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The Potential of Zinc Oxide Nanoparticles (ZnO NPs) to Alleviate Cadmium Heavy Metal Stress in Tomato: Estimation of Growth and Biochemical Events

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Abstract

Cadmium (Cd) contamination, especially in heavy metals, poses a substantial risk to agricultural productivity and food safety. Although zinc oxide nanoparticles (ZnO NPs) have demonstrated potential in reducing the harmful effects of cadmium toxicity, their specific impact on plant biochemical and physiological processes has not been extensively investigated. This research examined the possibility of using 100 mg. kg^{-1} ZnO NPs (foliar application) to reduce Cd stress (100 mg. kg^{-1}, applied to the roots) in two different varieties of tomato plants. A completely randomized design (CRD) was used, with four treatments: control, ZnO NPs, Cd stress and ZnO NPs +Cd interaction, each replicated five times. Growth parameters (fresh and dry biomass), photosynthetic pigments (chlorophyll a, b and total), biochemical attributes (soluble proteins, free amino acids, carbohydrates) and Cd accumulation in fruit were assessed. The results indicate that ZnO NPs significantly alleviated Cd toxicity, leading to a 35% increase in root biomass, 25% enhancement in soluble proteins and 20% higher chlorophyll content in combination as compared to Cd stress alone. Notably, ZnO NPs significantly decreased the accumulation of Cd in fruits by up to 85%, underscoring their potential in enhancing food safety. Principal component analysis (PCA) and correlation analysis provided additional evidence of strong positive connections between the use of ZnO NPs, enhanced biochemical responses and decreased Cd uptake. These findings indicate that ZnO NPs can be a valuable approach for reducing heavy metal toxicity in crops, which has important implications for sustainable farming practices and ensuring food security. Future studies should investigate the long-term impacts of ZnO NPs on plant growth, metabolism and potential nanoparticle bioaccumulation risks to ensure their safe application in agriculture.

Keywords: Cadmium; ZnO; Nanoparticles; Foliar spray; Tomato varieties; Alleviation

Introduction

Heavy metal stress has an unfavorable effect of different levels on plants through the production of reactive oxygen species (ROS), disturbance in vital activities including carbon assimilation, biochemical processes, membrane permeability, physiological activities and a decline in crop yield (Farid *et al.* 2013; Khan *et al.* 2017; White and Pongrate 2017). The excessive availability of Heavy Metal (HM) is one of the prominent factors in declining the yield of food quality and crops (Irshad *et al.* 2020; Javaid *et al.* 2020). Moreover, the primary limitation to food production supply for the caloric demographic has been noted to be 10 billion people by the year 2050. According to the population report published by the United Nations in 2019, there are around 10 to 12 million polluted areas in the universe and half of the pollution is a result of HMs. However, this pollution is on the rise with heavy metals as has been affirmed by numerous empirical studies reported by Li *et al.* (2014), Khalid *et al.* (2017) and Teng *et al.* (2014).

The four HMs (Cd, As, Hg and Pb) have been identified by The Agency for Toxic Substances and Disease Registry (ATSDR) (Duffs, 2002) as detrimental elements which have adverse effects on the growth rate and development processes of plants and the wellbeing of humankind (Mansoor *et al.* 2021). Cadmium is among the noble unreactive heavy metals which induce indirect oxidative stress by impeding the action of anti-oxidative enzymes or the production of ROS enzymes (Val *et al.* 2005; Bielen *et al.* 2013). The Agency for Toxic Substances and Disease Registry (ATSDR) describes such harmful trace metals as cadmium, or Cd, as one of the primary

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culprits. It is readily taken up from water or soil at the plant roots for movement to the shoots of plants (Liu and Lal 2015). This toxicity altered nutrient absorption, limited plant growth, improved crop yield and reduced quantity of fruit (Rizwan *et al.* 2019a).

Cadmium contamination is a confluence of aspects such as atmosphere, anthropogenic influences, volcanic activity, wind-driven dust particles, fertilizers, batteries, metallic coating, sewage sludge and some others (Thornton 1992; Sharma *et al.* 2015). As one of the recent sources suggests, Cadmium (Cd) is reported to be on the rise, especially in the soil and water. The ion dissolves in water, which is quite frequent and this somewhat facilitates its entry into the biological systems of plants and aquatic organisms as well, thereby assisting in bioaccumulation (Tripathi *et al.* 2020). The long-term application of pesticides and sludge to farmlands may be the top reason for the Cd concentration in soil (Ozyigi *et al.* 2016; Tabelin *et al.* 2018). Several studies have shown that the use of Zinc Oxide nanoparticles (ZnO NPs) can enhance certain aspects of plant growth. Specifically, there are reports on onions, wheat, soybeans and even ground nuts wherein certain concentrations of ZnO NPs enabled a corresponding increase in seed germination (Prasad *et al.* 2012; Sedghi *et al.* 2013; Ramesh *et al.* 2014; Raskar and Laware 2014). Again, it must be noted that a specific concentration increases its positive impact, while an increase from that level negatively affects germination. There are studies conducted by Rosa *et al.* (2013) that have reported the effects of using varying concentrations of ZnO NPs on tomatoes, alfalfa and cucumbers. According to this study, only the yield and germination of cucumbers were promising. In their study, Tarafdar *et al.* (2013) stated that the combination of nano zinc oxide and *Cylindraspis tetragonoloba* increased residual biomass production significantly.

In recent years, different NPs have been used by different workers for remediation of different abiotic stresses like drought, salinity and HMs toxicity. For instance, foliar supplement of Si nanoparticles was found to be effective in reduction of HMs accumulation and enhanced development in rice (Wang *et al.* 2016). The utilization of Ag nanoparticles in irrigation water has enhanced the production of biomass, chlorophyll pigment and increased activities of antioxidant enzymes like Superoxide Dismutase (SOD), Catalase (CAT) and Peroxidase (POD) in Chinese Bok choy under Cd toxicity stress (Li and Huang 2014). ZnO NPs have increased the barley against drought. Enhanced root and shoot growth and higher biomass and antioxidant enzyme levels have been reported in tomatoes against salinity stress (Faizan *et al.* 2021). Promoted growth was reported in rice crops against HM stress (Yan *et al.* 2021). Foliar application of ZnO NPs was successful in increasing biomass production and antioxidant enzymes in maize and wheat respectively. Rizwan *et al.* (2019a, b) improved photosynthetic pigments, enhanced growth and antioxidant activities were reported in soya been, under HMs toxicity (Ahmad *et al.* 2020). ZnO NPs are reported to have mitigation potential against Cd stress in chilli plants (Irfan and Bhatti 2023).

