliters of deionized water. Add 2 milliliters of Folin Ciocalteau's phenol reagent after 2 minutes, and then incubate for 40 minutes at 4 °C. The mixture was then given five milliliters of 20% Na2CO3, and double-distilled water was added to get the volume down to ten milliliters. Using a spectrophotometer, the absorbance of the reaction mixture was measured at 765 nm in comparison to a blank. The leaf's mg FW was used to compare the results to a standard known concentration.

Statistical Analysis

This investigation employed a three-way ANOVA. The impact of ZnO nanoparticles at various concentrations was assessed in relation to lead toxicity. Using Statistics 8.1, the means of all the parameters were compared using the LSD test.

RESULTS AND DISCUSSION

CHARACTERIZATION OF ZINC OXIDE NANOPARTICLES

Fourier-Transform Infrared Spectroscopy Analysis

The chemical characteristics of nanoparticles were determined by FTIR analysis, which involved scanning the sample with infrared light (Ashok et al., 2020). Wavelengths from 1634 to (600, 450) cm^{-1} are commonly examined in FTIR analysis. According to Fakhari et al. (2019), there may be stretching vibrations of Zn OH indicated by the big peak at about 3300 cm^{-1}. ZnO nanoparticles' FTIR spectra showed that the Zn OH functional group was represented by absorption peaks between 4000 and 650 cm^{-1}. Because of the hydroxal group, there are absorption peaks between 1300-800 cm^{-1}. It verified zinc's existence as well [**Figure 1**].

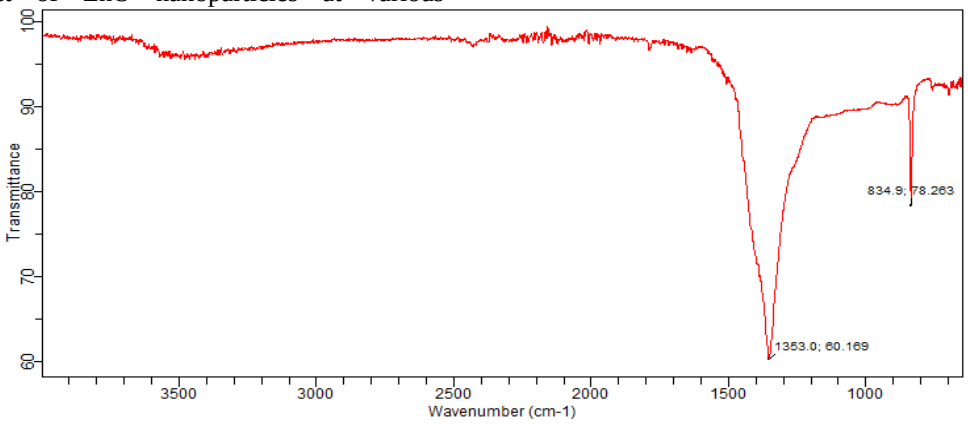

Figure 1: FTIR of ZnO nanoparticles

Effect of ZnO Nanoparticles on Morphological analysis under lead toxicity Germination Percentage, Speed and Mean time

Chilli plants were treated with Zinc oxide nanoparticles at different concentrations under lead toxicity and observed the effect on germination %.

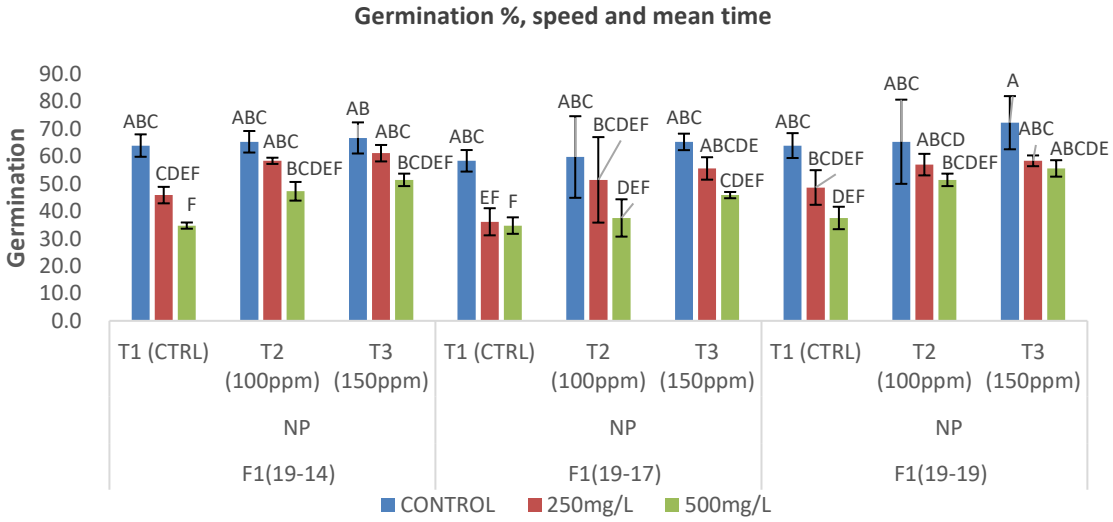

Figure 2: Effect of lead toxicity and different treatments on seed germination

The results suggested that when chilli plants were treated with Zinc oxide nanoparticles showed significant impact on the GP, Speed and Mean time. In comparison to the other varieties, the variety 3 treated plants showed more significant impact even under the lead toxicity. Highest germination %, speed and mean showed at the application of 150ppm solution of nanoparticles which is 72% [**Figure 2**]. It is found that ZnO nanoparticles have positive impact on chilli.

