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Original article

Nanoscale Zerovalent Iron Based Moderation of Chromium Stress in Tomato Seedlings is Related with Induced Antioxidants and Suppressed Cr Uptake

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Abstract

The nanoscale zerovalent iron (nZVI) has been widely used in remediation of environmental pollutants from the aqueous as well as soil media. The present study was conducted to evaluate the role of nZVI as a soil amendment in amelioration of chromium (Cr) toxicity in tomato seedlings. Three weeks exposure with low (10 mg kg-1) and high (100 mg kg-1) Cr(VI) was given to tomato seedlings grown in soil medium supplemented with or without 500 mg kg-1 nZVI in corresponding soils. The Cr exposure greatly reduced the biomass with high Cr(VI) lowering the plant height, root length, shoot and root biomass by 34, 24, 33 and 49%, respectively. However, nZVI significantly restored the growth retardation by increasing these parameters by 17, 14, 19 and 33%, respectively. The nZVI also lowered the Cr-induced MDA content, improved membrane stability index and increased relative water contents. The nZVI was also effective in improving the chlorophyll pigments and carotenoids contents. The antioxidant enzymes (viz. SOD, POD, CAT and APX) were slightly increased by Cr stress. The nZVI application together with Cr stressed soil further enhanced these enzyme activities. Application of nZVI further lowered the significant amount of Cr(VI) in shoots and roots tissues. The nZVI-induced tissue Cr concentration was lowered by 35% in shoots in case of low Cr exposure and 29% in roots by high Cr treatments. The amelioration of Cr-induced toxicity in tomato seedlings by nZVI application in soil seems to be the result of suppression of Cr uptake and enhancement in antioxidant enzyme system.

Keywords: nZVI, ROS, Antioxidant, Chromium, Tomato, Oxidative Stress, Biomass.

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INTRODUCTION

Human activities and industrial processes have led to the contamination of natural media including the soil and water, thus posing great threat to agricultural crops and human health via entry of the pollutants into food chains. The so called "heavy metals" are the potential toxic elements (PTEs) which originate from fossil fuel burning, municipal and industrial wastes, agricultural inputs and the sewage water; and make their way into agricultural soils and water thus limiting crop production and deteriorating quality of food crops (Malik et al., 2021; Oh et al., 2007). Among the most prevailing PTEs, chromium (Cr) is ranked 7th among the top 20 hazardous chemicals and the top most among carcinogenic PTEs (Oh et al., 2007). The hexavalent chromate Cr(VI) and the trivalent chromite Cr(III) are the most common and stable forms found in the natural environment (Ashraf et al., 2017). Due to its carcinogenicity, mutagenicity and teratogenicity nature, the Cr(VI) form is more mobile and toxic to biological processes. The researchers around the globe are particularly concerned about the environmental pollution of Cr(VI) because of its ever increasing levels in the water and soil media originating from natural and anthropogenic activities including metallurgical, refractories and chemicals processes (Ashraf et al., 2017; Z. Malik et al., 2021). In humans, Cr(VI) toxicity creates serious diseases including the nervous system disorder, renal failure, hematopoietic abnormalities and gastrointestinal diseases (Zeng et al., 2011). Cr(VI) toxicity also exerts various impairments in plants thus hampering the normal development and metabolism. It deteriorates the plant growth, imbalances in nutrient uptake and photosynthesis, induces synthesis of reactive oxygen species (ROS) and causing lipid peroxidation, and alters the antioxidant enzymatic and non-enzyme activities (Noli & Tsamos, 2016; Shaheen et al., 2016; Zeng et al., 2011). More importantly, Cr(VI) after entering into plant system accumulates in edible plant parts and thus poses severe health hazards for the ultimate consumers (Cherfi et al., 2015). Toxicity of Cr in biological processes of the plants and associated alteration in physiological activities relies directly on the concentration of Cr uptake by plant roots, its translocation to aboveground plant parts, and the accumulation in fluorescent tissues (Singh et al., 2021).

At cellular levels, Cr(VI) induces the synthesis of ROS leading to oxidative damage which can be indicated by overproduction of malondialdehyde (MDA), cellular membranes permeability and electrolyte leakage (EL) (Patra et al., 2019; Yu et al., 2018). The accumulation of ROS ultimately leads to the peroxidation of membrane lipids and disruption of membrane structure and function, which further causes the oxidation of cellular proteins and nucleic acids (Wakeel et al., 2020). Plants like other living organisms have evolved antioxidant system mainly comprising of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). These antioxidant enzymes detoxify the deleterious effects of ROS overproduction and confer the plants resistance against variety of stressful conditions (Zaheer et al., 2020). Here, it is worth mentioning that Cr toxicity also exerts negative impacts on the plants natural antioxidant defense system However, Cr toxicity also affects the

activities of the enzymes (Wakeel et al., 2020). Generally, higher Cr(VI) accumulation severely alter the plants structural, biochemical and physiological functioning of plants including the photosynthetic performance and ultimately results in poor production and yield decline (Singh et al., 2020).

