

Co-application of titanium nanoparticles and melatonin effectively lowered chromium toxicity in lemon balm (*Melissa officinalis* L.) through modifying biochemical characteristics

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Abstract

Chromium (Cr) toxicity can negatively affect plant growth and development, impacting agricultural productivity and posing risks to human health. Metallic nanoparticles (MNPs) such as titanium dioxide (TiO₂) and natural growth regulators such as melatonin (MT) become promising technology to manage heavy metal-contaminated soils and promote safe food production. The present work was conducted to find the effect of foliar application of TiO₂ NPs (15 Mg L⁻¹) and MT (100 μM) on growth, biochemical attributes, and Cr accumulation in plant tissues of *melissa officinalis* L. under Cr toxicity (50 and 100 mg Cr kg⁻¹ soil). The results showed that Cr toxicity led to decreased plant performance, where 100 mg Cr kg⁻¹ soil led to notable decreases in shoot weight (28%), root weight (27%), essential oil (EO) yield (34%), chlorophyll (Chl) a + b (33%), while increased malondialdehyde (MDA, 30%), superoxide dismutase (SOD) activity (51%), and catalase (CAT) activity (122%). The use of TiO₂ NPs and MT, particularly their co-application, remarkably reduced Cr toxicity by enhancing plant weight, Chl content, and lowered MDA and antioxidant activity. Total phenolic content (TPC), total flavonoid content (TFC), EO percentage, and rosmarinic acid in plants treated with Cr at 50 mg Cr kg⁻¹ soil and co-application of TiO₂ NPs and MT were relatively higher than in other treatments. Under 100 mg Cr kg⁻¹ soil, the synergic effect of TiO₂ NPs and MT enhanced rosmarinic acid content (22%) but lowered Cr accumulation in roots (51%) and shoots (72%). Heat map analysis showed that ACT, SOD, MDA, and EO yield had the maximum variability under Cr, TiO₂ NPs, and MT. Exogenous TiO₂ NPs and MT can be recommended to modulate Cr toxicity in lemon balm under soil Cr toxicity.

1 Introduction

Chromium (Cr) contamination in the environment poses a significant threat to plant growth and development. It can disrupt various biochemical processes, leading to oxidative stress and reduced productivity. Chromium toxicity can have various negative effects on plant physiology, including reduced growth, disrupted photosynthesis, impaired nutrient uptake, oxidative stress, altered water status, and hormonal imbalance (Ao et al. 2022). The specific physiological responses can vary depending on factors such as Cr concentration, duration of exposure, and plant species. Plants have developed defense systems to deal with Cr toxicity, but knowing these physiological responses is critical for creating strategies to lessen its negative consequences (Malik et al. 2021). In recent years, researchers have explored various strategies to mitigate the toxic effects of Cr on plants. One such approach involves the use of nanoparticles (NPs) and plant growth regulators like melatonin (MT) and metallic non-metallic nanoparticles (Sun et al. 2023; Khosropour et al. 2023).

Titanium dioxide nanoparticles (TiO₂ NPs) have gained attention for their potential to alleviate metal stress in plants. They have distinct physicochemical characteristics that make them useful at reducing metal toxicity. Exogenous TiO₂ NPs have showed promise in reducing Cr toxicity in plants (Emamverdian et al. 2021). These NPs can enhance antioxidant defenses, reduce oxidative damage, improve photosynthesis, enhance nutrient uptake, increase detoxification capacity, and alleviate morphological

abnormalities caused by Cr toxicity. Additionally, melatonin, a hormone found in plants and animals, has been reported to have protective effects against oxidative stress and enhance plant tolerance to various environmental stressors (Rizwan et al. 2019).

Melissa officinalis L., commonly known as lemon balm, is a perennial herbaceous plant belonging to the Lamiaceae family. It is known for its aromatic leaves and has been traditionally used for its medicinal properties, including its calming effects and potential as a natural remedy for various ailments (Stojanović et al. 2023). Lemon balm contains various chemical compounds including rosmarinic acid, citronellal, eugenol, linalool, geraniol, and triterpenes. These compounds contribute to its antioxidant, anti-inflammatory, antimicrobial, analgesic, calming, and neuroprotective properties (Ghasemian et al. 2021; Stojanović et al. 2023).

In this context, the favorable role of MT on subsiding Cr toxicity have been addressed on wheat (Sun et al. 2023), maize (Malik et al. 2023) and rapeseed (Ayyaz et al. 2021; Gatasheh et al. 2023). In addition, Kumar et al. (2021) showed that TiO₂ NPs potentially regulate the mechanism (s) for photosynthetic attributes and antioxidants defense machinery in *Helianthus annuus* plants exposed to Cr toxicity. However, there is no accessible data regarding the co-application of MT and TiO₂ NPs on modifying Cr toxicity in lemon balm, highlighting the study's novelty.

The hypothesis is exogenous MT along with TiO₂ NPs will result in a significant reduction of Cr toxicity in lemon balm compared to the control plants. This reduction will be evident through improved physiological and biochemical characteristics such as enhanced antioxidant activity, reduced lipid peroxidation, modified secondary metabolites, and improved plant growth parameters. The study aims to investigate how the synergic effect of exogenous MT and TiO₂ NPs affects the physiological and biochemical characteristics of lemon balm exposed to Cr, with the ultimate goal of potentially improving the plant's ability to cope with Cr-induced stress. The findings of this study highlight the potential of exogenous MT in boosting the efficiency of TiO₂ NPs in lowering Cr toxicity in lemon balm. The results are expected to contribute to the development of sustainable strategies for mitigating heavy metal stress in plants and enhancing their productivity in contaminated environments.

2 Materials and Methods

2.1 Growth conditions

This work was conducted as factorial based on a completely randomized block design (CRBD) with three replicates in 2023. The seeds were planted in 3-liter pots in a greenhouse under conditions of 15/9 photoperiod (light/darkness) and 65–75% relative humidity. The experimental soil was obtained from an agricultural field, which consisted of N: 0.31%, P: 11.8 mg kg⁻¹, K: 257 mg kg⁻¹, Cr: 0.11 mg kg⁻¹, pH: 7.2, and EC: 1.2 dS m⁻¹, and it had a sandy loam texture.

Before seed cultivation, the soils were mixed with low concentrations of Cr at 50 and 100 mg kg⁻¹ soil. The seeds were cultivated in plastic pots and all plants were normally irrigated. At 6-leaf stage, the plants were sprayed with MT (Merk, Germany) at 100 µM and TiO₂ (Sigma-Aldrich, USA) at 15 Mg L⁻¹ three times in 15-day intervals. TiO₂ (Sigma-Aldrich, USA) had CAS number: 1317-70-0; purity: 99.9%; particle size: 20–30 nm; true density: 3.9 g cm⁻³. Fully mature plants were harvested after 85 days of seed cultivation.

2.2 Plant weight and seed yield

Aerial parts (shoots) of the plants were removed from the roots after harvesting, and each plant's weight was recorded in g using a digital scale (0.001 g).

2.3 Chlorophyll (Chl) assay

Leaf Chl contents were quantified using Arnon's (1949) instruction. Liquid nitrogen was used to grind the 0.1 g of fresh leaves. A centrifuge was then used for 10 min at 6000 rpm after 10 mL of acetone (80%) had been added. A spectrophotometer (Shimadzu UV-160) was used to test the absorbance of samples at wavelengths of 645 and 663 nm. The results were used in the following equations:

$$\text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645}) V / 100W$$

$$\text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663}) V / 100W$$

Where A₆₄₅ and A₆₆₃ are the absorbance values read at 645 and 663 nm, respectively; V is the final volume of acetone consumed in mL; W is fresh tissue weight.