Tomato (*Solanum lycopersicum* L.) plants are grown as vegetables and fruit, and it is one of the major marketable vegetables and fruits globally. It is counted as a major species for various test crop research studies because of its fleshy fruit and active properties of genetic characters and genomic resources (Labate *et al.* 2007).

Recent research indicates that zinc oxide nanoparticles (ZnO NPs) have the potential to alleviate Cd stress by boosting antioxidant defense mechanisms and decreasing Cd accumulation in plants. Nevertheless, there are still several important aspects that have not been thoroughly examined. While most studies concentrate on the growth and yield of plants. Moreover, current studies has utilized little advanced statistical techniques, such as principal component analysis (PCA) and correlation analysis, to determine the significant plant traits affected by ZnO NPs under Cd stress.

Keeping in mind, the current study was designed to investigate the effects of different concentrations of ZnO NPs on plant growth and biomass accumulation under cadmium stress; to determine the effect of zinc oxide nanoparticles on growth and the biochemical parameters (chlorophyll contents *i.e*., a, b and total, soluble proteins, free amino acids and total carbohydrates); to quantify cadmium content in tomato fruit and find out the role of zinc oxide nanoparticles in reduction its uptake; to perform Principal Component Analysis (PCA) and correlation analysis to determine the pseudo-significant physiological and biochemical parameters influenced by ZnO NPs under Cd stress. By integrating nanotechnology with plant stress physiology, this study supports previous similar findings of different researchers. It may be helpful in establishing the mechanistic role of ZnO NPs in alleviation of Cd toxicity and may be adopted for sustainable agriculture and food safety.

Materials and Methods

Experimental site description

The experiment was carried out at the Department of Botany, University of Gujrat, Pakistan with GPS coordinates latitude: 32.6335° N and longitude: 74.1190° E, under controlled environmental conditions to minimize external variability. A controlled pot experiment was conducted to evaluate the potential of zinc oxide nanoparticles (ZnO NPs) in alleviation cadmium (Cd) toxicity in tomato (*Solanum lycopersicum* L.). The study focused on plant growth, physiological traits, biochemical responses and Cd accumulation in fruit. The experiment was conducted under controlled conditions with a temperature of 25–30°C (day) and 18–22°C (night), relative humidity of 65–75% and a 12-h light/dark photoperiod. The soil was loamy (40% sand, 35% silt and 25% clay), pH 7.2, with organic matter (1.8%) and macronutrients (N: 0.12%, P:

0.09%, K: 0.85%). Cadmium stress was induced using 100 mg. kg^{-1} $CdCl_2$. Watering was maintained at field capacity (~70%). These conditions ensured optimal growth to assess ZnO NPs' effects on Cd stress alleviation in tomato plants.

Green synthesis of nanoparticle ZnO NPs

ZnO NPs were blended by the method of green synthesis as described by Lee *et al.* (2011) with minor changes using green tea leaves (*Camellia sinensis*). The green tea leaves were produced from a local shop. To get rid of dust particles, the leaves were soaked, for approximately 2 minutes, in distilled water and then air dried in a shady area and sun-dried afterwards. The leaves were then pulverized into light powder. 25 g of the powder was combined in 500 ml distilled water in a beaker making sure that it was well mixed and kept in the water bath at 100°C for one h. The solution was allowed to reach room temperature after which it was successively filtered three times using Whatman filter paper. A total of 52.88 g of Zn $(CH_3COO)_2.2H_2O$ was dissolved in distilled water of 700 ml and the entire volume of the solution was adjusted to one litre.

On one hand, 690 ml of Zn $(CH_3COO)_2.2H_2O$ solution was mixed with 230 mL of green tea extract and a dark green color was observed. Prepared and kept in test tubes the colored solution was then centrifuged for 10 min at 9000 rpm and the pellet obtained was collected into petri dishes. The dishes were then transferred into an oven set at 60ºC and dried for twenty-four h. ZnO NPs powder was dried and stored in China dishes for further examination and therapeutic applications.

Seed beds preparation

A total of 4 seed beds (2 square ft. each) were arranged for the reason of planting both tomato assortments and were named. The soil was carried out and changed into light mass. Creature fertilizer was included in the soil with a proportion of 1:3 to extend soil fertility. The seeds of both varieties of tomato (Variety 1= 1602, Variety 2= 3040) were selected from the seed nourishment specialist of Dera Ghazi Khan, Punjab, Pakistan. A total of 2 seed beds were shaped for planting. The tomato seeds were surface sterilized using 5% NaOCl for 5 min and then thoroughly rinsed with purified water. A total of 250 tomato seeds were cultivated in their named seed beds. These were appropriately watered until their growth. The seeds were germinated after 12-15 days. The sprouting of tomato varieties was induced and inundated until they were transplanted into their pots. Medium-measured pots (24 × 20 cm) were put beneath the shed. Sufficient creature excrement was mixed with soil and water was included in these clay pots to create the soil immersed. The experimental clay pots were sited under a shady zone for two days. A total soil amount of 5.5 kg was placed in each clay pot. When the sprouting of both varieties of tomato reached 5-6 inches, seedlings of both varieties were uprooted from soil beds and kept in a shady zone for 2 days to accomplish steadiness.

Soil preparation methodology

The experiment was conducted in clay pots (24 × 20 cm) filled with 5.5 kg of soil, which was prepared to ensure uniformity in growth conditions. The soil was collected from an agricultural field and air-dried. The soil was sieved (2 mm mesh) to remove debris and large particles and characterized for pH (7.2), organic matter (1.8%), nitrogen (0.12%), phosphorus (0.09%) and potassium (0.85%), Cadmium (Cd) contamination was induced by applying 100 mg. kg^{-1} $CdCl_2$ solution to the soil one week before transplanting to allow proper absorption. For improved soil fertility, well-decomposed organic compost (1:3 ratio) was incorporated. Tomato seeds of two varieties (Variety 1602 & Variety 3040) were surface-sterilized (5% NaOCl, 5 min), rinsed and sown in seedbeds on March 1, 2021. After 14 days, uniform seedlings (~5–6 inches tall) were transplanted into experimental pots on March 14, 2021. The final harvest was conducted 45 days after transplanting, when fruit maturation was observed.

