1-15-2022

Chromium-resistant Staphylococcus aureus alleviates chromium toxicity by developing synergistic relationships with zinc oxide nanoparticles in wheat

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Ecotoxicology and Environmental Safety 230 (2022) 113142

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

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ARTICLE INFO

Edited by Dr Muhammad Zia-ur-Rehman

Keywords:
Zinc oxide
Nanoparticles
Microbes
Chromium
Toxicity
Wheat

ABSTRACT

Chromium (Cr) is a toxic heavy metal that contaminates soil and water resources after its discharge from different industries. It can act as carcinogen and mutagen for biological systems. Microbe-assisted phytoremediation is one of the most emergent and environment friendly technique used for detoxification of Cr from Cr-contaminated soils. In this study, wheat as a test crop was grown under varying stress levels (0, 50, 100 and 200 mg/kg) of Cr in a pot experiment under a complete randomized design. Alleviative role of *Staphylococcus aureus* strain K1 was assessed by applying as a treatment in different combinations of zinc oxide nanoparticles (0, 50, 100 mg/L). Growth and yield attributes data presented nurturing impact of bacterial inoculation and ZnO NPs in improvement of wheat defense system by decreasing Cr toxicity. Increase in chlorophyll and carotenoids contents, antioxidant enzymes (SOD, POD, APX, CAT) activities and nutrient uptake also confirmed the mitigative potential of bacterial inoculation when applied solely or in combination with ZnO NPs. The Cr accumulation in different parts of plant was significantly reduced with the application of NPs and *S. aureus* strain K1. Taken together, the results showed that combined application of *Staphylococcus aureus strain* K1 and ZnO NPs detoxifies the effects of Cr on wheat plants and boosts its growth, physiology and defense system.

1. Introduction

Globally, wheat (*Triticum aestivum* L.) is cultivated as a staple food and evidenced sensitive to various ecological stresses including heavy metals (Seleiman and Kheir, 2018a). In 2018, about 735.2 Mt of wheat grains was obtained from the cultivated area of 214.8 Mha worldwide (FAOSTAT, 2019). Environmental pollution induced by toxic metals has dramatically increased because of various anthropogenic activities during and after industrial revolution. These activities have substantially improved the human living standards, and deteriorated the environment at the same time (Singh et al., 2013). Soil contamination, due to toxic metals has evolved as a major environmental challenge worldwide (Seleiman et al., 2012, 2013a,b 2020, 2017; Seleiman and Kheir, 2018a,b). Direct or indirect discharge of sewage and industrial effluent into surface waterbodies has resulted in accumulation of chromium (Cr) and other toxic metals in soil (Kunjam et al., 2015), causing toxicity to plants (Habiba et al., 2015), animals and humans (Adrees et al., 2015).

In agricultural systems, Cr can easily move and accumulate in different parts of plants which is later on consumed by animals and

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https://doi.org/10.1016/j.ecoenv.2021.113142
Received 9 July 2021; Received in revised form 12 December 2021; Accepted 28 December 2021
Available online 3 January 2022

humans (Mathur et al., 2016). Pronounced influence of Cr contamination on the growth, physiology and yield of wheat plants has also been reported (Ali et al., 2018; Riaz et al., 2019). Numerous studies have reported restricted nutrient uptake in plants under exposure of Cr that eventually triggers the production of reactive oxygen species (ROS) and lipid peroxidation. These overexpressed ROS can desynchronize the antioxidant defense systems (enzymatic and non-enzymatic) besides malformation of chloroplast ultrastructure, and degradation of photosynthetic C-assimilation mechanism that ultimately leads to the reduction in plant growth and biomass (Ali et al., 2015a,b; Gill et al., 2015; Ulhassan et al., 2019). It is supposed that excessive production of ROS might be one of the main reasons of plant growth reduction under stressful regimes. Exogenous application of microbes is one of the applicable approaches that can help to significantly ameliorate the Cr-induced oxidative stress in plants by reducing electrolyte leakage (EL), malondialdehyde (MDA) and H_2O_2 formation, and increasing antioxidant enzyme activities under Cr-stress (Seleiman et al., 2020).

Phytoremediation is used to transform toxic metals from environment to less noxious forms (Muthusaravanan et al., 2018). During the few previous years, a well-known interest has been developed to explore the potential of phytoremediation for the reduction of pollution induced by substantial metals (Boopathy, 2000; Glick, 2010). Remediation of heavy metals from soil and water resources is not an easy process and plants alone cannot effectively remediate heavy metals from contaminated sites (Hrynkiewicz et al., 2018). Plant roots-associated microbes adopt different mechanisms to provide a considerable assistance for remediation of heavy metal contamination that can be used on large scale (Sharma, 2012).

Microbes can decrease the metal accumulation in plants via changing the metal species in the soil, thereby enhancing plant growth and increasing biomass. Chromium-reducing bacteria have capability to remediate Cr toxicity by reducing Cr^{6+} into Cr^{3+} in the rhizosphere through bioaccumulation and biosorption mechanisms (Mishra and Bharagava, 2016). The application of bacterial-assisted phytoremediation for the detoxification of Cr^{6+}, has been considered as safe, effective and economical over conventional physio-chemical techniques (Jing et al., 2014). The foliar spray of different stress alleviators further decreases metal stress in plants as mentioned by Seleiman et al. (2020) with foliar application of melatonin on the Cr-stressed wheat plants. Moreover, endophytic bacteria stabilized Cr in soil and reduced glutathione (GSH), glutathione peroxidase (GSH-Px) and glutathione s-transferase (GST) to their normal expression upon a combination with biogas slurry in Cr-stressed soil (Nafees et al., 2018).

Recently, nanotechnology has gained significant attraction because of its widespread application in numerous industries (Rizwan et al., 2017b). Nano-fertilizers could be a favorable methodology since chemical fertilizer are utilized in very small amounts by plants and other fertilizers left over in the soil can be involved in causing environmental risks (Liu and Lal, 2015; Raliya et al., 2017). The relationship between metal and zinc oxide nanoparticles (ZnO NPs) can affect the bioavailability of toxic metals such as Cd and Pb. Primarily, the studies on the application of NPs for the repair of metal uptake are lesser known (Venkatachalam et al., 2017). Nanotechnology provides a very large variety of techniques and devices to formulate NPs, detect biotic and abiotic stress in plants, and provide genetic manipulation that allows more precise plant breeding (Pérez-de-Luque and Hermosín, 2013; Fraceto et al., 2016). With unique physicochemical properties, NPs can enhance the biochemical processes of plants (Giraldo et al., 2014). However, the impact of nanoparticles on plants depends upon the plant species and the NPs variety (Servin and White, 2016; Singh et al., 2016; Vishwakarma et al., 2018; Anon, 2017; Rastogi et al., 2019).

In this research work, it was hypothesized that chromium-resistant bacteria (*i.e. Staphylococcus aureus* strain K1) may alleviate Cr toxicity in wheat by improving wheat growth and reducing Cr uptake in plants. This study will offer new insights to reduce toxic effect of Cr and its translocation via the combined application of ZnO NPs and chromium-resistant bacteria.

2. Materials and methods

2.1. Soil sampling and analysis

Soil was collected from the agriculture field and sieved through 2 mm sieve. Standard procedures were used for the initial characterization of the soil such as particle size (Bouyoucos, 1962), electrical conductivity (EC) and pH (soil to water ratio of 1:25, horizontal shaking for 2 h) of the soil extract. Pseudo total metals in the soil were determined with the standard procedure (Amacher, 1996). Walkley-Black method (Jackson, 1962) was used for the determination of soil organic matter and calcium carbonate was estimated with calcimeter method (Moodie et al., 1959). Detailed physico-chemical properties of soil are given in Table 1.