Root and Shoot Length

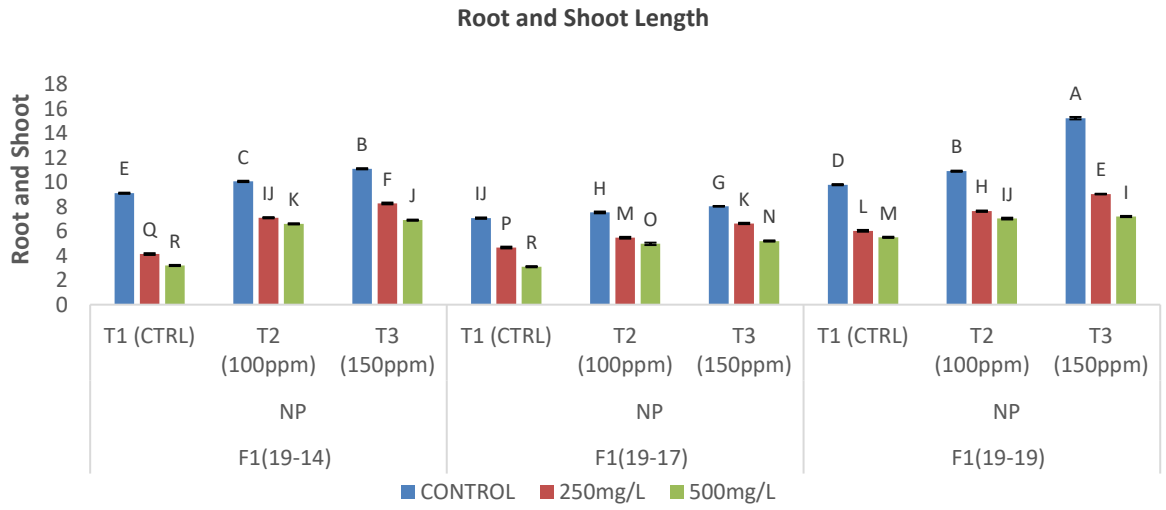

Figure 3: Impact of different treatments under lead toxicity on root and shoot length

The study assessed the effect of ZnO nanoparticles on chilli plants, with a particular emphasis on the root and shoot length parameter. As the concentration of ZnO nanoparticles increased, root and shoot length also increased. While the highest performance observed in concentration of 150ppm of ZnO nanoparticles in variety 3 and is classified as group A and it also performed well under stress. This variety has 15.2cm length of root and length of this shoot is 25.5cm [**Figure 3**].

Fresh Root and Shoot Weight

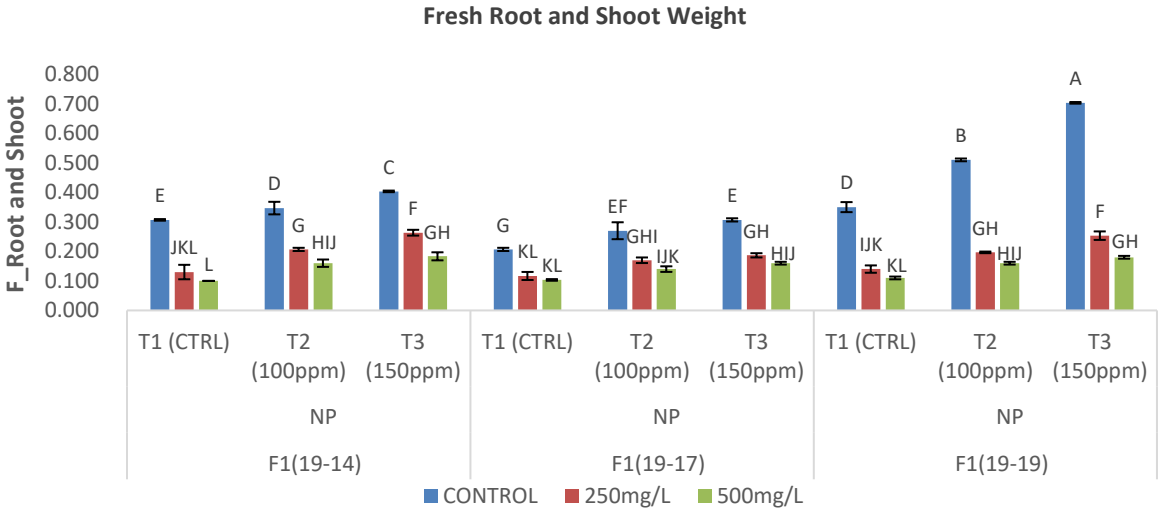

Figure 4: Impact of lead toxicity on FRW and FSW across treatments

With an emphasis on the fresh root and shoot weight parameter under lead poisoning, the study examined the impact of ZnO nanoparticles on chilli plants Fresh root weight increased proportionally with the amount of ZnO nanoparticles. The best performance was obtained in a 150ppm solution of nanoparticles from variety 3, which is categorized as group A, and it also performed well under stress. This fresh root weighs 0.70 grams and shoot weighs 3.8 grams [**Figure 4**].

