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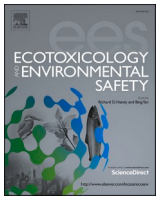

Interaction of titanium dioxide nanoparticles with PVC-microplastics and chromium counteracts oxidative injuries in *Trachyspermum ammi* L. by modulating antioxidants and gene expression

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ABSTRACT

The emergence of polyvinyl chloride (PVC) microplastics (MPs) as pollutants in agricultural soils is increasingly alarming, presenting significant toxic threats to soil ecosystems. Ajwain (*Trachyspermum ammi* L.), a plant of significant medicinal and culinary value, is increasingly subjected to environmental stressors that threaten its growth and productivity. This situation is particularly acute given the well-documented toxicity of chromium (Cr), which has been shown to adversely affect plant biomass and escalate risks to the productivity of such economically and therapeutically important species. The present study was conducted to investigate the individual effects of different levels of PVC−MPs (0, 2, and 4 mg L^{-1}) and Cr (0, 150, and 300 mg kg^{-1}) on various aspects of plant growth. Specifically, we examined growth and biomass, photosynthetic pigments, gas exchange attributes, oxidative stress responses, antioxidant compound activity (both enzymatic and nonenzymatic), gene expression, sugar content, nutritional status, organic acid exudation, and Cr accumulation in different parts of Ajwain (*Trachyspermum ammi* L.) seedlings, which were also exposed to varying levels of titanium dioxide (TiO_2) nanoparticles (NPs) (0, 25, and 50 μg mL^{-1}). Results from the present study showed that the increasing levels of Cr and PVC−MPs in soils significantly decreased plant growth and biomass, photosynthetic pigments, gas exchange attributes, sugars, and nutritional contents from the roots and shoots of the plants. Conversely, increasing levels of Cr and PVC−MPs in the soil increased oxidative stress indicators in term of malondialdehyde, hydrogen peroxide, and electrolyte leakage, and also increased organic acid exudation pattern in the roots of *T. ammi* seedlings. Interestingly, the application of TiO_2−NPs counteracted the toxicity of Cr and PVC−MPs in *T. ammi* seedlings, leading to greater growth and biomass. This protective effect is facilitated by the NPs' ability to sequester reactive oxygen species, thereby reducing oxidative stress and lowering Cr concentrations in both the roots and shoots of the plants. Our research findings indicated that the application of TiO_2−NPs has been shown to enhance the resilience of *T. ammi* seedlings to Cr and PVC−MPs toxicity, leading to not only improved biomass but also a healthier physiological state of the plants. This was demonstrated by a more balanced exudation of organic acids, which is a critical response mechanism to metal stress.

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1. Introduction

Heavy metals and metalloids are well known environmental pollutants, and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons (Yong et al., 2010; Song et al., 2019; Riaz et al., 2020; Adeyemi et al., 2021; Shaghaleh et al., 2024). Anthropogenic activities such as industrial effluents, mining, and sewage sludge as well as fertilizers and pesticides application are the major sources of heavy metals and metalloids in soils (Wang et al., 2022; Saleem et al., 2024a). Chromium is a toxic metal which does not have any essential metabolic function in plants, and its excess concentration in the soil may cause toxic effects in plants, growth reduction, lowered photosynthesis, nutritional imbalances, and quality of the crops (Ma et al., 2022; Alwutayd et al., 2023; Qureshi et al., 2024). Environmental contamination of Cr has gained substantial consideration worldwide because of its high levels in the water and soil originating from numerous natural and anthropogenic activities, and it is eventually accumulating in crops from contaminated soils and imparts severe health risks in humans via food chain contamination (Gautam et al., 2020; Basit et al., 2022). Higher Cr levels in plants cause ultra-structural alterations (Madhu and Sadagopan, 2020; Naz et al., 2021), oxidative stress in plants, and increased electrolyte leakage (EL) and malondialdehyde (MDA) concentrations, whereas induced alterations in antioxidant enzyme activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and ascorbate peroxidase (APX) (Sehrish et al., 2019; Qureshi et al., 2020). Previously, antioxidative enzymes played a significant role in the reduction of Cr phytotoxicity in *Lemna minor* (Sallah-Ud-Din et al., 2017), *Brassica napus* (Zaheer et al., 2020a), *Vigna radiata* (Gautam et al., 2020), *Spinacia oleracea* (Zaheer et al., 2020c), and *Triticum aestivum* (Ashraf et al., 2022) grown under excessive Cr concentrations. Hence, it is important to safeguard plants from high Cr levels to counter the phytotoxicity and oxidative stress triggered by the inevitable uptake of Cr in plants.