2.2. Seeds inoculation with Cr-resistant *Staphylococcus aureus* strain K1

For the preparation of bacterial inoculum, the individual bacterial colony was cultured in nutrient broth (250 mL) and incubated on rotary shaker (150 rpm) at 37 °C for 24 h (Zeng et al., 2020). Overnight culture was harvested using centrifuge (@ 10,000 rpm for 10 min) by discarding the supernatant. The obtained bacterial pallet was collected, washed with sterilized distilled water and resuspended in normal saline solution (0.85% NaCl) as described previously in Zeng et al. (2020). After inoculum preparation, seeds were surface sterilized by immersing the seeds in hydrogen peroxide (10% H_2O_2) for half an hour (Wu et al., 2006; Zeng et al., 2020). The bacterial inoculum was applied to these seeds through immersion of seeds in double volume of bacterial suspension on rotary shaker at 37 °C for 2 h (90 rpm). To facilitate the inoculum attachment to seeds, carboxymethyl cellulose (2%) was used. These inoculated seeds were added to a mixture of clay and peatmoss in equal proportion (1:1 w/w) which were shaken well for proper coating and incubated for 12 h in dark. Un-inoculated seeds were used as control (Zeng et al., 2020).

2.3. Pot experiment

Pot experiment was conducted under natural environmental conditions (28/20 °C day/night temperature, relative humidity 67 ± 5%) in the Botanical Garden of Government College University, Faisalabad, Pakistan. Plastic pots were filled with a sieved soil (5 kg/pot) spiked with different chromium stress levels (0, 50, 100 and 200 mg/kg) as per treatment plan. Five seeds of wheat were sown in each pot using a complete randomized design in triplicate. After germination, thinning was made and two plants were left in each pot. Salts of urea, di-ammonium phosphate and potassium sulfate were used as N, P & K source with ratio of 120:50:2 kg ha^{-1}, respectively. After 2 weeks of germination, plants were sprayed with different concentrations of ZnO nanoparticles (0, 50, 100 mg/L) and controls were provided with a

Table 1
Analysis of soil used for this experiment.

Soil	Units
Textural Class	Sandy Clay Loam
Sand	63.7%
Silt	14.4%
Clay	21.9%
pH	7.71
EC	1.93 dS m^{-1}
HCO_3^{-1}	3.15 mmol L^{-1}
Total nitrogen	0.07%
Available P	2.12 mg/kg
K^+	0.07 mmol L^{-1}
Cl^{-1}	5.2 mmol L^{-1}
Ca^{+2} + Mg^{+2}	14.92 mmol L^{-1}
Available Cr	0.04 mg/kg

simple canal water.

2.4. Plants harvesting

Plants were harvested 120 days after sowing. The plants were separated into spikes, grains, shoots and roots after measuring weight, height and spike length. Roots were washed with dilute HCl (0.1%) acid (to remove metals from the roots surface), and then with distilled water. Oven dried (72 h at 70 °C) roots and shoots weight was determined and such materials were crushed into small pieces for further analysis.

2.5. Growth, yield and photosynthetic pigments

Growth parameters such as shoot and root length (cm), fresh and dry weight of shoot and root (g), spike length (cm), no. of tillers per plant and grain weight (g) were determined.

Photosynthetic pigments (total chlorophyll, chlorophyll a & b, carotenoids) of fresh leaves collected from newly grown plants were determined spectrophotometrically following Lichtenthaler (1987). Samples were extracted in 85% acetone (v/v ratio) to estimate chlorophyll and carotenoid contents, After the extraction and centrifugation, readings were recorded at proposed wavelengths with the help of spectrophotometer.

2.6. Estimation of MDA, EL, H_2O_2 and antioxidants enzymes activities

Malondialdehyde (MDA) content was estimated using thiobarbituric acid (0.1%) following (Zhang and Kirkham, 1994; Abbas et al., 2017). For EL estimation, Dionisio-Sese and Tobita (1998) method was adopted. Extraction was performed in two steps. First, samples were extracted at 32 °C for 2 h to get initial EC of the solution. Secondly, the procedure was repeated at 121 °C for 20 min to get final EC of the solution. The contents of H_2O_2 were estimated by following Jana and Choudhuri's (1982) protocol. Shortly, before centrifugation, these samples were homogenized by adding phosphate buffer (50 Mm at pH 6.5) and centrifuged for 20 min. After centrifugation, H_2SO_4 (20%, vol/vol) was added in already ultra-spin extract and again centrifuged for 15 min. The absorbance was recorded at 410 nm.

For estimation of peroxidase (POD) and superoxide dismutase (SOD) activities, samples were homogenized in phosphate buffer (0.5 M at pH 7.8) (Zhang, 1992). Ascorbate peroxidase (APX) activity was evaluated using the protocol of Nakano and Asada (1981) and CAT activity was estimated following Aebi (1984) method. A comprehensive procedure was elaborated by Abbas et al. (2017).

2.7. Estimation of metal content

Zn and Cr contents were estimated in roots, shoots and grain samples. The samples were digested using diacid HNO_3: $HClO_4$ (4:1 v/v). After digestion, Zn and Cr contents in samples were measured using atomic absorption spectrophotometer (AAS) for detection of metal content (Rehman et al., 2015).

2.8. Data analysis

SPSS Statistics software Version 21 was used for data analyses, and analysis of variance (ANOVA) at 5% probability level was conducted. Tukey's HSD post hoc test was performed for multiple comparison of triplicates.

3. Results

3.1. Growth and yield attributes

To investigate the impact of chromium stress on various morphological, physiological and biochemical parameters of wheat plants and to explore the mitigation potential of chromium resistant bacteria (*S. aureus* K1) in combination with ZnO NPs, the present study was conducted. It was observed that application of 200 mg/kg Cr decreased the shoot length, root length, shoot fresh weight and shoot dry weight by 53.9%, 54.4%, 61.8% and 74.7%, respectively whereas, bacterial inoculation at same Cr stress level improved the outcome of these variables by 53.7%, 56.5%, 66.1% and 67.7%, respectively compared to their respective controls (Fig. 1). An increase of 20.6%, 27.4%, 36% and 38.9% was observed in plant shoot and root length, shoot fresh and shoot dry weight, respectively with the application of 100 mg/L ZnO NPs over respective control. However, combined application of 100 mg/L ZnO NPs and bacterial inoculation increased shoot and root length, shoot fresh and shoot dry weight to 13.3%, 17.1%, 22.9% and 33.8%, respectively over respective control (Fig. 1).

Statistically significant increase in shoot (24.5%) and root (29.17%) length, shoot fresh (43.7%) and dry (53.7%) weight was observed in wheat plants with 100 mg/L ZnO application under 200 mg/kg Cr level, while combined application of 100 mg/L ZnO and *S. aureus* K1 improved these growth parameters by 25.7%, 35.9%, 49.8% and 52.9%, respectively at the same level of Cr stress (Fig. 1).