Various remediation strategies have been being investigated for last two decades to clean up the soils from Cr pollution or at least limiting its entry into crop plants. These strategies include the chemical reduction of Cr(VI) to Cr(III), solvent extraction, chelation, absorption and adsorption (Li et al., 2019; Mitra et al., 2017). More recently, zerovalent iron nanoparticles (nZVI) have been employed successfully to clean up the contaminated media including the Cr(VI) due to their excellent removal potential of variety of pollutants from aqueous solutions as well as soil medium. The nZVI are the core-shell structures, where the core is generally made up of metallic iron while the shell is composed of Fe(II) and Fe(III) oxides and hydroxydes (Vilardi et al., 2018). The nZVI mediated removal/reduction of Cr(VI) has been investigated by various researchers (Di Palma et al., 2015; Vilardi et al., 2019). The nZVI are highly effective to adsorb, interact with, or reduce a variety of pollutants including the aromatic and chlorinated pollutants, and heavy metals (Yuvakkumar et al., 2011).

Not much work has been done in the area of effects of nZVI on plant growth performance under environmental stressful conditions. Recently, it has been shown that nZVI application increased the carotenoids contents, chlorophyll pigmentation and photosynthetic performance in tomato plants (Brasili et al., 2020). Though nZVI have been highly effective in remediating the pollutants from soil and water media, its impact on plant physiological and biochemical processes has yet to be investigated. The current study focusses on investigating the potential of nZVI as a soil amendment for alleviation of Cr(VI) toxicity in tomato plants. Tomato is one of the most important food crop mainly grown in suburban areas receiving a plenty of sewage water which normally has high amounts of Cr. Application of nZVI in Cr-contaminated agricultural fields can be a sustainable approach for healthier and optimum crop production of tomato.

MATERIAL and METHODS

Soil Preparation and Experimental Set up

The study comprised of a pot experiment, which was performed at the botanical garden of Bahauddin Zakariya University Multan, Pakistan. The sandy loam soil was collected from the agricultural farm of the university and prepared according to standard procedures required for the study. Before artificially spiking with Cr(VI), the soil was well dried, ground and sieved inorder to remove physical impurities. For artificially contamination with Cr(VI), the soil was spiked with two levels of Cr(VI) namely 10 mg kg^{-1} dry weight as low dose of Cr, and 100 mg kg^{-1} dry weight as high dose of Cr. The salt of potassium dichromate (K_2 Cr_2O_7) was used as the source of Cr(VI). Required doses of Cr(VI) were dissolved in deionized water and subsequently mixed in respective treatments by mixing the

required Cr(VI) dose in equal amounts of water. The same amount of water was applied to control soils having no Cr(VI) and nZVI alone soils. After mixing the Cr solution into respective soils, these were kept drying for week. On subsequent week, the soils were again irrigated and well mixed followed by another spell of drying. A total of 4 cycles of soil spiking were carried out in order to make the Cr well mixed and uniformly distributed in respective soil treatments. After incubation and Cr spiking, the respective treatments were supplemented with nZVI at the rate of 500 mg kg^{-1} soil. The nZVI was very kindly obtained by the soil science department of The Islamia University Bahawalpur, Pakistan, where it was synthesized by green synthesis process using mango peel. The selection of low and high levels of Cr (100 and 100 mg kg^{-1}) and level of nZVI (500 mg kg^{-1}) was made as per our preliminary experiments (not published yet). After supplementation with nZVI, the soil sets were again irrigated and kept for drying before loading into respective pots for the performing of experiment in glasshouse. The experiment contained five treatments and a control for normal conditions having no Cr or nZVI supplementation. The nZVI alone treatment was meant to evaluate any harmful impact upon tomato seedlings. While four treatments included the solo application of low and high doses of Cr(VI) as well as with the supplement of nZVI with both these Cr levels. The equal amounts of well pulverized soils (7.5 kg) as per respective treatments were loaded in plastic containers. Tomato seedlings were grown in separate trays containing peat moss; and uniform healthy seedlings (7 days post germination) were transplanted into plastic pots and immediately irrigated with equal amounts of water. All the pots were supplemented with half strength NPK solution to meet the basic nutrient requirements of the plant. All the treatments were replicated with three replication, each having five plants per pot. The young seedlings were kept growing for three weeks followed by measuring growth parameters and tissue sampling for biochemical analyses. The experiment was carried out in glasshouse uniform conditions with16/8 h day/night length and 30 °C/25 °C day/night temperature, 85% relative humidity, and 400 ± 25 μM photons m^{-2} s^{-1} light intensity, which was measured daily at the top of plant canopy by a portable light meter (Li-250 A; Li-Cor, Lincoln, NE, USA).

