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Elucidating melatonin-mediated distinct mechanistic of specific gene expression of coriander (*Coriandrum sativum* L.) under chromium stress

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Abstract: The current investigation demonstrates that the application of MEL (0, 1, and 2 µmol L^{-1}) mitigates the effects of Cr stress 0 (no Cr), 50 and 100 mg kg^{-1} on coriander (*Coriandrum sativum* L.) plants. Results from the present study showed that the increasing levels of Cr concentration in the soil induced a significant decrease in shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid contents, net photosynthesis, transpiration rate and stomatal conductance by 36%, 24%, 17%, 64%, 27%, 23%, 19%, 29%, 36%, 18%, 73%, 83%, and 43% respectively. Results findings also showed that the increasing Cr stress in the soil significantly ($p< 0.05$) decreases calcium, magnesium, iron, and phosphorus contents by 69%, 51%, 89%, and 109% respectively in the roots and decreased by 164%, 97%, 66%, and 124% respectively in the shoots. However, Cr toxicity boosted the production of reactive oxygen species (ROS) by increasing the contents of oxidative stress indicators. Although activities of various antioxidative enzymes and their specific gene expression and also the nonenzymatic antioxidants initially increased up to a Cr level of 50 µM but decreased gradually with the further increase in the Cr level of 100 µM in the medium, compared to those plants which were grown in the control treatment. Results also revealed that the soluble sugar, reducing sugar, and nonreducing sugar were decreased in plants grown under elevating Cr levels but increased the Cr accumulation in the roots and shoots of *C. sativum*. Although results also illustrated that the application of MEL also decreased Cr toxicity in *C. sativum* by increasing antioxidant capacity and their gene expression and thus improved the plant growth, photosynthetic pigments, and decrease oxidative stress in the roots and shoots of *C. sativum*.

Key words: Gene expression, herb, heavy metal toxicity, melatonin, organic acid, oxidative stress

1. Introduction

In recent decades, rapid increases in urbanization and industrialization have caused the excessive release of heavy metals in farmlands with damaging effects on ecosystems (Hashem et al., 2020; Saleem et al., 2020a). Heavy metal accumulation in soils is of great concern in agricultural production due to its adverse effects on food safety and marketability, crop growth due to phytotoxicity, and the environmental health of soil organisms (Saleem et al., 2020b; Imran et al., 2023; Wahab et al., 2023). Among various heavy metals, chromium (Cr) is an extremely noxious metal to living organisms, and negative effects have been reported in plants (Javed et al., 2021). Cr is a major toxic element discharged into the environment through various industries, such as tanning, electroplating, manufacturing of pigments, production of nuclear weapons, and corrosion control (Zaheer et al., 2019). This extensive industrial use of Cr composites and their subsequent release, without prior treatment, into the surrounding environment contaminates the entire ecosystem and can lead to catastrophic health risks (Ma et al., 2022). Hence, it is immensely required to safeguard the plants from Cr toxicity to counter the phytotoxicity and oxidative stress triggered by the uptake of Cr in plants.

Melatonin (MEL) is a crucial biological hormone associated with many physiological and biochemical processes in plants and also enhances resistance against various abiotic stresses (Ali et al., 2020). ML plays a vital part in plant growth and development by regulating plant physiology and root regeneration, antioxidant activity,

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photosynthesis, senescence of leaves, and immunological enhancement (Li et al., 2023). According to (Bhat et al., 2022), the utilization of MEL yielded an enhancement in the antioxidant mechanism within soybean plants, along with a simultaneous decrease in the uptake of arsenic (As), ultimately resulting in a noteworthy alleviation of As toxicity. The novelty of MEL application to plants lies in its multifaceted role as a versatile signalling molecule, demonstrating the potential to enhance plant resilience to environmental stressors, boost crop productivity, and improve overall agricultural sustainability, thus opening new horizons in the field of plant science (Yasmeen et al., 2022). In addition, the application of MEL under abiotic stress conditions represents a burgeoning area of research and innovation, harnessing the potential of this naturally occurring molecule to ameliorate the negative effects of environmental stressors on crop yield, offering novel solutions for sustainable agriculture (Mohamed et al., 2020). Previously, MEL has been reported to enhance resistance against salinity stress in various crops, but studies regarding ameliorating role of MEL in *C. sativum* under Cr stressed condition through exploring the physiological and biochemical characteristics are still scare.

Coriander (*Coriandrum sativum* L.), is an annual herb belonging to the family Umbelliferae (Apiaceae) (Saleem et al., 2021). *C. sativum* can be grown on a variety of soil and prefers well-drained, light, moist, heavy black,, and loamy soil and cultivated in the summer season (March–September) (Sardar et al., 2022). The essential oil of coriander contains significant amounts of natural elements (Ahmad et al., 2017). According to some research, making coriander a "biosorbent" or biomass absorbent that claims to remove heavy metals as an accumulator (Said-Al Ahl and Omer 2009). Numerous research studies have evaluated the impact of externally applied MEL on plants exposed to Cr stress, with a notable concentration of these investigations centered on rice and cucumber as plant species. However, further experiments are needed to comprehensively comprehend the influence and mechanisms of exogenous MEL on *C. sativum* and other vegetable crops. So, we aimed to designed this study which will increase our knowledge about the effects of different concentrations of Cr in the soil on plant growth and biomass, photosynthetic pigments, gas exchange characteristics, oxidative stress biomarkers, antioxidants machinery (enzymatic and nonenzymatic antioxidants) and their specific gene expression, ions uptake, organic acids exudation, and Cr uptake in different parts of plant under the application of MEL. The results from the present study gave a new insight that the use of MEL in heavy metals studies may be beneficial and can improve plant yield under Cr-contaminated soil.

2. Materials and methods

2.1. Experimental setup and materials collection

The present study was conducted in the botanical garden under greenhouse environment. The seeds of *C. sativum* were surface sterilized with (0.1%) bleaching powder for 10–20 min and washed gently with deionized water before starting an experiment. The soil used for this experiment was collected from the experimental station and was air dried, passed through a 5-mm sieve and was water-saturated twice before being used in pots and its characteristics are mentioned in Tables 1S. All the pots were categorized into three groups: (I), without any Cr treatment (II), addition of 50 µM of Cr, and (III) addition of 100 µM of Cr. Before sowing the plants, Cr was artificially supplied using potassium dichromate ($K_2Cr_2O_7$) at various concentrations (Hussain et al., 2021). After adding the Cr, the pots were equilibrated for 2 months with four cycles of saturation with distilled water and air drying. While melatonin was applied at MEL (0, 1, and 2 µmol L^{-1}) in the sole and integrated form, after 1 week of seedling's transplantation. The pots used in this study were rotated regularly in order to avoid environmental effects on the plants. All plants in the glass house territory received natural light, with a day/night temperature of 35/40 °C and day/night humidity of 60%/70%. The total duration of experimental treatments was two months under controlled conditions. Irrigation with Cr-free water and other intercultural operations were performed, when needed. All pots were placed in completely randomized design (CRD) having five plants in each pot with four replicates of each treatment.

2.2. Data collection

After two months, three seedlings were up rooted and washed gently with the help of distilled water to eliminate the aerial dust and deposition. Leaves and root samples from each treatment group were picked after one month for chlorophyll, carotenoid, and antioxidant analysis. The leaves were washed with distilled water, placed in liquid nitrogen, and stored at –80 °C for further analysis. The plants from each treatment were washed with tap water to remove debris and waste and then with distilled water. Plant length (shoot and root) was measured using a measuring scale. The number of leaves was measured by simply counting the leaves while the leaf area was also measured. For plant fresh weight (shoot and root) was determined by measuring the weight of the plant with a digital weighting balance. Later, root and shoot were dried in an oven at 105 °C for 1 h, then at 70 °C for 72 h to determine their dry weight. Roots were immersed in 20 mM Na_2EDTA for 15–20 min to remove Cr adhered to the surface of roots. Then, roots were washed thrice with distilled water and fnally once with deionized water and dried for further analysis. Although this experiment was conducted in pots, for the collection of organic acids, two seedlings were transferred to the rhizoboxes which

consist of plastic sheet, nylon net, and wet soil (Javed et al., 2013). After 48 h, plants were taken from the rhizoboxes and the roots were washed with redistilled water to collect the exudates from the root surface. The samples were fltered through a 0.45-μm filter (MillexHA, Millipore) and collected in eppendorf tubes. The collected samples were mixed with NaOH (0.01 M) in order to analyze the organic acids. However, the samples used for analysis of oxalic acid were not treated with NaOH (Javed et al., 2013).