In the current investigation, non-significant impacts of hexavalent Cr (Cr^{6+}) were observed at 50 mg/kg of Cr level. Fresh and dry weight of roots, spike length, no. of tillers per plant and grain weight were found to be 27%, 28.9%, 20.9%, 7.6% and 11.6% higher, respectively over control by applying 100 mg/L ZnO while this increase raised to 46.1%, 30.4%, 40.5%, 28.1% and 34.5%, respectively with the combined application of 100 mg/L ZnO NPs and *Staphylococcus aureus* strain K1 compared to their respective controls.

Dose level of 200 mg/kg Cr reduced fresh and dry weight of root, spike length, no. of tillers per plant and grain weight by 51.6%, 50.4%, 27.3%, 24.9% and 58.6%, respectively while inoculation of *S. aureus* K1 along with 200 mg/kg Cr reduced the stress that is discernable as 44%, 53.8%, 26.9%, 37% and 34.7% decrease, respectively over respective controls. In contrast, 37.9%, 33.1%, 33.1%, 1.8% and 42.5% increase was observed in 200 mg/kg Cr with 100 mg/L ZnO application, respectively. Moreover, combined application of 100 mg/L ZnO with *S. aureus* K1 enhanced these attributes by 25.7%, 37%, 2.6%, 17% and 18.9%, respectively compared to respective controls (Fig. 2).

3.2. Photosynthetic pigments

Data of chlorophyll a, b, total chlorophyll and carotenoid contents are presented in Fig. 3. Statistical analysis of photosynthetic pigments showed significantly higher values in *S. aureus* K1-inoculated wheat plants under varying levels of Cr stress and ZnO NPs application. With the foliar spray of uninoculated plants with 100 mg/L ZnO NPs, an increase of 10.6%, 2.2%, 5.6% and 4.7% was recorded in chlorophyll a, b, total chlorophyll and carotenoids, respectively while compared with their respective control. However, inoculation of *S. aureus* K1 along with 100 mg/L ZnO NPs exerted relatively more synergistic effects on chlorophyll a, b, total chlorophyll and carotenoids by improving their values to 13.7%, 4.3%, 6.9% and 11.1%, respectively over their respective controls. Under 200 mg/kg Cr stress, values of the mentioned pigments decreased by 62%, 45.6%, 29.8% and 43.1%, respectively while *S. aureus* K1 inoculation at this Cr stress level reduced the toxic effects of Cr by dropping the values to 58.3, 41.6, 29.2 and 37.1, respectively.

3.3. Estimation of MDA, H_2O_2 and EL

Malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and electrolyte leakage (EL) declined by 18.4%, 15.6%, and 3.6%, respectively in uninoculated plants sprayed with 100 mg/L ZnO NPs in 0 mg/kg Cr application, while 27.7%, 19.4%, and 7.6% decrease with the combined application of 100 mg/L ZnO NPs and *S. aureus* K1 inoculation was observed in the described activities as compared to their respective control. Statistically significant differences among treatment levels of

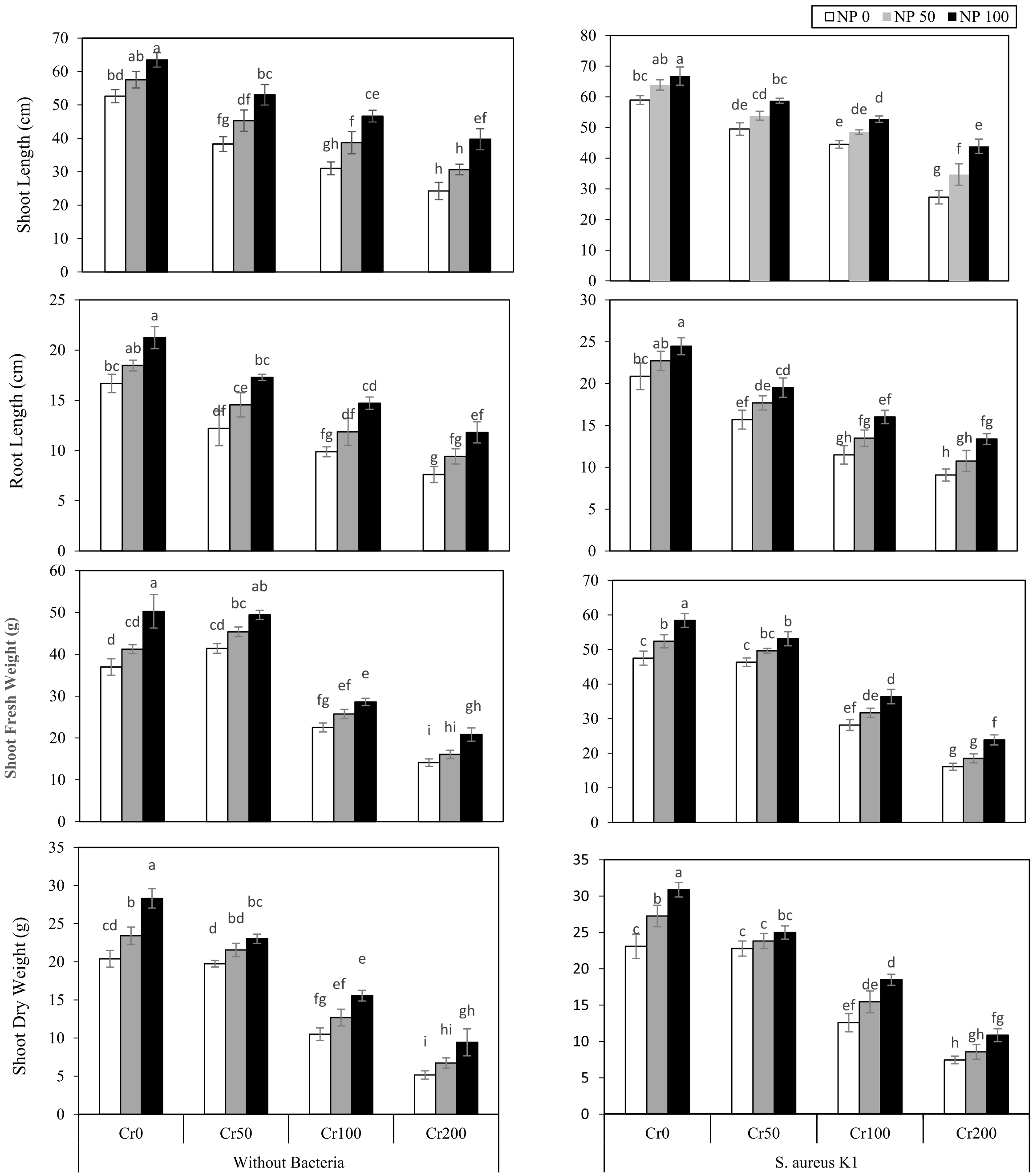

Fig. 1. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus K1 microbes on shoot length, root length, shoot fresh weight and shoot dry weight of wheat plant.

chromium, NPs and bacterial inoculation were observed for MDA, H_2O_2 and electrolyte leakage. Interaction among Cr stress x NPs levels, and Cr stress x microbe inoculation influenced the activities of the stated parameters. However, interaction of NPs x microbes, and Cr stress x NPs levels x microbe inoculation did not significantly persuade the MDA and H_2O_2 while EL values significantly changed under the interactive influence of these treatment factors.

At Cr stress level of 200 mg/kg, an increase of 207.6%, 134.2%, and 32.4% was recorded in MDA, H_2O_2 and EL values, respectively, while an increase of 237.1%, 138.1%, and 34.2% was observed with the bacterial inoculation at this Cr stress level. ZnO NPs of 100 mg/L concentration, on the other hand, improved these values by 164.9%, 103.5%, and 28.7%, respectively in uninoculated Cr-stressed (200 mg/kg) plants, but by 172%, 97.4%, and 30.4%, respectively in *S. aureus* K1 inoculated plants at the same Cr stress level (Fig. 4).