Experimental design

The experimental units were species = 1 × varieties = 2 × replicates = 5 × treatment = 4 = 40 pots. A total of 40 pots were arranged for the present study. These pots were arranged under the screening area and CRD completed randomized experimental design was applied. After 10 days, transplanted plants were treated with HMs to roots. The solution (100 mg. kg^{-1}) of nanoparticles was applied by foliar spray to the leaves of tomato plants (both varieties). The heavy metal Cd was applied in the form of a $CdCl_2$ solution. The HMs treatments were given weekly basis for six weeks and a foliar spray of NPs was applied after 2 h of heavy metals treated by the scheme of control (T_1), ZnO NPs (100 mg. kg^{-1}) foliar spray (T_2), Cd (100 mg. kg^{-1}) root solution (T_3) and ZnO NPs (100 mg. kg^{-1}) + Cd heavy metal (100 mg. kg^{-1}) (T_4).

Growth parameters

After treatment, a plant from each replication was uprooted without affecting the roots. The plants were labelled and put into the envelopes. The roots and shoots were segregated with the help of sterilized scissors. The growth variables of the tomato plant (both varieties) were weighted with the help of electric digital weight balance. The plant material including the Fresh weight of roots and shoots was measured separately and kept in an envelope. The envelopes of plant materials were kept in an oven for 3 days at 70°C to detach the moisture content plant material. After the dry oven steps, the dry weight of roots and shoots was measured. Similarly, the fruit was collected, dried and stored for estimation of heavy metal Cd of the fruit by ASS

(Atomic absorption spectroscopy).

Biochemical parameters

Chlorophyll contents: The chlorophyll contents of both varieties of tomato were determined by following the method of Witham *et al*. (1971). Fresh leaf tissues of 0.5 g was ground in acetone with 80% and then for 5 min, it was centrifugated at 3000 rpm at a temp. of 15-20ºC. The supernatant was then stored in test tubes. An 80% acetone was added to each tube to stabilize the volume of each tube. The prepared extract was transferred into the cuvette. The absorbance was taken at various wavelengths *i.e*., 645, 652 and 663 nm using a UV-1100 (spectrophotometer). The contents of chlorophyll a, b and total were estimated by given formulas:

$$\text{Chlorophyll-a mg/g tissue} = [12.7(D_{663}) - 2.69(D_{645})] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll-b mg/g tissue} = [22.9(D_{645}) - 4.68(D_{663})] \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll-total mg/g tissue} = \frac{D_{652} \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

V = Filtrate volume; W= Tissue Weight

Total carbohydrate parameter

The total carbohydrate content of both tomato varieties was determined according to the method of Ashwell (1957). A dried material of 0.2 g and extract samples were transferred into test tubes with the 6 N HCl compound amount of 10 mL mixed. All test tubes were mixed overnight using a shaker. The volume of the test tube substance increased to the amount of 100 ml with mixing distilled water. The solution was filtered and an amount of 5 mL of filtrate solution was transferred into a 100 mL flask. The total volume was balanced to 100 mL by totaling deionized water. All tubes were kept in a steaming water bath for 15 min, chilled and absorbance was calculated at 625 nm using a UV-1100 spectrophotometer and total carbohydrates with anthrone were determined using the following formula:

$$\text{Concentration of carbohydrates } (\mu g/g) = \frac{\textit{Value from standard curve} \times \textit{volume of extract} \times \textit{DF}}{\textit{Wt.of a material} \times \textit{volume of extract used for test}}$$

Total soluble protein (TPS)

The content of Total Soluble Proteins (TSP) of both varieties of tomato was used by following the method of Bradford (1976). For the determination of TSP, an amount of 0.2 g biomass of leaves was captured from individual plants and ground in 4 mL of 50 phosphate buffer maintained at 7.0 pH. The extracted extract was relocated after washing the pestle and the rubbing dish with an amount of 1 mL phosphate buffer. Then the sample was centrifuged for 20 min at 40°C at 13,000 rpm. The pellet was removed and the sample was kept at 40°C. Then the extract was utilized to estimate total soluble proteins according to the Bradford staining method (Bradford 1976). The 2.5 mL stain of Bradford was placed into a tube and 50 μL of extract was transferred to the tube. The tube was allowed to react for 10 min. The BSA (Bovine serum albumin) was utilized to generate the standard curve. The sample absorbance was determined at 595 nm in a spectrophotometer by following the method of Shah *et al.* (2010). The sample of the TSP was determined using the following formula:

$$\text{TSP (mg/g fresh leaf tissue)} = \frac{\text{Sample Absorbance (ppm)} \times \text{Sample Volume Used} \times \text{Diltuion Factor}}{\text{Sample wt.(g)} \times 1000}$$

Free amino acids (FAA)

The estimation of FAA of the samples was observed by using the method of Lowry *et al.* (1951). Total FAAs were determined by using the following formula:

$$\text{Total Free Amino Acids (mg/g fresh leaf tissue)} = \frac{\text{Absorbance of sample} \times \text{Volume of sample} \times \text{Diltuion Factor}}{\text{wt. of fresh tissue (g)} \times 1000}$$

Analysis of fruit heavy metals

The estimation of cadmium (Cd) HMs contents of both varieties of tomato fruits was determined by atomic absorption spectrometry at the University of Agriculture, Faisalabad, Pakistan.

Statistical analysis of the data

The data was compiled and computed and then subjected to ANOVA single factors as outlined by Gomez and Gomez (1984) applying the COSTAT software. To follow the ANOVA, a level of probability at 0.05 was used to test the difference between the treatments of means and to determine the significance of the various parameters and the DMRT test for Least Significant Difference (LSD) was used for the comparison of means. Principal Component Analysis (PCA) and Correlation Analysis were performed to identify key variables influencing plant responses to ZnO NPs and Cd stress.

Results

Growth and physiological parameters

Results for growth, biochemical attributes and fruit Cd content of two varieties of tomato are shown in Table 1. It has been shown that ZnO NPs and its interaction with Cd heavy metal have a non-significant effect on root and shoot fresh and dry biomass production at all levels of probabilities in both tomato varieties except tomato variety 3040 in which significantly increased root fresh and dry weights were observed as shown in Fig. 1.