Dry Root and Shoot Weight

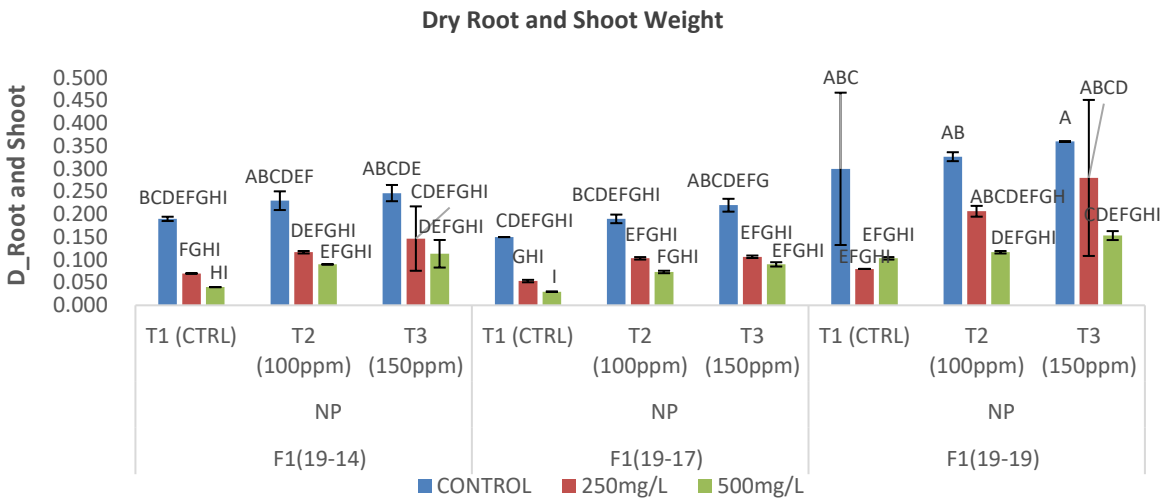

Figure 5: Effect of lead toxicity on DRW and DSW across treatments

The dry root and shoot weight parameter under lead poisoning was especially examined in the study to see how ZnO nanoparticles affected chilli plants. Group A nanoparticles of variety 3, which also exhibited good stress-behavior, produced the greatest results in a 150 ppm solution [**Figure 5**]. This root is 0.36 grams and shoot is 0.36 grams in weight. Group AB is assigned to variety 2, which employs nanoparticles to good effect. All species of the lead-poisoning plants showed extremely low mean values.

Effect of ZnO Nanoparticles on Biochemical analysis under lead toxicity

Under lead stress conditions, various biochemical parameters, such as TCC, Carotenoids, Peroxidase, Catalase, Flavonoids and TPC were evaluated.

Total Chlorophyll Content

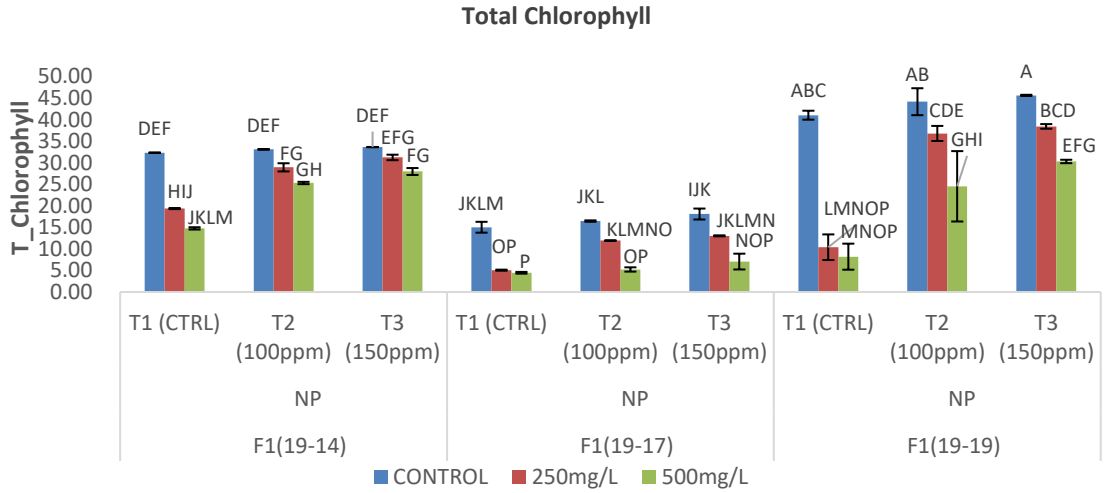

Figure 6: Effects of lead toxicity and various treatments on TCC

This graph shows that T3 of variety 3 has most significant impact of Zno nanoparticles and is placed into the group A. All the treatments of variety 3 have showed good result even under lead toxicity. In comparison to other varieties, the variety 2 exhibited relatively low significant impact of ZnO-Nps under lead stress [**Figure 6**]. These data show that applying zinc oxide nanoparticles under lead stress has various impacts on chlorophyll concentrations; larger concentrations of ZnO-NPs result in increased chlorophyll content, but lower concentrations may result in a drop in chlorophyll levels.