2.3. Photosynthetic pigments and gas exchange characteristics

Leaves were collected for the determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of Lichtenthaler (1987).

Net photosynthesis (N*p*), leaf stomatal conductance (G*s*), transpiration rate (T*r*), and intercellular carbon dioxide concentration (C*i*) were measured from 3 different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with a clear sky. Rates of leaf N*p*, G*s*, T*r*, and C*i* were measured with a LI-COR gas exchange system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set at 380 mmol $mol^{-1,}$ and LED light intensity was set at 1000 mmol m^{-2} s^{-1}, which was the average saturation intensity for photosynthesis in *C. sativum*.

2.4. Oxidative stress indicators

The degree of lipid peroxidation was evaluated as MDA contents. Briefly, 0.1 g of frozen leaves were ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1% polyethene pyrrole. The homogenate was centrifuged at 10,000 × g at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, United States) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532–A600)–0.56 A450. Lipid peroxidation was measured by using a method previously published by Heath and Packer (1968).

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1% titanium sulfate in 20% (v/v) H_2SO_4 and centrifuged at 6000 × g for 15 min. The yellow color intensity was evaluated at 410 nm. The H_2O_2 level was computed by the extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured by the method presented by Velikova et al. (2000).

Stress-induced EL of the uppermost stretched leaves and roots was determined by using the methodology of Dionisio-Sese and Tobita (1998). The leaves and roots were cut into minor slices (5 mm in length) and placed in test tubes containing 8 mL of distilled water. These tubes were incubated and transferred into a water bath for 2 h before measuring the initial electrical conductivity (EC1). The samples were autoclaved at 121 °C for 20 min and then cooled down to 25 °C before measuring the final electrical conductivity (EC2). EL was calculated by the following formula; EL = (EC1⁄EC2) × 100.

2.5. Determination of antioxidant enzyme activities and their gene expression

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0), including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000 × g for 10 min at 4 °C, and the supernatant was used for measurement of SOD and POD activities. SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate bufer (pH 7), 56 mM nitro-blue tetrazolium, 1.17 mM ribofavin, 10 mM methionine, and 100 μL enzyme extract. Finally, the sample was measured by using a spectrophotometer (× Mark™ Microplate Absorbance Spectrophotometer; Bio-Rad). Enzyme activity was measured by using a method by Chen and Pan (1996) and expressed as U g^{-1} FW.

POD activity in the leaves was estimated by using the method of Cai et al. (2008) by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1% H_2O_2, and 0.1 mL of 4% guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation were recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme.

CAT activity was analyzed according to Aebi (1983). The assay mixture (3.0 mL) was composed of 100 μL enzyme extract, 100 μL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM ETDA (pH 7.0). The CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss (ε = 39.4 $mM^{-1}cm^{-1}$).

APX activity was measured according to Nakano and Asada (1981). The mixture containing 100 μL enzyme extract, 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm (ε = 2.8 $mM^{-1}cm^{-1}$).

A quantitative real-time PCR (RT-qPCR) assay was applied to investigate the expression levels of 4 stress-related genes, including Fe-SOD, POD, CAT, and APX. Total RNA was extracted from leaf tissue samples using RNeasy Plant

Mini kits (Qiagen, Manchester, UK). Contaminating DNA was then removed and first-strand cDNAs were prepared using Reverse Transcription kits (Qiagen, Manchester, UK). RT-qPCR analysis was conducted as reported in the protocol of QuantiTect SYBR Green PCR kit (Qiagen, Manchester, UK). Reaction volume and PCR amplification conditions were adjusted as mentioned by El-Esawi et al. (2020). The gene amplifications of Sirhindi et al. (2016) of the following genes are given in Table 2S.

2.6. Osmolytes, sugar, and proline assays

Plant ethanol extracts were prepared for the determination of nonenzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of dry plant material was homogenized with 10 mL ethanol (80%) and filtered through Whatman No. 41 filter paper. The residue was reextracted with ethanol, and the 2 extracts were pooled together to a final volume of 20 mL. The determination of flavonoids, phenolics, ascorbic acid, anthocyanin (Gillespie et al., 2007), and total sugars (Irigoyen et al., 1992) was performed from the extracts.

Fresh leaf material (0.1 g) was mixed thoroughly in 5 mL of aqueous sulphosalicylic acid (3%). The mixture was centrifuged at 10,000 × g for 15 min, and an aliquot (1 mL) was poured into a test tube having 1 mL acidic ninhydrin and 1 mL glacial acetic acid. The reaction mixture was first heated at 100 °C for 10 min and then cooled in an ice bath. The reaction mixture was extracted with 4 mL toluene, and test tubes were vortexed for 20 s and cooled. Thereafter, the light absorbance at 520 nm was measured by using a UV-VIS spectrophotometer (Hitachi U-2910, Tokyo, Japan). The free proline content was determined on the basis of the standard curve at 520 nm absorbance and expressed as μmol (g FW)$^{-1}$ (Bates et al., 1973).

2.7. Determination of nutrients uptake

For nutrient analysis, plant roots and shoots were washed twice in redistilled water, dipped in 20 mM EDTA for 3 s, and then, again, washed with deionized water twice for the removal of adsorbed metal on the plant surface. The washed samples were then oven dried for 24 h at 105 °C. The dried roots and shoots were digested by using a wet digestion method in HNO_3: $HClO_4$ (7:3 V/V) until clear samples were obtained. Each sample was filtered and diluted with redistilled water up to 50 mL. The root and shoot contents of calcium, magnesium, iron, and phosphorus were analyzed by using Atomic Absorption Spectrophotometer (AAS) model Agilent 240FS-AA.

2.8. Root exudates analysis and Cr uptake

In order to determine the concentration of organic acids, freeze-dried exudates were mixed with ethanol (80%), and 20 μL of the solutions was injected into the C18 column (Brownlee Analytical C-183 μm; length 150 mm × 4.6 mm2, USA). Quantitative analysis of organic acids in root exudates was executed with high-performance liquid chromatography (HPLC), having a Flexer FX-10 UHPLC isocratic pump (PerkinElmer, MA, USA). The mobile phase used in HPLC was composed of an acidic solution of aceto-nitrile containing aceto-nitrile:H_2SO_4:acetic acid in ratios of 15:4:1, respectively, and pH of 4.9. The samples were analyzed at a flow rate of 1.0 mL min^{-1} for a time period of 10 min. The inner temperature of the column was fixed at 45 °C, and quantification of organic acids was carried out at 214 nm wavelength with the help of a detector (UV-VIS Series 200, USA). Freeze dried samples were dissolved in redistilled water, and the pH of the exudates was recorded with an LL micro-pH glass electrode by using a pH meter (ISTEK Model 4005-08007 Seoul, South Korea).

Plant samples were vigilantly digested via di-acid (HNO_3- $HClO_4$) technique. A 0.5 g dry sample of roots and shoots of the plants were taken into the flask having 10 mL of HNO_3- $HClO_4$ (3:1, v:v); this collection was then retained overnight. The final digestion of these plants' samples was completed after the addition of HNO_3 (5 mL) and then placed on the hot plate for complete digestion as described by Ali et al. (2015). Atomic absorption spectrophotometer (AAS) was used to investigate the exact amount of Cr in shoots and roots of the plant.

2.9. Statistical analysis

The normality of data was analyzed using IBM SPSS software (Version 21.0. Armonk, NY, USA: IBM Corp) through a multivariate posthoc test, followed by Duncan's test in order to determine the interaction among significant values. Thus, the differences between treatments were determined by using ANOVA, and the least-significant difference test ($p < 0.05$) was used for multiple comparisons between treatment means where significant Tukey's HSD posthoc test was used to compare the multiple comparisons of means. The analysis showed that the data in this study were almost normally distributed. The graphical presentation was carried out using Origin-Pro 2017.