Results for chlorophyll a, b and total are shown in Fig. 2. It has shown that ZnO NPs nanoparticles alone and their combination with Cd heavy metal (ZnO NPs + Cd) have decreasing effects for chl. a and chl. b for tomato variety 1602 as compared to the control value but total chlorophyll

Table 1: F-values derived from ANOVA for growth and biochemical attributes

Parameters	Varieties	F-ratio	*P*-Values	$LSD_{0.05}$
	Growth Attributes			
Dry Weight Shoot	Var. 1	1.143	0.3617 ns	4.424
	Var. 2	0.994	0.4208 ns	6.094
Dry Weight Root	Var. 1	2.371	0.1087 ns	0.746
	Var. 2	6.545	0.0042**	0.694
Fresh Weight Shoot	Var. 1	0.807	0.5077 ns	19.901
	Var. 2	0.151	0.9273 ns	17.937
Fresh Weight Root	Var. 1	1.137	0.3638 ns	4.165
	Var. 2	9.247	0.0008**	2.16
	Biochemical Attributes			
Chlorophyll ‘a’	Var. 1	79.25	0.0000***	0.155
	Var. 2	1.729	0.2379 ns	0.421
Chlorophyll ‘b’	Var. 1	155.765	0.0000***	0.147
	Var. 2	0.676	0.5906 ns	0.444
Chlorophyll ‘Total’	Var. 1	57.334	0.0000***	0.195
	Var. 2	0.502	0.6910 ns	0.312
Carbohydrates (Shoot)	Var. 1	113.429	0.0000***	0.004
	Var. 2	5.513	0.0129*	0.028
Carbohydrates (Root)	Var. 1	14.534	0.0002***	0.066
	Var. 2	19.638	0.0000***	0.042
Free Amino Acid	Var. 1	0.212	0.8861 ns	0.159
	Var. 2	37.373	0.0000***	0.041
Soluble Protein	Var. 1	16.199	0.0001***	2.56
	Var. 2	42.542	0.0000***	2.571
Fruit Cadmium content	Var. 1	55.928	0.0000***	0.246
	Var.2	30.119	0.0000***	0.43

F= F-ratios were obtained from ANOVA tables, **LSD=**Least significant difference at *P = 0.05*, NS = Non significance; *, **, ***, significant at *0.05*, *0.01* and *0.001*, respectively

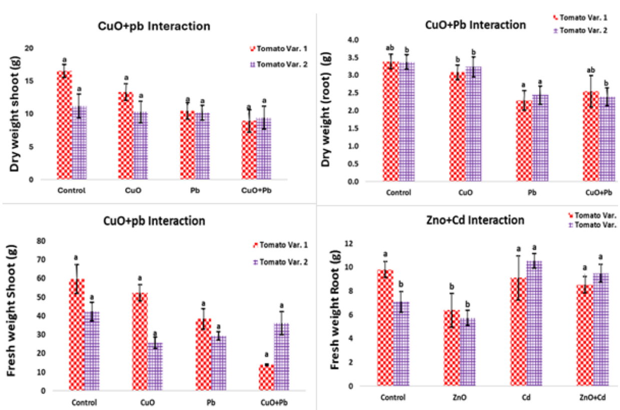

Fig. 1: Fresh and Dry, Shoot and Root weights of Tomato varieties in response to different treatments with Standard Error (SE) bar and lettering shows the significance level of DMRT

was increased significantly. Whereas, the same parameters remained unaffected for tomato variety 3040.

Results for shoot and root total carbohydrates are given in Fig. 3. It has been shown that ZnO NPs and Cd alone have significantly decreased effect on shoot carbohydrates, but their combination was successful in improving the values. In contrast, the combination of both, *i.e.*, ZnO NPs + Cd interaction has maintained the sugar values for root in both tomato varieties.

Results for soluble proteins of the plants are given in Fig. 4 It has shown that alone ZnO NPs and Cd treatments

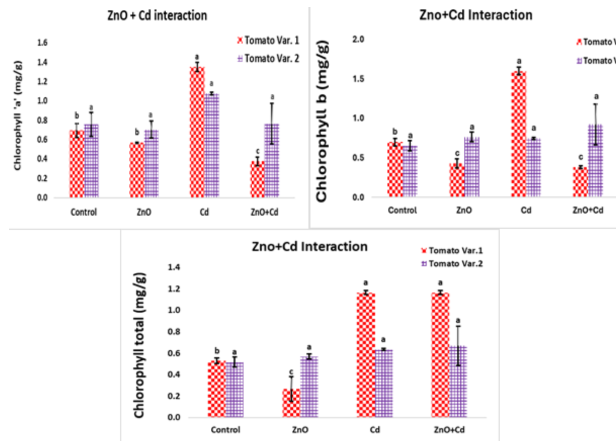

Fig. 2: Chlorophyll a, b & total of Tomato varieties in response to different treatments with Standard Error (SE) bar and lettering shows the significance level of DMRT

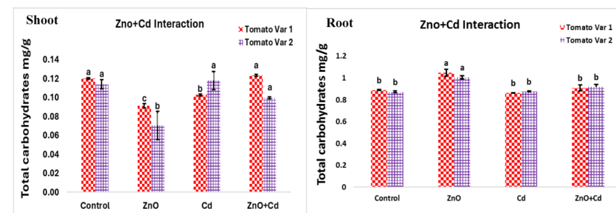

Fig. 3: Total Carbohydrate contents of Shoot and Root of Tomato varieties in response to different treatments with Standard Error (SE) bar and lettering shows the significance level of DMRT

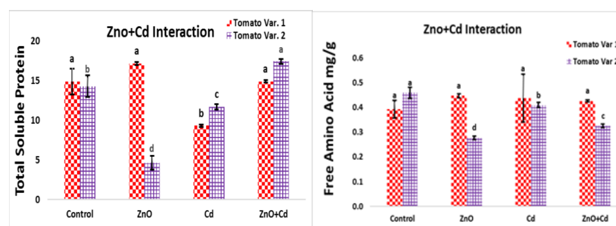

Fig. 4: Total Soluble Protein (mg/g fresh leaf tissue) and Free Amino Acid contents of Tomato varieties in response to different treatments with Standard Error (SE) bar and lettering shows the significance level of DMRT

have significantly decreasing effects on soluble proteins value but ZnO NPs + Cd interaction has increased values as compared to all other treatments for both tomato varieties. Results for free amino acids of the plants of two varieties of tomato are given in Fig. 4. It has shown that both ZnO NPs and Cd alone treatments have significant decreasing effects on free amino acids values for tomato variety 3040 but a significant increase was shown in combination (ZnO NPs + Cd) treatment. Whereas, values for variety 1602 remained non-significant for all treatments.