3.4. Antioxidant enzyme activities

Interaction of Cr stress levels x NPs x microbe inoculation did not produce significant change in the antioxidant enzymes activity however, interaction of Cr stress levels x microbe interaction was found to be more influential for significant change in SOD and POD activities. An increase of 12.8%, 24.2%, 6.5% and 9.3% in SOD, POD, APX and CAT activities, respectively, was observed with the foliar application of 100 mg/L ZnO NPs in uninoculated wheat plants. However, this increase further improved by 15.2%, 17.9%, 7.5% and 9.5%, respectively in inoculated wheat plants at the same level of ZnO NPs (100 mg/L). On the other hand, chromium stress reduced the antioxidant enzymes activity but this reduction was less pronounced in *S. aureus* K1-inoculated wheat plants compared to uninoculated ones. At 200 mg/kg Cr stress, SOD, POD, APX and CAT activities decreased in uninoculated plants by 96.4%, 88.5%,

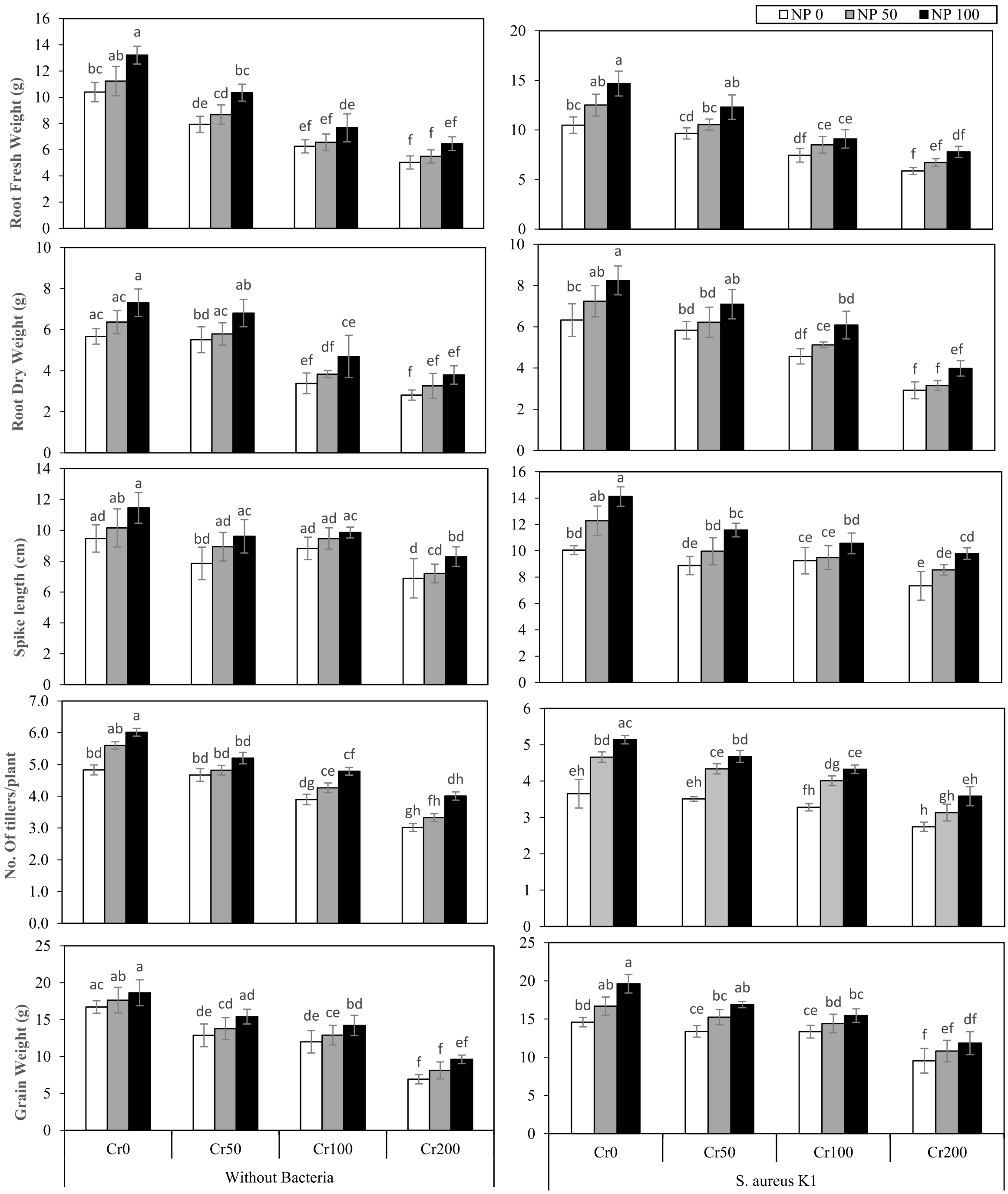

Fig. 2. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus K1 microbes on Root fresh weight, Root dry weight, Spike length, No. of tiller's/ plant and Grain weight of wheat plant.

57.4% and 67.7%, respectively while this decrease improved to 90.8%, 77.5%, 50.3% and 61.9%, respectively in *S. aureus* K1 inoculated plants at the same Cr stress level (200 mg/kg). Application of 100 mg/L ZnO NPs at this Cr stress level also influenced the antioxidant enzymes activity by reducing the extent of activity decline which further improved with additional inoculation of *S. aureus* K1. About 90.9%, 73.4%, 49.9% and 61.3% decrease in SOD, POD, APX and CAT activities, respectively was observed with the application of 100 mg/L ZnO NPs at 200 mg/kg Cr stress which improved to be 85.2%, 66.8%, 45.2% and 56.1%, respectively with the inoculation of *S. aureus* K1 (Fig. 5).

3.5. Accumulation of metals

Zinc contents in roots, shoots and grains increased to 8.5%, 15.5%, and 18.8%, respectively by applying 100 mg/L ZnO NPs in uninoculated wheat plants while these values were further improved to 10.9%, 10.1% and 17.4%, respectively with the combined application of 100 mg/L ZnO NPs and *S. aureus* K1 inoculation at 0 mg/kg Cr stress. Likewise, Zn contents in roots, shoots and grains of 200 mg/kg Cr stressed uninoculated plants decreased to 75.1%, 70.8%, and 87.3% which improved to 63.4%, 63.3%, and 78.5%, respectively with *S. aureus* K1 inoculation. Zinc contents in roots, shoots and grains also increased to 61.3%, 58.3%, and 79.9%, respectively with the application of 100 mg/L ZnO NPs at 200 mg/kg Cr stress level. However, inoculation with *S. aureus* K1 along