3. Results

3.1. Effect of exogenous application of MEL on plant growth and photosynthetic measurements in C. sativum under the toxic concentrations of Cr

In the present study, various growth and photosynthetic parameters were also measured in *C. sativum* grown under the different levels of Cr 0 (no Cr), 50, and 100 μM in the soil which were also supplied with the different exogenous levels of MEL, i.e. 0 (no MEL), 1, and 2 μmol L^{-1}. The data regarding shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight are presented in Figure 1, and the data regarding the chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid content, net photosynthesis, stomatal conductance, transpiration rate, and intercellular CO_2 are presented in Figure 2. Results from the present study showed that the increasing levels of Cr concentration in the soil induced a

significant decrease in shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid contents, net photosynthesis, transpiration rate and stomatal conductance by 36%, 24%, 17%, 64%, 27%, 23%, 19%, 29%, 36%, 18%, 73%, 83%, and 43% respectively. The exogenous application of MEL was also applied at different levels 0 (no MEL), 1, and 2 µmol L^{-1}, to measure various growth (Figure 1) and photosynthetic attributes (Figure 2) in *C. sativum* grown under the elevating levels of Cr in the soil. The application of MEL nonsignifcantly increased root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight are presented in Figure 1, and the data regarding the chlorophyll-a, chlorophyll-b, total chlorophyll, carotenoid content, net photosynthesis, stomatal conductance, transpiration rate at all levels of Cr in the soil, compared to the plants which were grown without the application of MEL (µmol L^{-1}). We have also noticed that Cr toxicity did not significantly affect intercellular CO_2 and also application of MEL did not significantly influence intercellular CO_2 in *C. sativum* under all levels of Cr in the soil (Figure 2H).

3.2. Effect of exogenous application of MEL on oxidative stress and response of antioxidant compounds in C. *sativum* under the toxic concentrations of Cr

Malondialdehyde (MDA) contents, hydrogen peroxide (H_2O_2) initiation, and electrolyte leakage (%) increased in

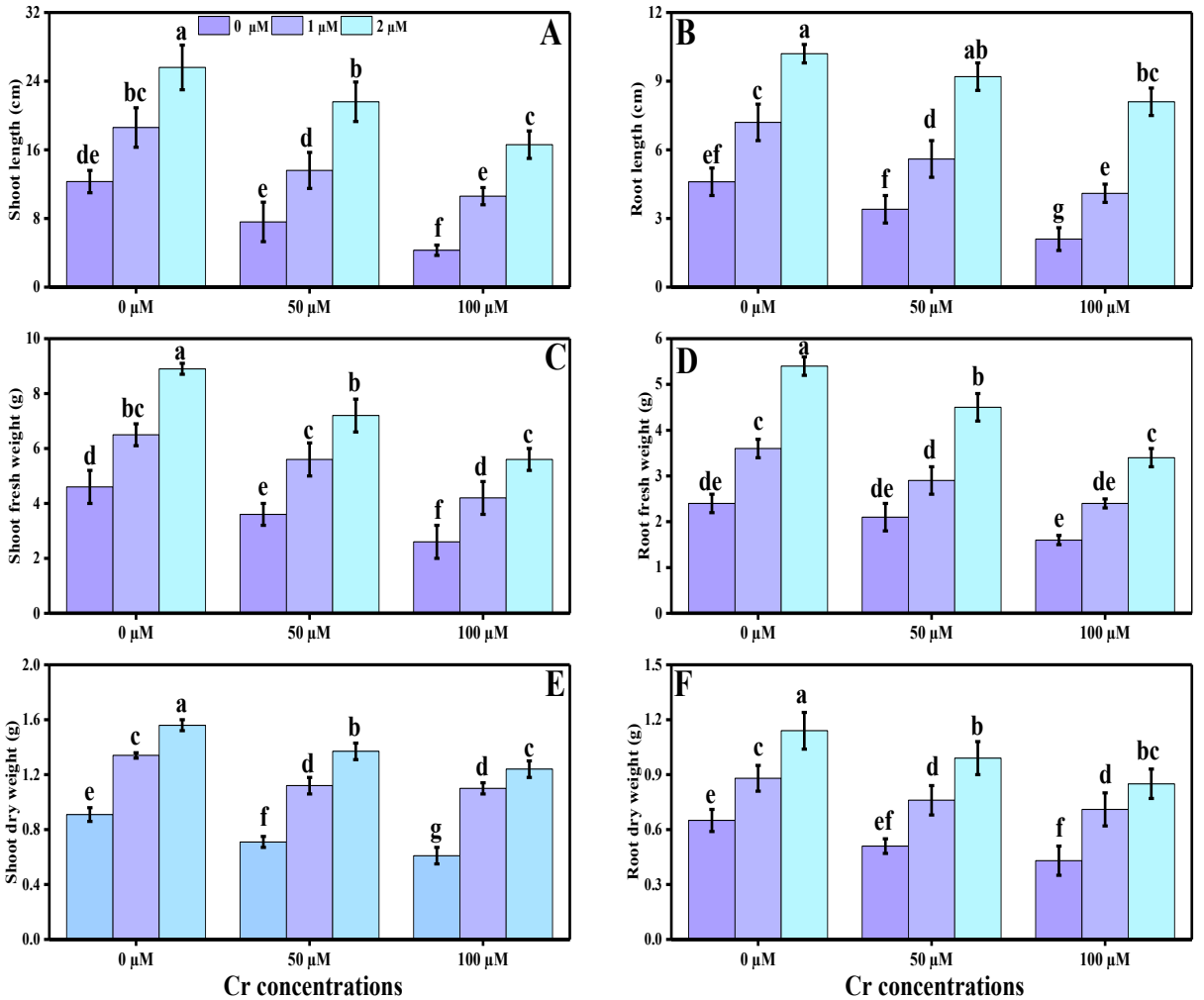

Figure 1. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on various growth-related attributes i.e. shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E), and root dry weight (F) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50 and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p< 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