ANOVA results for cadmium HMs accumulation by fruit in tomato varieties find a highly significant difference impact in all treatments. Fig. 5 has shown an increased

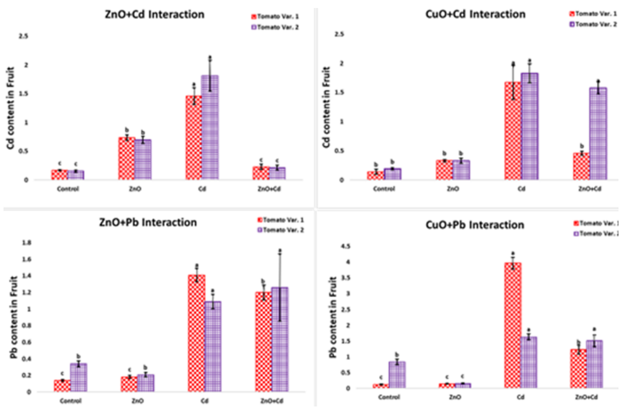

Fig. 5: Effect of Zinc Oxide and CuO NPs interactions with Cd and Pb HMs and accumulation (mg. kg^{-1} dry matter) in the fruit of different varieties of Tomato crop with Standard Error (SE) bar and lettering shows the significance level of DMRT

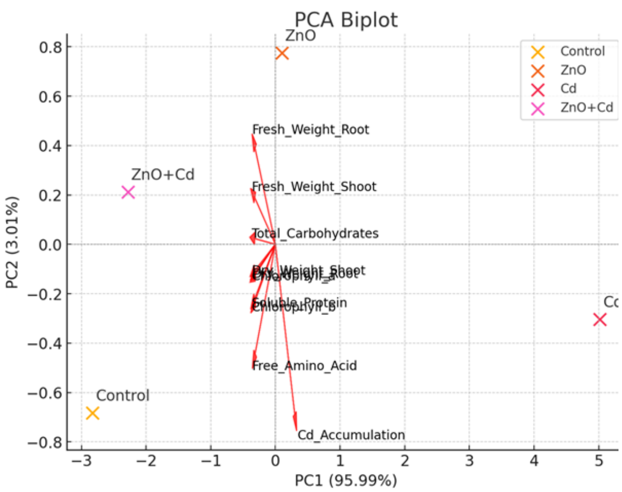

Fig. 6: Principal Component Analysis (PCA) of different plant parameters in the response of various treatments

value for fruit Cd Results for Cd heavy metal accumulated in the fruit of both varieties are shown in Fig. 5. It has been shown that both ZnO NPs and alone Cd HM treatments have a significantly increasing effect on fruit Cd content, whereas a maximum reduction was noted in both tomato varieties treated with the combination of both ZnO NPs +Cd interaction. It has shown that ZnO NPs may have the potential to alleviate heavy metal toxicity by uptake inhibition of Cd and its accumulation in plant parts including the fruit of tomato. Reduction in Cd cumulation in tomato fruit varieties was up to 83 to 85%.

PCA and correlation analysis

Principal component analysis (PCA) revealed that the first principal component (PC1) accounted for 95.99% of the variance in the data and appears to differentiate treatments based on biomass (fresh/dry weight) and biochemical

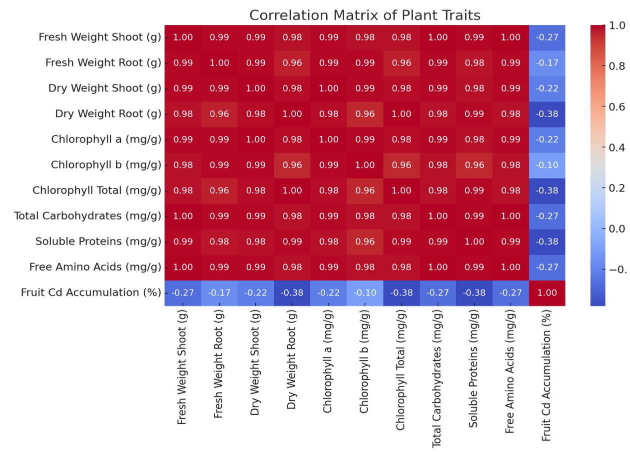

Fig. 7: Correlation heatmap of key relationships of tomato plant parameters

responses, while the second (PC2) explained only 3.01%, suggesting that PC1 effectively represents the majority of the data's variability and highlights the contrast between Cd accumulation and plant growth. The PCA biplot (Fig. 6) further demonstrated clear treatment clustering, with the cadmium (Cd) treatment group exhibiting a distinct separation from the zinc oxide nanoparticles (ZnO NPs) and ZnO NPs + Cd groups, implying a mitigative effect of ZnO NPs on Cd toxicity. The ZnO NPs + Cd treatment clusters closer to the control, suggesting ZnO helps alleviate Cd toxicity.

The correlation analysis results in Fig. 7 revealed key relationships in the correlation heatmap. Specifically, chlorophyll content showed a positive correlation with growth attributes, namely the fresh and dry weights of both roots and shoots. Furthermore, soluble proteins and free amino acids exhibited a positive correlation with biomass, indicating a protective biochemical function of ZnO NPs. Conversely, cadmium (Cd) accumulation in the fruit displayed a negative correlation with plant health parameters, suggesting that treatment with ZnO NPs and Cd reduces Cd toxicity.

Discussion

The present results strongly suggest that zinc oxide nanoparticles (ZnO NPs) alleviate cadmium (Cd) toxicity in tomato plants. Specifically, ZnO NPs promoted plant growth, improved biochemical responses and substantially decreased Cd accumulation in the fruit. These findings corroborate and build upon earlier studies. This conforms to the findings of various workers that ZnO NPs nanoparticles increase biomass and photosynthetic pigments in various plants such as increased value in the wheat crop reported by Awasthi *et al.* (2017) in *Coriandrum sativum* (Disfani *et al.* 2017; Yuan *et al.* 2018; Bhatt *et al.* 2020) and in various plants.