Estimation of Plant Biomass

After three weeks of growth, plant heights were recorded using a normal scale. The SPAD (soil–plant analyses development) value was recorded by using SPAD meter (SPAD-502, Zhejiang Top Instruments Co., Ltd., China) on the topmost second fully opened leaf. The plants were carefully uprooted from the pots without harming the roots in fully wet conditions. After measuring root lengths, these were cut into roots and shoot tissues. For estimation of membrane stability index, relative water content, MDA and antioxidant enzymes, top 2^{nd} fully opened leaves were separated and used for biochemical assays accordingly. The roots and shoots (stem and leaves) were kept for oven drying before acid digestion and estimation of Cr contents.

Plant Tissues Chromium Determination

For measurement of Cr concentration in shoot and root tissues, freshly harvested seedlings were washed thoroughly with water followed by 20 min immersing in 20 mM EDTA solution in order to clean it from any adsorbed metal ions on plant surfaces . Separated shoots and roots were oven dried at 80 °C for 24 hours. After grinding about 1.0 g plant material was digested with H_2SO_4 and H_2O_2. Finally, the Cr contents in shoots and roots tissues was measured by atomic absorption spectrophotometer (Shimdazoo AA 6300) (Malik et al., 2021).

Estimation of Relative Water Content, Membrane Stability Index and MDA Content

The relative water contents (RWC) of the leaves was measured according to the method described by Lazcano-Ferrat and Lovatt (1999). Briefly, the youngest leaf of tomato plants kept sealed in plastic bags and quickly transferred to the laboratory. After noting the fresh weight (FW) by using simple electrical balance, the turgid weight (TW) was recorded after soaking the leaves in distilled water for 24 h at room temperature. Finally, the dry weight (DW) was measured after drying at 65 °C for 72 h. For calculation of RWC, following equation was used:

$$\text{RWC (\%)} = (\text{FW}-\text{DW}) / (\text{DW}-\text{TW}) \times 100$$

For determination of Membrane stability index (MSI), 0.1 g of the leaf discs were crushed, followed by 30 min heating in 10 ml distilled water at 40 °C. Firstly, initial electrical conductivity (C_1) was determined by electrical conductivity (EC) meter and then the electrical conductivity (C_2) was recorded by keeping the same sample for 10 min in water bath at 100 °C (Sairam et al. 2002). The MSI was calculated using the following Eq. (2):

$$\text{MSI} = [1-(C_1/C_2)] \times 100$$

Chlorophyll a and b and carotenoid contents were measured as per the methods described by Arnon (1949) and Wellburn (1994), respectively. Briefly, 0.05 g fresh leaf material was ground in mortar. After extraction in 10 ml dimethylsulphoxide (DMSO), these samples were kept for 4 h in oven at 65 °C. After heating, the absorbance of extract was recorded by spectrophotometer (Halo DB-20/DB-20S, Dynamica Company, London, UK) at 645 nm, 665 nm and 470 nm for chlorophyll a, chlorophyll b and carotenoid contents, respectively. Extinction constants and equation were used to finally calculate the respective chlorophyll and carotenoid contents. For MDA contents determination in shoots/leaves, 0.5 g fresh leaf samples were ground in 10 ml TBA solution (0.25%), already prepared in trichloroacetic acid (TCA) (10%). The extracted material was heated at 95 °C for 30 min, followed by ice-cooling to stop the reaction. After 10 min centrifugation at 10,000 g, the absorbance of supernatant's absorbance was noted at 532 nm. A simultaneous absorbance at 600 nm was also taken, which was then subtracted

for correction of non-specific turbidity. Finally the MDA content in leaves/shoots was determined by using 155 mM^{-1} cm^{-1} as extinction coefficient (Ali et al., 2018).

Estimation of Antioxidant Enzymatic Activity

For antioxidant enzymes extraction, plant leaves/shoots were firstly rinsed with distilled water. After that, plant extract (0.5 g) was placed on chilled plaster. The phosphate buffer solution (pH of 7.8) of about 2-3 ml was added, followed by distilled water addition to make the final volume of 1000 ml. a 5 ml buffer solution was added to make the samples homogenized. After centrifugation (8000–13,000 rpm) for 20 min at 4 °C, resultant supernatants were placed in 5 ml centrifuge tubes. The activities of antioxidant enzymes were measured by spectrophotometer (Halo DB-20/DB-20S, Dynamica, UK). The SOD activity determination was done by homogenizing 100 µl enzyme extract in 3 ml reaction mixture which had 50 mM potassium phosphate buffer (pH 7.8), 75 µM nitro-blue tetrazolium (NBT), 2.0 µM riboflavin, 13 mM methionine and 0.1 mM EDTA. Activity was recorded at 560 nm, (Zhou et al., 1997). The POD activity of was assayed according to Zhang (1992) with minor modifications. For POD activity, the reaction mixture contained of potassium phosphate buffer (pH 7.0) (50 mM), guaiacol (1%), H_2O_2 (0.4%) and enzyme extract (100 µl). The activity was recorded at 470 nm absorbance at. For the activity of CAT was assayed in reaction mixture (3 ml) having potassium phosphate buffer (pH 7.0) (50 mM), EDTA-Na_2 (2 mM), H_2O_2 (10 mM) and enzyme extract (100 µl). The CAT activity was measured with the use of H_2O_2 (as extinction coefficient) for 1 min at A240. In case of APX activity, reaction mixture (3 mL) was consisted of phosphate buffer (pH 7) (100 mM) EDTA-Na_2 (0.1 mM), ascorbic acid (0.3 mM), H_2O_2 (0.06 mM) and 100 µL enzyme extract. The change in absorption was taken at 290 nm for 30 s after H_2O_2 addition (Nakano and Asada, 1981).