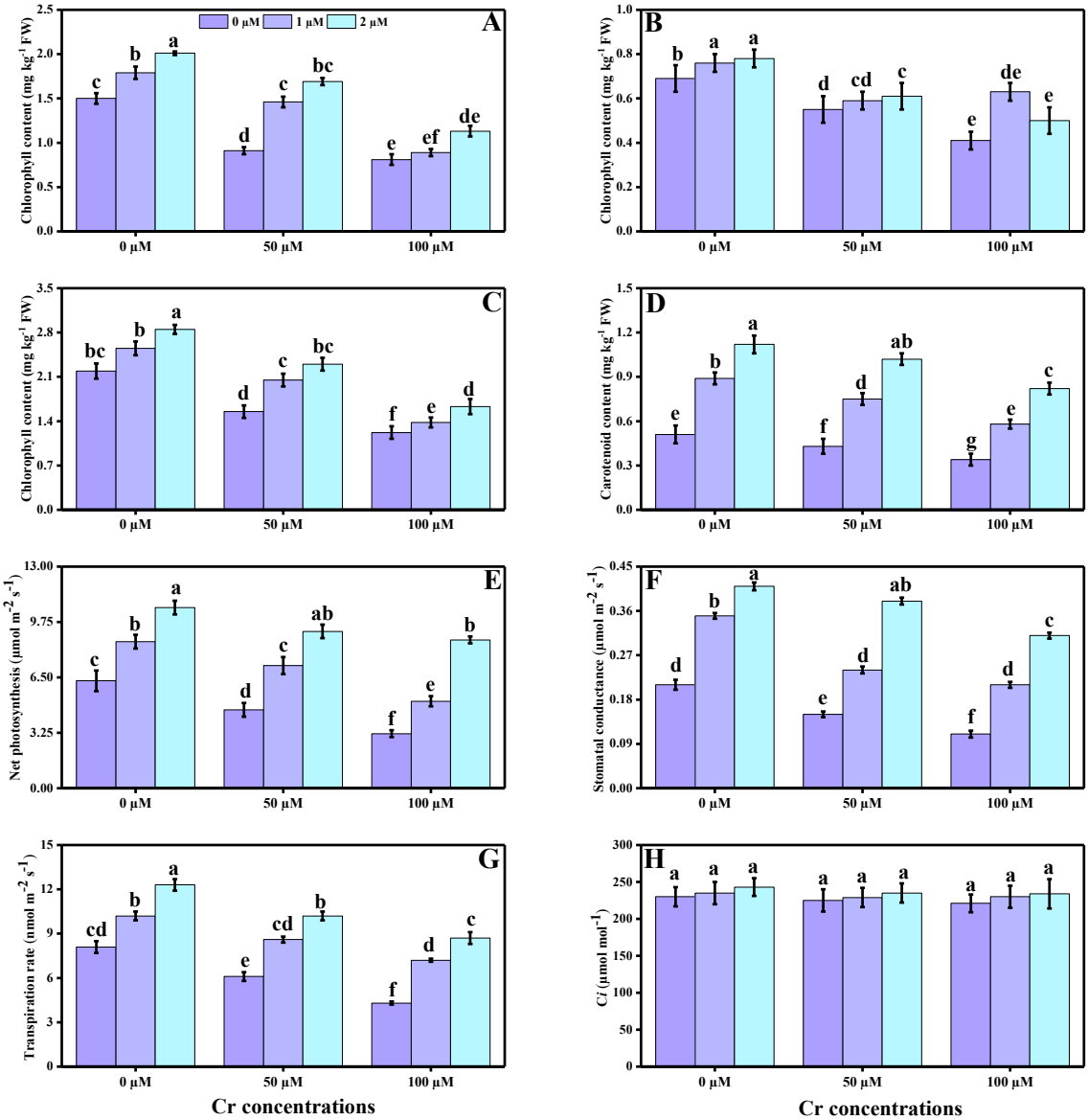

Figure 2. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on photosynthetic pigments and gas exchange characteristics i.e. chlorophyll-a content (A), chlorophyll-b contents (B), total chlorophyll contents (C), carotenoid contents (D), net photosynthesis, (E) stomatal conductance (F), transpiration rate (G), and intercellular CO_2 (H) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50 and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p< 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

the roots and shoots of *C. sativum* under the increasing Cr i.e. 50 and 100 µM in the soil medium without MEL application as compared to the plants grown in 0 mM of Cr. The data regarding oxidative stress indicators in the roots and shoots of *C. sativum* are presented in Figure 3. It was observed that the contents of MDA, H_2O_2, and EL (%) were in the roots and also in the shoots grown in 100 µM of Cr without the application of MEL as compared to those plants grown in 0 µM of Cr in the soil without the application of MEL. Application of MEL significantly decreased the contents of MDA, H_2O_2, and EL (%) in roots and also in the shoots of the plants grown with Cr level of 100 µM under MEL application of 2 µmol L^{-1} as compared to those plants grown with 100 µM of Cr without the application of MEL.

Various antioxidative enzymes like superoxidase dismutase (SOD), peroxidase (POD), catalase (CAT),

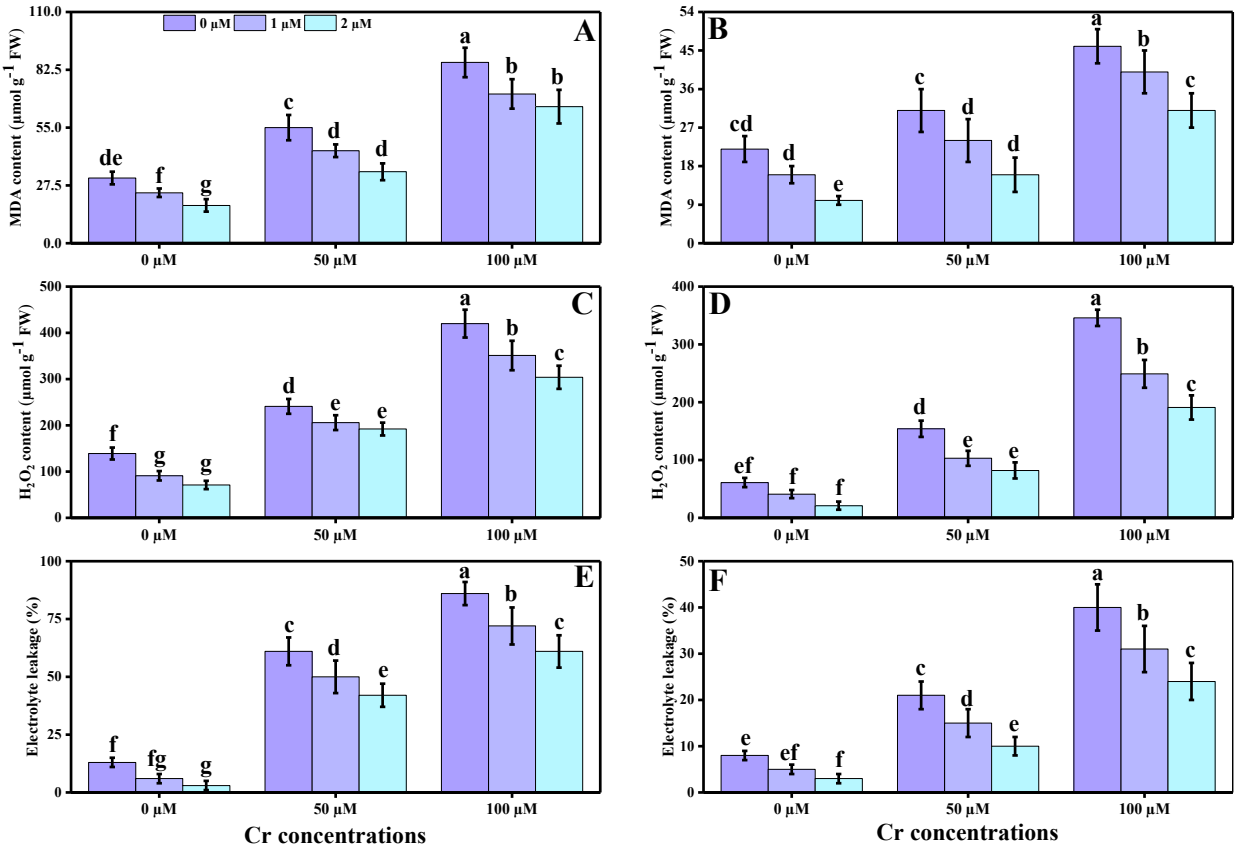

Figure 3. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on oxidative stress indicators i.e. MDA contents in the roots (A), MDA contents in the leaves (B), H_2O_2 contents in the roots (C), H_2O_2 contents in the leaves (D), EL percentage in the roots (E), and EL percentage in the leaves (F) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50 and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p< 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

and ascorbate peroxidase (APX) in the roots and shoots of *C. sativum* and their specific gene expression i.e. Fe-SOD, POD, CAT and APX in the shoots of *C. sativum* and nonenzymatic compounds such as phenolic, flavonoid, ascorbic acid, and anthocyanin contents were also measured in the present study. The data regarding the activities of enzymatic antioxidants (SOD, POD, CAT, and APX) are presented in Figure 4, and their specific gene expression i.e. Fe-SOD, POD, CAT, and APX are presented in Figure 5 and also the results regarding the compounds of nonenzymatic antioxidants (phenolic, flavonoid, ascorbic acid, and anthocyanin) are presented in Figure 6. The results showed that the activities of enzymatic antioxidants (SOD, POD, CAT, and APX) and their specific gene expression i.e. Fe-SOD, POD, CAT, and APX and also the compounds of nonenzymatic antioxidants (phenolic, flavonoid, ascorbic acid, and anthocyanin) were initially increased up to a Cr level of 50 µM in the soil, but decreased significantly ($p < 0.05$) with the maximum increased of Cr 100 µM in the soil, compared to the plants grown without the addition of Cr in the soil. Results also showed that the addition of MEL in the soil nonsignificant increased the activities of enzymatic antioxidants (SOD, POD, CAT, and APX) and their specific gene expression i.e. Fe-SOD, POD, CAT, and APX and also the compounds of nonenzymatic antioxidants (phenolic, flavonoid, ascorbic acid, and anthocyanin) at all levels of Cr (no Cr), 50 and 100 µM in the soil, compared to the plants which were not applied by the MEL treatment (0 mM).