Carotenoids Content

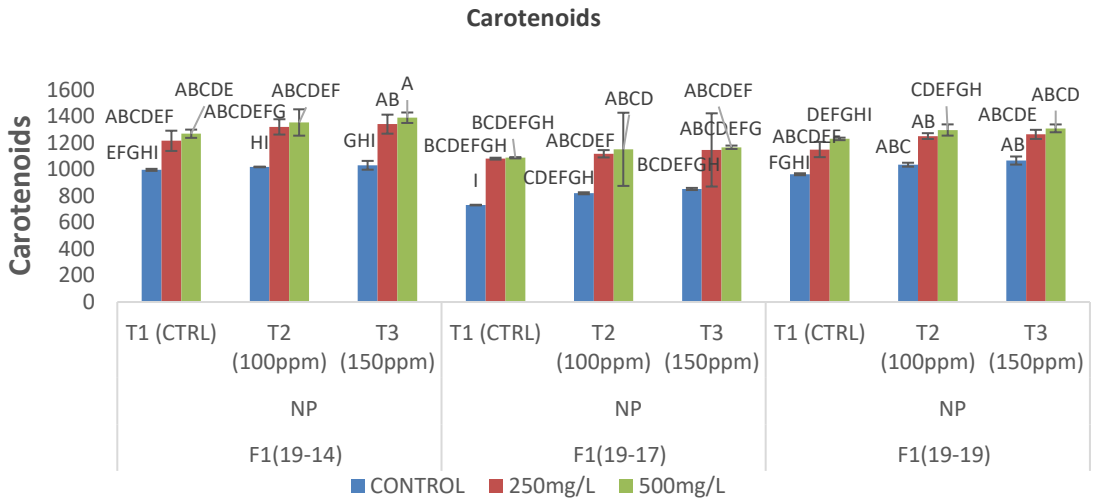

Figure 7: Effect of lead toxicity and different treatments on carotenoids

The total carotenoid content had a significant impact in T3 of variety 1, placing it in the A category. When ZnO nanoparticles were applied at a concentration of 150ppm, increased carotenoid levels were found in chilli plants under lead stress. Chilli plants exposed to lead stress had comparatively high carotenoids levels when treated with nanoparticles [**Figure 7**]. It has been shown that raising the concentration of ZnO nanoparticles increases the content of carotenoids in plants. **Peroxidase Activity**

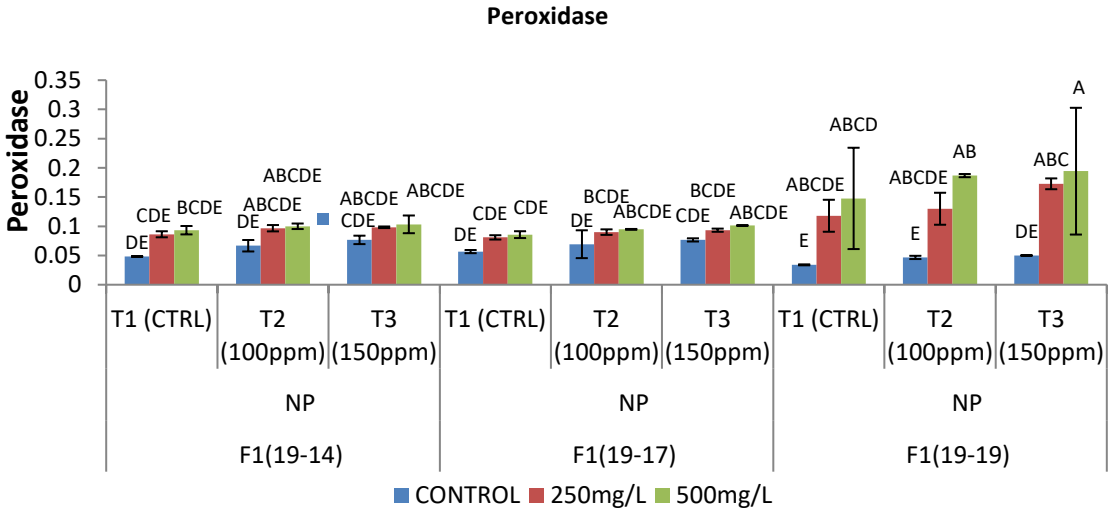

Figure 8: Impact of lead toxicity and treatments on peroxidase

The peroxidase activity shown by T3 of variety 3 was significantly high and was considered into group A. When ZnO nanoparticles applied at the application of 150ppm solution with 500mg of lead concentration then the peroxidase activity showed most significant result because the enzymes activities are increased in stress condition [**Figure 8**].It suggests that under lead stress, supplementation of zinc oxide nanoparticles at different concentrations had great influence on the peroxidase activity.

Catalase Activity

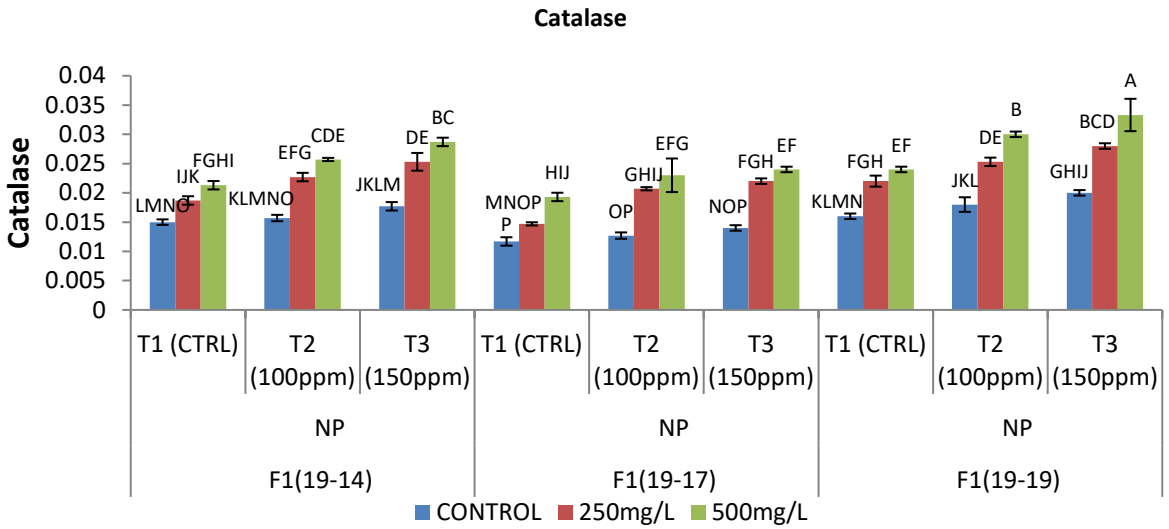

Figure 9: Lead toxicity and treatment effects on catalase

T3 from variety 3 demonstrated very strong catalase activity and was classified as group A. When ZnO nanoparticles were applied at the concentration of 150ppm solution containing 500mg of lead, catalase activity increased significantly. Variety 2 has not produced substantial outcomes under stress [**Figure 9**]. It implies that under lead stress, supplementation with zinc oxide nanoparticles at various doses had a significant impact on catalase activity.