2.4 Malondialdehyde (MDA) concentration

Leaf MDA content was measured by centrifuging 0.5 g of fresh leaves for 30 min at 14000 rpm after they were combined with 4 mL of 20% w/v trichloroacetic acid (TCA) that contained 0.5% thiobarbituric acid. The mixture was then maintained in a hot water bath at 95°C for 25 min. After 30 min in a hot water bath, the samples were centrifuged for 10 min at 10000 rpm. 532 nm was the wavelength at which samples were read (Heath and Packer 1969).

2.5 Enzymes assay

Fresh leaf samples were used to obtain enzyme extract in 5 mL of cold 50 mM K-phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1% Polyvinylpolypyrrolidone (PVPP). Catalase (CAT) activity was determined by calculating the initial rate of H₂O₂ disappearance (Dhindsa et al. 1981). In 0.05 M Na-phosphate buffer (pH 7), the reaction mixture contained 3% H₂O₂ and 0.1 mM ethylenediaminetetraacetic acid (EDTA). The decrease in H₂O₂ concentration was detected as a decrease in optical density at 240 nm, and the activity was calculated as mol H₂O₂ decomposed as an enzyme unit (U) per mg protein. Superoxide dismutase (SOD) activity was measured using the method described by Beyer and Fridovich (1987). 1.17 × 10⁻⁶ M riboflavin, 0.1 M methionine, 2 × 10⁻⁵ M KCN, and 5 × 10⁻⁵ M nitroblue tetrazolium salt (NBT) were dissolved in 3 mL of 0.05 M sodium phosphate buffer

(pH 7.8). One mL of enzyme extract was mixed with 3 mL of reaction. Illumination was turned on for 60 min to kickstart the reaction at 30°C. The blanks were identical solutions stored in the dark. The absorbance was measured using a spectrophotometer at 560 nm relative to the blank.

2.6 Determination of total phenolic content (TPC)

The Folin-Ciocalteu reagent was used to detect leaf TPC spectrophotometrically. The 100 µl of the MeOH solution of the precisely determined weight of studied plant 1–10 (2–15 mg mL⁻¹) were mixed with 0.75 mL of Folin-Ciocalteu reagent and left to stand at 22 °C for 5 min. Then 0.75 mL of NaHCO₃ was added to the mixture. After 90 min at, absorbance was measured at 725 nm using a UV-VIS spectrophotometer (Varian Cary 50). Gallic acid (GA) at 0-100 mg mL⁻¹ was used to calibrate the standard curve. The results were expressed as mg GA g⁻¹ dry weight (DW).

2.7 Determination of total flavonoid content (TFC)

The aluminum chloride colorimetric method was used to measure TFC in lemon balm leaves. In brief, 2.8 mL of distilled water was mixed with 0.5 mL of extract solution containing 1.5 mL of 95% ethanol, 0.1 mL of aluminum chloride 10%, and 0.1 mL of 1 M potassium acetate. The mixture was vortexed for 10 s and then set aside at 25 °C for 30 min. The absorbance of the combination was measured at 415 nm.

Quercetin concentrations ranging from 0 to 1200 g mL⁻¹ were generated, and the standard curve was calibrated using a linear fit.

2.8 EO content

The EO content of shoots was determined using the method given by Sefidkon et al. (2006). In brief, 100 g of dried aboveground plant parts were hydro-distilled for 3 h in a Clevenger-type device. After that, the percentage of EO (w/w) was determined as weight of EO per 100 g of plant DW.

2.9 Rosmarinic acid measurement

Lemon balm leaves were air-dried and crushed. The crushed leaves were then powdered using a mixer mill with a steel ball. 50 mg of the ground leaf material was weighed and mixed with 1.5 mL of 50% aqueous ethanol. The mixture was vortexed and sonicated, and then centrifuged. The supernatant was transferred to a volumetric flask, and the remaining residue was extracted twice more with 1.5 mL of 50% aqueous ethanol. The resulting extracts were combined and adjusted to a total volume of 10 mL with 50% aqueous ethanol. A portion of the solution was filtered and stored for analysis. The analysis of rosmarinic acid was performed using a high-performance liquid chromatography (HPLC) system. The extracts were injected onto a C18 column and separated using a gradient of aqueous formic acid and methanol as eluents. The column temperature was maintained at 40°C. Rosmarinic acid was detected at 320 nm and quantified using an external standard calibration (Wang et al. 2004).

2.10 Cr concentration in plant tissues

The plants were uprooted and separated into roots and shoots. They were then washed and dried in an oven at 60°C. The dried plant sample (1 g) was crushed into a powder and passed through a 0.5-mm sieve. The powdered sample was then digested using a mixture of nitric acid and hydrogen peroxide in high temperature. After digestion, the solutions were cooled, filtered, and diluted to a final volume of 50 mL using distilled deionized water (DDW). The concentration of Cr in the digested solutions was quantified using an atomic absorption spectrophotometer (AAS, SpectrAA 220).

2.11 Data analysis

Using SAS software, LSD test was employed to compare means with a 5% statistical significance level. Heat map analysis was done by CIMMiner online at <https://discover.nci.nih.gov/cimminer/home.do>.

3 Results

3.1 Plant weight

The Cr toxicity decreased plant weight, while Ti NPs and MT increased it. There were 13 and 28% decreases in shoot weight (Fig. 1a) and 17 and 27% in root weight (Fig. 1b) of lemon balm when exposed to 50 and 100 mg kg⁻¹ soil, respectively, relative to the control. However, TiO₂ NPs and MT, particularly their co-application, enhanced plant weight. Foliar applied TiO₂, MT, and TiO₂ + MT raised shoot weight (Fig. 1c) by 8, 13, and 16% and root weight (Fig. 1d) by 10, 11, and 17%, respectively, compared with the control.

3.2 Chl and MDA

The interaction effect of Cr toxicity and foliar-applied TiO₂ and MT was significant ($P \leq 0.05$) on Chl and MDA. Cr toxicity decreased Chl content, but TiO₂ NPs and MT had a positive role in enhancing it. Without TiO₂ and MT, Cr toxicity at 50 and 100 mg Cr kg⁻¹ soil diminished Chl *a + b* by 23 and 35%, respectively, relative to the control. Under Cd stress at 100 mg kg⁻¹ soil, the use of TiO₂, MT, and TiO₂ + MT increased Chl *a + b* by 11, 10, and 16%, respectively (Fig. 2a). MDA was raised by Cr toxicity but reduced with TiO₂ and MT. Without TiO₂ and MT, the use of Cr concentration at 50 and 100 mg Cr kg⁻¹ soil increased MDA content by 41 and 59%, respectively. However, foliar application of TiO₂ NPs and MT lowered MDA. Under moderate Cr toxicity (50 mg Cr kg⁻¹ soil), TiO₂, MT, and TiO₂ + MT reduced MDA by 16, 15, and 23%, respectively (Fig. 2b).

3.3 SOD and CAT activity

The acidity of antioxidant enzymes was significantly ($P \leq 0.05$) influenced by the interaction of Cr toxicity and foliar-applied TiO₂ and MT. The maximum SOD activity was obtained in plants exposed to 100 mg Cr kg⁻¹ soil without TiO₂ NPs and MT with a 97% increase relative to the control. The interesting results

were obtained under severe drought stress with significant declines of SOD activity under TiO₂ NPs and MT. Under severe Cr toxicity, the use of TiO₂, MT, and TiO₂ + MT lowered SOD activity by 23, 25, and 29%, respectively, relative to the non-application of TiO₂ and MT (Fig. 3a). The trend of CAT was similar to the SOD, with the highest activity at severe Cr toxicity without TiO₂ and MT to be 253% increases relative to the control. Co-application of TiO₂ and MT lowered CAT activity by 30% in plants exposed severe Cr toxicity relative to the treatment without TiO₂ and MT (Fig. 3b).