Compared to cadmium stress alone, applying ZnO

NPs resulted in a 35% increase in root biomass and a 25% increase in shoot dry weight in the ZnO NPs +Cd treatment. This observation aligns with earlier research indicating that ZnO NPs stimulate root and shoot development by facilitating nutrient absorption and alleviating oxidative stress in crops like wheat and maize (Rizwan *et al*. 2019b; Faizan *et al.* 2021). The enhanced bioavailability of zinc, an essential micronutrient, in its nano-form contributes to improved plant health under stress (Awasthi *et al*. 2017). Similar responses have been reported by Noman *et al.* (2020).

ZnO NPs significantly increased chlorophyll a, b and total chlorophyll content by 20%, mitigating Cd-induced degradation of photosynthetic pigments. This supports with the findings of Ahmad *et al.* (2020), who reported that ZnO NPs enhance chlorophyll stability and photosynthetic efficiency in Cd-stressed plants by limiting oxidative damage. Cd toxicity disrupts chloroplast structure and leads to chlorophyll degradation (Ali *et al.* 2019), but ZnO NPs appear to counteract this effect by improving metal homeostasis and antioxidant enzyme activity. ZnO NPs significantly increased chlorophyll a, b and total chlorophyll content by 20%, mitigating Cd-induced degradation of photosynthetic pigments. It is in conformity with the findings of Ahmad *et al.* (2020), who reported that ZnO NPs enhance chlorophyll stability and photosynthetic efficiency in Cd-stressed plants by limiting oxidative damage. Cd toxicity disrupts chloroplast structure and leads to chlorophyll degradation (Ali *et al.* 2019), but ZnO NPs appear to cope with this effect by improving metal homeostasis and antioxidant enzyme activity. These increased values match with the findings of various workers, *i.e*., use of ZnO NPs having potential to alleviate toxic effects and providing tolerance against heavy metals (Li and Huang 2014; Singh and Lee 2016; Venkatachalam *et al.* 2017; Ali *et al.* 2019; Rizwan *et al.* 2019c; Ahmad *et al.* 2020; Katiyar *et al.* 2020; Yan *et al.* 2021; Irfan and Bhatti 2023).

Biochemical analysis revealed a 25% increase in soluble proteins and a 20% enhancement in free amino acids in ZnO NPs + Cd-treated plants. Similar responses have been reported in rice and soybean, where ZnO NPs modulated proline and soluble sugar metabolism, helping plants adapt to heavy metal stress (Katiyar *et al.* 2020; Yan *et al.* 2021). Increased soluble protein content in the presence of ZnO NPs may be attributed to their role in stabilizing enzymatic functions and enhancing protein synthesis (Venkatachalam *et al.* 2017).

Results for fruit cadmium content of the tomato varieties have shown significant difference effect for all levels of probabilities. One of the most significant findings of this study is the 85% reduction in Cd accumulation in tomato fruit under ZnO NPs + Cd treatment. This supports earlier studies by Hussain *et al.* (2019), who reported that ZnO NPs inhibit Cd uptake and translocation in various crops. The mechanism behind this effect likely involves ZnO NPs competing with Cd ions for root absorption sites and enhancing chelation and sequestration mechanisms, thereby limiting Cd transport to aerial parts (Rizwan *et al.* 2019b). This has critical implications for food safety, as excessive Cd in edible crops poses serious health risks. This finding matches the results of some earlier workers (Baybordi 2005). Venkatachalam *et al.* (2017) and Hussain *et al.* (2019) where a reduction in the uptake of HM in different plants growing under stress by application of NPs.

Principal Component Analysis (PCA) demonstrated that applying ZnO nanoparticles (NPs) is strongly associated with improved plant growth, increased chlorophyll levels and reduced cadmium (Cd) uptake. This comprehensive analysis highlights the beneficial impact of ZnO NPs. The subsequent correlation heatmap corroborated these findings, showing a strong inverse relationship between Cd accumulation and indicators of plant health, thus supporting ZnO NPs' function in Cd detoxification. According to the recommendation of Singh and Lee (2016) that these results underscore the importance of employing sophisticated statistical models to fully elucidate the complex interactions between nanomaterials and plants.

This research suggests that zinc oxide nanoparticles (ZnO NPs) hold promise as a sustainable solution, alleviating cadmium (Cd) toxicity in agricultural crops. The observed reduction in Cd uptake (up to 85%) and improved plant vigor following ZnO NP application highlights their potential for foliar spray use in contaminated farmlands. It provides safer food sources including plant products and different vegetables. Their contribution to enhanced nutrient acquisition and stress resistance further supports their value in eco-friendly farming, diminishing their dependence on conventional chemical amendments. Further research is needed, including field-level validation, dosage optimization and thorough safety analysis, to facilitate the integration of ZnO NPs into practical crop management practices for heavy metal remediation. Further studies are being conducted by similar authors to assess the various antioxidants and physiological activities of the same crop to provide more comprehensive results to support in establishment of the mechanism of regime that ZnO NPs are potent in enhancing HM tolerance by foliar application of ZnO NPs in different crops in future.

Conclusion

It is concluded that zinc oxide nanoparticles (ZnO NPs) significantly alleviate cadmium (Cd) toxicity in tomatoes, leading to improved plant development and biochemical function. Notably, ZnO NPs reduce Cd accumulation in tomato fruit by as much as 85%. Consequently, ZnO NPs present a valuable tool for sustainable agriculture, enhancing crop resilience in Cd-polluted soils and promoting food safety. Their impact extends to improved nutrient use, increased stress tolerance and reduced heavy metal uptake, thereby supporting eco-friendly and climate-resilient agriculture. Further research is needed to evaluate the long-term environmental effects of

ZnO NPs, optimize their application for safety and efficacy and validate these findings in field settings. The integration of nanotechnology for sustainable crop management offers transformative potential in combating heavy metal contamination, guaranteeing safer food production and environmental protection.