Flavonoids Content

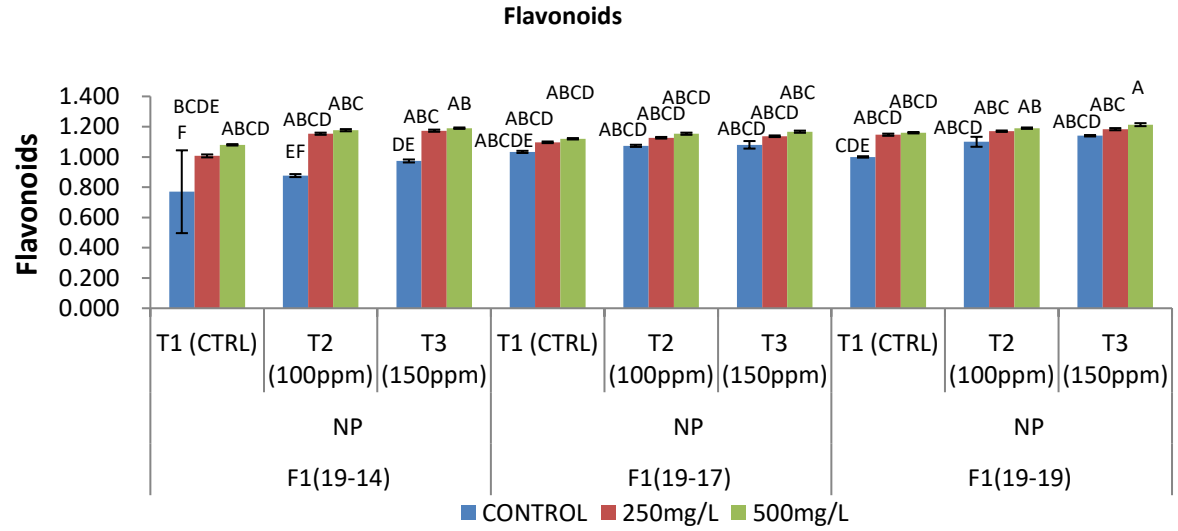

Figure 10: Lead toxicity and various treatments affect on flavonoids

All the varieties showed significant results but variety 3 was categorized as group A due to its extremely high flavonoid activity under lead toxicity. Flavonoid activity rose dramatically when ZnO nanoparticles were added at a concentration of 150 ppm solution containing 500 mg of lead [**Figure 10**]. It suggests that supplementing with zinc oxide nanoparticles at different dosages significantly affected flavonoid activity under lead stress.

Total Phenolic Content

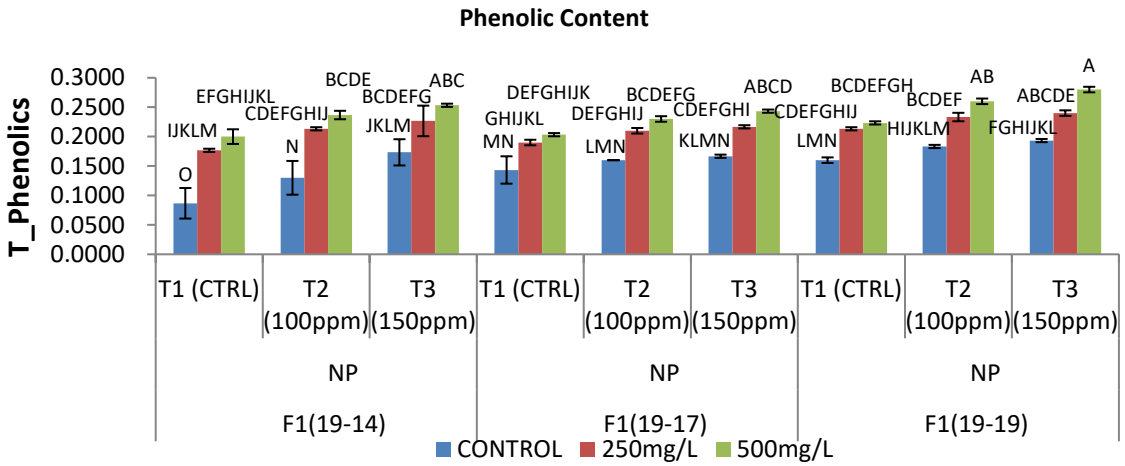

Figure 11: Effects of lead toxicity and treatments on TPC

The effect of ZnO nanoparticles on phenolic contents under lead stress conditions was investigated. The results showed that the use of ZnO nanoparticles had a substantial impact on TPC levels. The variety 3 was classified as group A due to its strong TPC activity at a lead concentration of 500mg [**Figure 11**]. These findings show that using ZnO nanoparticles can boost the accumulation of phenolic chemicals in chilli plants, perhaps improving the plants' ability to endure stress.

Analysis of Variance of Biochemical Parameters under Lead Stress

Table 1: Analysis of variance of biochemical parameters under lead stress

	TCC	Carotenoids	Peroxidase	Catalase	Flavonoids	TPC
P-value	0.01084	0.00999	0.9997	0.7943	0.9989	0.9510
Error	17.79	32455	0.00374	3.651*10^-6	0.01306	0.00063
Grand mean	23.057	1135.7	0.0962	0.0211	1.0996	0.2017
CV	18.30	15.86	63.58	9.06	10.39	12.45
Standard Error	3.4442	147.09	0.0500	1.560*10^-3	0.0933	0.0205

P-Values

The p-values indicated the effect of ZnO nanoparticles on biochemical analysis of chilli plant. This finding indicated that ZnO nanoparticles influenced the Chlorophyll and carotenoids level in plant. However, ZnO-Nps showed no significant effect on peroxidase, catalase, flavonoids and total phenolic contents [**Table 1**].

