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A nano-bioengineered cobalt oxide biostimulant mediated regulation of physiological, biochemical, and antioxidant mechanisms in *Zea mays*

Yun Wang[1], Tuba Tariq[2], Faisal Mahmood[3], Mansour Ghorbanpour[4]✉, Ghazala Mustafa[5]✉ & Murtaza Hasan[6]✉

Biogenic synthesized cobalt nanoparticles (NPs), dose optimization toxicity, and metabolic studies of *Zea mays* are very important before their application in the field. Here, we investigate the morphological, biochemical, and metabolic response of summer maize (*Zea mays*) against bulk cobalt chloride and *Withania-assisted* bioengineered cobalt NPs. It was found that cobalt chloride as bulk and concentration of 100 mg/L NPs inhibits growth *via* significant changes their metabolic and biochemical molecules. While biogenic assisted with *Withania*, cobalt NPs with concentrations of 50 and 100 mg/L have shown a significant increase in shoot length by 15% and 9% respectively. Root length was found to be decreased at 25 mg/L and 50 mg/L as compared to control. Fresh and dry weight was found to be increased at 25 mg/L and 50 mg/L. However, chlorophyll contents seemed to decline at 25 mg/L and increased at 50 mg/L. Carbohydrate content was found to be decreased at 50 mg/L and 25 mg/L by 76% and 70% respectively. Starch content was found to be increased at 25 mg/L and 50 mg/L by 28% and 33% respectively. Nitrate content was found to be decreased at 50 mg/L by 17%. However, higher tested concentrations showed a very much decrease in these compounds. Results displayed that a small quantity of cobalt oxide nanoparticles had a stimulatory impact on the seedling development while a higher quantity encouraged an inhibitory effect. 100 mg/L also showed an increase in activities when comparison was done against control. At 25 mg/L all activities were found to be maximum. This increased level suggests that the congregation of these secondary metabolites generates an oxidative response in plants when exposed to Cobalt oxide nanoparticles and cobalt chloride. However, further mechanistic research should be adopted as our experimental findings ruled out the generalized phytotoxicity of plants.

Keywords Nano fertilizers, Anti-oxidative, Metabolomics, Green synthesis, Nanobiotechnology

The agricultural sector is a vital component in a country's economic progress and sustenance of food security[1]. The scarcity of natural assets such as arable land, water as well as soil, coupled with the rapid growth of the world's population[2], poses a significant obstacle to the sustainable and efficient growth of agriculture[3–5]. To ensure that agriculture is both economically viable and environmentally sustainable, it is necessary to address several challenges[6,7], such as the non-renewable use of resources, impacts of climate change, and the overuse of chemical fertilizers[8]. An estimated 56% of the global population depends on agriculture to meet their basic

[1]Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China. [2]Department of Biochemistry, The Institute of Biochemistry, Bioinformatics and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. [3]Department of Botany, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. [4]Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-8-8349, Iran. [5]Depatment of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan. [6]Department of Biotechnology, The Institute of Biochemistry, Bioinformatics and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan. ✉email: m-ghorbanpour@araku.ac.ir; mghazala@qau.edu.pk; murtaza@iub.edu.pk

sustenance needs. Belonging to the grass family, *Z. mays* is a genus of flowering plants and is the most well-known species[9,10].

Corn (*Z. mays*) has been cultivated for over 10,000 years, making it one of the oldest domesticated crops, and remains a key staple for many regions. Its ability to withstand low-moisture conditions and nutrient-deficient soil makes it a valuable strategic crop for arid regions[11], where agriculture relies entirely on rainfall[12]. The impact of NPs on the growth of crops needs to be examined to ensure sustainable agricultural development[13]. Nanotechnology has become known as a possible remedy, offering innovative techniques to enhance crop productivity by introducing new agrochemical agents and delivery mechanisms while minimizing the need for pesticides[14,15]. Additionally, leaching, pollutants, and soil microbes can all be reduced using nanotechnology[16–18]. To maximize crop yield, it is crucial to explore new methods for synthesizing and applying nanoparticles that can enhance their effectiveness in agriculture[19,20]. This entails investigating the relationship between nanoparticles and agriculture by adopting innovative approaches. In addition to improving environmental quality, nanomaterials can aid in the detection and remediation of polluted areas, with only a few nanoparticles exhibiting adverse effects[21,22]. Because NPs are used continuously in so many different industries, there is a chance that they will leak into the environment and endanger ecosystems and negatively impact living things there[23–25]. Due to the intriguing characteristics of cobalt oxide, many researchers are interested in exploring its potential uses in agriculture and biomedicine[26]. Cobalt oxide (CoO) NPs possess antibacterial, antioxidant, and anti-inflammation properties, while biogenic cobalt oxide NPs demonstrate DPPH free radical scavenging potential[27–29]. They can also operate as an energy store, a cofactor for vitamin B12, and a nano-pesticide[30].

The precise mechanism underlying the ecotoxicity of these NPs is yet unknown. However, it seems to be primarily influenced by their form and other physical and chemical characteristics[16,31,32]. The toxicity of Co-NPs can result from either their direct uptake by cells or the presence of solubilized metal ions in the surrounding media. Cobalt oxide nanoparticles possess unique material properties due to their small size, which distinguish them from their bulk counterparts. Yet, achieving size homogeneity and proper dispersion of these NPs can be challenging. To synthesize CoO NPs, a green method was employed utilizing *W. coagulans* (Paneer), which is a magical herb that helps in curing diabetes[33,34]. This ancient plant is known to contain bioactive molecules such as withanolide, withaferin, and withacoagin[35,36], because of their decreased adverse effects, which have been utilized to create therapeutic medications for the prevention and treatment of numerous illnesses[37,38]. Using biological entities like bacteria, fungi, and plant extracts[39] in biosynthetic methods is a simple and cost-effective alternative to more complex chemical procedures for producing nanomaterials. This approach allows for the rapid, efficient, and low-cost generation of desired metal or metallic oxide nanoparticles, making it a promising avenue for nanomaterial synthesis. To date, the number of NPs present in the environment is still below toxic levels. However, potential health risks and environmental concerns associated with NPs should be thoroughly investigated before their widespread commercial use. In plants, there are two confirmed pathways for the uptake and translocation of nanoparticles: root-to-leaf and leaf-to-root pathways[40]. In some cases, nanoparticles have been reported to translocate from seeds or mature tubers. Despite this, the fate of nanoscale materials in terrestrial ecosystems has been the subject of very few investigations. It has become imperative to research the toxicity, transformation, solution, mobility, diffusion, and the presence of CoO NPs in complicated systems such as soil and plant systems. So, the focus of the present study was to evaluate the morphological, biochemical, metabolic, and adverse response of summer *Z. mays* against bulk $CoCl_2$ and biologically synthesized CoO NPs.

Materials and methods

Reagents, chemicals and glassware

Sigma company provided all of the chemicals and reagents needed for the biological production of cobalt oxide (CoO) nanoparticles, including *Z. mays*, ethanol (CH_3CH_2OH), methanol (CH_3OH), sodium hydroxide (NaOH), and DPPH.

Preparation of plant extract

The fresh plant of *Withania coagulans* (authenticated by Dr. M. Hasan. the Islamia University of Bahawalpur, and a voucher specimen (Code No: 1501 H) was deposited at the Herbarium of University) was cleaned three to four times with Mili Q water followed by drying up in the shade until it was completely dehydrated. The dehydrated plant material is crushed and ground with an electric grinder to get a refined powder for further use. Aqueous leaf extract was prepared using the plant material homogenized with 20 g of crushed leaves and 100 mL of distilled water. Subsequent heating was done for 30 min at 90–100 °C. The extract was filtered through Whatman filter paper to remove particulate material. The filtrate was then dried in an incubator at 37 °C and kept at 4 °C for further usage in the formation of nanoparticles through the biological method.