Statistical Analysis

All the data were subjected to one-way analysis of variance (ANOVA). Statistical difference among treatments were determined by Duncan's multiple range test by using SPSS17.0 statistical package at probability level $p < 0.05$ (SPSS. Chicago, IL, USA).

RESULTS

Effect of nZVI on Plant Biomass under Cr Stress

Biomass of the tomato seedlings was severely affected by 3 weeks exposure to Cr(VI) in nutrient medium. Plant height was reduced by 21% and 33% by exposure to low Cr (10 mg kg^{-1}) and high Cr (100 mg kg^{-1}), respectively. Similarly, the root lengths were also shortened by 16% and 24% in case of low and high Cr treatments, respectively. Compared to non-Cr conditions (control), the shoot and root dry weights were reduced by 25% and 33%, and 28% and 50% as the result of low and high Cr exposure, respectively. The decline in biomass characteristics was more pronounced in high Cr stressed conditions showing that higher Cr stress can be lethal for growth and development. The Cr-induced stress was

moderated by exposure to nZVI as soil amendment and the relative biomass characteristics were improved in case of nZVI presences with Cr stressed conditions, compared to Cr alone treatments (non-nZVI). Compared to Cr alone, the presence of nZVI improved 17% plant height in case of low Cr conditions; while only 9% increase in plant height occurred by nZVI compared to high Cr alone treatment. Root lengths were improved by 14% by nZVI in case of low Cr conditions. However, high Cr exposure along with nZVI could not improve the root lengths. The nZVI caused 19% and 33% increase in shoot and root dry weights in case of low Cr exposure. While nZVI amelioration of shoot and root dry weights were limited to 11% and 20% only in case of high Cr exposure. It can be deduced that nZVI was more effective in restoring biomass related parameters when under low Cr stress as compared to high Cr exposure (Figure 1).

Shoot and Root Cr Concentration

Tissue Cr concentrations have been shown in Figure 2. Cr concentration mainly contained in roots, however a reasonable amount was also transferred to aboveground portion. In shoots Cr concentration reached to 36 mg kg^{-1} and 49 mg kg^{-1} DW in low and high Cr treatments. However, nZVI presence significantly lowered shoot Cr by 35% and 9% in 10 and 100 mg kg^{-1} Cr exposure, respectively. Similarly, root Cr concentration reached upto 62 mg kg^{-1} DW and 129 mg kg^{-1} DW in low and high Cr treatments. The nZVI effectively lowered root Cr concentration by 6% in low and 29% in high Cr conditions, respectively. The results indicate that nZVI was more effective in lowering shoot Cr concentration in case of low Cr exposure; while it gave more pronounced results in terms of root Cr concentration under high stressed conditions.

Effect of nZVI on Chlorophyll and Carotenoids Contents of Tomato Leaves under Cr Stress

Low Cr stress yielded 14% and 36% decline in chlorophyll a and b contents of leaves, as compared to non-stressed control leaves. Similarly, high Cr stress caused 33% and 55% decline in chlorophyll a and b contents in comparison with the control. nZVI markedly restore the chlorophyll a nd b contents in both low and high Cr stressed leaves. However, nZVI induced restoration of chlorophyll a and b was more pronounced in high Cr stressed plants (42% and 73%). Carotenoids contents of the leaves were also showed to decline in response to Cr exposure; where it was reduced to 25% and 41% compared to control, in case of low and high Cr treatments, respectively. Like chlorophyll contents, the carotenoid contents were also restored by nZVI. It was significantly improved by 17% compared to low Cr alone treatment. Likewise, SPAD value was laos declined by 24% and 40% by low and high Cr, as compared to control. The nZVI improved the SPAD value by 13% in low Cr treatment and 40% by high Cr treatment. The results clearly demonstrated that nZVI was very effective in restoring plants pigmentation and carotenoids contents even in the presence of Cr exposure (Figure 3).

Effect of nZVI on RWC, MSI and MDA of Tomato Leaves under Cr Stress

Cr stress negatively affected RWC and MSI of tomato leaves/shots and also induced lipid peroxidation which was evident in terms of greater production of MDA (Figure 4). Cr exposure caused wilting in plant leaves which was shown by 19% decline in RWC in low Cr treatment; while this decline was 44% in high Cr treatments as compared to the control plants. The nZVi caused 13% increase in RWC of low Cr treatment, while 26% increase in high Cr treatment which brough the water status to near normal conditions. Similarly, the MSI was 20% lowered by low Cr exposure and 35 decline in high Cr treatment, compared to the control plants. Again, the nZVI restored membrane stability by increasing MSI by 13% and 11% in low and high Cr treatments, respectively. The MDA content of the leaves which indicates the creation of lipid peroxidation as the result of stress, was also induced by both low and high Cr treatments. Low and high Cr treatments caused 1.6 and 2.4 times greater MDA in leaves, compared to non-stressed plants. Yet, the nZVI was effective in significantly lowering the Cr-induced MDA overproduction. nZVI produced 41% and 9% lowering of shoot MDA contents in low and high Cr treatments, respectively. Our results support the idea that Cr stressed medium causes stress indicators/biomarkers in plants while the nZVI moderated the stress indicators.