Experimental Error

The precision of the experimental measurements was demonstrated by the relatively minimal error values for these parameters.

Grand Mean

The total chlorophyll content exhibited mean value of 23.05, determining the quantity of this essential pigment responsible for photosynthetic activity. Carotenoids content showed a grand mean of 1135.7, marked the concentration of these important pigments in the chilli plants. Additionally, the peroxidase activity had a mean value of 0.0962, indicating the concentration of peroxidase activity in the chilli plants subjected to ZnO nanoparticles. Catalase activity exhibited mean value of 0.0211. It indicates the low concentration of catalase activity in the chilli plants subjected to ZnO nanoparticles. Flavonoid contents, with a grand mean of 1.0996, indicates a greater level of catalytic activity. The total phenolic contents had a mean value of 0.2017, indicating an average degree for this activity.

CV values

The degree of variability is exhibited by the coefficient of variation (CV). In particular, the peroxidase activity displayed highest CV value 63.58. TCC and Carotenoids have comparatively low CV values, indicating that the measurements of Total Chlorophyll Content and Carotenoids are more accurate and consistent than the other parameters. Contrarily, peroxidase activity exhibited higher CV value which indicates greater variability in the measurements relative to the mean. It is suggested that the influence of zinc oxide nanoparticles on lead stress has a greater impact on peroxidase variability than other parameters.

Standard Error

The standard error values provided information about the precision of measurements, with TCC, Carotenoids, Peroxidase activity, Catalase activity, Flavonoids and Total Phenolics Contents having standard error values of 3.4442, 147.09, 0.0500, 1.560*10^-3, 0.0933 and 0.0205 respectively [**Table 1**].

Correlation of Biochemical Parameters under Lead Stress

Table 2. After supplimentation of ZnO nanoparticles to lead-stressed chilli plants, a variety of significant relationships between biochemical parameters (TCC, Carotenoids, Peroxidase, Catalase, Flavonoids and TPC) were investigated by using computer software statistic 8.1.

	Chlorophyll	Carotenoid	Catalase	Peroxidase	Flavonoids
Carotenoid	0.07364				
Catalase	0.42593*	0.82039			
Peroxidase	-0.10986	0.63722*	0.5962*		
Flavonoids	-0.18341	0.57325*	0.4188*	0.6008*	
Phenolics	-0.12907	0.77378	0.65446*	0.76031	0.91545

Chlorophyll and Catalase Content Correlation

The correlation between catalase and carotenoids (r=0.42593) was found to be positive and significant. This shows that the application of ZnO nanoparticles stimulated CAT activity, increasing chlorophyll contents in the plant. The findings suggest a defensive mechanism because catalase has antioxidant capabilities.

Carotenoids and Peroxidase Correlation

Carotenoids showed positive relationship with peroxidase (r=0.62722) under the application of ZnO nanoparticles. It is observed that application of ZnO-NPs enhanced peroxidase contents in lead stressed chilli plants. During stress conditions, peroxidase activates the defense mechanism of plants.

Carotenoids and Flavonoids Correlation

The most significant and positive association (0.57325) was found between carotenoids and flavonoids. This relationship suggested that ZnO nanoparticles have a beneficial effect on flavonoids, which in turn regulates the buildup of carotenoids levels. Carotenoids show significant impact on flavonoids content.

Catalase and Flavonoid Contents Correlation

Catalase activity also had a positive connection (r=0.4188) with flavonoids. It implies that ZnO nanoparticles increased catalase and flavonoids concentration. The addition of zinc oxide nanoparticles greatly improved photosynthetic efficiency and plant growth. It helps chilli plants continue photosynthesis under stressful conditions.

Total Phenolic Contents and Catalase Activity Correlation

A high positive association exists between total phenolics content (r=0.65446) and catalase activity. This positive connection implies that ZnO nanoparticles enhanced TPC levels and catalase contents in chilli plants during stress. A complex interaction between these two parameters demonstrates that chilli plants need higher catalase content to combat oxidative damage.

Peroxidase and Flavonoids Content Correlation

On the other hand, the correlation between peroxidase and flavonoid contents show significant impact (r=0.6008). This shows that ZnO nanoparticles enhanced peroxidase and flavonoids activity [**Table 2**].

Discussion

Agriculture is the cornerstone of most developing countries, and the vast majority of their citizens rely on it for a living. Every year, the global population expands by about 83 million people, needing increased agricultural output. Crop losses occur as a result of biotic and abiotic stressors, which eventually limit production. It is estimated that biotic and abiotic stressors will be responsible for 50% of the production loss. Weather extremes, droughts, salt, and flooding are the most common abiotic factors that cause yield loss (Ashraf et al., 2012). Heavy metals occur in soil in many forms, each with a unique solubility/bioavailability profile. Soil physicochemical properties influence heavy metal geochemistry in soil, plant absorption, and crop yield.