Biological synthesis of CoO NPs

Utilizing extract from *Withania coagulans* seeds as a reducing agent, CoO NPs were created through a green synthesis method. By using a UV-vis spectrophotometer and precipitate formation, the reduction of Co to Co + was verified. 10 milliliters of plant extract was combined with a 0.5 M cobalt chloride solution in flasks to create CoO NPs artificially. A color shift indicated that the mixture had undergone nanoparticle production after it had been heated to 80 °C for four hours on a hot plate with a magnetic stirrer. When the solution turned dark brown after 4 h, it was clear that CoO NPs were forming. Following a 20-minute centrifugation at 5000 rpm, the pellet was cleaned with ethanol and dried in a hot air oven at 80 °C for 24 h. A black powder of CoO NPs was the end product and was characterized for additional examination.

Characterization of CoO NPs

UV – Visible spectrometry

For the confirmation of the successful synthesis of CoO NPs using the green synthesis method, UV-visible spectrometry was conducted. The reduction of cobalt ions was constantly monitored by sampling aliquots at regular intervals and measuring the spectra on a UV-vis spectrophotometer (Epoch-BioTek Instruments USA) with a range of 300–700 nm. Spectra were recorded every 30 min to monitor changes over time, and a positive indication of nanoparticle synthesis was observed through the appearance of peaks between 550 –510 nm.

X-Ray diffraction analysis

For the confirmation of the size and structural properties of CoO NPs crystals, the diffracted intensities of the dried CoO NPs powder were measured using an X-ray diffractometer (D8, Advance, Germany). Measurements were conducted at 40 kV and 30 mA current with a scanning range of 20–80 degrees and a counting time of 0.3 s per count.

Zeta potential (ζ)

The zeta potential was calculated to assess the behavior and solubility of cobalt NPs in a solution. Deviations in the zeta potential (ZP) from normal values could result in interparticle attraction due to Van der Waals forces, leading to aggregation, coagulation, or flocculation.

Scanning electron microscopy

For morphology and microstructure identification of prepared CoO NPs, SEM (Quanta 250, 30 kV, FEI, Czech Republic, magnification up to 2000 times), was used. The shape and grain size of the produced cobalt oxide nanoparticles were assessed using SEM.

Experimental setup

Growth of Z. mays, in the existence of CoO NPs at different concentrations

To conduct the experiment, *Z. mays* seeds were purchased from Pioneer Seed Industries Limited ®. Lahore Pakistan. As a solid hydroponic medium, coconut peat was used in the seedling tray where the seeds were first planted. (Fig S1). Over the organic medium, a sufficient amount of water is sprinkled. Seedling trays were placed under hydroponic trays, ensuring that the required amount of water was available to the plant, which is a prerequisite for its optimal germination. Trays for seedlings were set up in a naturally sunny, 38–43 °C region. Seventy-eight seedlings were moved to net pots after three days. Additionally, these net pots were set inside unique plastic bottles that act as a nutrient pool. One thousand milliliters (1000 mL) of Hoagland solution were put into plastic bottles. To guarantee that the hydroponic medium interacts with the plant roots, the volume of liquid medium inside these plastic containers is changed every day. Every test plantlet received 10 milliliters of varying concentrations of cobalt oxide nanoparticles. Concentrations of CoO NPs were generated at 25, 50, and 100 mg/L in distilled water then ultra-sonication was utilized for dispersion. Cobalt oxide nanoparticles were applied continuously for 25 days, accompanied by a control and 0.1 M concentration of cobalt chloride. The plants were maintained at 40 °C with a 14-hour light/10-hour dark cycle in an open atmosphere. Following a Milli Q-water wash, *Z. mays* plant samples were promptly frozen at – 80 °C in preparation for additional testing. We investigated how *Z. mays* growth was impacted by cobalt oxide nanoparticles at concentrations of 25, 50, and 100 mg/L.

Determination of morphological parameters

Once the treatments were finished, plants were picked up and washed with Milli Q-water. Comparison between different physiological characteristics of plants treated with varying concentrations of cobalt oxide nanoparticles, cobalt chloride, and control was performed (Fig. S2). These features included leaf area, plant height, shoot and root lengths, fresh and dried weights, and chlorophyll content. Plant morphological measurements, such as height (cm), shoot length (cm), and root length (cm), were made with a ruler scale; the number of leaves and branches on the plants was counted visually. A computerized weight balance was used to measure the fresh weight of the plants. Plant samples were baked for three days at 70 °C to determine the dry weight.

Chlorophyll content exposed to CoO NPs

The chlorophyll content of a 0.2 g leaf extract homogenized with 80% (v/v) ice-cooled acetone using a pestle and mortar was calculated. The homogenate was centrifuged for 15 min at 10,000 rpm following two hours of dark refrigeration, as per Arnon's 1949 instructions. In comparison to an 80% acetone blank, the green supernatant's optical density was measured in a UV-vis spectrophotometer at wavelengths of 663 and 645 nm[41]. The following formula was used to measure the chlorophyll contents.

$$Chlorophyll\ a\ = 12.7\,(A663) - 2.69\,(A645)$$

$$Chlorophyll\ b = 22.9\,(A645) - 4.68\,(A663)$$

$$Total\ Chlorophyll = 20.0\ D\,(A645) + 6.10\ D\,(663)$$

Estimation of antioxidant potential

Using a pestle and mortar, a dried plant sample was mashed. In Eppendorf tubes, suspension was made using DMSO (10 mg/ml). Tubes were centrifuged for 10 min at 10,000 rpm. Using the produced supernatant, various

antioxidant activities (total antioxidant potential[42], total reducing power[8], and DPPH-based free radical scavenging activity[43] and non-catalytic antioxidants (phenolic and flavonoids) were assessed[42].

Statistical analysis

The varied concentrations of biologically generated CoO NPs were evaluated for relevance using the least significant difference (LSD) test. When the *P*-value was less than 0.05, differences were deemed statistically significant. Tukey's significant difference (HSD) and two-way Analysis of Variance (ANOVA) appropriate for CRD and correlation coefficient were used to assess a significant difference between treatments at $P \leq 0.05$. All the data was analyzed by using IBM SPSS statistics software version 20.

Results and discussions

Cobalt oxide NPs were prepared using *Withania coagulans* plant extract. Figure 1 summarizes the whole synthesis, characterization, and experiment with physiological data.

In this study, CoO NPs were fabricated with the use of *W. coagulans* as a bio-reducing agent. The synthesized CoO NPs, are thoroughly characterized by techniques such as UV-vis spectrometry, scanning electron microscopy (SEM), and X-ray diffraction (XRD). Furthermore, the application of CoO NPs for biochemical metabolomics and antioxidant potential in *Z. mays* was investigated.

Characterization of CoO NPs

To ensure the amalgamation of cobalt oxide NPs by biological process, UV-vis spectroscopy was carried out. Because the reaction mixture had begun and there was no reducing agent present, the control group did not exhibit any peak (black spectra) as its precursor of CoO NPs. Initially, the extract had a light-yellow color which turned to dark brown as it mixed with the cobalt oxide precursors, indicating the formation of CoO NPs. UV-vis spectra of the reaction mixture primarily showed a weak absorbance (red and green spectrum), which became sharper and clearer with time (1–5 h), showing an absorption band at 500–510 nm as shown in blue spectra (Fig. 2a). The fabrication of CoO NPs was achieved by adding the extract of plant (*W. coagulans*) to a solution of cobalt chloride ($CoCl_2$), resulting in the solution color changing from yellow to brown due to the reduction of $CoCl_2$ to cobalt ions (Co+). The cobalt nanostructure's surface plasmon response at several time points was identified as the cause of the absorption band in the 300–700 nm range, and stable nanoparticles were obtained after 5 h. The Present results support and endorse the former absorption spectral profile[26,44–46].