3.3. Effect of exogenous application of MEL on sugar, proline, and nutrients uptake in C. *sativum* under the toxic concentrations of Cr

Soluble sugar, reducing sugar, nonreducing sugar, proline, and various nutrients such as calcium (Ca^{2+}), magnesium (Mg^{2+}), iron (Fe^{2+}), and phosphorus (P) contents from the roots and shoots of *C. sativum*, were also measured in the present study under the different levels of Cr 0 (no Cr), 50 and 100 µM in the soil which

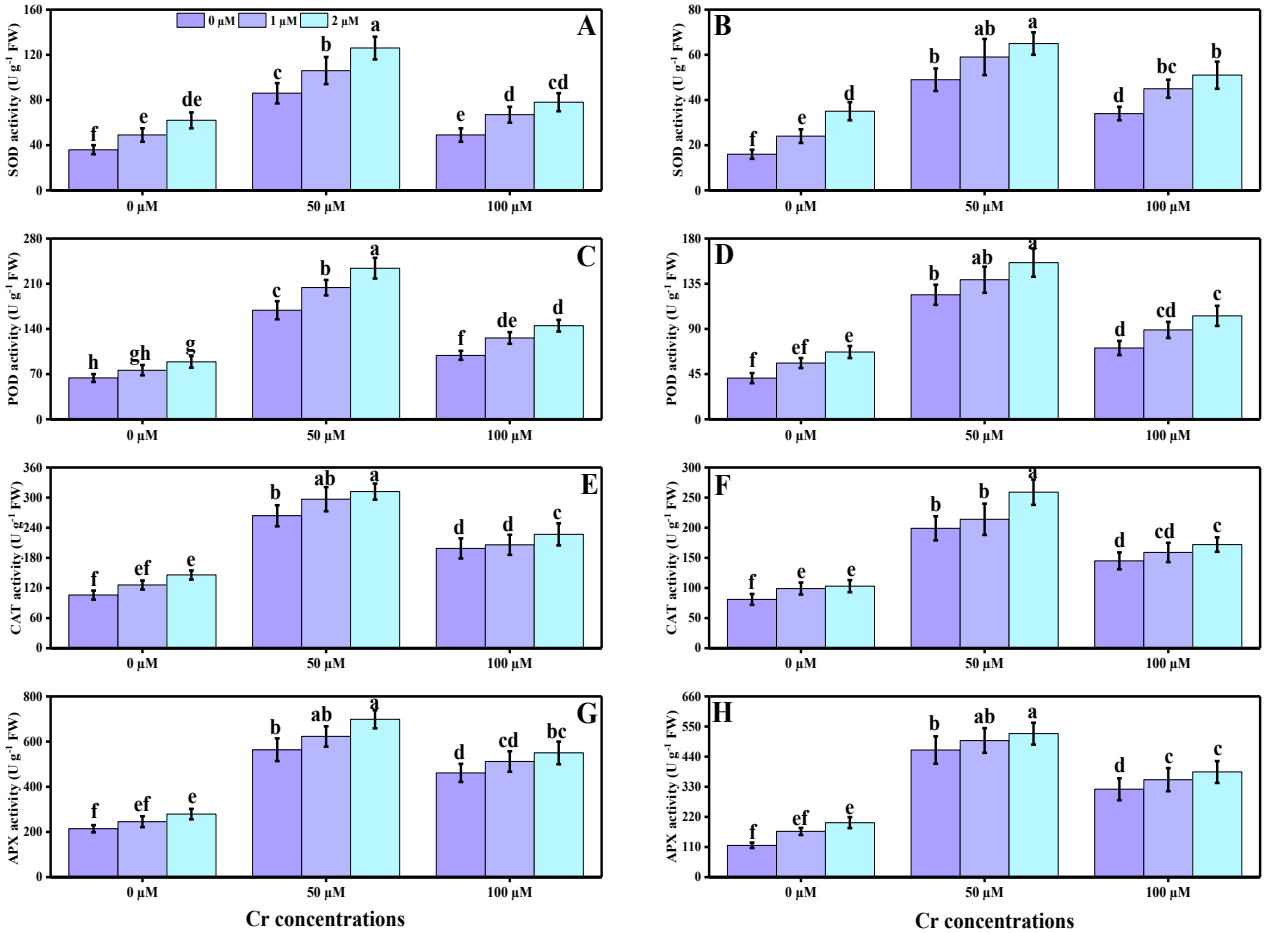

Figure 4. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on enzymatic antioxidants i.e. SOD activity in the roots (A), SOD activity in the leaves (B), POD activity in the roots (C), POD activity in the leaves (D) CAT activity in the roots (E), CAT activity in the leaves (F), APX activity in the roots, (G) and APX activity in the leaves (H) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50, and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p< 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

were also supplied with the different exogenous levels of MEL, i.e. 0 (no MEL), 1 and 2 µmol L^{-1}. The data regarding the content of soluble sugar, reducing sugar, nonreducing sugar, and proline are presented in Figure 6 and the data regarding the content of Ca^{2+}, Mg^{2+}, Fe^{2+}, and P from the roots and shoots of the plants are presented in Figure 7. Results from the present study show that the increasing levels of Cr in the soil significantly ($p < 0.05$) decreased the contents of nutrients (Ca^{2+}, Mg^{2+}, Fe^{2+}, and P) in the roots and shoots of the plants and also decreased the sugar content (soluble sugar, reducing sugar, nonreducing sugar), compared to the plants which were grown in the soil which was not treated with Cr. However, the content of proline was increased by increasing the levels of Cr in the soil, compared to the plants which were not treated with Cr (Figure 6H). The application of MEL was also added in the soil and determined various sugar (Figure 6), proline, and nutrient content (Figure 7) from the roots and shoots of the plants. Results from the present study suggested that the application of MEL nonsignificantly increased sugar content (soluble sugar, reducing sugar, nonreducing sugar), proline and significantly also nutrients (Ca^{2+}, Mg^{2+}, Fe^{2+}, and P) in the roots and shoots of the plants, compared to the plants grown without the treatment of MEL, at all the levels of Cr in the soil.

3.4. Effect of exogenous application of MEL on organic acids and Cr accumulation in C. *sativum* under the toxic concentrations of Cr

The contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots and Cr concentration in the roots and shoots of *C. sativum* grown

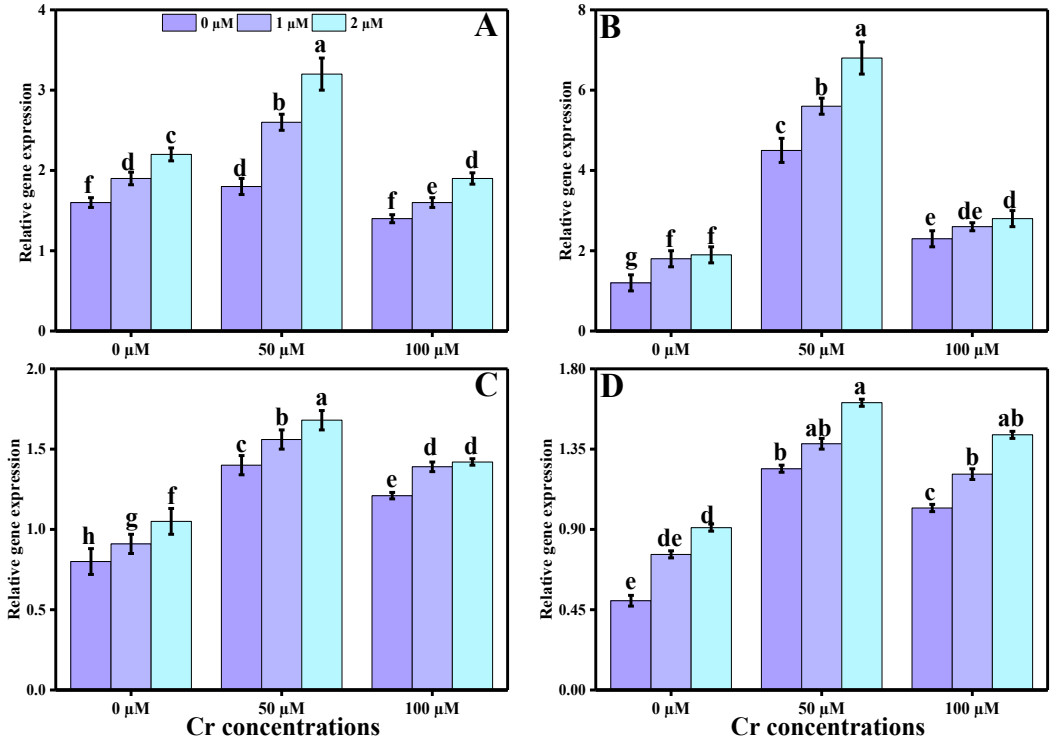

Figure 5. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on relative gene expression i.e. gene expression of Fe-SOD (A), gene expression of POD (B), gene expression of CAT (C), gene expression of APX (D) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50, and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