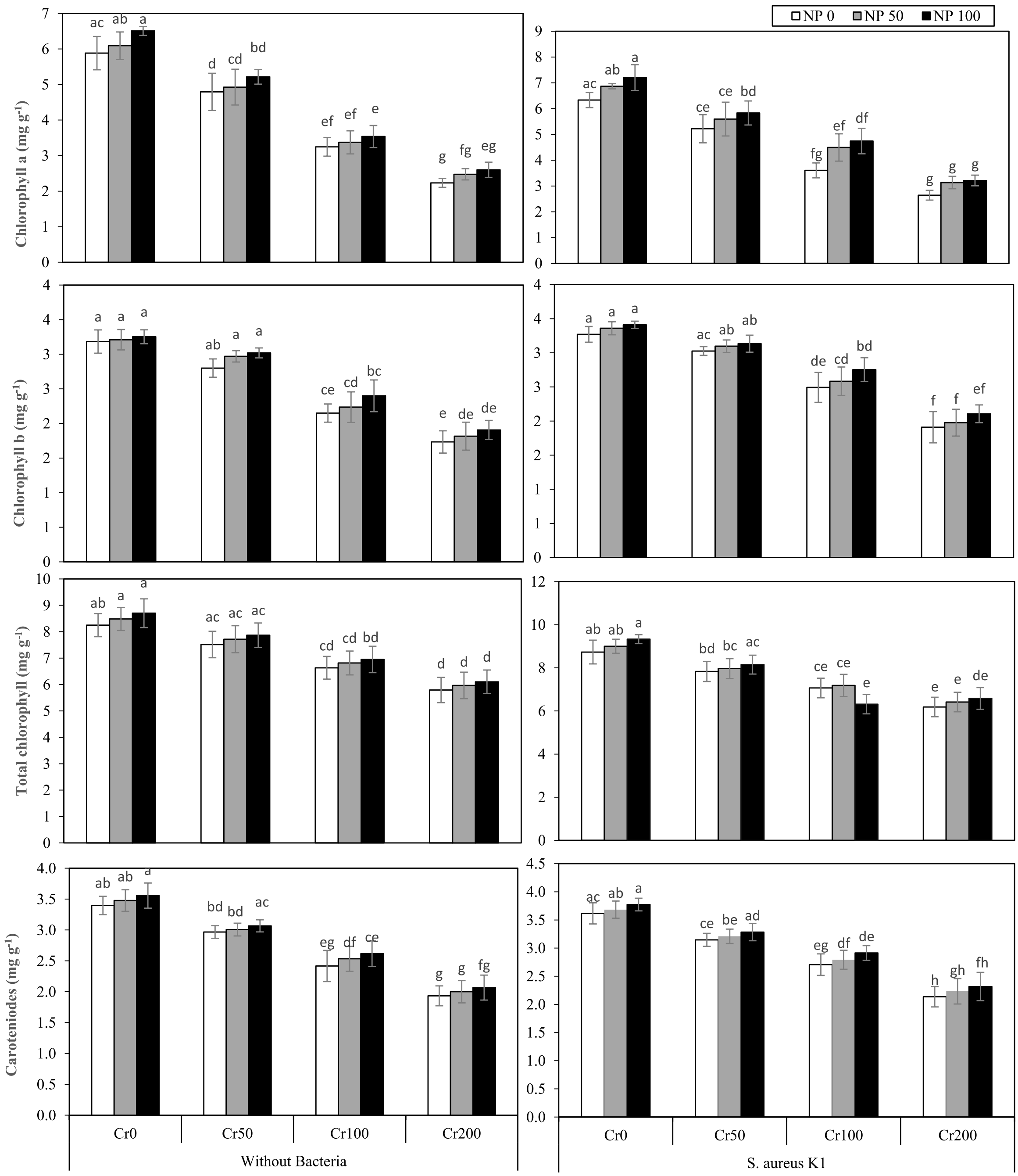

Fig. 3. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus K1 microbes on chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of wheat plant.

100 mg/L ZnO NPs reduced the extent of increase in these parameters by improving them to 52.9%, 50.8%, and 70.8%, respectively at Cr stress level of 200 mg/kg (Fig. 6).

A decrease of 8.1% and 76.5% in Cr content was observed in roots and shoots, respectively in uninoculated plants sprayed with 100 mg/L ZnO NPs, while *S. aureus* K1 inoculation along with 100 mg/L ZnO NPs decreased these values to 5.5% and 28.7%, respectively which shows the positive impact of *S. aureus* K1 inoculation. Similarly, Cr contents in roots and shoots were 171 mg/kg and 97 mg/kg in Cr stressed (200 mg/kg) uninoculated plants while inoculation of *S. aureus K1* showed a decrease of 152 mg/kg and 85 mg/kg respectively. It is also worth stating that more pronounced increase was observed in Cr concentration of roots compared to that of the shoots. At Cr stress level of 200 mg/kg and 100 mg/L ZnO NPs, an increase of 156 mg/kg and 90 mg/kg was observed in uninoculated root and shoot, respectively. However, inoculation with *S. aureus* K1 reduced the values to 141 mg/kg and 80 mg/kg, respectively at the same levels of Cr stress and ZnO NPs (Fig. 7).

4. Discussion

Chromium, having high redox potential, can easily change its oxidation states (Shahid et al., 2017), and damage the cell integrity by striking on membranes, lipids, proteins and DNA of cell (Tchounwou et al., 2012; Stambulska et al., 2018). Its translocation in plants depends upon its oxidation state, concentration and exposed plant species (Shahid et al., 2017). Moreover, its concentration is usually found higher in below ground plant biomass compared to above ground biomass due to its low mobility and lack of any explicit mechanism of its uptake in