Over the last three decades, researchers have particularly focused on environmental safety and well-being; identifying the various contaminants that will cause negative impacts on ecosystems and liveability (An et al., 2020; Sun et al., 2023). A growing body of evidence has illustrated the fate, behavior, and impacts of these contaminants in both terrestrial and aquatic ecosystems (Liu et al., 2022; Li et al., 2023). These contaminants vary in nature and size and have diverse impacts on living organisms. Residues of metals, metalloids, salts, plastics, and other materials enter the food chain and progress from one trophic level to another (Yong et al., 2010; Liu et al., 2012; Khan et al., 2022; Al-Huqail et al., 2024; Shaghaleh et al., 2024). A detailed study of these contaminants reveals that microplastics (MPs) are of special interest due to their phytotoxicity and the associated health hazards for animals and humans (Li et al., 2023; Wang et al., 2023). Various researchers have examined carefully on the broad-range effects of small-sized particles on organisms at different trophic levels. The toxicity of these MPs ranges from individual organisms to the ecosystem level, including changes in microbial activities, retardation in plant growth, nutritional imbalances, and losses in productivity (Wang et al., 2020b; Fan et al., 2022). In soil, MPs can destructively influence physical and chemical properties such as structure, porosity, and water-holding capacity, along with hampering plant-soil interactions and microbial diversity (Wang et al., 2020a; Lee et al., 2022). As plants are considered a prime biotic component of terrestrial ecosystems, a careful exploration of plant-microplastic interactions and associated changes at all levels is highly crucial (Lee et al., 2022; Sun et al., 2023). Nanotechnology application in agriculture has been increasing over recent years and constitutes a valuable tool in reaching the goal of sustainable food production worldwide (Ahmar et al., 2021; Wahab et al., 2023). A wide array of nanomaterials has been used to develop strategies of delivery of bioactive compounds aimed at boosting the production and protection of crops (Irshad et al., 2021; Patowary et al., 2023). Among nanoparticles (NPs), titanium dioxide (TiO_2) NPs have been widely used in daily life and can be synthesized through various physical, chemical, and green methods (Irshad et al., 2021; Alshegaihi et al., 2023). Under normal condition, TiO_2 is insoluble in water and is highly stable and possess high refractive index of $n = 2.4$ which makes it applicable as a white pigment. TiO_2 is normally found in anatase, rutile and brookite crystalline polymorphs forms (Ma et al., 2023; Al-Huqail et al., 2024). Owing to large use of TiO_2 global markets, it is vital to understand its impacts on public health and the environment and its huge demand in various industrial sectors. TiO_2-NPs are widely use in cosmetics, sunscreens, food preparation, and drug delivery systems (Burke et al., 2015; Sardar et al., 2022). Application of TiO_2-NPs was also studied for wheat plants as it was seen beneficial to enhance chlorophyll content in cowpea plant under Cu stress. NPs showed potential for minimizing Cu content in plants which may be responsible for causing stress in plants (Alshegaihi et al., 2023). Instead, NPs promoted the availability of micronutrients in plants and activities of stress related enzymes were also promoted by application of TiO_2-NPs (Kiany et al., 2022; Ma et al., 2023).