XRD was used to determine the cobalt oxide nanoparticle's crystalline structures. The CoO NP's (220), (400), and (422) planes were identified as the diffraction peaks at 2θ degrees, which were measured at 31.2, 40.1, and 45.4°. CoO NPs displayed superior development along the z-axis direction, as seen by the stronger peak on the

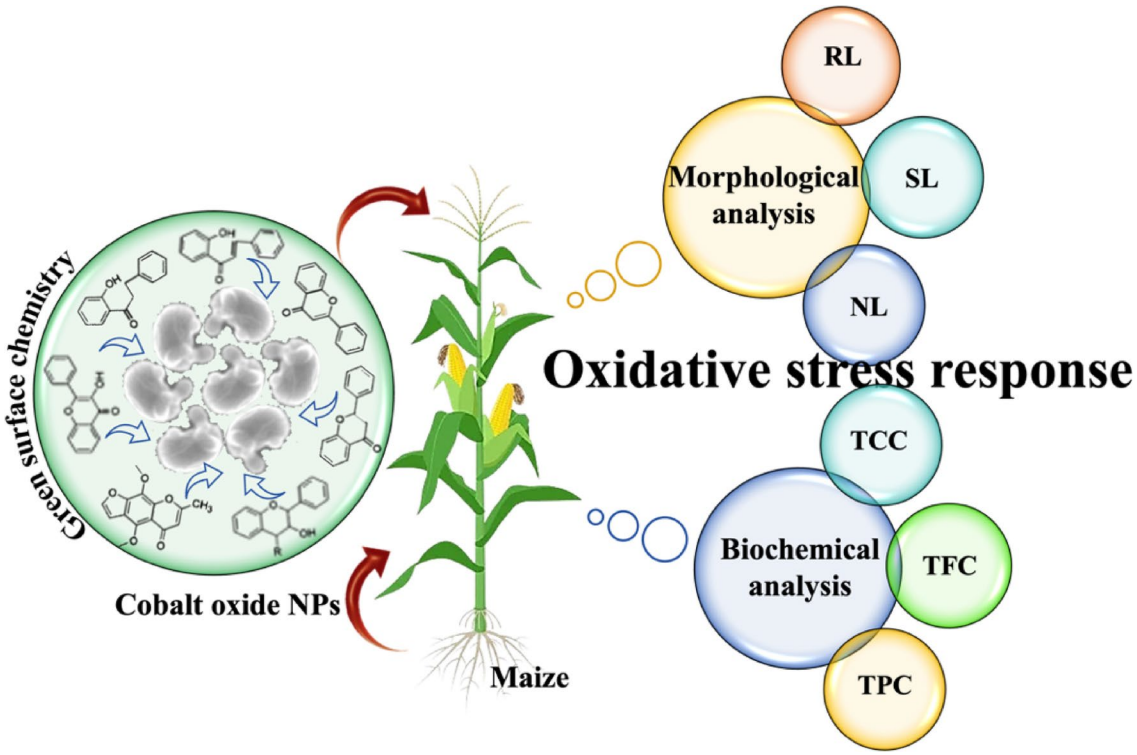

Fig. 1. Illustration of *W. coagulans* assisted Cobalt oxide NPs to regulate the physiological, biochemical, and antioxidant mechanism in *Zea mays*. [RL (root length), SL (shoot length) and NL (number of leaves), TCC (total chlorophyll contents), TFC (total flavonoid contents), TPC (total phenolic contents)].

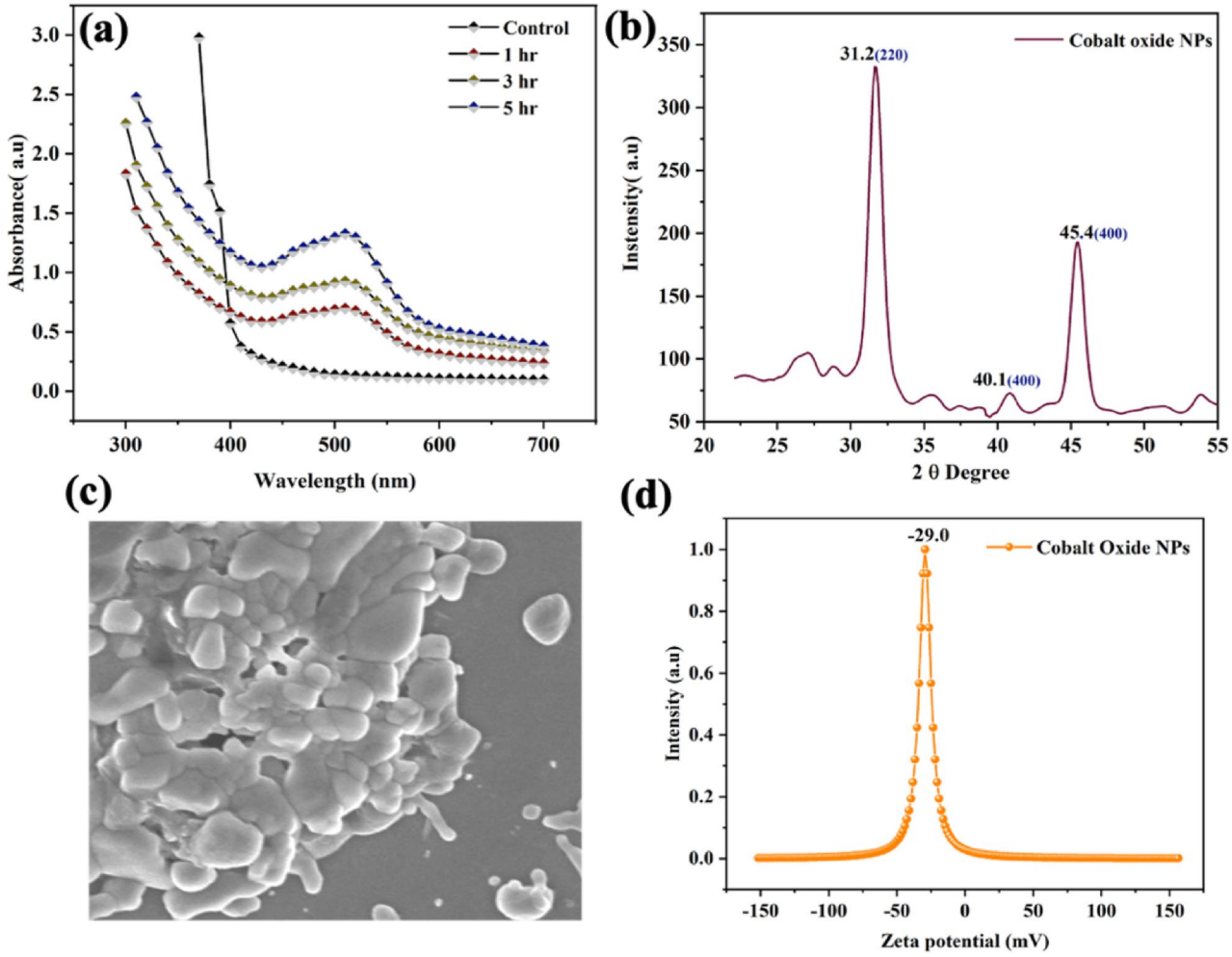

Fig. 2. Characterization of CoO NPs from *W. coagulans* extract. (**a**) UV – vis spectrum of CoO NPs. (**b**) XRD pattern of CoO NPs. (**c**) Scanning electron micrographs of CoO NPs. (**d**) Zeta Potential of CoO NPs.