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Author Contributions

SMI and KHB planned the experiments, interpreted the results and made the write up and ABG statistically analyzed the data and made illustrations.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

References

Ahmad P, MN Alyemeni, AA Al-Huqail, MA Alqahtani, L Wijaya, M Ashraf, C Kaya, A Bajguz (2020). Zinc oxide nanoparticles application alleviates Arsenic (As) toxicity in soybean plants by restricting the uptake of as and modulating key biochemical attributes, antioxidant enzymes, ascorbate-glutathione cycle and glyoxalase system. *Plants* 9:825-842

Ali S, M Rizwan, A Hussain, MZ Ur Rehman, B Ali, B Yousaf, L Wijaya, MN Alyemeni, P Ahmad (2019). Silicon nanoparticles enhanced the growth and reduced the cadmium accumulation in grains of wheat (*Triticum aestivum* L.). *Plant Physiol Biochem* 140:1–8

Ashwell G (1957). Colorimetric analysis of sugars. *In: Methods in enzymology,* pp:3–87. Colowick SP, NO Kaplan (Eds.). Academic Press Inc., New York

Awasthi A, S Bansal, LK Jangir, G Awasthi, KK Awasthi, K Awasthi (2017). Effect of ZnO nanoparticles on germination of *Triticum aestivum* seeds. *Macromol Symp* 376:1700043-1700051

Baybordi A (2005). Effect of zinc, iron, manganese and copper on wheat quality under salt stress conditions. *J Water Soil* 140:150–170

Bhatt D, MD Bhatt, M Nath, R Dudhat, M Sharma, DS Bisht (2020). Application of nanoparticles in overcoming different environmental stresses. *In: Protective Chemical Agents in The Amelioration of Plant Abiotic Stress: Biochemical and Molecular Perspectives*, pp:635–654. Roychoudhury A, DK Tripathi (Eds.). Hoboken: Wiley-Blackwell

Bielen A, T Remans, J Vangronsveld, A Cuypers (2013). The influence of metal stress on the availability and redox state of ascorbate and possible interference with its cellular functions. *I J Mol Sci* 14:6382–6413

Bradford MM (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principal of protein-dye binding. *Anal Biochem* 72:248–254

Disfani NM, A Mikhak, MZ Kassaeec, AH Magharid (2017). Effects of nano Fe/SiO2 fertilizers on germination and growth of barley and maize. *Arch Agron Soil Sci* 63:817–826

Duffs JH (2002). Heavy metals –a meaningless term? *Pure Appl Chem* 74:793–807

Faizan M, JA Bhat, C Chen, MN Alyemeni, L Wijaya, P Ahmad, F Yu (2021). Zinc oxide nanoparticles (ZnO-NPs) induce salt tolerance by improving the antioxidant system and photosynthetic machinery in tomato. *Plant Physiol Biochem* 161:122–130

Farid M, MB Shakoor, S Ehsan, S Ali, M Zubair, MA Hanif (2013). Morphological, physiological and biochemical responses of different plant species to Cd stress*. Intl J Chem Biochem Sci* 3:53–60

Gomez KA, AA Gomez (1984). *Statistical procedures for agricultural research*, 2nd edn. Wiley, New York, USA

Hussain A, M Rizwan, S Ali (2019). Seed priming with Silicon nanoparticles improved the biomass and yield while reduced the oxidative stress and Cadmium concentration in wheat grains. *Environ Sci Pollut Res* 26:7579–7588

Irfan SM, KH Bhatti (2023). Zinc Oxide Nanoparticles Mitigate Toxic Effects of Cadmium Heavy Metal in Chili (*Capsicum annuum* L.). *Proc Pak Acad Sci B Life Environ Sci* 60:477–487

Irshad MK, A Noman, HA Alhaithloul, M Adeel, Y Rui, T Shah, S Zhu, J Shang (2020). Goethite-modified biochar ameliorates the growth of rice (*Oryza sativa* L.) plants by suppressing Cd and As-induced oxidative stress in Cd and as co- contaminated paddy soil. *Sci Total Environ* 717:137086-137096

Javaid S, QU Zaman, K Sultan, U Riaz, A Aslam, S Sharif, N Ehsan, S Aslam, A Jamil, S Ibraheem (2020). Heavy metals stress, mechanism and remediation techniques in rice (*Oryza sativa* L.): A review. *Pure Appl Biol* 9:403–426

Katiyar P, B Yadu, J Korram, ML Satnami, M Kumar, S Keshavkant (2020). Titanium nanoparticles attenuates arsenic toxicity by up-regulating expressions of defensive genes in *Vigna radiata* L. *J Environ Sci* 92:18–27

Khalid S, M Shahid, NK Niazi, B Murtaza, I Bibi, C Dumat (2017). A comparison of technologies for remediation of heavy metal contaminated soils. *J Geochem Exp* 182:247–268

Khan MIR, NA Khan (2017). *Reactive Oxygen Species and Antioxidant Systems in Plants: Role and Regulation under Abiotic Stress*. Singapore: Springer Singapore

Labate JA, S Grandillo, T Fulton, S Munos, AL Caicedo, I Peralta, Y Ji, RT Chetelat, JW Scott, MJ Gonzalo, D Francis, W Yang, E van der Knaap, AM Baldo, B Smith-White, LA Mueller, JP Prince, NE Blanchard, DB Storey, MR Stevens, MD Robbins, JF Wang, BE Liedl, MA O'Connell, JR Stommel, K Aoki, Y Iijima, AJ Slade, SR Hurst, D Loeffler, MN Steine, D Vafeados, C McGuire, C Freeman, A Amen, J Goodstal, D Facciotti, JV Eck, M Causse (2007). Tomato. *In: Genome Mapping Molecular Breeding in Plants,* pp:1–125. Kole C (Ed.). Verlag Berlin Heidelberg: Springer

Lee HG, G Lee, NR Jang, JH Yun, JY Song, BS Kim (2011). Biological synthesis of copper nanoparticles using plant extract. *Nanotechnology* 1:371–374

Li M, X Xi, G Xiao, H Cheng, Z Yang, G Zhou, Z Li (2014). National multi-purpose regional geochemical survey in China. *J Geochem Exp* 139:21–30

Li Z, J Huang (2014). Effects of nanoparticle hydroxyapatite on growth and antioxidant system in Pakchoi (*Brassica chinensis* L.) from cadmium contaminated soil. *J Nanomater* 2014:470962-470968

Liu R, R Lal (2015). Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. *Sci Total Environ* 514:131–139

Lowry OH, NH Rosebraugh, AL Farr, RJ Randel (951). Protein measurement with Folin phenol reagent. *J Biol Chem* 193:265–275

Mansoor S, N Kour, S Manhas, S Zahid, OA Wani, V Sharma, L Vijaya, MN Alyemeni, AA Alsahli, HA El-Serehy, BA Paray, P Ahmad (2021). Biochar as a tool for effective management of drought and heavy metal toxicity. *Chemosphere* 271:29458

Noman MT, M Petru, N Amor, P Louda (2020). Thermophysiological comfort of zinc oxide nanoparticles coated woven fabrics. *Sci Rep* 10:21080-21091

Ozyigi İI, S Yilmaz, I Dogan, MS Sakcali, G Tombuloglu, G Demir (2016).