Ajwain (*Trachyspermum ammi* L.), a member of Apiaceae, is a herbaceous crop plant and widely cultivated in Pakistan, India, Egypt, Iran, and many European countries (Javed et al., 2020). Seeds of *T. ammi* contain beneficial essential oil, traditionally used for different ailments and applications, such as antiseptic, diuretic, antimicrobial, antiviral, bronchodilatory, and hepatoprotective (Rao and Ikram, 2015). *T. ammi* has also been established as an important medicinal plant. Additionally, *T. ammi* plants exhibit remarkable resilience against environmental stressors, including MPs and Cr contamination, showcasing an inherent capacity to maintain growth and vitality under such challenging conditions. This stability is attributed to *T. ammi* 's efficient stress response mechanisms, which include enhanced antioxidant activity and selective organic acid exudation, effectively mitigating the toxic effects of pollutants and sustaining its physiological and biochemical functions (Sun et al., 2022). During recent years, certain heavy metals and MPs have received considerable attention on plant morphology and physiology because of increasing environmental exposure, which also likely to have an negative impact on medicinal plants, including *T. ammi* (Javed et al., 2020). Previously, few studies on *T. ammi* were executed to investigate its morphology and physiology under metal and MPs stress (Javed et al., 2020; Sun et al., 2022); but the application of NPs on various morpho-physiological characteristics, ionomics, and organic acid exudation potential of *T. ammi* was rarely investigated under metal and MPs stressed regimes. Therefore, the present study was conducted to study (1) the effect of different levels of TiO_2−NPs on plant growth, biomass, and gaseous exchange parameters of *T. ammi* seedlings under Cr and MPs stress, (2) oxidative stress and the responses of different antioxidative enzymes (enzymatic and non-enzymatic), as well as the response of the specific gene expression; (3) essential minerals uptake, organic acids exudation, and Cr accumulation in different organs of *T. ammi* seedlings under Cr and MPs stress. Assessing the impacts of TiO_2−NPs under Cr and MPs stress will provide new insights into the application of nanotechnology in metal and MPs contaminated soils.

2. Materials and methods

2.1. Experimental setup

A pot experiment was conducted in the Botanical Garden of the School of Public Administration, Hohai University, Nanjing 211100, China, under the glass house. Pots were placed under a glass house environment where they received natural sunlight, day/night humidity (60/70%), and day/night temperature (35/40 °C), respectively. Before sowing, the seeds were carefully washed 10% (v/v) commercial bleach for 10–20 min and then washed with distilled water. Also, before sowing, the seeds were primed with TiO_2−NPs at various concentrations (0, 25 and 50 μg mL^{-1}) for 20 h at room temperature in the dark, with continuous aeration for each treatment. Meanwhile, the control seeds were primed with deionized water for the same duration. The soil

sample was air dried, passed through a 5-mm sieve, and was water saturated two times before being used in pots. The soil used for this study was collected from the experimental stations of Hohai University, and its properties was as follow: pH-6.9, EC-0.9 dS cm^{-1}, organic matter-17 g kg^{-1}, EK-21 mg kg^{-1}, TP-0.17 g kg^{-1} and TN-16 g kg^{-1} (further details are mentioned in Table S1). The Cr concentration in the natural soil was 36.3 mg kg^{-1} as reported by Lu et al. (2003). After spiking the soil with Cr by using potassium dichromate ($K_2Cr_2O_7$) at different levels, i.e., 0 (no Cr), 150, and 300 mg kg^{-1} in the soil, plastic pots (30-cm-tall * 40-cm-wide) were filled with 10 kg of amended soil and had undergone four different cycles of water equilibration for 2 months and then drying in the air. The same levels of soil were used in a pot experiment previously reported by Levizou et al. (2019). After the addition of $K_2Cr_2O_7$ to the soil, PVC–MPs were added as 3.5% w/w of the depth of soil (20 cm) at different concentrations, i.e., 0, 2, and 4 mg L^{-1} PVC–MPs as discussed in the review of literature by (Ge et al., 2021). A moisture level of 70% was maintained throughout the experiment by irrigating the plants with deionized water free of Cr and PVC–MPs. The total duration of experimental treatments was 4 weeks under controlled conditions. Experiment was conducted in completely randomized design (CRD) with four replications of each treatment and one plant was used in each pot. All chemicals used were of analytical grade, procured from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2. Synthesis and characterization of TiO_2

Trachyspermum ammi L. seeds were sterilized through soaking in 0.5% sodium hypochlorite for 5 min. The seeds were washed thrice by using distilled water. The sterilized seeds were primed with varying concentrations of titanium dioxide nanoparticles (TiO_2-NPs). For this purpose, TiO_2-NPs of analytical grade were procured from Sigma-Aldrich (Saint-Louis, MO, USA) in the form of nano-powder with purity higher than 99.5%. These spherical TiO_2-NPs had an average particle size of distilled water for seed exposure, to final concentrations (Sardar et al., 2022). Seeds were primed for 24 h while placed under dim light at 25 ± 1 °C. During priming, seeds were aerated with the help of air pump. Following the process of priming, seeds were washed by using distilled water and air dried at room temperature (Sardar et al., 2022) (Fig. S1).