(220) plane compared to the other peaks. The green synthesis based on *W. coagulans* produced slightly larger peaks, which at a glance indicates a tiny particle size[47].

Mathematically, the lattice constants *a* and *c* prove the hexagonal wurtzite structure of the biologically synthesized CoO NPs in relation[48–50]. The average ball size obtained for CoO NPs was 26 nm in the green synthesis method. Based on the diffraction peaks, it is clear that the formed CoO NPs were in a hexagonal phase, which was recorded with the standard hexagonal wurtzite crystalline CoO NPs with the reported JCPDS card no. 00-089-7102. Furthermore, the XRD results show that none of the samples have any characteristic peaks due to impurities, which shows that the grown samples have an outstanding crystalline nature (Fig. 2b). The absorption peak of the green synthesized CoO NPs was broad, which can be ascribed to the poly isolated nature of the CoO NPs[51].

The surface morphology of the synthesized cobalt oxide nanoparticles was examined using SEM at a resolution of 500 nm. Figure 2c shows the SEM image of cobalt oxide NPs at a resolution of 1 µm. The prepared cobalt oxide nanoparticles were observed to be in the form of nanoballs (Nbs). The SEM images revealed non-uniform size and distribution of the nanoballs, with variations in shape and size. This finding is consistent with a previous study[52,53].

Zeta potential (ZP) measurement, an analytical technique used to determine the surface charge in the colloidal solution of NPs with values > + 25 and < – 25 mV, generally has a high degree of stability. In our study, the green-synthesized CoO NPs exhibited a ZP value of – 29 mV, which falls within the stable range (Fig. 2d). This indicates that the CoO NPs have a high degree of stability, consistent with previous research[50,54,55].

Effect of CoO NPs on morphological properties of Zea mays

The application of CoO NPs on *Z. mays* seedlings showed an insignificant impact on their biomass properties. The adverse effect of CoO NPs was conspicuous for both root and shoot length Fig. S3a). The optimal response for shoot length was observed at 100 mg/L over control. For 50 mg/L a slight increase was observed in shoot length by 15% and for 25 mg/L and $CoCl_2$ reduction was observed to be 8% and 9% respectively (Fig. S3b).

Reduction in stem elongation was found to be associated with lower availability of the above-ground dry biomass when compared with control. However, no lethal effects were observed even at higher concentrations[56].

For root length a 13% reduction was observed in $CoCl_2$ treated plants. At 25 mg/L reduction was 16% and for 50 mg/L the reduction was 4% as compared with control (Fig. S3c). Effect of Cobalt oxide NPs on chlorophyll content (chlorophyll a and b, total chlorophyll) of *Zea mays* showed significant increase above control. At 25 and 50 mg/L doses of cobalt oxide NPs, chlorophyll **a** increased by 5% and 51% as compared to control (Fig. 3a–c). A conspicuous difference was observed between 25 and 50 mg/L. Nevertheless, at the highest tested concentration of 100 mg/L, chlorophyll **a** decreased by 46%, and chlorophyll b increased by 29%. In the case of cobalt chloride, chlorophyll **b** content increased by 34%, and chlorophyll **a** decreased by 84% over control, same trend was seen in an already published work[57]. A similar trend was seen in total chlorophyll content where 50 mg/L showed an increase of 23%. While 100 mg/L and cobalt chloride showed a significant decrease over control.

The fresh and dry weight of *Z. mays* seedlings is increased with the increased in concentration of CoO NPs. At 50 mg/L and 100 mg/L the fresh weight was found to be increased by 15% and 9% respectively over control (Fig. 3c). No major increase was observed in the dry weight of *Z. mays* seedlings at the concentrations of 25 mg/L and 100 mg/L. At 100 mg/L the fresh weight was increased by 9% but the dry weight decreased by 5%. A severe decrease in both fresh and dry weight was observed for $CoCl_2$ by 18% and 48% over control. It has been observed that CoO NPs toxicity increases with an increase in the concentration of nanoparticles[58,59].

For plant height, the application of cobalt oxide nanoparticles increases plant height when it is given at 50 mg/L. At 100 mg/L plant height was also increased as compared to control. There wasn't a significant decrease between CoO NPs and $CoCl_2$ treated plants. At 50 mg/L increase in plant height (16%) was observed as compared to control (Fig. 4a). At 25 mg/L and 100 mg/L, no significant increase was observed which was approximately 3% and 8% respectively (Fig. 4a). Current work also matched with previous reports regarding the nanoparticles[60].

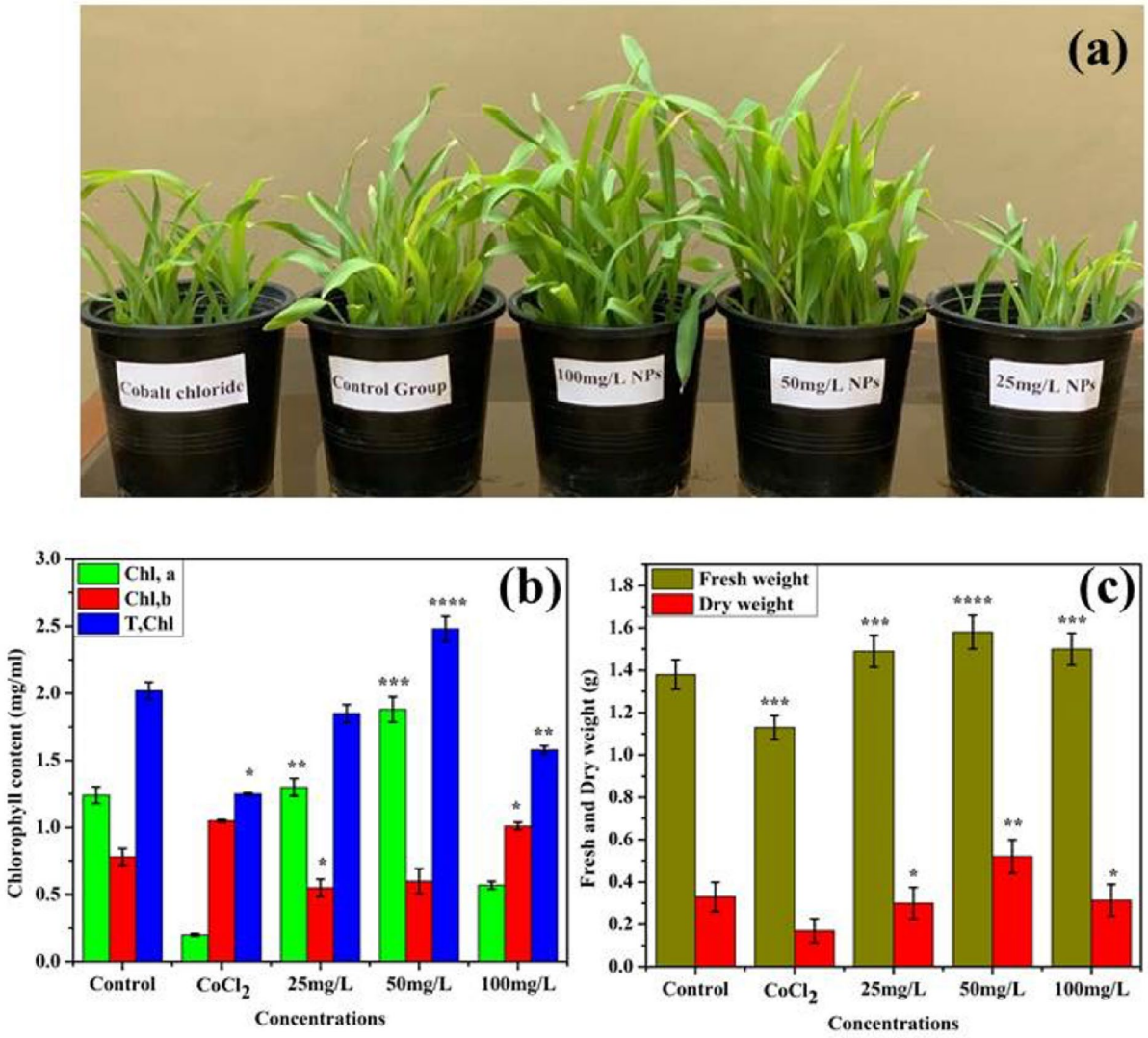

Fig. 3. The impact of CoO NPs treatments on whole plant (**a**), chlorophyll content (**b**) and (**c**) fresh and dry weight of Zea mays.