3.4 TPC and TFC

Although TPC and TFC showed no significant difference under 100 mg Cr kg⁻¹ soil, they showed a significant increase under 50 mg Cr kg⁻¹ soil. Relative to the control, the 16 and 14% increases for TPC (Fig. 4a) and TFC (Fig. 4b), respectively, were obtained in plants experienced 50 mg Cr kg⁻¹ soil. The data showed a significant increase in TPC and TFC particularly when plants treated with con-application of TiO₂ NPs and MT. The use of TiO₂ + MT led notable increases of 13 and 16% in TPC (Fig. 4c) and TFC (Fig. 4d), respectively, when compared with the control.

3.5 EO and EO yield

Cr toxicity changed the EO content and EO yield of lemon balm plants. Moderate Cr toxicity led to a notable boost of EO to 24% relative to the control (Fig. 5a). Although moderate Cr toxicity didn't show a significant difference from the control, severe Cr stress (100 mg Cr kg⁻¹ soil) lowered EO yield by 34% relative in comparison with the control (Fig. 5b). However, the use of TiO₂ NPs and MT led to remarkable increases in EO content and EO yield. relative to the control, the co-application of Ti NPs and MT enhanced EO content by 17% (Fig. 5c). In addition, the use of TiO₂ NPs, MT, and TiO₂ + MT enhanced EO yield by 16, 19, and 36%, relative to the control (Fig. 5d).

3.6 Rosmarinic acid content

The interaction of Cr toxicity and foliar-applied MT and TiO₂ was significant in rosmarinic acid content. Moderate Cr stress enhanced rosmarinic acid but severe Ct toxicity lowered its content. The minimum was obtained under server Cr toxicity without MT and TiO₂ with a 31% decline relative to the control. Foliar spraying with MT and TiO₂ NPs enhanced rosmarinic acid. Under severe Ct toxicity, by 20, 24, and 21%, relative to non-MT and TiO₂ (Table 1).

Table 1

Plant weight and rosmarinic acid of lemon balm under chromium toxicity and titanium dioxide nanoparticle and melatonin;

Cr toxicity	TiO ₂ & MT	Root Cr content	Shoot Cr content	Rosmarinic acid
Non-Cr	Control	0.05 ± 0.01f	0.04 ± 0.01f	47.7 ± 0.95ab
	TiO ₂	0.05 ± 0.01f	0.03 ± 0.01f	48 ± 0.01ab
	MT	0.05 ± 0.02f	0.03 ± 0.02f	49 ± 0.02a
	TiO ₂ + MT	0.04 ± 0.01f	0.02 ± 0.01f	48.3 ± 1.57ab
50 mg Cr/kg soil	Control	27.33 ± 2.53d	4.1 ± 0.35c	46 ± 1.25c
	TiO ₂	17.67 ± 2.95e	1.8 ± 0.17e	47.7 ± 0.95ab
	MT	14 ± 1.25e	1.83 ± 0.26e	48 ± 1.25ab
	TiO ₂ + MT	13.33 ± 0.95e	1.33 ± 0.1e	47 ± 0.63bc
100 mg Cr/kg soil	Control	74.33 ± 5.64a	11.43 ± 1.06a	33 ± 1.25e
	TiO ₂	42.33 ± 1.57b	5.17 ± 0.38b	41 ± 0.63d
	MT	39.33 ± 3.15bc	4.63 ± 0.48bc	39.7 ± 0.95d
	TiO ₂ + MT	36 ± 2.25c	3.13 ± 0.22d	40 ± 1.08d

3.7 Cr concentration in leaf and root

Cr content in leaves and roots was significantly affected by the interaction of Cr toxicity and foliar-applied MT and TiO₂. As expected, the amount of Cr in roots and leaves increased by increasing soil Cr levels. However, the use of MT and TiO₂ diminished the accumulation of Cd in roots and shoots. Although the high amount of Cr can be uptake by roots their transferee to aerial parts will be hindered by MT and TiO₂. Under severe Cr toxicity, the use of MT + TiO₂ lowered Cr accumulation by 51 and 72% in roots and leaves, respectively, relative to non-MT and TiO₂ applications (Table 1).

3.8 Heat map analysis

According to the heat map study, CAT, MDA, and SOD were clustered in a distinct cluster and showed the greatest variability under Cr toxicity. TPC, TFC, rosmarinic acid, EO, and EO yield, on the other hand, exhibited the least fluctuation under Cr stress. Based on the clustering, 100 mg Cr kg⁻¹ soil is considerably distinct from other threats in andiron (Fig. 6a). EO yield had the greatest variability in MT and TiO₂ NPs, followed by SOD, CAT, and MDA. The clustering revealed that TiO₂ and MT were in one cluster, control was in another, and TiO₂ + MT was in yet another (Fig. 6b).

4 Discussion

Chromium toxicity decreased plant weight of lemon balm. It can decrease soil fertility by affecting nutrient availability and uptake by plants. Cr ions can enter plant cells and cause cellular damage. It can disrupt cellular structures and impair various physiological functions, leading to cellular dysfunction and ultimately hindering plant growth. Cr toxicity can induce oxidative stress in plants. High levels of Cr can generate ROS such as superoxide radicals and hydrogen peroxide, leading to oxidative damage to plant cells. Oxidative stress can disrupt cellular membranes, impair enzyme activities, and hinder plant growth (Javed et al. 2021). It can interfere with essential plant nutrients such as phosphorus, iron, and calcium, leading to nutrient imbalances and reduced plant growth. High levels of Cr in soils can inhibit plant growth and reduce crop productivity. Cr can accumulate in plant tissues and eventually transfer to higher trophic levels through consumption. This bioaccumulation and biomagnification phenomenon can pose risks to wildlife and humans who consume contaminated plants or animals, leading to potential health hazards. Therefore, Cr toxicity negatively affects plant growth by impairing seed germination, stunting growth, causing chlorosis, inducing cellular damage, interfering with nutrient uptake and assimilation, disrupting water and nutrient transport, and inducing oxidative stress. These effects collectively reduce plant vigor, biomass production, and overall plant health (Ahmad et al. 2020). Exogenous MT and TiO₂ NPs increased plant weight. Melatonin acts as a powerful antioxidant, and its exogenous application can enhance the plant's antioxidant defense system when used in conjunction with TiO₂ NPs. This combination can provide added protection against oxidative stress, reducing the damage caused by ROS and improving overall plant health. The synergistic action of MT and TiO₂ NPs can boost the plant's ability to scavenge ROS more effectively (Giglou et al. 2023; Sun et al. 2023). Exogenous MT application has been shown to enhance nutrient uptake in plants. By promoting the activity of nutrient transporters and improving root development, melatonin can increase the absorption and translocation of essential nutrients in the presence of TiO₂ NPs. This improved nutrient uptake facilitates better plant growth and biomass accumulation. Melatonin has been reported to enhance photosynthesis in plants. When combined with TiO₂ NPs, which can also enhance the efficiency of light absorption and energy transfer, exogenous melatonin can further increase photosynthetic activity (Emamverdian et al. 2022). This combination leads to improved carbon assimilation, higher carbohydrate production, and ultimately, enhanced plant growth and weight. In addition, foliar-applied MT can regulate the balance of various plant hormones, including auxins, cytokinins, and gibberellins. This hormone modulation in the presence of TiO₂ NPs can influence cell division, elongation, and overall plant growth, resulting in greater biomass accumulation and increased plant weight (Rizwan et al. 2023). Previously, the positive role of TiO₂ NPs on *Pleiblastus pygmaeus* (Emamverdian et al. 2022) and MT on *Zea mays* (Malik et al. 2021) and *Brassica napus* (Ayyaz et al. 2021). However, there is no prior evidence on the co-application of MT and TiO₂ NPs to improve plant biomass under Cr toxicity that can be presented in this study.