Detection of physiological and genotoxic damages reflecting toxicity in kalanchoe clones. *J Glob Nest* 18:223–232

Prasad TNVKV, P Sudhakar, Y Sreenivasulu, P Latha, V Munaswamy, KR Reddy, TS Sreeprasad, PR Sajanlal, T Pradeep (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. *J Plant Nutr* 35:905–927

Ramesh M, K Palanisamy, K Babu, NK Sharma (2014). Effects of bulk & nano-titanium dioxide and zinc oxide on physio-morphological changes in *Triticum aestivum* Linn. *J Glob Biosci* 3:415–422

Raskar SV, SL Laware (2014). Effect of zinc oxide nanoparticles on cytology and seed germination in onion. *Intl J Curr Microbiol Appl Sci* 3:467–473

Rizwan M, S Ali, B Ali, M Adrees, M Arshad, A Hussain, MZU Rehman, AA Waris (2019a). Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. *Chemosphere* 214:269–277

Rizwan M, S Ali, MZU Rehman, M Adrees, M Arshad, MF Qayyum, L Ali, A Hussain, SAS Chatha, M Imran (2019b). Alleviation of cadmium accumulation in maize (*Zea mays* L.) by foliar spray of zinc oxide nanoparticles and biochar to contaminated soil. *Environ Pollut* 248:358–367

Rizwan M, S Noureen, S Ali, S Anwar, MF Qayyum, A Hussain (2019c). Influence of biochar amendment and foliar application of iron oxide nanoparticles on growth, photosynthesis and cadmium accumulation in rice biomass. *J Soil Sed* 19:3749–3759

Rosa GDL, ML López-Moreno, DD Haro, CE Botez, JR Peralta-Videa, JL Gardea-Torresdey (2013). Effects of ZnO nanoparticles in alfalfa, tomato and cucumber at the germination stage: Root development and X-ray absorption spectroscopy studies. *J Pure Appl Chem* 85:2161–2174

Sedghi M, M Hadi, SG Toluie (2013). Effect of nano zinc oxide on the germination parameters of soybean seeds under drought stress. *Ann West Univ Timisoara Ser Biol* 16:73-78

Shah AM, MS Memon, AN Memon, AW Ansari, BA Arain (2010). Analysis of Protein by Spectrophotometric and Computer Colour Based Intensity Method from Stem of Pea (*Pisum sativum*) at Different Stages. *Pak J Anal Environ Chem* 11:63–71

Sharma H, N Rawal, BB Mathew (2015). The characteristics, toxicity and effects of cadmium. *Intl J Nanotechnol Nanosci* 3:17–26

Singh J, BK Lee (2016). Influence of nano-TiO2 particles on the bioaccumulation of Cd in soybean plants (*Glycine max*): A possible mechanism for the removal of Cd from the contaminated soil. *J Environ Manage* 170:88–96

Tabelin CB, T Igarashi, M Villacorte-Tabelin, I Park, EM Opiso, M Ito, N Hiroyoshi (2018). Arsenic, selenium, boron, lead, cadmium, copper and zinc in naturally contaminated rocks: A review of their sources, modes of enrichment, mechanisms of release and mitigation strategies. *Sci Total Environ* 645:1522–1553

Tarafdar JC, R Raliya, H Mahawar, I Rathore (2014). Development of zinc Nano fertilizer to enhance crop production in pearl millet (*Pennisetum americanum*). *Agric Res* 3:257–262

Teng Y, J Wu, S Lu, Y Wang, X Jiao, L Song (2014). Soil and soil environmental quality monitoring in China: A review. *Intl J Environ* 69:177–199

Thornton I (1992). Sources and pathways of cadmium in the environment. *IARC Sci Publ* 118:149–162

Tripathi S, P Srivastava, RS Devi, R Bhadouria (2020). Influence of synthetic fertilizers and pesticides on soil health and soil microbiology. *In: Agrochemicals detection, treatment and remediation*, pp:25–54. Butterworth- Heinemann

Val ME, M Bouvier, J Campos, D Sherratt, F Cornet, D Mazel, FX Barre (2005). The single-stranded genome of phage CTX is the form used for integration into the genome of *Vibrio cholerae*. *J Mol Cell* 19:559–566

Venkatachalam P, M Jayaraj, R Manikandan, N Geetha, ER Rene, NC Sharma, SV Sahi (2017). Zinc oxide nanoparticles (ZnONPs) alleviate heavy metal-induced toxicity in *Leucaena leucocephala* seedlings: A physiochemical analysis. *Plant Physiol Biochem* 110:59–69

Wang WP, E Lombi, FJ Zhao, PM Kopittke (2016). Nanotechnology: a new opportunity in plant sciences. *Trends Plant Sci* 21:699–712

White PJ, P Pongrac (2017). Heavy-metal toxicity in plants. *In: Plant Stress Physiology*, pp:300–331. Shabala S (Ed.). Wallingford: CABI

Witham FH, DF Blaydes, RM Devlin (1971). *Experiments in Plant Physiology,* pp:55–58. Van Nostand Reinhold Co., New York

Yan S, F Wu, S Zhou, J Yang, X Tang, W Ye (2021). Zinc oxide nanoparticles alleviate the arsenic toxicity and decrease them accumulation ofarsenic in rice (*Oryza sativa* L.). *BMC Plant Biol* 21:150-160

Yuan J, Y Chen, H Li, J Lu, H Zhao, M Liu, GS Nechitaylo, NN Glushchenko (2018). New insights into the cellular responses to iron nanoparticles in *Capsicum annuum. Sci Rep* 8:1–9