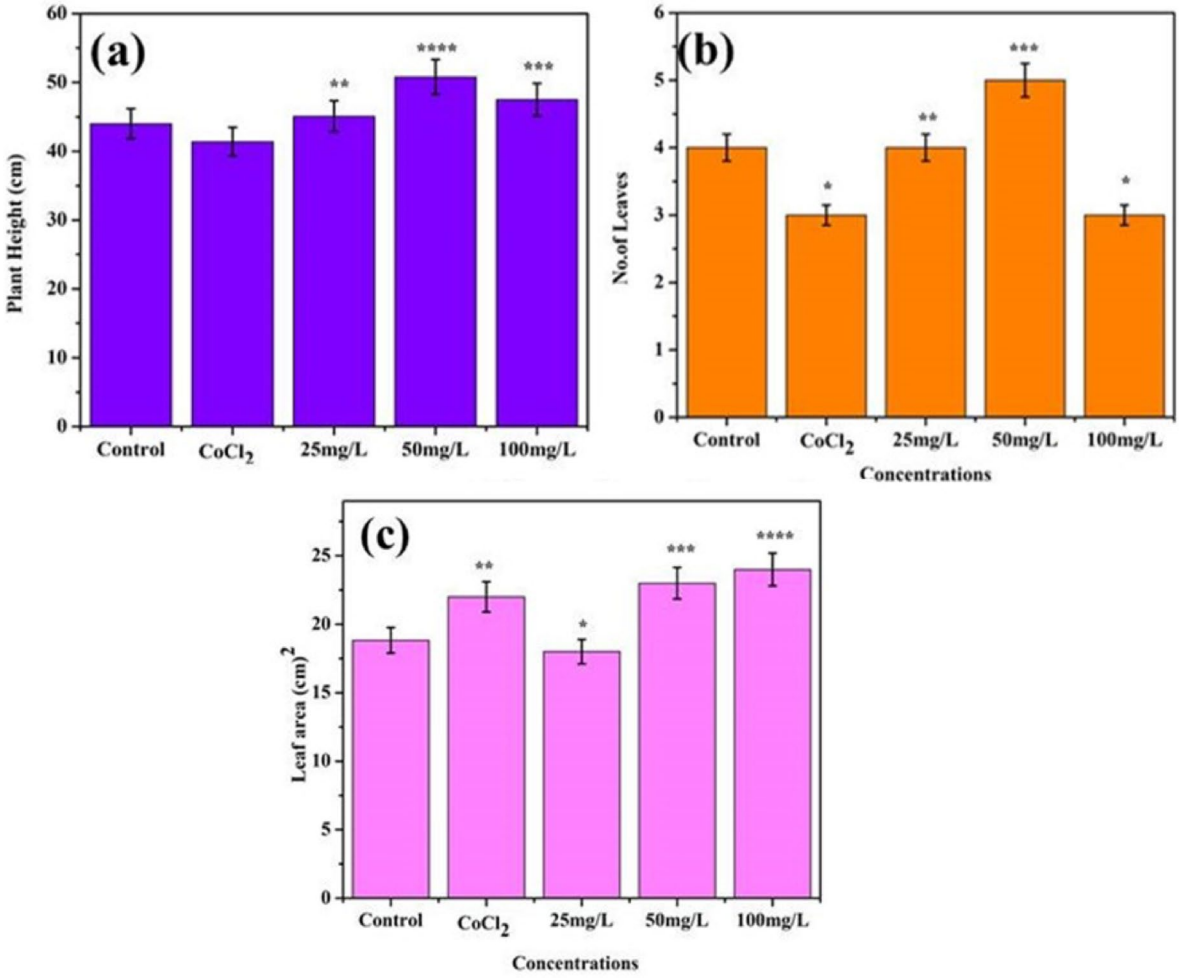

Fig. 4. Effect of CoO NPs on plant height of *Z. mays* (**a**), No. of leaves (**b**), leaf area (**c**).

In the case of leaves number per plant, it was found that at 50 mg/L there was no significant increase in leaves as compared to control (Fig. 4b). The leaf area was found to be increased at 50 mg/L as compared to the control (Fig. 4c). At 25 mg/L the leaf area was found to be decreased by 5% as compared to the control. While at 100 mg/L and $CoCl_2$ leaf area was increased by 28% and 17% respectively as compared to control. These results were supported by previous study[61].

Effect of CoO NPs and $CoCl_2$ on the biochemical parameters of *Z. mays*

For 25 and 50 mg/L cobalt oxide NPs treatment, the plant showed a 70% and 76% decrease over the control. At the highest tested concentration of 100 mg/L cobalt oxide NPs treatment substantial decrease in carbohydrate content (96%) was observed (Fig. 5a). On the contrary cobalt chloride treatment showed significant reduction by 51% when compared to the control. The reduction in carbohydrate content of the tested plant at higher doses may be attributed to an increase in the toxicity of nanoparticles thus causing a decline in growth[62].

The effect of $CoCl_2$ and cobalt oxide NPs on starch content is illustrated in (Fig. 5b). Two tested concentrations of cobalt oxide NPs, 25 and 50 mg/L showed significant change over control. The highest tested treatment of cobalt oxide NPs, 100 mg/L showed a significant decrease (9%) compared to control. $CoCl_2$ treatment showed a significant decline (32%) in starch content. At 25 and 50 mg/L starch content increases by 28% and 34% respectively. The decrease in carbohydrate content and increase in starch content represent the possible stress indicators that could provide energy and solutes for osmotic regulation[62,63].

At 25 mg/L nitrate content decreased by (6%) (Fig. 5c) and at 50 mg/L the decrease was (18%). While highest tested treatment of cobalt oxide NPs, 100 mg/L showed a significant reduction (22%) compared to the control. $CoCl_2$ treatment showed significant decay (34%) in nitrate content. The decrease in nitrate content may be attributed to increased toxicity in plants[64–66].