Cr toxicity reduced Chl content in lemon balm plants. Cr toxicity can disrupt Chl synthesis, which is essential for the production of Chl molecules. Cr interferes with the activity of enzymes involved in the biosynthesis of Chl, such as chlorophyll synthase and magnesium chelatase. This disruption hampers

the normal production and turnover of Chl molecules, leading to a decrease in Chl content. Cr toxicity can disrupt the structure and function of photosystem components, specifically photosystem II (PS II). PS II is responsible for capturing light energy and initiating the process of photosynthesis (Bruno et al. 2020). Chromium can impair the activity of PS II reaction centers and reduce the efficiency of light energy conversion, which can reduce the synthesis of Chl molecules. Cr toxicity can promote the generation of ROS in plants, which can damage cell membranes, proteins, and pigments, including chlorophyll. Chromium can disrupt the integrity and permeability of cell membranes in plants (Kumar and Seth 2022; Singh et al. 2023). This disturbance affects the efficiency of nutrient uptake, water transport, and metabolic processes, including Chl synthesis and maintenance. As a result, the plants may experience reduced availability of minerals and impaired photosynthetic machinery, leading to a decrease in Chl content. Exogenous melatonin, when combined with titanium nanoparticles TiO₂ NPs, can enhance the efficiency of TiO₂ NPs on Chl content under Cr toxicity. Melatonin acts as an antioxidant, protecting chlorophyll molecules from oxidative damage caused by Cr toxicity. It also regulates gene expression and hormone levels related to Chl synthesis and photosynthesis. Additionally, melatonin enhances nutrient uptake, supporting Chl production. The synergistic effects of melatonin and TiO₂ NPs lead to a more significant increase in Chl content, providing a more efficient approach to counteract the negative effects of Cr toxicity on Chl content in plants (Raja et al. 2023). Gatasheh et al. (2023) reported that synergistic application of MT and Si NPs alleviates Cr stress in *Brassica napus* through improving Chl content.

The foliar application of MT and TiO₂ NPs decreased MDA content under Cr toxicity. Cr toxicity increases the production of ROS in plants. These ROS attack and oxidize lipids in cell membranes, leading to lipid peroxidation. This peroxidation process results in the formation of MDA, a byproduct of lipid peroxidation. Thus, Cr toxicity increases MDA levels as a consequence of oxidative damage to cell membranes (Kumar et al. 2023). Melatonin and TiO₂ NPs possess antioxidant properties and can scavenge ROS generated under Cr toxicity. ROS can cause oxidative damage to cell membranes and increase MDA levels. By neutralizing these ROS, MT and TiO₂ NPs prevent lipid peroxidation and decrease MDA production. Cr toxicity can disrupt the integrity and fluidity of cell membranes. This disruption can lead to increased lipid peroxidation and MDA levels. Melatonin and TiO₂ NPs can help maintain the integrity of cell membranes by preventing lipid peroxidation and stabilizing membrane structure. This action reduces the release of MDA into the extracellular space and decreases MDA levels. MT and TiO₂ NPs can enhance the activity of antioxidant enzymes, which play a crucial role in the detoxification of ROS and the prevention of lipid peroxidation (Chen et al. 2021; Malik et al. 2021; Mao et al. 2023). MT and TiO₂ can modulate signaling pathways involved in oxidative stress responses. These substances can regulate the expression of genes related to antioxidant defense systems and lipid metabolism. By influencing these signaling pathways, MT and TiO₂ can mitigate the production of MDA under Cr toxicity.

Soil Cr at 100 mg Kg⁻¹ soil had toxic effects on lemon balm plants. Cr toxicity leads to increased ROS production in cells, which damage cellular components. In response to this oxidative stress, cells upregulate the production of antioxidant enzymes such as SOD and CAT to counteract the detrimental

effects of ROS. Cr toxicity can modulate gene expression, resulting in increased synthesis and production of SOD and CAT enzymes (Albqmi et al. 2023). This regulation involves the activation or inhibition of specific transcription factors that control the expression of these enzymes. Cr toxicity can also affect the post-translational modifications of SOD and CAT enzymes, thereby impacting their activity. For instance, Cr can induce phosphorylation, acetylation, or nitration of these enzymes, which can either enhance or impair their catalytic functions. These modifications can alter enzyme stability, substrate specificity, or interaction with cofactors, ultimately affecting their enzymatic activities. In addition, Cr ions can directly interact with SOD and CAT enzymes, impacting their activity. Cr can bind to metal centers within these enzymes, such as the copper and zinc (Cu/Zn) active site of SOD or the iron (Fe) center of CAT, leading to changes in their enzymatic properties. This interaction can either enhance or inhibit the catalytic efficiency and stability of these enzymes, depending on the Cr species and concentration (Tang et al. 2023). SOD and CAT activity was higher overall than in the control, but it can be lower in stressed plants. It could be because TiO₂ and MT can control Cr toxicity, resulting in decreased ROS generation. As a result, when ROS levels in the cell are low, enzyme activity is decreased. Foliar application of TiO₂ and MT is a powerful antioxidant molecule known for its ability to scavenge ROS and reduce oxidative stress in plants (Deng et al. 2024). It can directly neutralize ROS or indirectly facilitate the activities of antioxidant enzymes like SOD and CAT. Melatonin is believed to stimulate the expression and activity of these enzymes by modulating gene expression pathways (Sun et al. 2023). It can activate specific transcription factors or signaling molecules, leading to upregulation of the SOD and CAT genes. Similar to our results, the decreased antioxidant enzyme activity was obtained in stressful plants when treated with NPs (Zadegan et al. 2023).

Cr toxicity at 50 mg kg⁻¹ soil led to the maximum TPC and TFC in lemon balm plants. Moderate Cr toxicity can induce the production of TPC and TFC. This is primarily achieved through the activation of defense responses, including the synthesis of phenolic compounds as part of the plant's antioxidant defense system (Khosropour et al. 2021). These phenolic compounds, such as flavonoids, can scavenge ROS, induce antioxidant enzymes, chelate metal ions, and regulate gene expression pathways. Overall, the increase in TPC and TFC helps plants cope with the toxic effects of Cr by enhancing their antioxidant capacity and protection against oxidative stress. Similarly, the increased TPC and TFC have been reported on coriander (Baabashpour et al. 2022), summer savory (Alavian et al. 2023), barberry (Khosropour et al. 2021) under moderate amounts of heavy metals. Both MT and TiO₂ NPs can stimulate the synthesis of secondary metabolites, including phenolic compounds and flavonoids, in plants. These compounds are synthesized through various biochemical pathways, such as the phenylpropanoid and flavonoid biosynthetic pathways. The application of Ti NPs or melatonin can activate the expression of key enzymes involved in these pathways and subsequently increase TPC and TFC. Melatonin and TiO₂ have antioxidant properties and can scavenge ROS in plants. ROS accumulation can result in oxidative stress, leading to the synthesis of phenolic compounds and flavonoids as a defense response (Vafadar et al. 2020). The scavenging of ROS by MT and TiO₂ NPs reduces the oxidative stress burden on plants, allowing them to allocate resources towards the synthesis of TPC and TFC. Melatonin and TiO₂ can activate specific genes associated with the biosynthesis of phenolic compounds and flavonoids. These

molecules can interact with various signaling pathways, including transcription factors and protein kinases, to enhance the expression of genes encoding key enzymes involved in TPC and TFC synthesis. By upregulating the expression of these genes, MT promote the accumulation of TPC and TFC in plants (Tousi et al. 2020; Nourozi et al. 2021). MT and TiO₂ NPs and melatonin can modulate the activity of enzymes involved in the biosynthesis of TPC and TFC. For instance, they may enhance the activity of key enzymes such as phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) in the phenylpropanoid and flavonoid pathways, respectively. This increased enzyme activity leads to higher production and accumulation of phenolic compounds and flavonoids, thereby increasing TPC and TFC levels (Vafadar et al. 2020).