2.3. Plant harvesting

After 4 weeks, the remaining three seedlings were carefully uprooted and gently washed with distilled water to remove any airborne dust and surface deposition. Soil parameter determinations were conducted at Hohai University laboratories. Functional leaf in each treatment was picked at a rapid growth stage during 09:00–10:30. The sampled leaves were washed with distilled water, immediately placed in liquid nitrogen, and stored in a freezer at −80 °C for further analysis. All of the harvested plants were divided into two parts (i.e., roots and shoots) to study different physio-biochemical traits. Leaves from each treatment group were picked for chlorophyll, carotenoid, oxidative stress, and antioxidants analysis. Root and shoot lengths were measured straightway after the harvesting by using measuring scale and digital weighting balance to measure fresh biomass. Number of leaves were measured by simple counting the leaves while leaf area was also measured. Roots were uprooted and immersed in 20-mM ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) for 15–20 min to remove Cr adhered to the root surfaces. Then, roots were washed thrice with distilled water and, finally, once with de-ionized water, and dried for further analysis. The different parts of the plant (i.e., roots and shoots) were oven-dehydrated at 65 °C for 72 h for Cr determination, and the total plant dry weight was also measured. Although this experiment was conducted in pots, for the collection of organic acids, two seedlings were transferred to the rhizoboxes, which consist of a plastic sheet, a 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 (the process of distilling a liquid, such as water, for purification purposes, ensuring a high level of purity by eliminating impurities or contaminants through repeated distillation) to collect the exudates from root surface. The samples were filtered through a 0.45-mm filter (Millex-HA, Millipore) and collected in eppendorf tubes (Greger et al., 2016). The collected samples were mixed with sodium hydroxide (NaOH) (0.01 M) to analyze the organic acids. However, the samples used for analysis of oxalic acid were not treated with NaOH.

2.4. Determination of photosynthetic pigments and gas exchange parameters

Leaves were collected at four weeks for the determination of chlorophyll content. For chlorophyll content analysis, 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 (Arnon, 1949). On the same experimental days, foliar gaseous exchange was also measured on mature leaves. Net photosynthesis (N*p*), leaf stomatal conductance (G*s*), transpiration rate (T*r*), and intercellular carbon dioxide concentration (C*i*) were measured from three different plants in each treatment group (Song et al., 2020). Measurements were conducted between 11:30 and 13:30 on days with a clear sky. The rates of leaf N*p*, G*s*, T*r*, and C*i* were measured with an open system infra-red gas-exchange system (LI-6400; LICOR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the gas exchange cuvette, CO_2 concentration was set as 380 mmol mol^{-1}, and LED light intensity was set at 1000 mmol m^{-2} s^{-1}, which is the average saturating photosynthetic active radiation for photosynthesis in *T. ammi and other species* (Yong et al., 2010).

2.5. Determination of oxidative stress biomarkers and antioxidants

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) content. 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} using the following formula: 6.45 (A532− A600) − 0.56 A450. Lipid peroxidation was measured using methods previously published (Heath and Packer, 1968; Wu et al., 2023).

For hydrogen peroxide (H_2O_2) assay, leaf and root samples were homogenized with 50 mM phosphate buffer at pH 6.5. After that, homogenized samples were centrifuge at 6000×g for 25 min, followed by the addition of H_2SO_4 (20% v/v) and again centrifuged at 6000×g for 15 min H_2O_2 contents were estimated by taking the absorbance at 410 nm and calculations were completed with the help of extinction coefficient (0.28 μmol^{-1} cm^{-1}) (modifed from Wu et al., 2023).