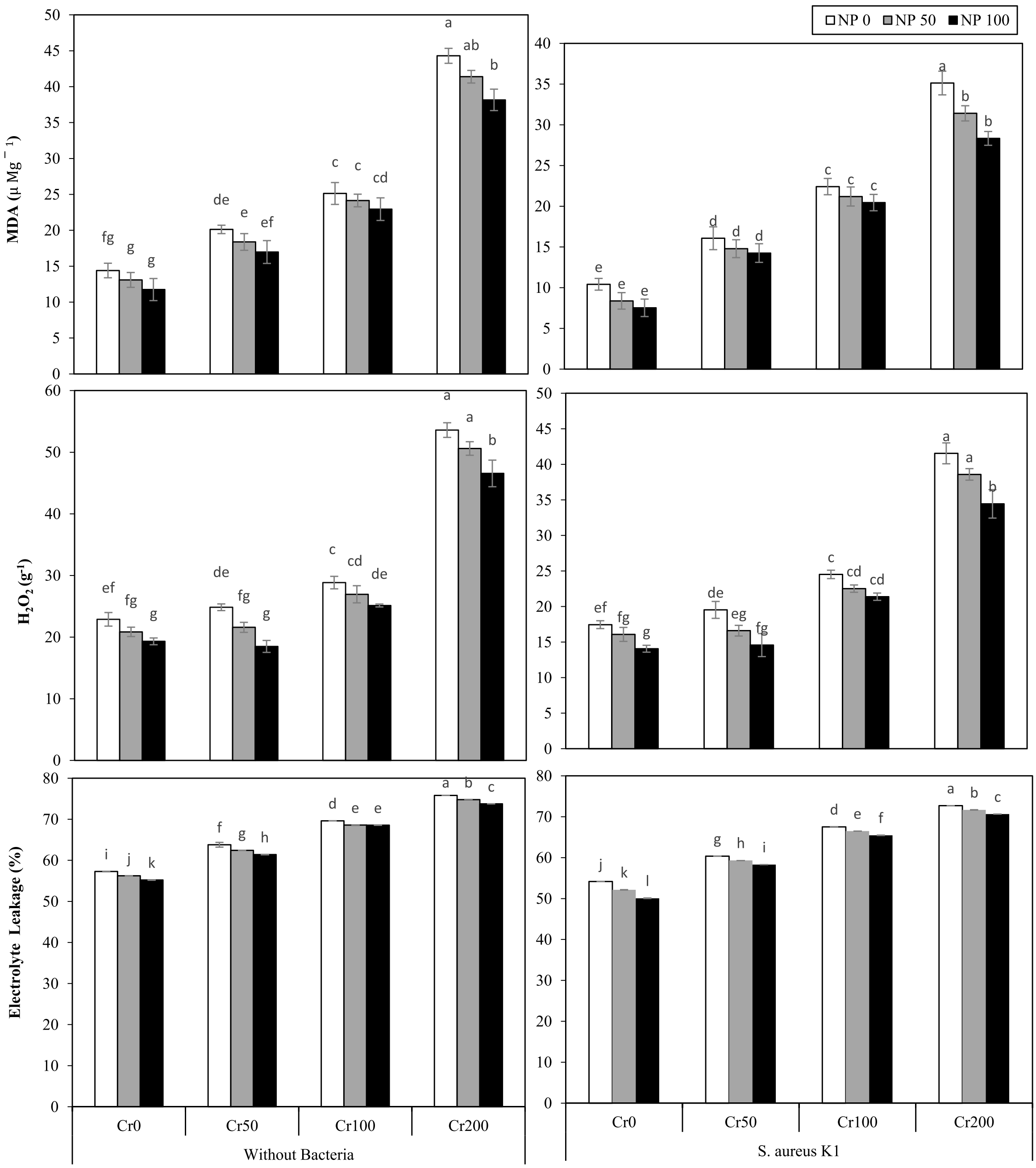

Fig. 4. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus microbes on Malondialdehyde (MDA), Hydrogen peroxide (H_2O_2) and Electrolyte leakage of wheat plant.

plants (Oliveira, 2012). Wheat variety Punjab 2011 being used in our study was found to be Cr tolerant at lower dose levels as reported by Datta et al. (2011) who recorded tolerance ability of various wheat varieties against Cr^{6+}. However, gradual reduction in plant growth responses was observed by increasing Cr dose levels that might be due to its toxic effects on various plant growth processes including modifications in ultrastructure, imbalance in water transportation, alteration in enzymatic activities and disturbance in mineral nutrients uptake (Chigonum et al., 2019; Anjum et al., 2017; Reale et al., 2016; Farooq et al., 2016; Ali et al., 2015a,b). In the current research scenario, reduced root size may have resulted due to higher levels of Cr to be accumulated into roots or structural and functional impairment of root endings (Ali et al., 2013, 2015a). Stunted shoot length, on the other hand, may have appeared due to the impaired water absorption and transport that affects nutrient absorption, plant height, biomass, oxidative stress and ultrastructure of chloroplast leading to reduced photosynthesis (Asati et al., 2016). Disturbance in growth parameters of wheat plants led to reduction in total chlorophyll contents that work as indicator of toxicity initiated by Cr. High levels of Cr stress reduce photosynthetic efficiency either by inhibiting chlorophyll biosynthesis or deteriorating chlorophyll contents as observed in many plants (Sharma et al., 2019). The reduced contents of chlorophyll which have direct impact on photosynthesis process due to chromium toxicity ultimately minimize the overall crop yield and production (Subrahmanyam, 2008; Chatterjee and Chatterjee, 2000; Wang et al., 2014). Similar findings of Cr toxicity were communicated by Panda and Choudhury (2005) showing compromised shoot metabolism on cellular level. Notable decrease in shoots length under Cr stress may have been resulted due to high concentration of Cr in roots cells and restricted cell division (Woolhouse, 1983; Shanker et al., 2004). In the current study, carotenoid content was

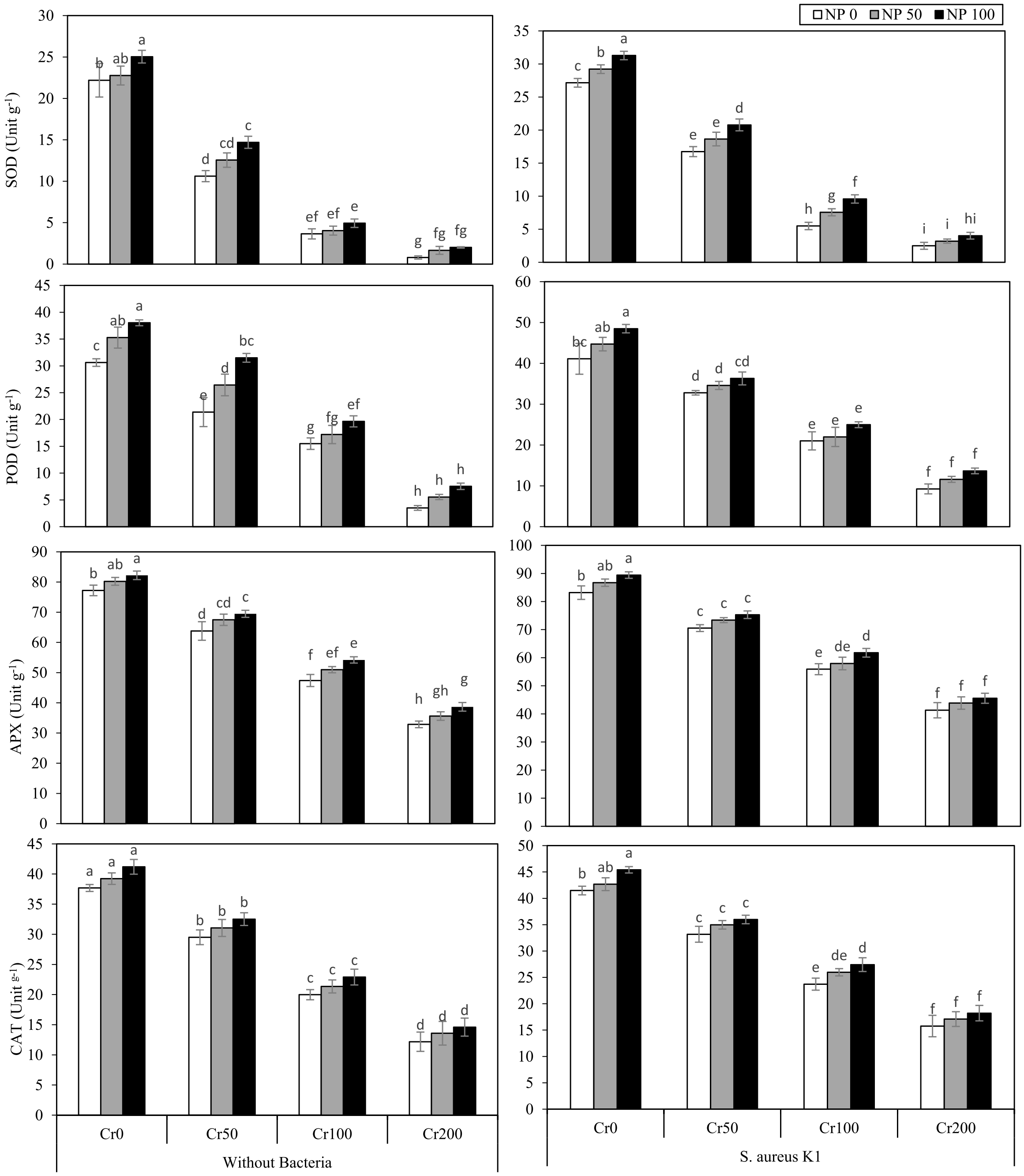

Fig. 5. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus K1 microbes on SOD, POD, APX and CAT of wheat plant.

also reduced due to stress caused by Cr heavy metal. Carotenoids are naturally helpful in preventing the chlorophyll from the damage caused by oxidation (Stahl and Sies, 2005). Chlorophyll and carotenoid contents can fall down in wheat crop due to the production of reactive oxygen species under heavy metal stress (Ehsan et al., 2014). It was also noted that MDA formation started to increase according to imposed Cr stress level which indicate the destruction or instability of cell membrane and initiation of oxidative stress that is associated with H_2O_2 and O^{2-} production (Wang et al., 2008). Oxidation of protein, peroxidation of lipids and disruption of the DNA and RNA strands along with obstruction in the enzyme's activities may result from the over-production of ROS (Shahbaz et al., 2018). ROS are generated under oxidative stress caused by Cr metal which produces MDA leading to disintegration of fatty acids in cell membranes (Malecka et al., 2001; Mittler, 2002). Under chromium stress conditions, intensive H_2O_2 generation with elevated MDA formation was observed in wheat plants that is in line with the findings of Zhang et al. (2010).