Although moderate Cr toxicity increased EO content, its severe stress decreased EO yield. Chromium is a heavy metal that can have both beneficial and detrimental effects on plants, depending on its concentration and the duration of exposure. The response of plants to Cr toxicity can vary, and the effects on EO production may be influenced by multiple factors, including the plant species, growth stage, and Cr concentration. Under moderate levels of Cr toxicity, some studies have suggested that certain plant species may exhibit an increase in EO production as a defense mechanism against the stress induced by Cr (Prasad et al. 2010). This increase in EO production could be a response to counteract oxidative stress and alleviate the toxic effects of Cr. On the other hand, higher concentrations or severe levels of Cr toxicity are known to have detrimental effects on plant growth and development. This can lead to physiological disorders, such as reduced photosynthesis, impaired nutrient uptake, and oxidative damage. In such cases, the plant's overall health and productivity can be negatively affected, potentially leading to a decrease in EO production (Patra et al. 2019).

MT and TiO₂ NPs have been reported to stimulate the synthesis of secondary metabolites, including essential oils, in plants. These compounds are produced through various biochemical pathways, such as the terpenoid and phenylpropanoid pathways. The application of MT and TiO₂ NPs can activate the expression of key enzymes involved in these pathways, leading to an increase in essential oil production. MT and TiO₂ can activate specific genes associated with the biosynthesis of essential oils. They can interact with various signaling pathways, such as transcription factors and protein kinases, to enhance the expression of genes encoding enzymes involved in essential oil synthesis (Gohari et al. 2020). By upregulating these genes, MT and TiO₂ and melatonin promote the accumulation of essential oils in plants. TiO₂ NPs and melatonin can induce the release of volatile compounds, including essential oils, from plant tissues. This could be attributed to their influence on the regulation of stomatal opening and closure, which can affect the release of essential oils. The increased release of volatile compounds leads to a higher concentration of essential oils in plants treated with co-application of MT and TiO₂ (Bidabadi et al. 2020; Gohari et al. 2020).

The moderate Cr toxicity had a positive impact but severe Cr stress possessed a negative impact on the production and accumulation of rosmarinic acid in lemon balm. Cr is a heavy metal that can be taken up by plant roots and subsequently transported to various plant tissues, where it can disrupt normal cellular

processes and biochemical pathways. One way in which Cr toxicity can reduce rosmarinic acid in lemon balm is by interfering with the activity of key enzymes involved in its biosynthesis (Adamczyk-Szabela et al. 2023). Rosmarinic acid is synthesized through the phenylpropanoid pathway, which begins with the conversion of phenylalanine into cinnamic acid by the enzyme phenylalanine ammonia-lyase (PAL). The increased PAL and rosmarinic acid were reported under salinity stress in lemon balm by Ghasemian et al. (2021). Studies have shown that severe Cr can inhibit the activity of PAL and other enzymes involved in this pathway, thereby reducing the synthesis of rosmarinic acid (Gho et al. 2020). Furthermore, Cr toxicity can disrupt the antioxidant defense system in lemon balm. Rosmarinic acid acts as a potent antioxidant, protecting the plant against oxidative stress. However, heavy metals like Cr can ROS and impair the plant's ability to scavenge and neutralize them, causing oxidative damage (Adamczyk-Szabela et al. 2023). This oxidative stress can lead to a reduced production of rosmarinic acid, as the plant allocates its resources towards combating Cr-induced oxidative damage. Cr ions can compete with essential nutrients like nitrogen, phosphorus, and iron, leading to nutrient deficiencies and impaired rosmarinic acid production. Abiotic stress can interfere with gene expression and transcription factors involved in the biosynthesis of rosmarinic acid. Studies have shown that abiotic stresses can downregulate the expression of genes encoding key enzymes like PAL, affecting the overall synthesis of rosmarinic acid (Ghasemian et al. 2021). Foliar application of MT and TiO₂ NPs has been investigated for their potential to enhance rosmarinic acid production in lemon balm and other plant species. MT and TiO₂ NPs can stimulate the phenylpropanoid pathway and activate key enzymes involved in rosmarinic acid synthesis, while MT acts as a signaling molecule to enhance gene expression in the phenylpropanoid pathway. Both MT and also have antioxidant properties, reducing oxidative stress and redirecting plant resources towards secondary metabolite production (Vafadar et al. 2020). The combination of MT and TiO₂ NPS has shown synergistic effects on rosmarinic acid production. However, the optimal concentration and application method should be determined for efficient results without negative impacts on plant growth and development.

5 Conclusions

The study demonstrated that the combined application of exogenous melatonin and titanium dioxide nanoparticles resulted in an enhanced reduction of chromium toxicity in lemon balm plants and improved the plant's biochemical characteristics. Foliar-applied melatonin and titanium dioxide nanoparticles effectively mitigated chromium-induced oxidative stress, as evidenced by decreased chlorophyll and plant biomass and increased activities of antioxidant enzymes such as catalase, superoxide dismutase, as well as reduced malondialdehyde content. Furthermore, the combination treatment significantly improved the total phenolic content and flavonoid concentration in lemon balm leaves, indicating an enhancement in the plant's production of secondary metabolites. This upregulation of secondary metabolites corresponded with an increase in rosmarinic acid content in lemon balm. Overall, this study highlights the potential of exogenous melatonin in combination with titanium dioxide nanoparticles to alleviate chromium toxicity and enhance the biochemical characteristics of lemon balm, suggesting a promising approach for sustainable cultivation and increased yield of valuable phytochemicals in lemon

balm. Further investigation and optimization of the dosage and application methods of melatonin and titanium dioxide nanoparticles are warranted to fully understand their potential in agricultural and environmental applications.

Declarations

Ethical Approval Ethical approval was not required for this work

Consent to Participate The author agreed to submit the manuscript to *Environmental Science and Pollution Research*

Consent to Publish The author approved the final manuscript to publish in *Environmental Science and Pollution Research*

Authors Contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Mona H. Soliman, Suliman M.S. Alghanem, Ibtisam Mohammed Alsudays. The first draft of the manuscript was written by Abdullah Alaklabi, Basmah M. Alharbi, Hadba Al-Amrah, Ehab Azab, Ghalia S.H. Alnusairi and edited by others. All authors read and approved the final manuscript.

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Competing Interests There is no competing interest