Effect of nZVI on Antioxidant Enzymes in Shoots of Tomato Seedlings under Cr Stress

Antioxidant enzyme system is the plants' internal detoxification mechanism to cope with the stressful conditions. However, stressed condition itself affects negatively this defense mechanism. Activities of antioxidant enzymes (SOD, POD, CAT and APX) have been shown in Figure 5. Generally, in our study the antioxidant enzymes activities were increase with CR exposure. However, nZVI further boosted the activities and improved the enzymatic activities combating against ROS. The SOD activity was increase by 29% and 43% by low and high Cr treatments, respectively. This enhancement was further induced by 15% and 20% by nZVI in low and high Cr treatments. The POD activity was increased by 44% and 67% by low and high Cr stress, which was further enhanced by nZVI by 56% in case of low Cr treatment. POD activity was not further increased by nZVI in case of high Cr exposure. The CAT activity was increased by 1.5 and 1.7 times the control treatment in low and high Cr treatments. This supplementation in CAT shoot activity was further boosted by nZVI by 59% and 54% in low and high Cr treatments. Similarly, the APX activity was increased by 34% and 20% in low and high Cr exposure, which were further induced by 25% and 64% by nZVI presence in low and high Cr conditions (Figure 5). The nZVI-induced supplementation of already enhanced antioxidant enzymes activities could further strengthen the plants intrinsic potential to cope with the environmental stresses.

DISCUSSION

Cr is highly toxic to living organisms including the crop plant which could severely hamper the normal growth and development. In our study, growth retardation owned to Cr stress has been evident

in terms of biomass reduction. The biomass reduction was Cr dose dependent where 10 mg kg^{-1} Cr(VI) exposure greatly reduced the plant height, root length and shoot and root dry biomass. This decline might be due to the plant wilting, inhibition of cell division in roots, plasmolysis and hampering of nutrients and water uptake (McCarroll et al., 2010).

Under stressful conditions, production of ROS is one of the most important indicator produced at metabolic levels that also is the case with Cr toxicity. We found that higher Cr toxicity caused greater production of MDA and also associated membrane injury in terms of lowered MSI and plant wilting as reduced percentage of RWC. These parameters were in consistency with the growth parameters indicating that MDA production, membrane injury and lowered water status caused decline in growth parameters (Ali et al., 2018). Stress creation at metabolic levels may cause injury to photosynthetic apparatus and leaves pigmentation that can lead to abnormalities in photosynthesis efficiency of the plants. Parallel with the stress indicators, chlorophyll contents, total carotenoids and the SPAD values were also badly affected by Cr exposure. Detoxification of the ROS by plants internal antioxidants is natural defense mechanism of the living organisms. Under normal stressed conditions, elevation of antioxidant enzymes is a normal defensive approach, which was evident in our study. The SOD, POD, CAT and APX activities in shoots were induced by sole Cr exposure. However, the nZVI presence in the soil further ensured the boosting of these enzymes. This is very interesting from the point of view of understanding the phenomenon behind nZVI induced Cr toxicity tolerance. One possible interpretation might be the capacity of nZVI to reduce the uptake of Cr from soil to roots and then further translocation to aboveground shoot tissues. Reducing the mobility might have lowered the ROS generation and other stress indicators thus moderating the Cr-induced injury. Ultimately, low stressed conditions within the plants might have boosted antioxidant system to work with its full potential and conferred the plants a great deal of resistance against Cr(VI) stress. This was further supported by overall reduction in Cr uptake by tomato seedlings and mainly by reduction in Cr mobility from the roots to shoots. Apparently, it seems that the soil application of nZVI has converted toxic forms of Cr(VI) into less toxic forms, Cr(III) and further reduced the uptake by roots and translocation into aboveground plant parts. Lowering of Cr contents in plant parts by nZVI presence in soil might have moderated the stress and further amplified the antioxidant enzymes to cope with the stress and flourish growth parameters. However, to understand the actual phenomenon involved, further studies are needed to actually specify the role of Cr suppression or antioxidant induction, involved behind Cr toxicity resistance