Lead acetate, a dangerous heavy metal, is found in leafy greens at higher levels than other plants. Lead uptake was regulated by soil concentration, pH, type, organic matter content, plant species, and hazardous agricultural practices. The mechanism by which lead inhibits plant growth involves a decline in the quantity of dividing cells, decreasing chlorophyll production, causing plants to experience water stress, lowering NO-3 uptake, lowering nitrate and nitrite reductase activity, influencing protein synthesis directly, and lowering the amount of nutrients that plants absorb and concentrate (Feleafel and Mirdad, 2013). After a thorough analysis, the scientists concluded that nanoparticles could offer remarkably high flexibility for hazardous heavy metal (HHM) removal from the environment, both in-situ and ex-situ. By developing transgenic plants, these techniques could be utilized to enhance the intrinsic genetic potential and phytoremediation capabilities of plants (Kumar et al., 2019)

Nanotechnology is making important contributions to tackling a variety of environmental concerns by developing novel and practical answers. Nutritious value, oxidative stress system activation, and the enhancement of plant physiological and biochemical activity are some of its mitigating tactics (Zhou et al., 2020). ZnO-NPs have the potential to dramatically improve food plant growth by enhancing chlorophyll content and yield generation. The use of ZnO-NPs in heavy metal-exposed plants greatly increased plant growth rate, biomass, metal accumulation, and levels of MDA, photosynthetic pigments, and protein, as well as favorable genetic alterations and upregulation of antioxidant defense enzymes. Using optimal amounts of phycomolecule-coated ZnO nanoparticles may decrease the harmful

impacts of Cd and Pb accumulation in plants (Venkatachalam et al., 2017).

This experiment performed on ZnO-NPs at various doses (20, 40, 60, 80, and 100mg) significantly improved seed germination after 4 hours of treatment. According to the results, the control showed 80% germination, whereas zinc oxide nano particles treated seeds showed a rise in germination at different concentrations: 20mg showed 100%, 40mg-95%, 60mg-90%, 80mg-90%, and 100mg of ZnO NPs showed 85% germination in Mungbean seeds. Seed germination rates decreased as the concentration of ZnO NPs increase (Jayarambabu et al., 2014) while our results showed that ZnO-NPs applied at the concentration of 150ppm improved seed germination. The control plants revealed 64% germination whereas ZnO nanoparticles treated seeds showed 75% germination at the application of 150ppm solution in chilli plant.

This investigation on plants treated with ZnO-NPs increased leaf chlorophyll content, favonoid content, phenolic content, and total antioxidant capacity in Portulaca oleracea. The group treated with 500 mg L−1 of ZnO-NPs experienced the highest rise. In comparison to the control group, all treatments increased chlorophyll content, flavonoid content, total phenolic content, and antioxidant enzyme (POX) of the leaves. On the other hand, decrease the activity of catalase at this concentration. The group treated with 500 mg L−1 ZnO NPs showed the highest increase (Iziy et al., 2019) While our data revealed that ZnO-NPs sprayed at a concentration of 150ppm enhanced total chlorophyll content, flavonoids content, total phenolic content, and antioxidant enzymes (POX and CAT) of the plants when compared to the control group in C.annuum.

The effects of Pb and ZnO-NPs on the biochemical levels of tomato seedlings were investigated in this work. It is noteworthy that in tomato seedlings that were treated and those that were not, biosynthesized ZnO-NPs raised the levels of carotenoids and chlorophyll. When compared to the control, the green-produced ZnO-NPs (50 mg/L) + Pb (100 mg/L) dramatically increases the activities of peroxidase and catalase (Azim et al., 2022) while ZnO-NPs increased the amount of carotenoids and chlorophyll in the chilli at a concentration of 150 ppm solution, our results also showed that, in comparison to the control group, ZnO-NPs (150 ppm) plus Pb (500 mg/L) increased the activities of peroxidase and catalase.

Conclusion

In conclusion, ZnO-NPs had a substantial effect on lead acetate uptake, metabolic responses, early growth, and germination in chillies. The findings indicate that ZnO-NPs could be employed as an efficient agrichemical to increase plants' development while reducing Pb buildup in chilies. 150 ppm solution was found to be the optimal ZnO-NPs concentration; this solution greatly lowers Pb concentrations during germination and early growth phases without causing phytotoxicity. The biochemical research also revealed that nanoparticle application influenced total chlorophyll contents, carotenoid contents, total phenolic contents, flavonoid contents, peroxidase, and catalase activity. It dramatically boosted antioxidant enzyme activity to counteract lead stress.

References

Ahmed, F., Fakhruddin, A., Fardous, Z., Chowdhury, M., Rahman, M., and Kabir, M. (2021). Accumulation and Translocation of Chromium (Cr) and Lead (Pb) in Chilli Plants (Capsicum annuum L.) Grown on Artificially Contaminated Soil. *Nature Environment & Pollution Technology* **20**.DOI 10.46488/nept.2021.v20i01.007

Al Jabri, H., Saleem, M. H., Rizwan, M., Hussain, I., Usman, K., and Alsafran, M. (2022). Zinc oxide nanoparticles and their biosynthesis: overview. *Life* **12**, 594. DOI 10.3390/life12040594

Aldalbahi, A., Alterary, S., Ali Abdullrahman Almoghim, R., Awad, M. A., Aldosari, N. S., Fahad Alghannam, S., Nasser Alabdan, A., Alharbi, S., Ali Mohammed Alateeq, B., and Abdulrahman Al Mohsen, A. (2020). Greener synthesis of zinc oxide nanoparticles: Characterization and multifaceted applications. *Molecules* **25**, 4198. DOI 10.3390/molecules25184198

Ashenafi, E. L., Nyman, M. C., Shelley, J. T., and Mattson, N. S. (2023). Spectral properties and stability of selected carotenoid and chlorophyll compounds in different solvent systems. *Food Chemistry Advances* **2**, 100178. DOI 10.1016/j.focha.2022.100178

Ashok, B., Hariram, N., Siengchin, S., and Rajulu, A. V. (2020). Modification of tamarind fruit shell powder with in situ generated copper nanoparticles by single step hydrothermal method. *Journal of Bioresources and Bioproducts* **5**, 180-185. DOI 10.1016/j.jobab.2020.07.003

Ashraf, M., Ahmad, M. S. A., Öztürk, M., and Aksoy, A. (2012). Crop improvement through different means: Challenges and prospects. *Crop production for agricultural improvement*, 1-15. DOI 10.1007/978-94-007-4116-4_1