Availability of data and materials The data are available on request

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Figures

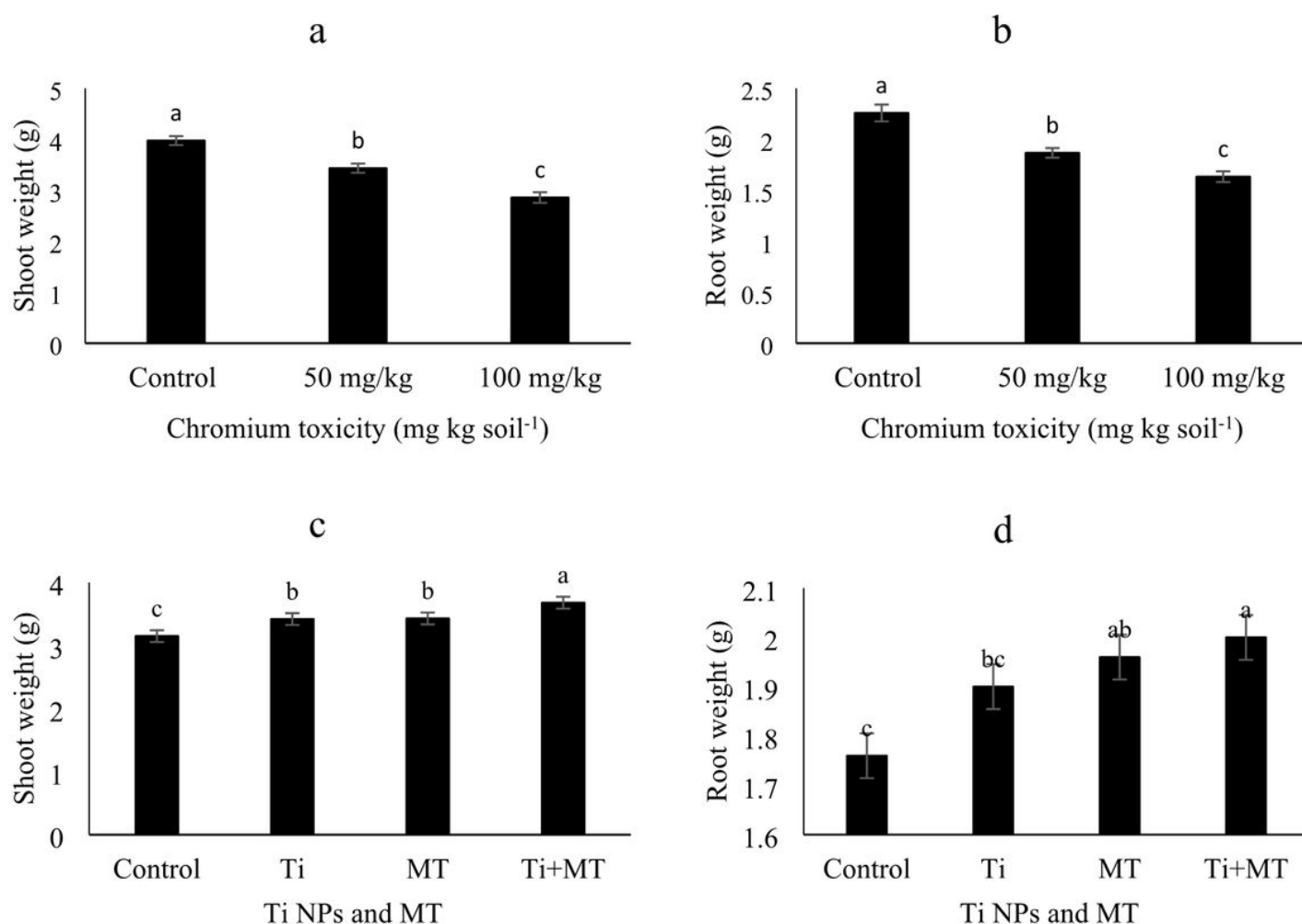


Figure 1

Shoot and root weight of lemon balm under chromium toxicity (a and b) and titanium dioxide nanoparticle and melatonin (c and d). Lowercases letters show significance between treatments. Bars are standard error (SE) of the data.

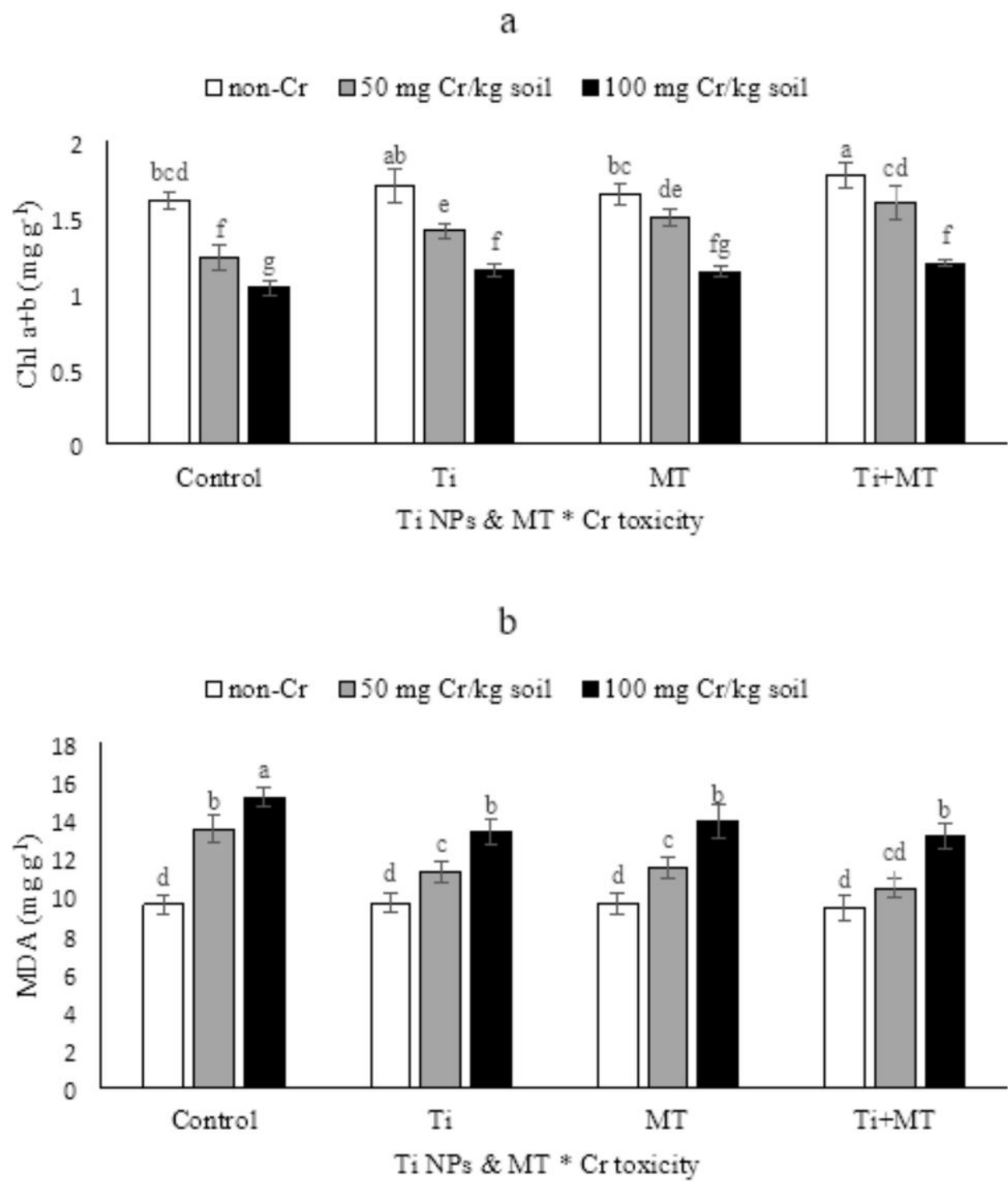


Figure 2

Chlorophyll (Chl, a) and malondialdehyde (MDA, b) of lemon balm plants under the interaction of chromium toxicity and titanium dioxide nanoparticle and melatonin. Lowercases letters show significance between treatments. Bars are standard error (SE) of the data.