Antioxidative response of *Z. mays* to CoO NPs and $CoCl_2$

The immune response of plants to stress and other abiotic factors is very important to cope with their system. Nanoparticles can boost the immunity of plants against environmental factors, for this, the total reducing potential was found to be increased in 25 mg/L by (4%) as compared to the control. A 30% significant reduction

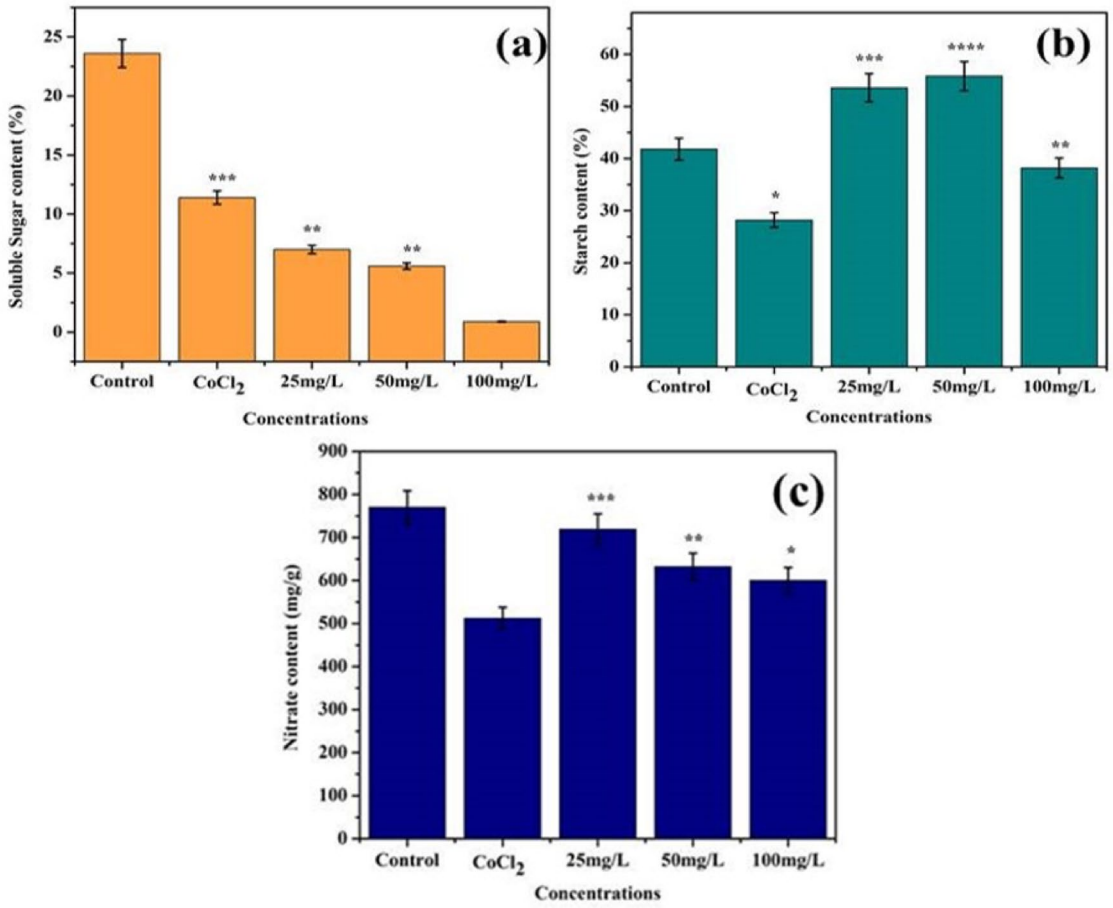

Fig. 5. Effect of CoO NPs on sugar contents (**a**), starch content (**b**), nitrat content (**c**).

was observed at 50 mg/L (Fig. 6a) in TRP. No change was observed in control and cobalt chloride-treated plants. In the case of free radical scavenging activity at 25 mg/L free radical scavenging activity increased (5%) as compared to control (Fig. 6b). For Cobalt chloride and 100 mg/L, this increase was non-significant. At 50 mg/L this activity was decreased by (12%).

Total Phenolic Content is very important for plants to enhance the immune system. Total phenolic content was increased by 25 mg/L (10%) as compared to control (Fig. 6c). No change was observed for 50 mg/L as compared to control but at 100 mg/L and cobalt chloride-treated plants, a significant increase was observed 11% and 18% respectively. Total Flavonoid Contents are also important, Total flavonoid contents were increased by 7% in 25 mg/L as compared to control. At 50 mg/L and 100 mg/L, the flavonoid content decreased by 9% and 13% respectively (Fig. 6d). For cobalt chloride, the decrease was 10% in flavonoid contents. The reduction in all antioxidant potentials causes toxicity in *Z. mays* plants due to CoO and $CoCl_2$[67–69].

Correlation

The correlation analysis was compared in terms of morphological, physiological, biochemical, and antioxidant response in *Z. mays* in response to Withania-assisted oval-shaped cobalt NPs (Fig. 7). In morphological parameters, such as root, length, shoot length, and other parameters were correlated and it was found that plant height in *Z. mays* was observed non-significantly ($p < 0.01$) associated with number of leaves. As the number of leaves increased, plant height also increased with the strength of correlation − 0.05. Plant height was observed positively significantly correlated with the number of branches. The number of branches was improved with the strength of correlation − 0.11 as the height of the plant increased. Plant height was observed non-significant correlated with shoot length-0.04.

As plants grew taller, there was a stronger link between the plant's branch length and height. A significant positive association was found between root length and plant height. Plant height was positively correlated with an increase in root length, with an asset of 0.14. The relationship between plant height and fresh weight was found to be non-significant. The fresh weight of plants was reduced with the strength of correlation − 0.57 as the height of the plant increased. Plant height was observed positively significantly correlated with the dry weight of plants. The dry weight of plants was decreased with the strength of correlation − 0.61 as the height of the plant increased. Chlorophyll content was observed non-significantly correlated with plant height. As plant height increased the chlorophyll content which is a physiological parameter, also reduced significantly by − 0.12. Chlorophyll b and