Stress-induced electrolyte leakage (EL) of uppermost and mature leaves was determined by the method reported by (Dionisio-Sese and Tobita, 1998). The leaves were cut into minor slices (5 mm length) and placed in test tubes having 8 mL distilled water. These tubes were incubated and transferred into a water bath for 2 h prior to 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). Electrolyte leakage was measured using pH/conductivity meter (model 720, INCO-LAB Company, Kuwait) and calculated as:

$$EL = (EC1 / EC2) = \times 100.$$

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 the measurement of superoxidase dismutase (SOD) and peroxidase (POD) activities. SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 μL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMark™ microplate absorbance spectrophotometer; Bio-Rad). Enzyme activity was measured using a method by Chen and Pan (1996) and expressed as U g^{-1} FW. POD activity in the leaves was estimated using the method of (Sakharov and Ardila, 1999) 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 was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme. Catalase (CAT) activity was analyzed according to (Aebi, 1984). The assay mixture (3.0 mL) was comprised 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 ($\varepsilon = 39.4\ mM^{-1}\ cm^{-1}$). Ascorbate peroxidase (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 ($\varepsilon = 2.8\ mM^{-1}\ cm^{-1}$).

Quantitative real-time PCR (RT-qPCR) assay was applied to investigate the expression levels of 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 S2.

2.6. Determination of amino acid, sugar and non-enzymatic compounds

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 (Pękal and Pyrzynska, 2014), phenolics (Bray and Thorpe, 1954), ascorbic acid (Azuma et al., 1999), anthocyanin (Lewis et al., 1998), and total sugars (Dubois et al., 1956) was performed from the extracts. Fresh leaf material (0.1 g) was mixed thoroughly in 5 mL 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).

To determine the total free amino acids in a sample, first prepare your sample, which could be plant or animal tissue, cells, or biological fluids. Begin by hydrolyzing the sample in 6 N hydrochloric acid (HCl) to release the amino acids. This step typically involves heating the sample in the acid for several hours. After hydrolysis, the acid needs to be neutralized; this is usually done using 6 N sodium hydroxide (NaOH). Finally, the results are analyzed by comparing the sample's amino acid profile with the standards to determine the concentration of each amino acid present in the original sample (Bowne et al., 2012).

2.7. Determination of nutrients, root exudates and Cr uptake

For nutrient analysis, plant roots and shoots underwent a thorough washing procedure: twice in redistilled water, a brief 3-second immersion in 20 mM EDTA, followed by two additional washes with deionized water to eliminate adsorbed metals from the plant surfaces. The washed samples were then oven-dried at 105 °C for 24 hours. Subsequently, the dried roots and shoots were subjected to wet digestion in HNO_3: $HClO_4$ (7:3 V/V) until clear samples were obtained. Each resulting sample was filtered, and the filtrate was diluted with redistilled water to a final volume of 50 mL. The root and shoot contents of Ca^{2+}, Fe^{2+}, Mg^{2+} and P were analyzed by using Atomic Absorption Spectrophotometer (AAS) model Agilent 240FS-AA (Shi et al., 2019). 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 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 comprised of 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 using a spectrometer (UV–VIS Series 200, USA) as described by (UdDin et al., 2015). Freeze-dried samples were dissolved in redistilled water and the pH of the exudates was recorded with LL micro-pH glass electrode by using a pH meter (ISTEK Model 4005–08007 Seoul, South Korea). Vigilant digestion of plant samples was performed using the di-acid (HNO_3−$HClO_4$) technique. Specifically, 0.5 g of dry samples from the roots and shoots were placed in a flask containing 10 mL of HNO_3−$HClO_4$ (3:1, v-v), left overnight, and finally digested after the addition of HNO_3 (5 mL) by heating on a hot plate until complete digestion, following the method described by (Rehman et al., 2015). The exact amount of Cr in plant shoots and roots was determined using an AAS.

2.8. Statistical analysis

Data analysis was carried out using one−way analysis of variance (ANOVA) with computer based Co-stat version Cohorts Software 6.2, 2003 (Monterey, CA, USA). The differences among treatments were evaluated by least significant difference method (Fisher s LSD) at p value of ≤ 0.05 level. The data was standardized by logarithmic or inverse transformations prior to analysis. The graphical presentation was carried out using Origin-Pro 2017.