under toxic levels of Cr in the soil, with or without the application of MEL are presented in Figure 8. According to the given results, we have noticed that increasing the concentration of Cr in the soil (50 and 100 µM) induced a significant ($p < 0.05$) increase in the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots and also Cr concentration in the roots and shoots of *C. sativum*, compared to those plants, which were grown in Cr level of 0 µM in the soil. Results also illustrated that the application of MEL decreased the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots and also Cr concentration in the roots and shoots of *C. sativum*, compared with those plants, which were grown without the exogenous application with MEL in the soil. In addition, at all levels of Cr stress (50 and 100 µM), the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid, and also Cr concentration in the roots and shoots decreased with the increasing levels of MEL (1 and 2 µmol L^{-1}) in the soil, compared with those plants, which were grown without the application of MEL.

4. Discussion

Exposure to Cr may induce toxic effects in several biochemical processes in plants, such as plant germination, root growth and length, stem growth, and leaf development (Ahmad et al., 2022). Moreover, Cr stress is also known to negatively influence photosynthesis in terms of electron transport, CO_2 fixation, enzyme activities, and photophosphorylation and causes a decrease in chlorophyll-a, chlorophyll-b, total chlorophyll, and carotenoids which have been well established (Hussain et al., 2021). It has been previously shown that Cr stress negatively affects the plant biomass and photosynthetic efficiency in different plant species which depends upon a number of factors including plant species, dose, and duration of Cr application (Li et al., 2021; Abeed et al., 2021; Salama et al., 2022). Stress conditions can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants (Abeed and Dawood, 2020; Ahmed et al., 2023; Alshegaihi et al., 2023). It was reported that an excess of Cr can increase lipid peroxidation and MDA, an oxidized product of membrane lipids, indicating the prevalence

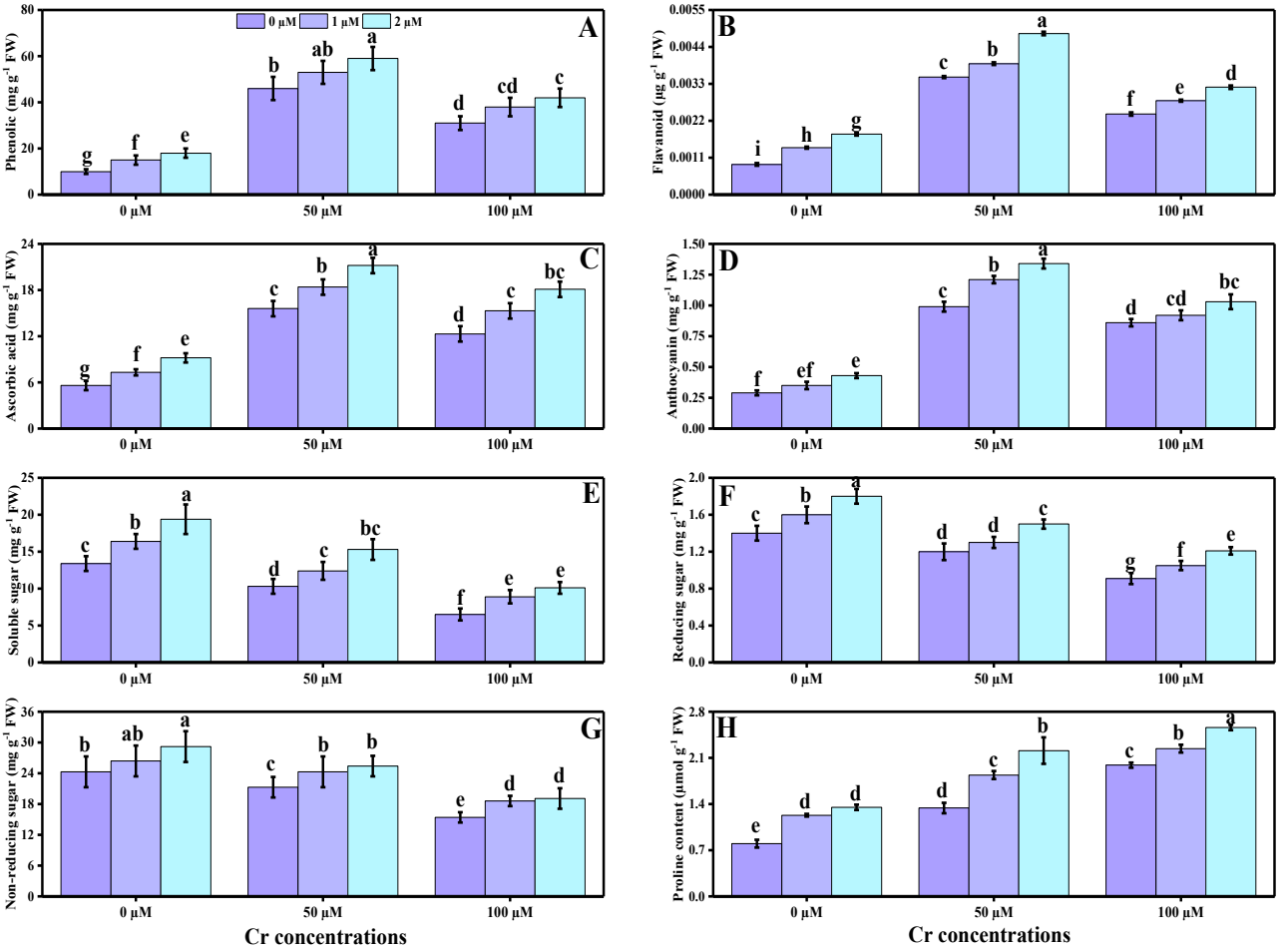

Figure 6. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on nonenzymatic compounds, sugar and proline i.e. phenolic contents (A), flavonoid contents (B), ascorbic acid contents (C), anthocyanin contents (D), soluble sugar contents (E), reducing sugar contents (F), nonreducing sugar contents (G), and proline contents (H) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50, and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and means differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

of oxidative stress and membrane damage (Abeed et al., 2023). A high concentration of Cr in the soil induced oxidative damage by increasing the contents of MDA, initiation of H_2O_2, and increased percentage of EL which was observed. It was also noticed that severe Cr toxicity (100 µM) decreased the various antioxidants, possibly due to the severe concentration of Cr in soil which induces alterations in gene expression and function of various proteins in plant tissues. Plants produce a variety of secondary metabolites such as proline, flavonoids, and phenolics that improve tolerance against metal toxicity. However, proline accumulation in plant tissue/organs is a response to metal toxicity, which might be associated with signal transduction and prevents membrane distortion, which has been observed in many plant species (Alsafran et al., 2022). Essential nutrients are required for the normal growth of plants. Numerous reports demonstrated that the uptake and translocation of essential elements in plants were restricted under Cr stress (Ma et al., 2022; Zaheer et al., 2020). Excess Cr decreased the Ca^{2+}, Mg^{2+}, Fe^{2+}, and P contents in the roots and shoots of the plants, which was also noticed in the present study (Figure 7). It is well known that Cr toxicity in crops depends on the bioavailability of Cr in soils and the concentration of elements, which can compete with Cr during plant uptake.