Zinc oxide is found nontoxic, inexpensive and safe to be used for multiple purposes compared to other metal oxides (Sakir et al., 2020; Kim et al., 2020). In agriculture, ZnO-NPs are supposed to act as a slow releasing Zn-fertilizer (Ditta and Arshad, 2016; Monreal et al., 2016) that can provide important microelement which improves soil fertility and plant growth as observed in soybean (Yusefi-Tanha et al., 2020), cotton (Esper Neto et al., 2020), coffee (Rossi et al., 2019) and wheat plants (Dimkpa et al., 2020). Foliar application of ZnO NPs act as Zn source to the plant leaves and take part in metabolic activities in plants as observed in soybean and tomato (Li et al., 2018) and sunflower (Li et al., 2019). Improved nutritional values and physiological attributes of

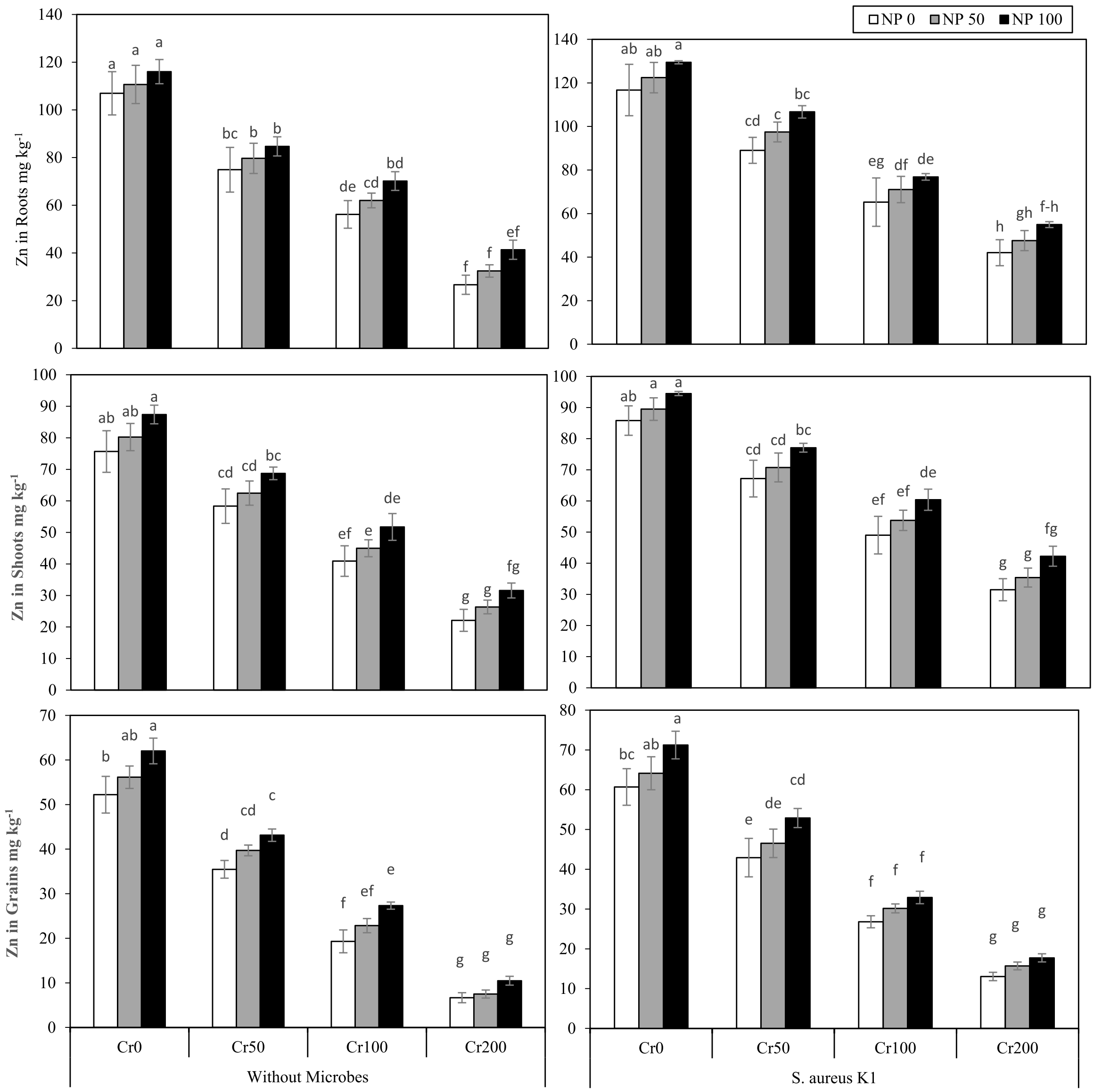

Fig. 6. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus K1 microbes on Zn in shoot, Zn in root and Zn in grain of wheat plant.

foxtile millet were also reported by foliar spaying with ZnO NPs (Kolenčík et al., 2019). Nanoparticles have ability to develop chloroplast, repair the photosystem II under stress conditions (Govorov and Carmeli, 2007; Singh et al., 2015; Salama et al., 2019). Increase in the growth of mung bean and chickpea has been reported with the application of 1–20 ppm ZnO NPs (Mahajan et al., 2011). Notable increase in cotton production by decreasing plant oxidative stress has also been reported with ZnO NPs application (Sing Brar et al., 2021). The relationship between heavy metals and ZnO-NPs can affect the bioavailability of toxic metals such as Cd and Pb as reported in lettuce (Sharifan et al., 2019) and cotton (Priyanka et al., 2021), however, it is needed to further explore the mechanism that works with the application of NPs (Venkatachalam et al., 2017). In the present work, applications of ZnO NPs enhanced SOD, POD, APX and CAT activities in Cr-stressed wheat plants and these outcomes are concurrent to the findings on maize (Rizwan et al., 2019), wheat (Adrees et al., 2021) and mustard plants (Rao and Shekhawat, 2014) where up-regulation of antioxidant enzyme and plant growth was concomitantly observed. To control the oxidative injury and initialize a defense system under heavy metals stress, plants have the mechanism of antioxidant enzymes like superoxide dismutase, peroxidase, catalases and ascorbate peroxidases (Nounjan and Theerakulpisut, 2012; Yildiz and Terzi, 2013). Increased content of Zn by the application of ZnO NPs substantially improves the antioxidants enzymes activities in plant leaves and can minimize the oxidative stress (Pavithra et al., 2017). NPs can effectively modify the enzyme activity. Inhibitory influence on ROS by application of metal NPs along with improved antioxidant activity and plant growth has also been reported in cotton (Priyanka and Venkatachalam, 2016).