Conclusion

Our results demonstrate that Cr toxicity exerted harmful impacts on the growth of tomato seedlings, where higher Cr treatment was more detrimental in lowering plant height, root length and the biomass. The growth retardation was also accompanied with the membrane injury, leaf wilting and oxidative stress creation in terms of lowered membrane stability index, relative water content and MDA

production. Chlorophyll contents, carotenoids contents and SPAD values were also lowered by Cr exposure. These stressed parameters were more pronounced in higher (100 mg kg^{-1}) Cr exposure, where shoot and root Cr concentrations were greatly higher than low Cr stress treatment. The tissue specific Cr concentration was mainly retained in roots. Application of 500 mg kg^{-1} nZVI greatly reduced the plant injury and effectively restored growth and stressed parameters. In case of growth and biomass parameters, nZVI performed better when applied together with low Cr stress. However, in case of stress indicators, nZVI performance was better when applied with high Cr stressed conditions. Antioxidant enzymes activities were increased slightly by Cr exposure, but nZVI addition in the soil further boosted the activities which might have contributed greatly in conferring tolerance to Cr stress in tomato seedlings.

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FIGURES

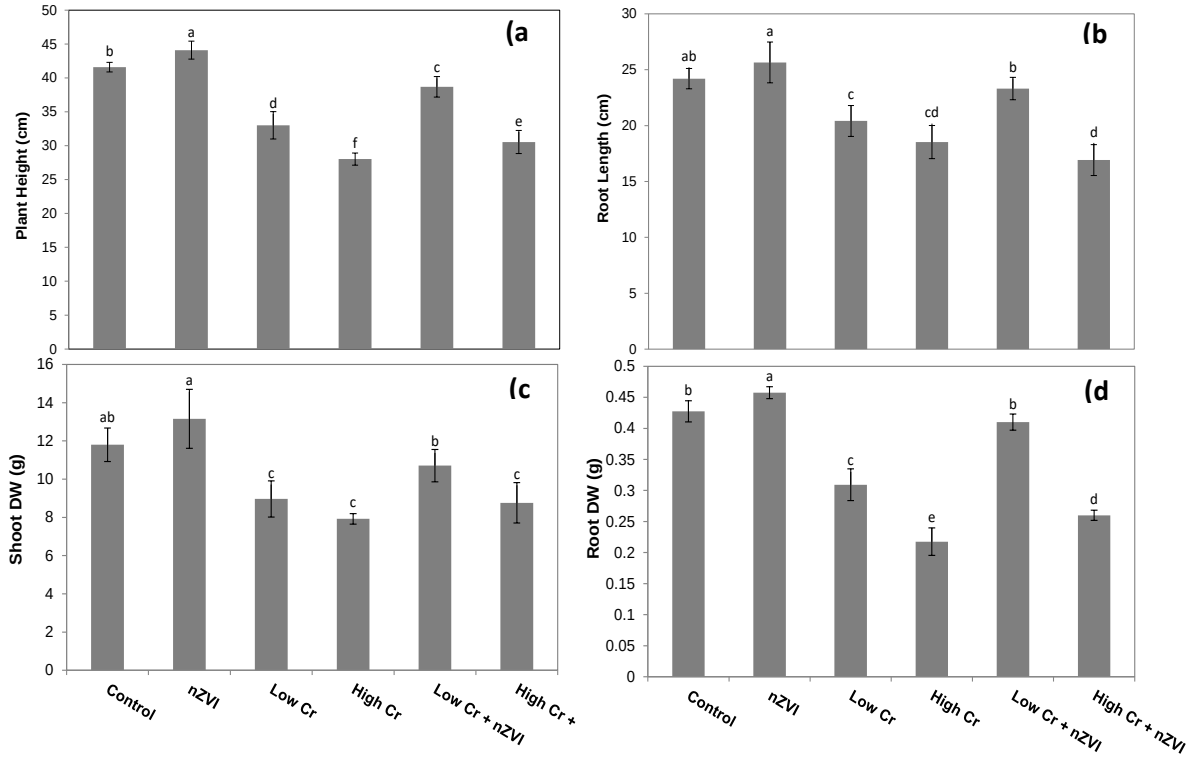

Figure 1. Effect of solo and combined application of nZVI and Cr on biomass (plant height (a); root length (b); shoot dry weight (c); root dry weight (d)) of tomato seedlings. Individual bars represent treatment means ± standard deviation (n=3). Treatment means not sharing a common letter are significantly different from each other ($p \leq 0.05$).

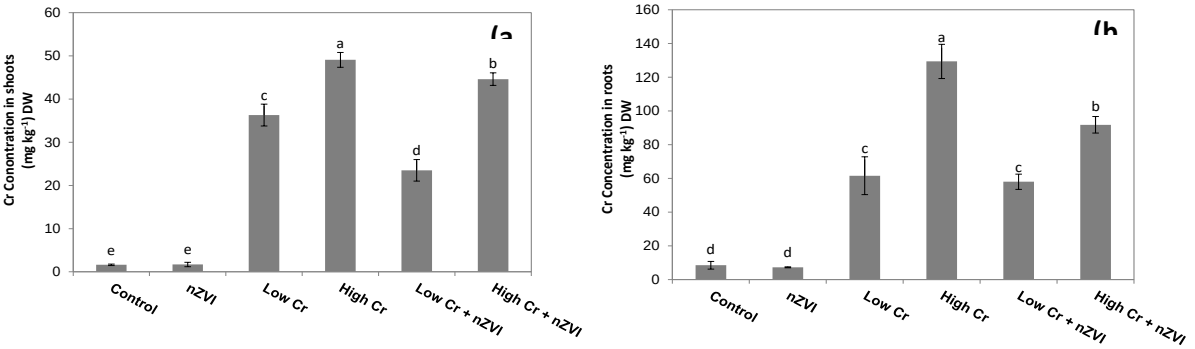

Figure 2. Effect of solo and combined application of nZVI and Cr on Cr concentration in shoots (a) and roots (b) of tomato seedlings. Individual bars represent treatment means ± standard deviation (n=3).