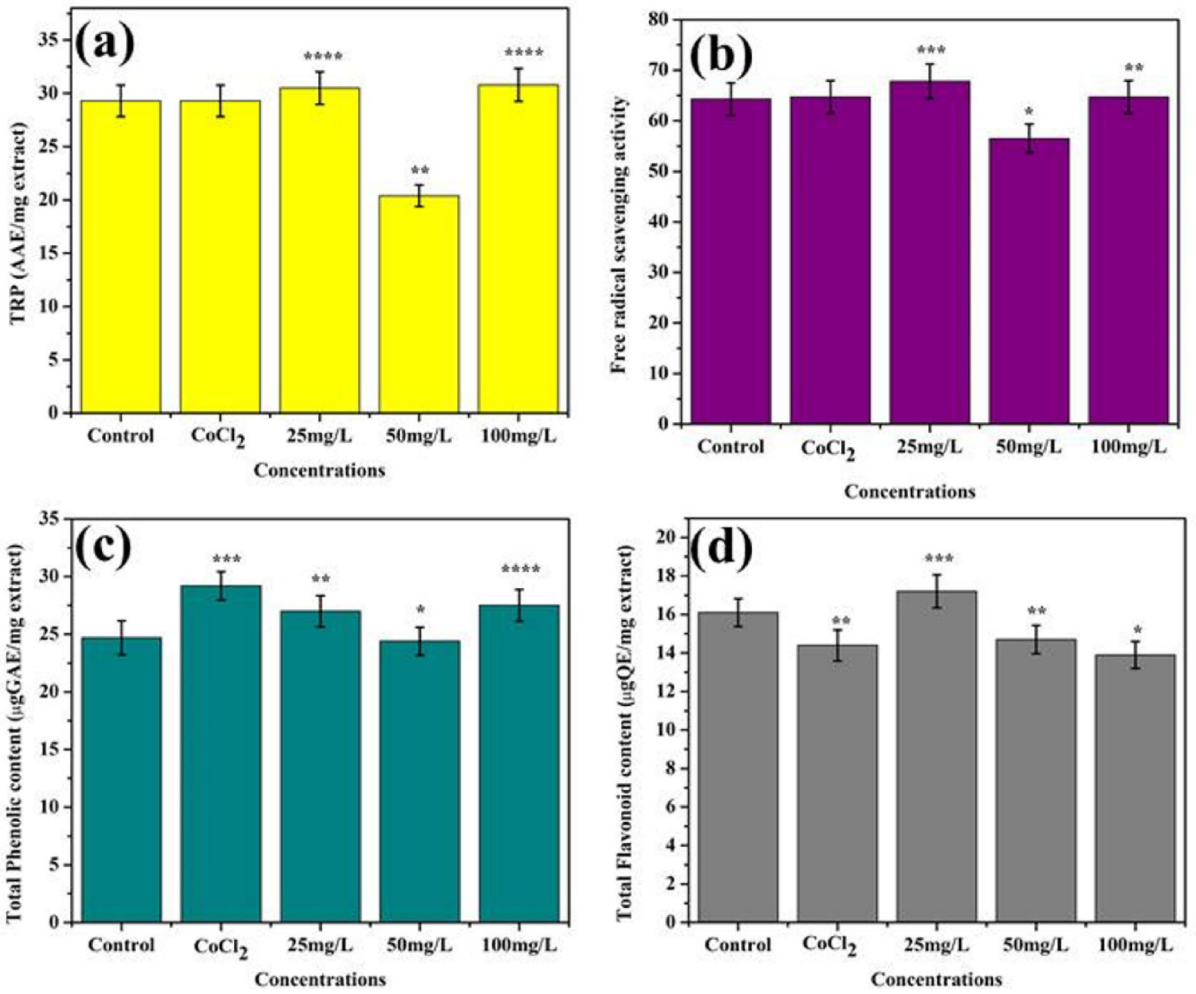

Fig. 6. Effect of CoO NPs on TRP total antioxidant content (**a**) Free radical scavenging activity (DPPH) (**b**), TPC total phenolic content (**c**), TFC total flavonoid content (**d**).

total chlorophyll levels were estimated significantly higher as plant height increased from 0.22 to 0.24. TPC, TFC, DPPH, TAC, and TRC were observed to reduce with the strength of an increase in plant height as estimated at −0.22, −0.27, −0.38, −0.02, and −0.27 respectively. The current result was also compared to recent studies comparison[51,70,71]. Increased salt accumulation was noted as significantly correlated with plant height at 0.25. The number of leaves showed a positively significant correlation with the number of branches. The number of branches increased with the strength of correlation 0.51 as the number of leaves increased. The number of leaves was significantly correlated with shoot length. Shoot length increased with the strength of correlation 0.40 as the number of leaves increased. The number of leaves was observed positively significantly correlated with root length. Root length showed a remarkable change with the strength of correlation −0.56 as the number of leaves increased. The number of leaves was noticed expressively correlated with fresh weight. As fresh weight increased the number of leaves also increased to 0.23. The number of leaves was observed significantly correlated with the dry weight of the plant. The dry weight of plants showed a remarkable change as the number of leaves increased by 0.16. The number of leaves also showed a positive correlation with chlorophylls a, b, and total chlorophyll levels were noted as 0.71, 0.44, and 0.52 respectively. As the number of leaves increased the chlorophyll contents positively increased and showed a significant correlation. TPC, TFC, DPPH, TAC, and TRP contents which are antioxidant parameters, showed no remarkable or significant correlation with the increase in the number of leaves as previous results reported[14,15,44,72–75]. Salt absorption by plants showed a significant correlation with the strength of the number of leaves as −0.42. The number of branches was non-significantly correlated with the length of shoot, fresh weight, root length, and dry weight of plants at −0.15, −0.20, 0.11, and 0.08 respectively. A non-significant decline was observed in chlorophyll a, b, and total chlorophyll contents as correlated to the number of branches 0.03, −0.02, and 0.10 respectively. TPC, TFC, DPPH, TAC, and TRP showed no significant correlation with the increase in the number of branches at 0.05, −0.27, −0.32, −0.24, and 0.21 respectively. Shoot length was positively significantly correlated with root length. Root length increased with the strength of correlation 0.74 as shoot length increased. Shoot length was negatively significantly correlated with the fresh weight of the plant. The fresh weight of plants was observed to increase with the strength of correlation −0.30 as shoot length increased. Shoot length was positively significantly correlated with the dry weight of plants. The

For each parameter:
N = 15
df (two-tail) = 15-2 = 13
Critical value ($r_{0.05}$) = ± 0.31

(a) *Zea mays*

SOV	PH	NL	NB	SL	RL	FWP	DWP	Chl a	Chl b	Total Chl	TPC	TFC	DPPH	TAC	TRP
NL	-0.05														
NB	-0.11	0.51													
SL	-0.04	-0.40	-0.15												
RL	0.14	-0.56	-0.20	0.74											
FWP	-0.57	0.23	0.11	-0.30	-0.40										
DWP	-0.61	0.16	0.08	-0.21	-0.30	0.98									
Chl a	-0.12	0.71	0.03	-0.55	-0.46	0.48	0.47								
Chl b	0.22	0.44	-0.02	-0.51	-0.34	0.11	0.13	0.70							
Total Chl	0.24	0.52	0.10	-0.62	-0.62	0.37	0.32	0.71	0.77						
TPC	-0.22	0.05	0.05	0.30	0.09	-0.11	-0.10	-0.17	-0.55	-0.48					
TFC	-0.27	-0.28	-0.27	-0.10	0.06	0.26	0.24	0.07	-0.30	-0.11	-0.06				
DPPH	-0.38	0.00	-0.32	0.33	0.20	0.36	0.45	0.30	0.03	-0.06	0.44	-0.15			
TAC	-0.02	0.01	-0.24	-0.15	-0.02	-0.18	-0.15	0.32	0.17	0.10	-0.23	0.55	-0.20		
TRP	-0.27	0.20	0.21	-0.08	-0.47	0.23	0.22	-0.04	-0.36	-0.20	0.53	-0.09	0.13	-0.13	
Cd	0.25	-0.42	-0.18	0.37	0.38	0.00	0.05	-0.40	0.00	-0.11	-0.19	-0.35	0.08	-0.49	-0.26

Max. 0.98
Med. 0.01
Min. -0.62

Fig. 7. Pearson's correlation among all the studied parameters in *Z. mays* absolute critical value r = ± 0.242 beyond which the calculated correlation is found significant (p-value < 0.05) [SOV = Source of variance, PH = Plant height (cm), NL = Number of leaves, NB = Number of branches, SL = Shoot length (cm), RL = Root length (cm), FWP = Fresh weight of plant (g), DWP = Dry weight of plant (g), Chl a = Chlorophyll a contents, Chl b = Chlorophyll b contents, Total Chl = Total chlorophyll contents, TPC = Total phenolic contents, TFC = Total flavonoid contents, DPPH = 2,2- diphenylpicrylhydazyl, TAC = Total antioxidant contents and TRP = Total reducing potential].