The application of exogenous MEL plays a multifunctional role in improving plant physiology under stress and a nonstressed environment (Mir et al., 2020). Reduced Cr concentration, acclimation of oxidative stress, up-regulation of the antioxidant defense system, and enhanced photosynthetic activity are important MEL

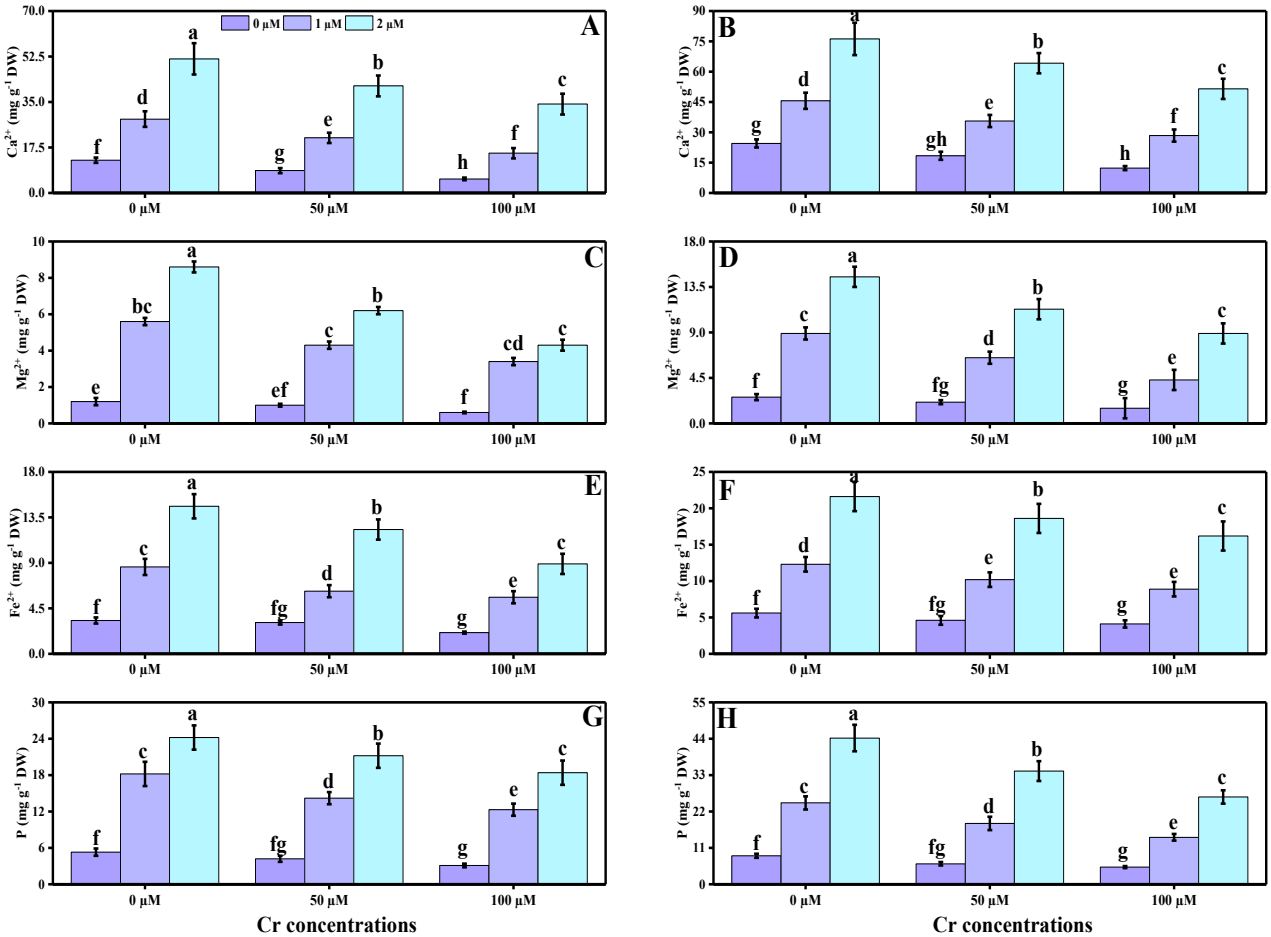

Figure 7. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on essential nutrients i.e. calcium contents in the roots (A), calcium contents in the shoots (B), magnesium contents in the roots (C), magnesium contents in the shoots (D), iron contents in the roots (E), iron contents in the shoots (F), phosphorus contents in the roots (G), and phosphorus contents in the shoots (H) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50, and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant difference between the treatments.

ramifications in improving metal stress (Imran et al., 2021). MEL application can promote plant growth, leading to increased biomass and better overall plant development by reducing stress and enhancing growth, melatonin can lead to higher crop yields, making it valuable for agriculture (Imran et al., 2021) which reported in the presented study (Figure 1). Moreover, MEL application markedly enhanced the plant water contents by improving the growth and ability of plant roots to absorb more water from the growth medium, balance osmotic adjustment, regulate transpiration rate with stomatal conductance, and regulate plant water potential (Kaya et al., 2020; Li et al., 2023). MEL enhanced antioxidant machinery by acting as a scavenger against H_2O_2 and O_2^- ensured ROS equilibrium at a cellular level, which promoted membrane stability and permeability (Castañares and Bouzo, 2019). As a result of MEL application, the overall oxidative stress in plants is overcome, and their antioxidant defense system is increased along with their specific gene expression and nonenzymatic compounds. This can lead to improved plant growth and productivity, and better tolerance to various environmental stresses, ultimately contributing to increased agricultural productivity and the sustainability of plant-based ecosystems (Bhat et al., 2022). In Figure 5, we present the detailed outcomes of our study, focusing on the impact of melatonin (MEL) application in chromium (Cr) stressed soil conditions on coriander. Figure 9 encapsulates a range of crucial parameters that were assessed, including growth and biomass, gas exchange characteristics, and the activities of key antioxidant enzymes. Additionally, it