3. Results

3.1. Effects of PVC–MPs and Cr stress on growth and photosynthesis in T. ammi under the application of TiO_2–NPs

The various growth parameters and photosynthetic pigments and also the gas exchange parameters in *T. ammi* under the PVC-MPs and Cr stress with the application of TiO_2-NPs, were measured. Growth and biomasses of *T. ammi* are presented in Fig. 1, while the gas exchange attributes are presented in Fig. 2. Root length exhibited a significant ($P < 0.05$) decrease by 29% under PVC-MPs stress and 25% under Cr stress compared to the control. However, the application of TiO_2-NPs led to a

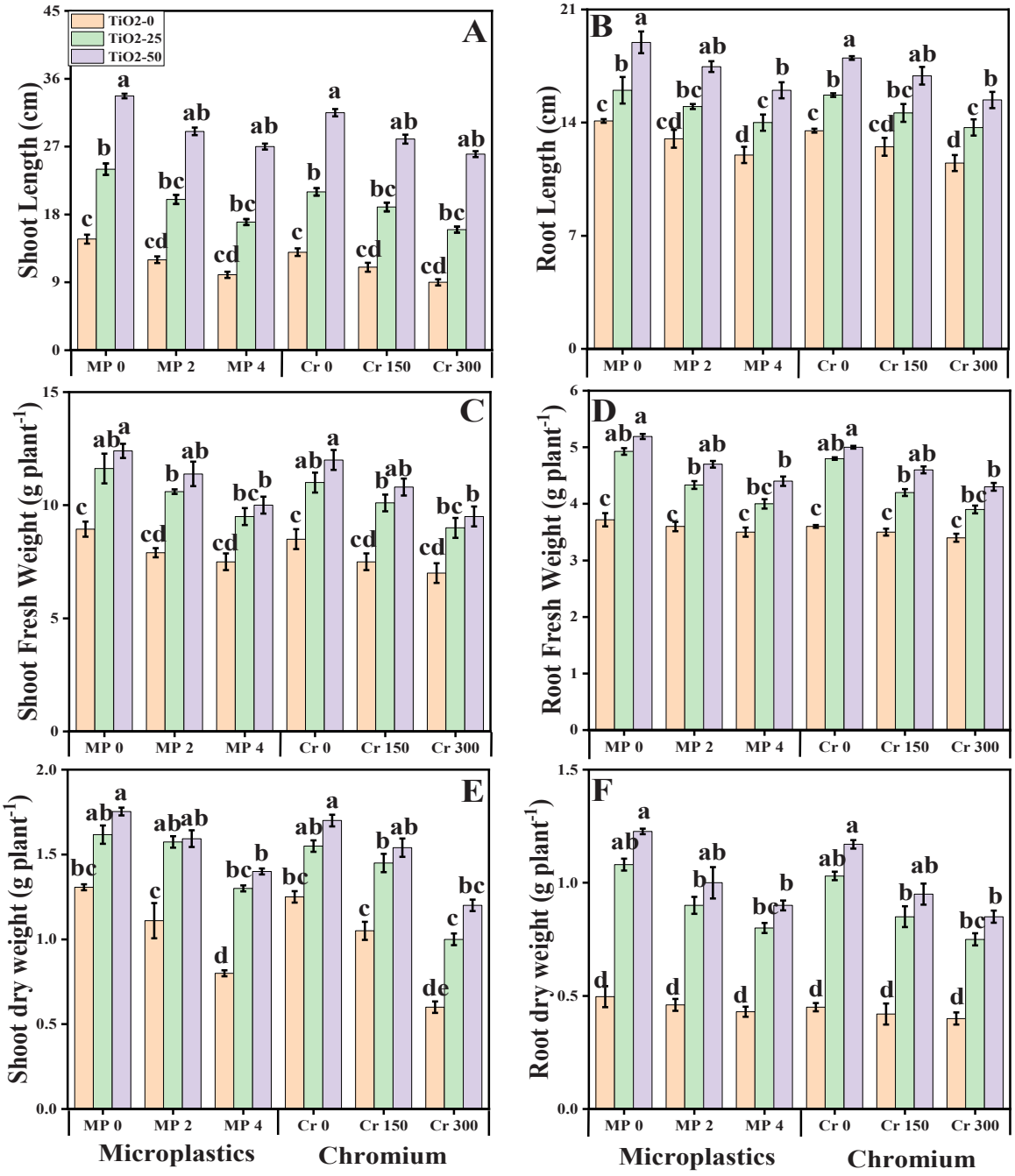

Fig. 1. Impact of PVC–microplastics (PVC–MPs), namely 0 (no PVC–MPs), 2, and 4 mg L^{-1}, along with chromium (Cr) levels of 0 (no Cr), 150, and 300 mg kg^{-1} in the soil on morphological traits, i.e., shoot length (A), root length (B), shoot fresh weight (C), root fresh weight (D), shoot dry weight (E), and root dry weight (F) is concurrently applying titanium dioxide–nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 μg mL^{-1} in *Trachyspermum ammi* L. Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