In the current study, it is also anticipated that chromium-resistant *S. aureus* K1 may have reduced the hexavalent form of chromium into trivalent form. Particular mechanism of chromium reduction from hexavalent to trivalent form is not identified yet. However, the assumption of metal reduction may be supported by a number of prevailing mechanisms like Cr^{6+} detoxification by participation of intracellularly metabolism where chromate acts as electron acceptor for the sake of energy gain (Wani et al., 2007). Moreover, metabolic byproducts such as H_2S and enzymatic actions performed by bacteria may possibly help to reduce Cr^{6+} into Cr^{3+} (Fude et al., 1994; Cheung and Gu, 2003). Potential of *Pseudomonas olevorans* to reduce Cr^{6+} into Cr^{3+} in Cr-contaminated soil was reported by Mistry et al. (2009).

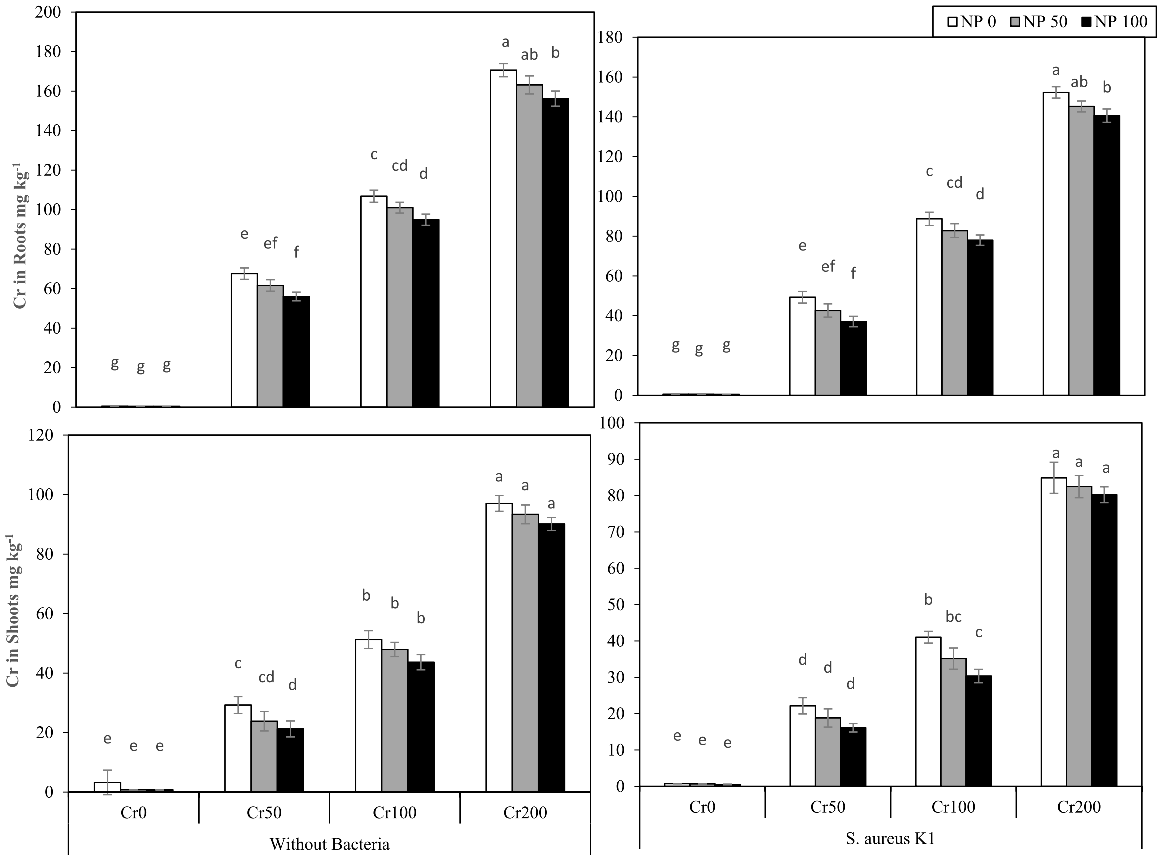

Fig. 7. Effect of different chromium levels (0, 50, 100, 200 mg/kg) and foliar spray of ZnO NPs (0, 50,100 mg/l) with and without S. aureus K1 microbes on Cr accumulation in roots and shoots of wheat plant.

Morales and Eliseo (2007) also reported capability of *Streptomyces sp.* strain CG252 to reduce Cr uptake. Plant growth promotion, Cr reduction from Cr^{6+} into Cr^{3+} and decline in Cr uptake were also reported in sunflower (Faisal and Hasnain, 2005). Recently, Silva et al. (2021) also reported improved plant growth and reduced Cr translocation in PGPR inoculated maize plant under Cr stress conditions. Mitigation of chromium toxicity with the combined application of PGPR and metal (Fe) fortification was reported by Danish et al. (2019) whose findings agreed with that obtained in the current investigation upon the application ZnO NPs and *S. aureus* K1 under Cr metal stress. Application of ZnO NPs along with PGPR inoculation has also positively influenced growth and yield of soybean as reported by Sharifi and Khoramdel (2016). The outcomes of the current study indicated the positive impacts of ZnO NPs and *S. aureus* K1 on nutrient uptake, antioxidant enzymes, physiological attributes, growth and yield of Cr stressed wheat plants. This indicates that the application of biotechnological approaches (i.e. microbes) along with nanotechnology can provide new opportunities to develop innovative bioformulations that can be exploited as environment-friendly and sustainable remedy for the management of plant stresses.

5. Conclusion

Our study exploited the potential of ZnO nanoparticle in a microbe (*S. aureus* K1) assisted phytoremediation which explored new avenues to deal with Cr-contaminated soils. This study indicated that *S. aureus* K1 and ZnO nanoparticles could reduce Cr stress by improving defense system and antioxidant enzymes activities. It can be concluded that application of *S. aureus* K1 and ZnO nanoparticles can be used to remediate the Cr-contaminated soil to get better quality and productivity of wheat.

CRediT authorship contribution statement

Shoaib Ahmad: Data curation, Resources, Formal analysis, Writing – original draft. **Shafaqat Ali**: Supervision, Resources, Funding acquisition, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Muhammad Rizwan**: Project administration, Data curation, Methodology, Software, Writing – original draft, Writing – review & editing**. Manar Fawzi Bani Mfarrej:** Conceptualization, Writing – review & editing. **Mohamed A. El-Esawi**: Methodology, Formal analysis, Writing – review & editing. **Muhammad Nafees**: Conceptualization, Writing – original draft, Writing – review & editing. **Muhammad Hamzah Saleem:** Formal analysis, Writing – original draft. **Aishah Alatawi:** Formal analysis, Investigation, Writing – review & editing. **Tahira Yasmeen**: Statistical analyses, Writing – original draft, Writing – review & editing. **Muhammad Waseem**: Resources, writing – review & editing. **Alia Anayat:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors are grateful for the financial support from the Government College University, Faisalabad and Higher Education Commission, Pakistan under HEC Project No. 20-3653/NRPU/R&D/HEC/14/437 and NRPU Project No. 5634/Punjab/NRPU/R&D/HEC/2016. The authors would like to express their deepest gratitude to University of Tabuk, for the technical support for this study.

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