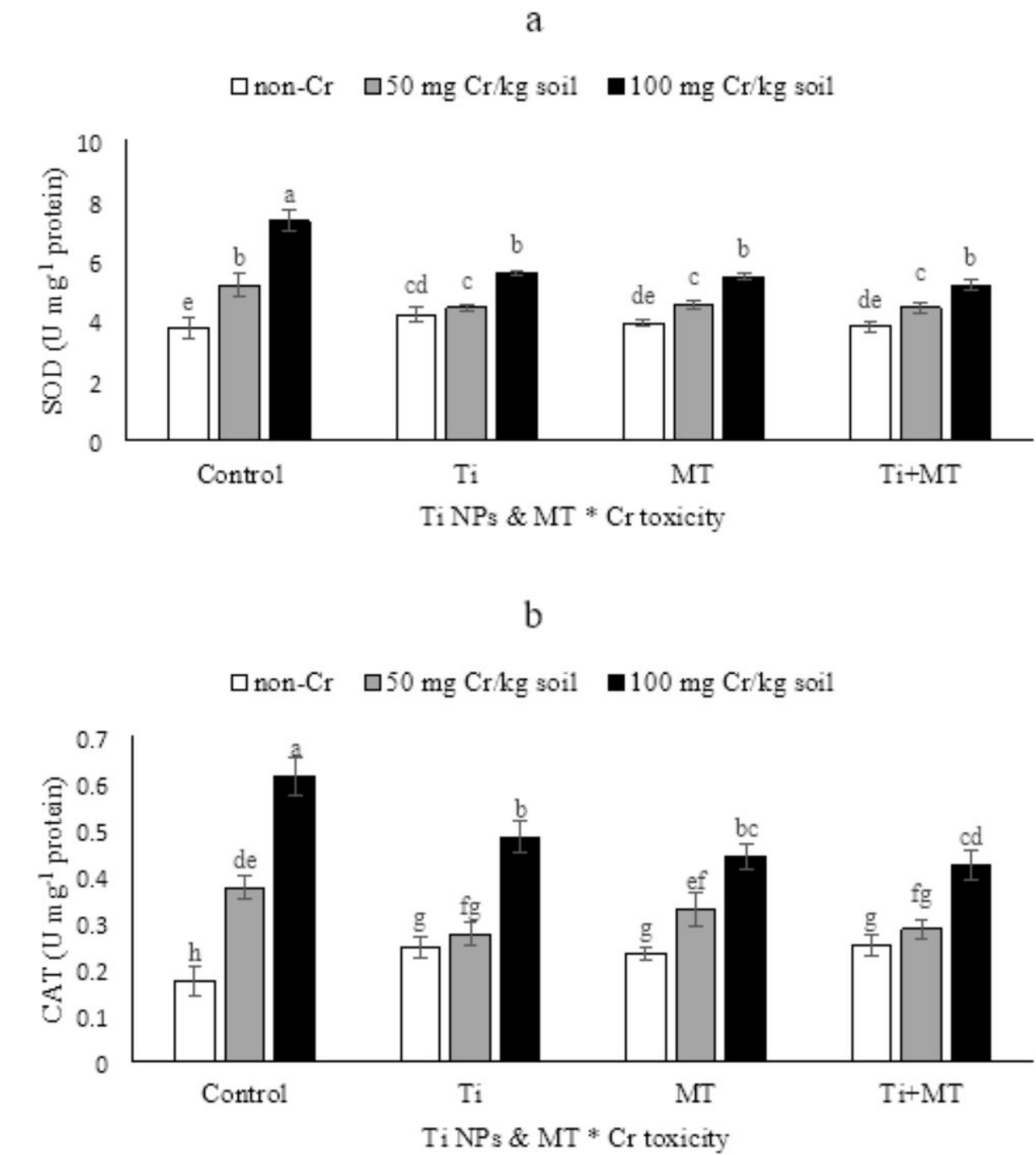


Figure 3

Superoxide dismutase (SOD, a) and catalase (CTA, b) of lemon balm plants under the interaction of chromium toxicity and titanium dioxide nanoparticle and melatonin. Lowercases letters show significance between treatments. Bars are standard error (SE) of the data.

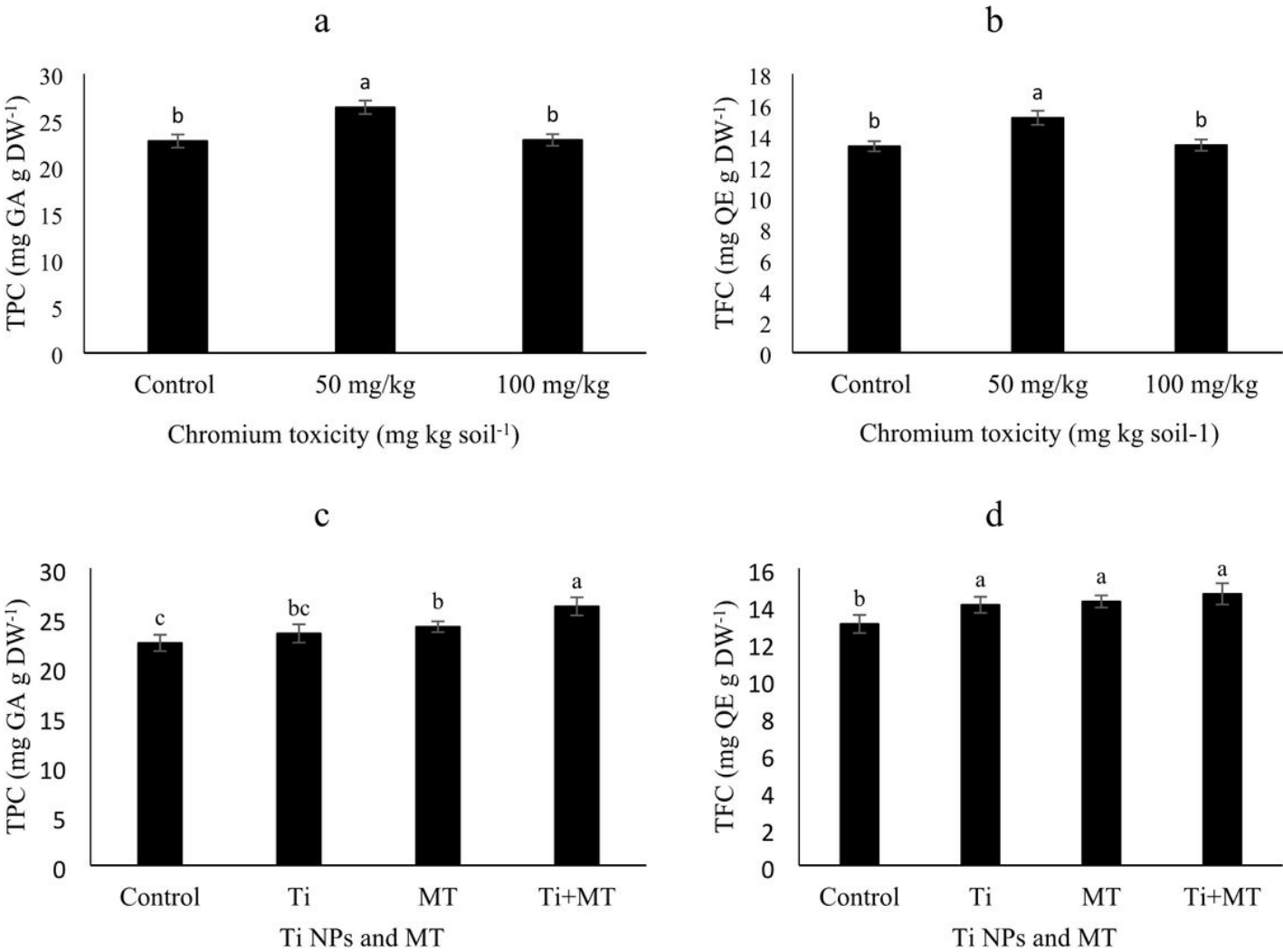


Figure 4

Total phenolic content (TPC) and total flavonoid content (TFC) of lemon balm under chromium toxicity (a and b) and titanium dioxide nanoparticle and melatonin (c and d). Lowercases letters show significance between treatments. Bars are standard error (SE) of the data.