significant increase in root length, compared to the control. Shoot length experienced a reduction of 21% under PVC-MPs stress and 24% under Cr stress, with TiO_2-NPs application demonstrating a noteworthy increase. Similarly, shoot fresh weight showed a significant decrease of 16% under PVC-MPs stress and 19% under Cr stress, while TiO_2-NPs application resulted in a substantial improvement. Root fresh weight declined by 10% and 9% under PVC-MPs and Cr stress, respectively, but significantly increased with TiO_2-NPs application. Shoot dry weight, under PVC-MPs and Cr stress, exhibited a decrease of 15% and 16%, respectively, while the application of TiO_2-NPs led to a marked increase. Root dry weight decreased by 25% and 20% under PVC-MPs and Cr stress, respectively, but significantly increased with TiO_2-NPs application. Chlorophyll a, chlorophyll *b*, and total chlorophyll content experienced reductions under PVC-MPs and Cr stress, ranging from 6% to 30%. However, TiO_2-NPs application resulted in further increased while compared to the control (which were not supplied with TiO_2-NPs. Carotenoid content decreased by 6–9% under PVC-MPs and Cr stress but increased significantly with TiO_2-NPs application. The net photosynthesis, stomatal conductance, transpiration rates, and intercellular CO_2 showed reductions ranging from 4% to 30% under PVC-MPs and Cr stress, while TiO_2-NPs application induced further reductions. These individual parameter analyses provided a detailed understanding of the