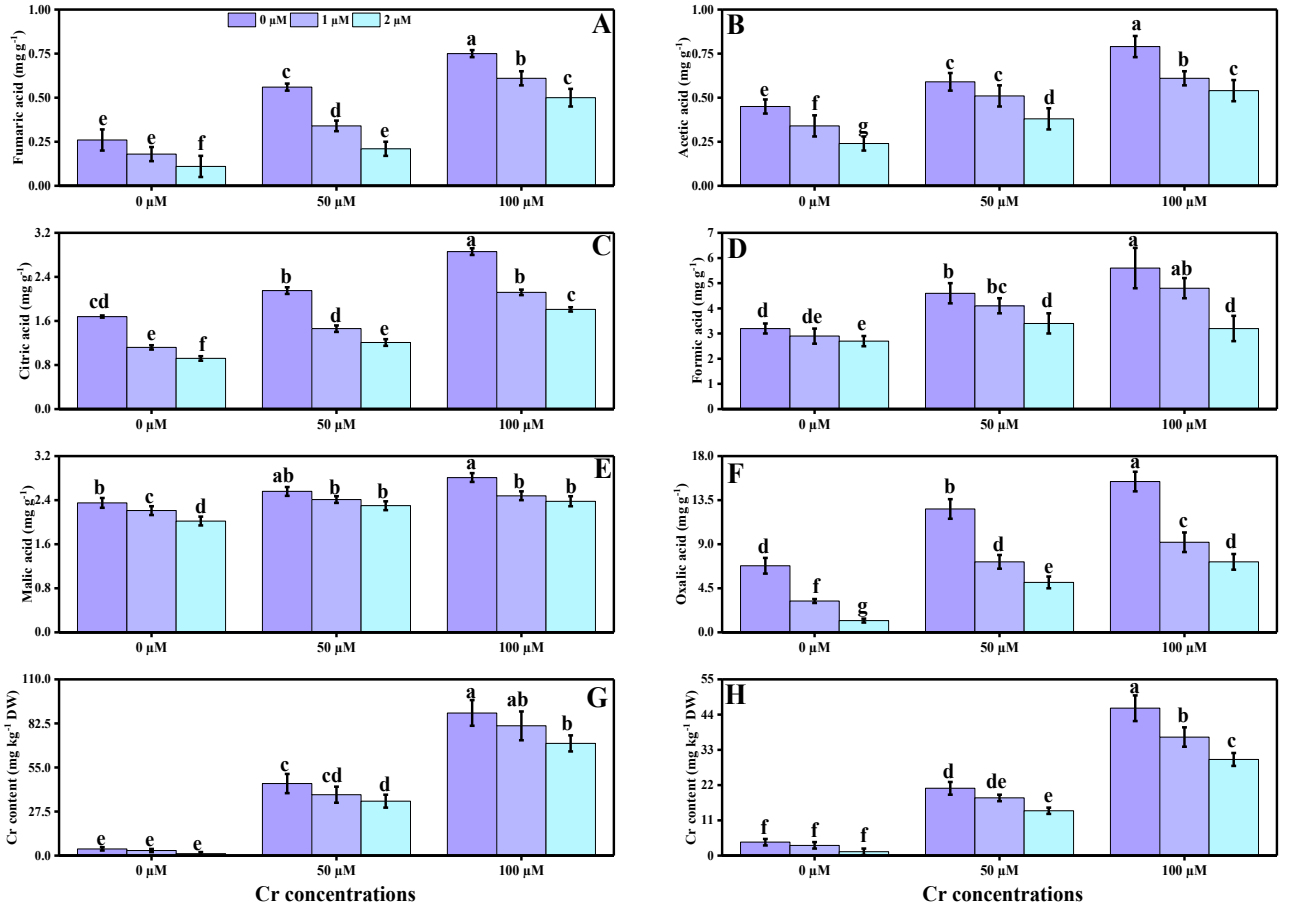

Figure 8. Effect of exogenous application of melatonin [0 (no MEL), 1, and 2 µmol L^{-1}) on organic acids and Cr uptake i.e. fumaric acid contents (A), acetic acid contents (B), citric acid contents (C), formic acid contents (D), malic acid contents (E), oxalic acid contents (F), in the roots and Cr contents in the roots (G), and Cr contents in the shoots (H) of coriander (*Coriandrum sativum* L.) grown under the various levels of Cr stress i.e. [0 (no Cr), 50, and 100 µM] in the soil. Values are demonstrated as means of four replicates along with standard deviation (SD; n = 4). Two-way ANOVA was performed and mean differences were tested by HSD ($p < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments.

illustrates the variations in Cr accumulation in different parts of the plant under the influence of MEL. This visual representation provides a clear and comprehensive overview of the ameliorative effects of MEL on coriander exposed to Cr stress, highlighting its potential role in mitigating heavy metal toxicity in plants

5. Conclusion

On the basis of these findings, it can be concluded that the negative impact of Cr toxicity can be overcome by the application of MEL. Our results depict that Cr toxicity induced severe metal toxicity in *C. sativum* by increasing the generation of ROS in the form of oxidative stress and also increased the concentration of Cr in the roots and shoots of the plants. Furthermore, Cr toxicity also increased organic acids exudation and imbalanced the nutritional status of the plants, which ultimately decreased plant growth and yield and photosynthetic efficiency. Hence, Cr toxicity was eliminated by the external application of MEL, which also decreased the Cr concentration in the plant tissues, degenerated ROS, and organic acids exudation, and increased the activities of antioxidants, their specific gene expression and nonenzymatic compounds and also the essential nutrients in the plants. Therefore, long-term feld studies should be executed to draw parallels among plants/crops root exudations, metal stress, nutrient mobility patterns, and plant growth to gain insights into underlying mechanisms.

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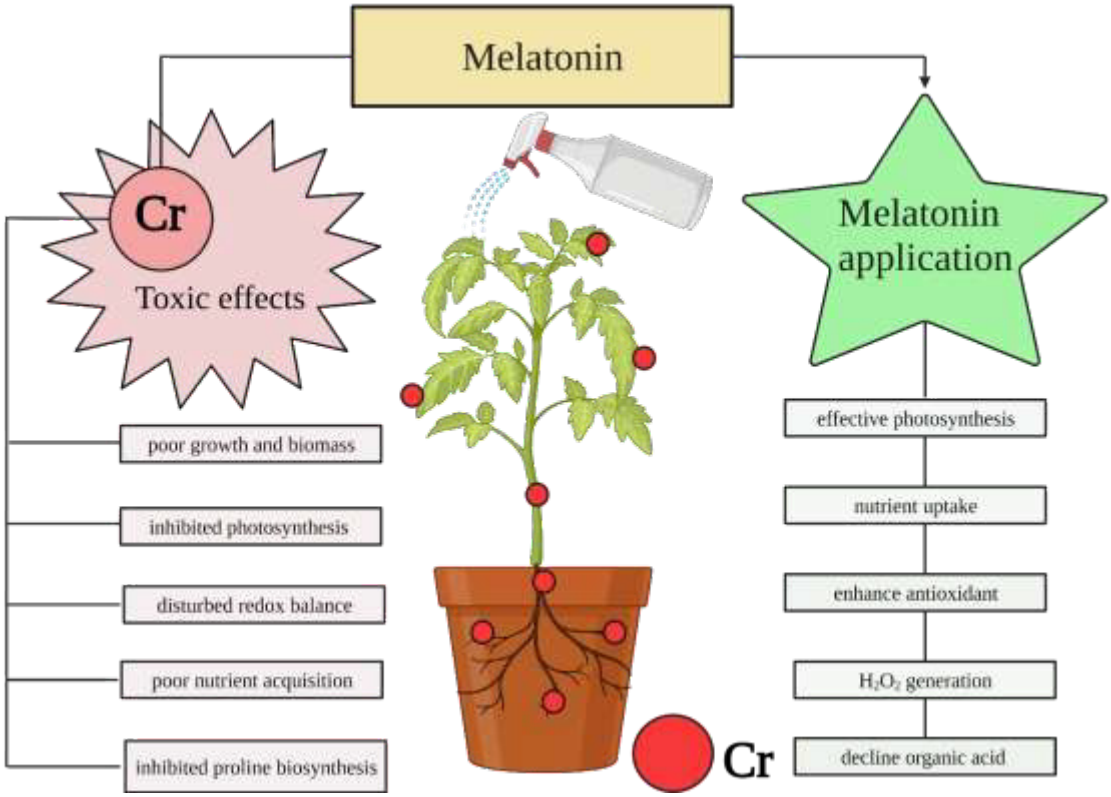

Figure 9. Detailed results of Melatonin (MEL) application in chromium (Cr) stressed soil on coriander: This figure presents comprehensive data demonstrating the effects of varying concentrations of MEL on coriander (*Coriandrum sativum* L.) grown in Cr-contaminated soil. Key parameters including plant growth metrics, photosynthetic pigment levels, antioxidant enzyme activities, and metal accumulation are depicted under different treatment conditions.

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Table 1S. Physical and nutritional properties of the soil used in this experiment.

Physical properties	
Organic matter	>70% of total solids
Density	350 kg/m^3
pH	7.6
Electrical conductivity	20mS/m
Organic nitrogen	1400 mg/L
Nutrients	**g/m^3**
Nitrogen (NO_3-N + NH_4-N)	150
Phosphorus (P)	75
Potassium (K)	160
Magnesium (Mg)	250
Calcium (Ca)	1600
Sulphur (S)	85
Copper (Cu)	2.5
Zinc (Zn)	1.8
Molybdenum (Mo)	2.7
Iron (Fe)	5.6

Table 2S. Gene-specific primer sequences were used in the present investigation.

Gene	**Primer sequence (5′-3′)**	**Gene accession number**	**Reference**
Fe-SOD	F: ATCTTAGTTATGGTTCTCTTTGT R: ATGGTGTAGAGCCTTTTCATAT	M64267	(Sirhindi et al., 2016)
POD	F: TTGAAATAAAC CAAAGGAGTAGT R: AATAATTATTTGAATCTCTTTAAGG	AF145349	(Sirhindi et al., 2016)
CAT	F: AGCATCTCACCTGAACTTGAA R: AGGTGAGAGGTTTGTGGCC	AF035252	(Sirhindi et al., 2016)
APX	F: CGTGACGATGATTGGGAAGT R: TGATAGTGATCTTTCGGACCT	NM_001354113	(Sirhindi et al., 2016)