dry weight of the plant increased with the strength of correlation − 0.21 as the shoot length increased. Shoot length was observed highly correlated to chlorophyll contents as chlorophyll a − 0.55, chlorophyll b − 0.51, and total chlorophyll levels observed at − 0.62 respectively. TPC, TFC, DPPH, TAC, and TRP contents showed no positive correlation concerning the increase in shoot length at 0.30, − 0.10, 0.33, − 0.15, and − 0.08 respectively. Root length was noticed positively significantly correlated − 0.40, − 0.30, − 0.46, − 0.34, and − 0.62 in plant fresh weight, dry weight of the plant, chlorophyll a, chlorophyll b, and total chlorophyll contents respectively[17,31,76–78]. Root length was observed positively significant with TPC, TFC, DPPH, TAC, and TRP as 0.09, 0.06, 0.20, − 0.20, and − 0.47. The fresh weight of the plant increased with the strength of correlation as root length increased. Root length was positively significantly correlated with the dry weight of plants. The dry weight of the plant increased with the strength of correlation as chlorophyll a, b, and total chlorophyll contents increased at the rate of 0.47, 0.13, and 0.32 respectively. Dry weight was observed to be positively significantly correlated with TPC, TFC, DPPH, TAC, and TRP with the rate of − 0.10, 0.24, 0.45, − 0.15, and 0.22 respectively. Chlorophyll content was significantly correlated with chlorophyll b, total chlorophyll, TPC, TFC, DPPH, TAC, and TRP at the rate of 0.70, − 0.71, − 0.17, 0.07, 0.17, and − 0.36 respectively. Chlorophyll b content was observed positively correlated with total chlorophyll, TPC, TFC, DPPH, TAC, and TRP at a rate of 0.77, − 0.55, − 0.30, 0.03, 0.17, and − 0.36 respectively. Total chlorophyll showed a non-significant correlation with TPC, TFC, DPPH, TAC, and TRP as − 0.48, − 0.11, − 0.06, 0.10, and − 0.20 respectively as the total chlorophyll contents increased there was no significant increase was observed in antioxidant contents. Total phenolic contents were non-significantly correlated with TFC, DPPH, TAC, and TRP with rates of − 0.15, 0.55, − 0.09, and − 0.35 respectively. Total flavonoid contents showed a non-significant correlation with DPPH, TAC, and TRP as − 0.20, 0.13, and 0.08.

Mechanism of *Z. mays* in salinity stress response

Withania assisted cobalt NPs applied on *Z. mays*, due to which the cells got signals from the genetic materials in the form of activation of stress-responsive genes which initiate the process of osmoprotectants expression and up-regulation of those genes and proteins[79,80] which play a key role for the exchange of ions within the cell and avoid it from plasmolysis Fig. 8. In the absence of these green cobalt NPs, the stress showed its result and it did

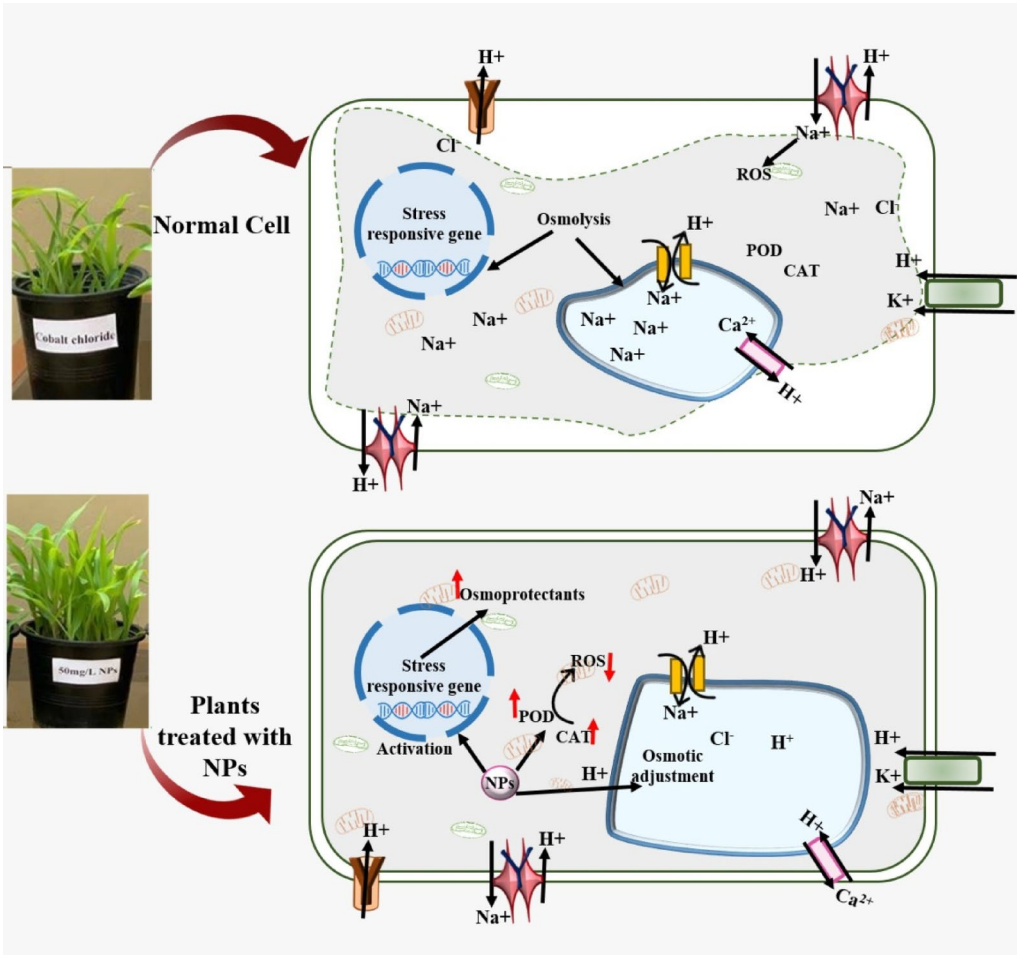

Fig. 8. *Withania*-assisted cobalt NPs regulate the osmotic balance in *Z. mays*.

regulate the ions exchange mechanism as a result the cell became shrunk[81,82], and it strongly influenced the cell physiology and anti-defense system which directly or indirectly decreased the growth in *Z. mays*. The above result showed that nanoparticles with the biological coating are very economical, eco-friendly, and productive enhancing plant growth for boosting agriculture products. Furthermore, this study also needs more detail in the field experiment regarding the optimization of different concentrations, time points, and other related parameters that bring these NPs utilization practically at the industrial level.

Conclusions

The biological synthesis of CoO NPs, generally considered life-friendly, will provide insight into the material and for future applications. Moreover, this approach can help establish safe synthesis limits for future applications and potentially replace many existing tedious and time-consuming methods. However, the elevated level of secondary metabolites in plants exposed to CoO NPs and $CoCl_2$ indicates that the aggregation of these compounds triggers an oxidative response. In addition, this research found mild toxic, phytotoxic, and environmental toxic effects of CoO NPs, as well as a poor phytotoxic effect of $CoCl_2$ on the morphology of *Z. mays* and its metabolic reactions. This current study suggests that lower concentrations of CoO NPs are more favorable for plants, and plants show better growth and less toxicity at lower concentrations.

Data availability

All the data generated or analyzed during the current study were included in the manuscript. The raw data is available from the corresponding author on reasonable request.

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Author contributions

Tuba Tariq and Yun Wang: Methodology, Investigation, data collection, formal analysis. Murtaza Hasan and Faisal Mahmood: Supervision, Draft for writing manuscript, visualization, interpretation. Ghazala Mustafa, Tuba Tariq and Faisal Mahmood: Conceptualization, Supervision, data interpretation. Mansour Ghorbanpour: writing, review and editing.

Declarations

Competing interest

The authors declare no competing interests.

Statement of compliance

The authors confirm that all the experimental research and field studies on plants, including the collection of plant material, complied with relevant institutional, national, and international guidelines and legislation.

Statement specifying permissions

Authors acquired permission to study plant sample by the Agricultural and Natural Resources Ministry of Pakistan.

Statement on experimental research and field studies on plants

The plants sampled comply with relevant institutional, national, and international guidelines and domestic legislation of Pakistan.

Additional information

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Correspondence and requests for materials should be addressed to M.G., G.M. or M.H.