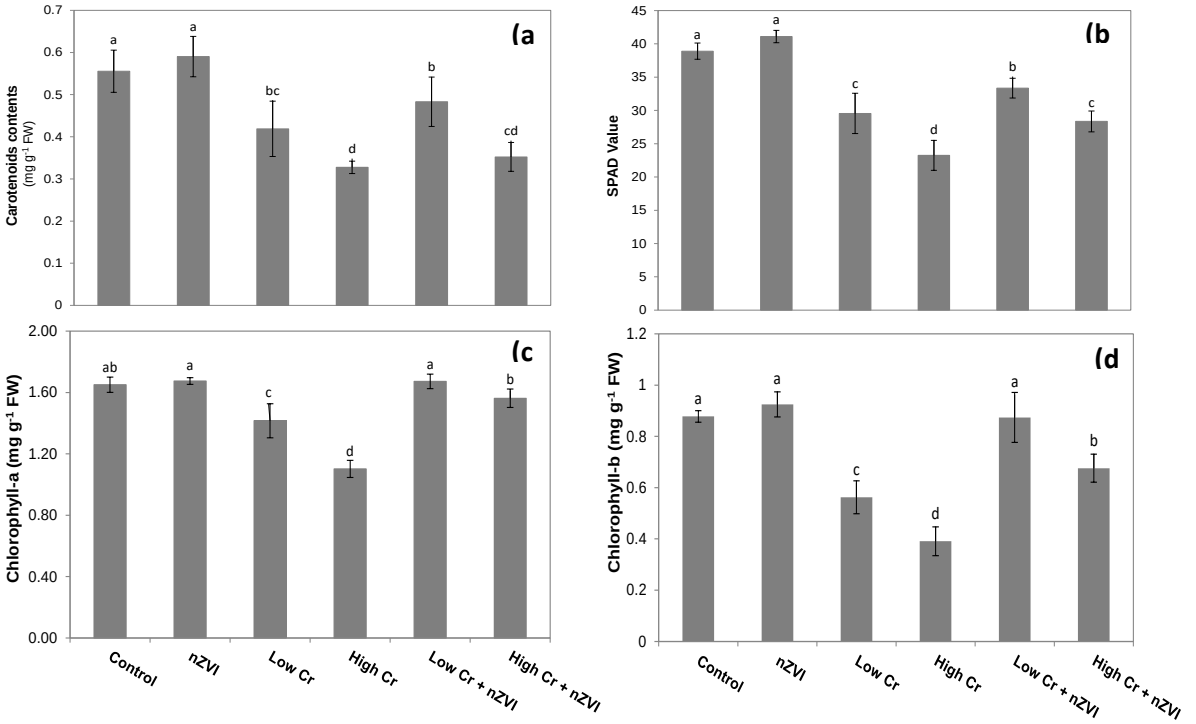

Figure 3. Effect of solo and combined application of nZVI and Cr on carotenoid contents (a), SPAD value (b), chlorophyll-a (c) and chlorophyll-b (d) of tomato seedlings. Individual bars represent treatment means ± standard deviation (n=3). Treatment means not sharing a common letter are significantly different from each other ($p \leq 0.05$).