Azim, Z., Singh, N., Khare, S., Singh, A., Amist, N., Yadav, R. K., and Hussain, I. (2022). Potential role of biosynthesized zinc oxide nanoparticles in counteracting lead toxicity in Solanum lycopersicum L. *Plant Nano Biology* **2**, 100012. DOI 10.1016/j.plana.2022.100012

Bal, S., Sharangi, A. B., Upadhyay, T. K., Khan, F., Pandey, P., Siddiqui, S., Saeed, M., Lee, H.-J., and Yadav, D. K. (2022). Biomedical and antioxidant potentialities in chilli: Perspectives

and way forward. *Molecules* **27**, 6380. DOI 10.3390/molecules27196380

Cao, L., Jin, X., and Zhang, Y. (2019). Melatonin confers drought stress tolerance in soybean (Glycine max L.) by modulating photosynthesis, osmolytes, and reactive oxygen metabolism. *Photosynthetica* **57**. DOI 10.32615/ps.2019.100

Deepa, B., Yuvaraj, K., Jayaprada, M., Ramana, C. V., and Sireesha, Y. (2022). Studies on yield potentiality and yield contributing characters of different chilli genotypes. DOI 10.5962/bhl.title.27468

del Pilar Sánchez-Camargo, A., Gutiérrez, L.-F., Vargas, S. M., Martinez-Correa, H. A., Parada-Alfonso, F., and Narváez-Cuenca, C.-E. (2019). Valorisation of mango peel: Proximate composition, supercritical fluid extraction of carotenoids, and application as an antioxidant additive for an edible oil. *The Journal of Supercritical Fluids* **152**, 104574. DOI 10.1016/j.supflu.2019.104574

Feleafel, M., and Mirdad, Z. (2013). Hazard and effects of pollution by lead on vegetable crops. *Journal of agricultural and environmental ethics* **26**, 547-567. DOI 10.1007/s10806-012-9403-1Gan, C., Liu, Q., Zhang, Y., Shi, T., He, W.-S., and Jia, C. (2022). A novel phytosterols delivery system based on sodium caseinate-pectin soluble complexes: Improving stability and bioaccessibility. *Food Hydrocolloids* **124**, 107295. DOI 10.1016/j.foodhyd.2021.107295

Iziy, E., Majd, A., Vaezi-Kakhki, M. R., Nejadsattari, T., and Noureini, S. K. (2019). Effects of zinc oxide nanoparticles on enzymatic and nonenzymatic antioxidant content, germination, and biochemical and ultrastructural cell characteristics of Portulaca oleracea L. *Acta Soc. Bot. Pol* **88**, 3639. DOI 10.1155/2023/9387016

Jayarambabu, N., Kumari, B. S., Rao, K. V., and Prabhu, Y. (2014). Germination and growth characteristics of mungbean seeds (Vigna radiata L.) affected by synthesized zinc oxide nanoparticles. *Int. J. Curr. Eng. Technol* **4**, 3411-3416. DOI 10.19045/bspab.2020.90085

Kumar, S., Prasad, S., Yadav, K. K., Shrivastava, M., Gupta, N., Nagar, S., Bach, Q.-V., Kamyab, H., Khan, S. A., and Yadav, S. (2019). Hazardous heavy metals contamination of vegetables and food chain: Role of sustainable remediation approaches-A review. *Environmental research* **179**, 108792. DOI 10.1016/j.envres.2019.108792

Lim, J. G., Park, H. M., and Yoon, K. S. (2020). Analysis of saponin composition and comparison of the antioxidant activity of various parts of the quinoa plant (Chenopodium quinoa Willd.). *Food science & nutrition* **8**, 694-702. DOI 10.1002/fsn3.1358

Shang, Y., Hasan, M. K., Ahammed, G. J., Li, M., Yin, H., and Zhou, J. (2019). Applications of nanotechnology in plant growth and crop protection: a review. *Molecules* **24**, 2558. DOI 10.1016/0261-2194(84)90014-0

Singh, R., and Kumar, G. (2020). Ionizing radiation mediated effect on morphological, biochemical and microsporogenesis behavior of Artemisia annua L. *Journal of Environmental Biology* **41**, 1046-1053. DOI 10.22438/jeb/41/5/mrn-1246

Sruthi, S., Ashtami, J., and Mohanan, P. (2018). Biomedical application and hidden toxicity of Zinc oxide nanoparticles. *Materials today chemistry* **10**, 175-186. DOI 10.1016/j.mtchem.2018.09.008

Venkatachalam, P., Jayaraj, M., Manikandan, R., Geetha, N., Rene, E. R., Sharma, N., and Sahi, S. (2017). Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in Leucaena leucocephala seedlings: a physiochemical analysis. *Plant Physiology and Biochemistry* **110**, 59-69. DOI 10.1016/j.plaphy.2016.08.022

Zhou, P., Adeel, M., Shakoor, N., Guo, M., Hao, Y., Azeem, I., Li, M., Liu, M., and Rui, Y. (2020). Application of nanoparticles alleviates heavy metals stress and promotes plant growth: An overview. *Nanomaterials* **11**, 26. DOI 10.3390/nano11010026

Declaration

Data Availability statement

All data generated or analyzed during the study have been included in the manuscript.

Funding

There were no sources providing support for this research.

Conflict of interest

The authors assure that there were no financial relationships involved that could be perceived as a conflict of interest.

Acknowledgments

Not Applicable.

Author, Disclosure Statement

Ethics approval

Not applicable.

AutAuthhor's Contributions

RM did the experiments and wrote the manuscript. MS, and MID conceived the study and design, AB, AS reviewed the manuscript. All the authors participated in the experimentation & optimizations

Consent for Publication

Not applicable