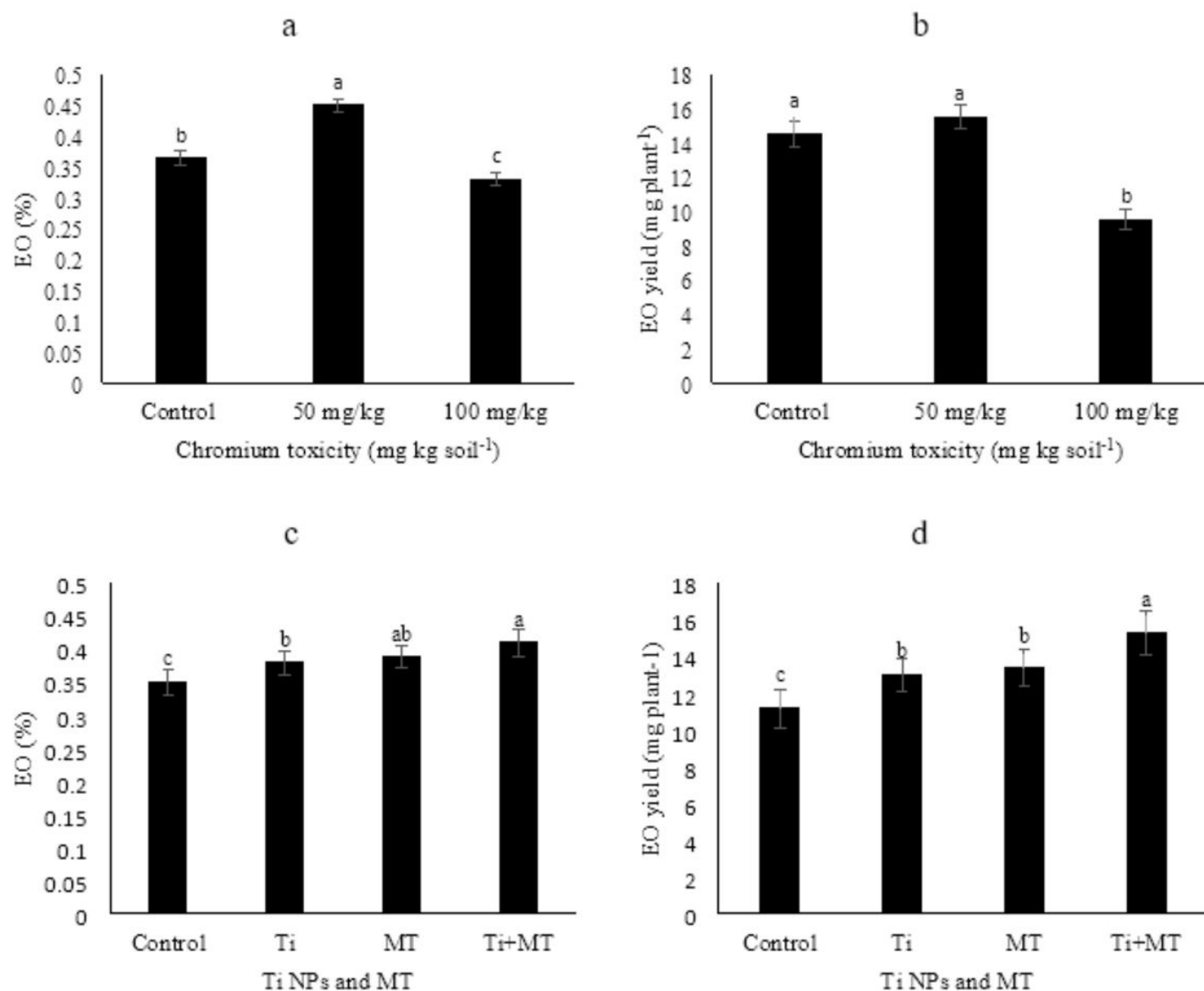


Figure 5

Total phenolic content (TPC) and total flavonoid content (TFC) of lemon balm under chromium toxicity (a and b) and titanium dioxide nanoparticle and melatonin (c and d). Lowercases letters show significance between treatments. Bars are standard error (SE) of the data.

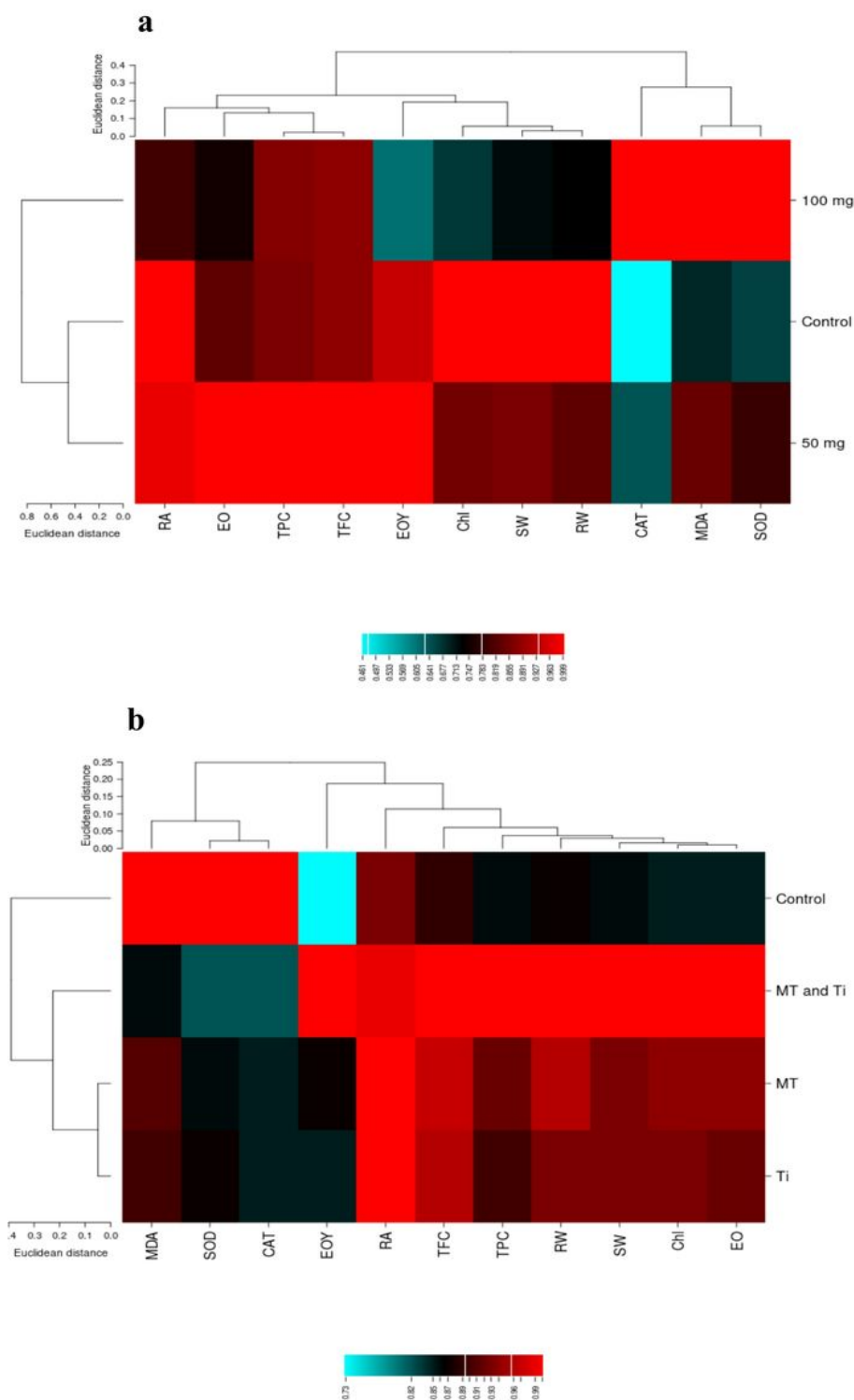


Figure 6

Heat map of studied traits under chromium toxicity (a) and titanium dioxide nanoparticle and melatonin (b). MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, EOY: essential oil yield, RA: rosmarinic acid, TPC: total phenolic content, TFC: total flavonoid content, RW: root weight, SW: shoot weight, Chl: chlorophyll, EO: essential oil content.