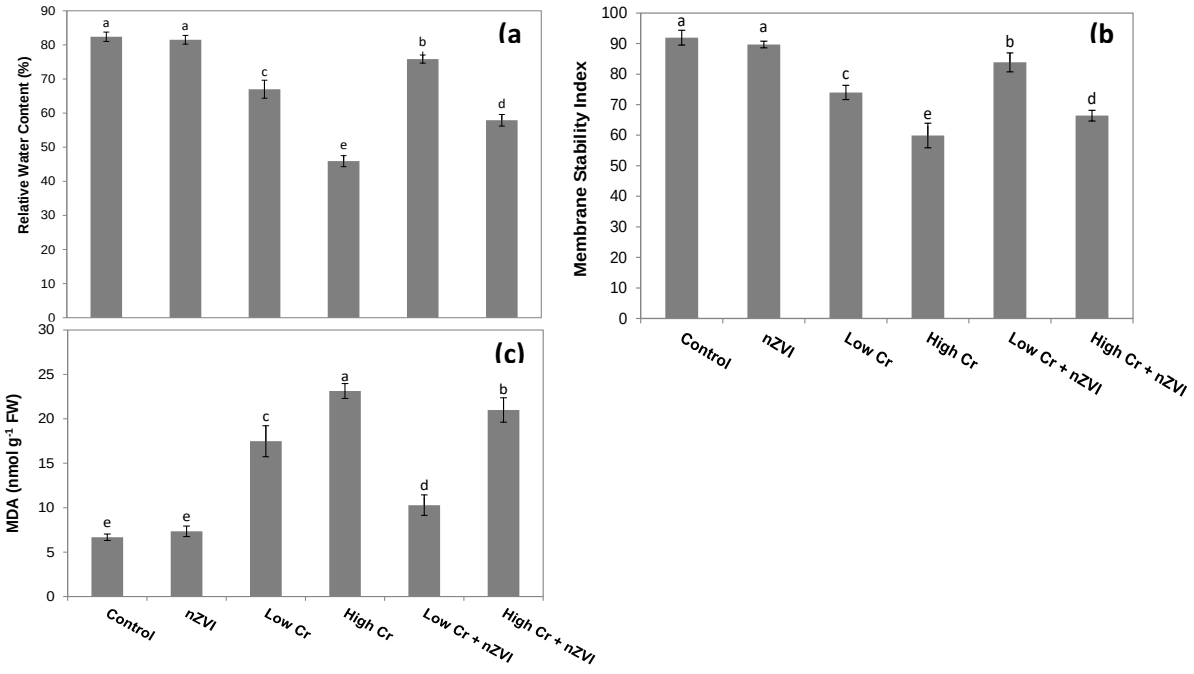

Figure 4. Effect of solo and combined application of nZVI and Cr on relative water content (a), membrane stability index (b) and MDA content (c) of tomato seedlings. Individual bars represent treatment means ± standard deviation (n=3). Treatment means not sharing a common letter are significantly different from each other ($p \leq 0.05$).

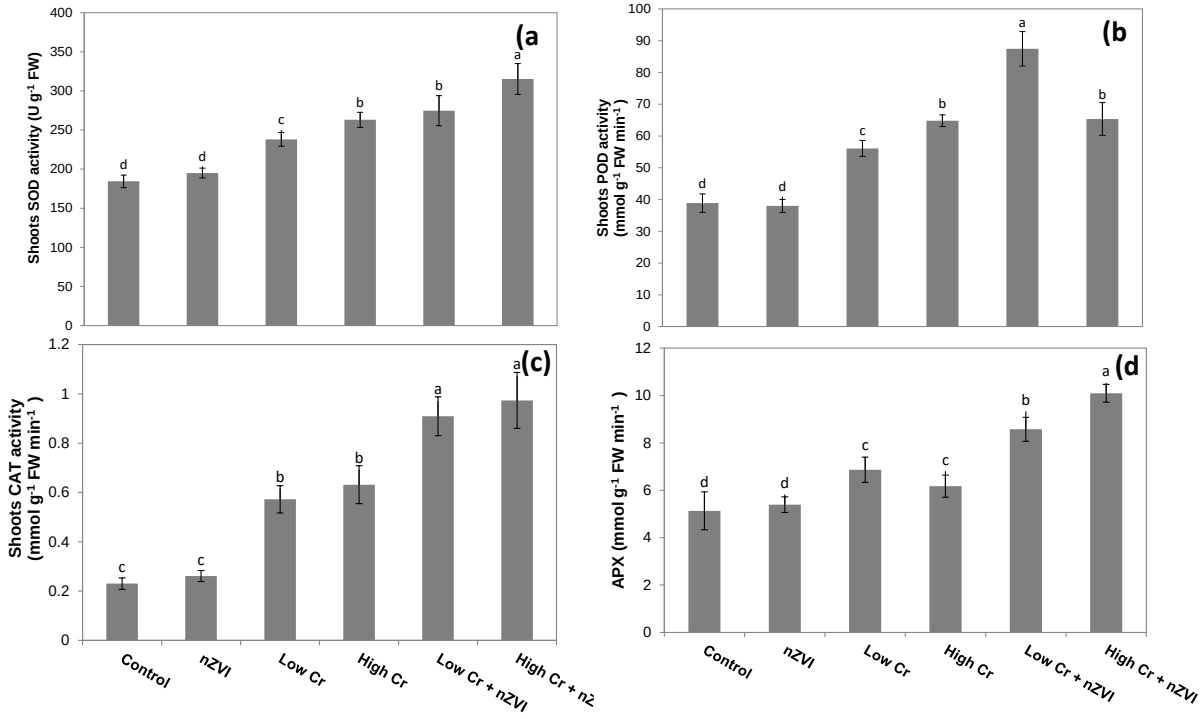

Figure 5. Effect of solo and combined application of nZVI and Cr on antioxidant enzyme activities in shoots (SOD activity (a), POD activity (b), CAT activity (c) and APX activity (d) of tomato seedlings. Individual bars represent treatment means ± standard deviation (n=3). Treatment means not sharing a common letter are significantly different from each other ($p \leq 0.05$).