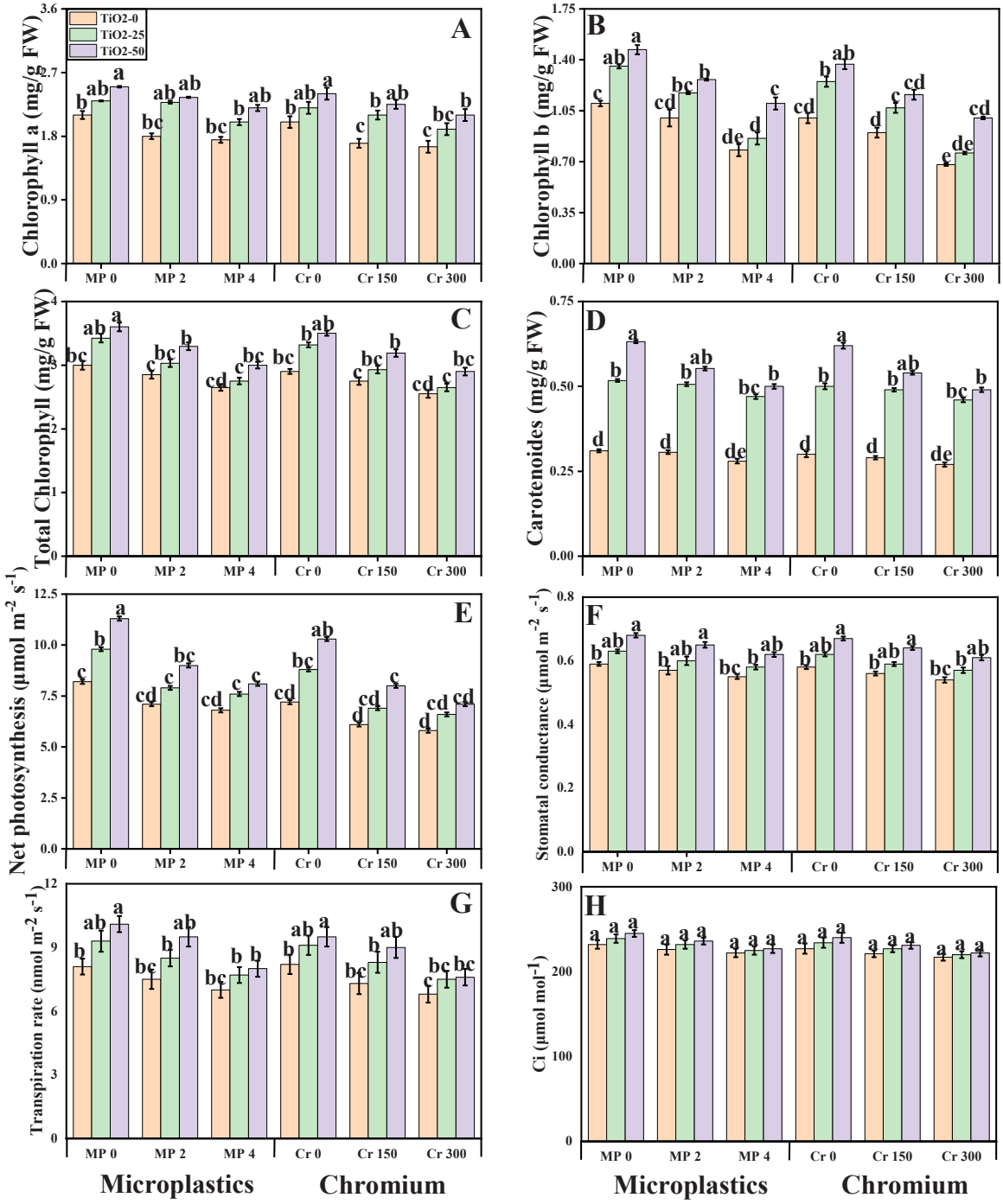

Fig. 2. Impact of PVC–microplastics (PVC–MPs), namely 0 (no PVC–MPs), 2, and 4 mg L^{-1}, along with chromium (Cr) levels of 0 (no Cr), 150, and 300 mg kg^{-1} in the soil on photosynthetic pigments and gas exchange attributes, i.e., chlorophyll-a (A), chlorophyll-b (B), total chlorophyll (C), carotenoid (D), net photosynthesis, µmol m^{-2} s^{-1} (E), stomatal conductance, mol m^{-2} s^{-1} (F), transpiration rate, mmol m^{-2} s^{-1} (G) and intercellular CO_2, µmol mol^{-1} (H) is concurrently applying titanium dioxide–nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 µg mL^{-1} in *Trachyspermum ammi* L. Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

distinct responses of *T. ammi* to PVC-MPs and Cr stress, highlighting the nuanced effects of TiO_2-NPs application on each measured parameter.

3.2. Effects of PVC–MPs and Cr stress on oxidative stress and antioxidants in T. ammi under the application of TiO_2–NPs

In the present study, the different oxidative stress biomarkers, i.e., malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and electrolyte leakage (EL) were measured from the roots and leaves of *T. ammi* as presented in (Fig. 3). MDA levels in the roots increased by 32% under PVC−MPs stress and 29% under Cr stress compared to the control. TiO_2−NPs application reduced MDA by 12% and 15% under PVC−MPs and Cr stress, respectively. In leaves, MDA content rose by 28% and 24% under PVC-MPs and Cr stress, with TiO_2−NPs leading to a decrease of 10% and 12%. Root H_2O_2 content saw a 24% rise under both PVC-MPs and Cr stress. TiO_2−NPs reduced H_2O_2 by 10% and 12%. In leaves, H_2O_2

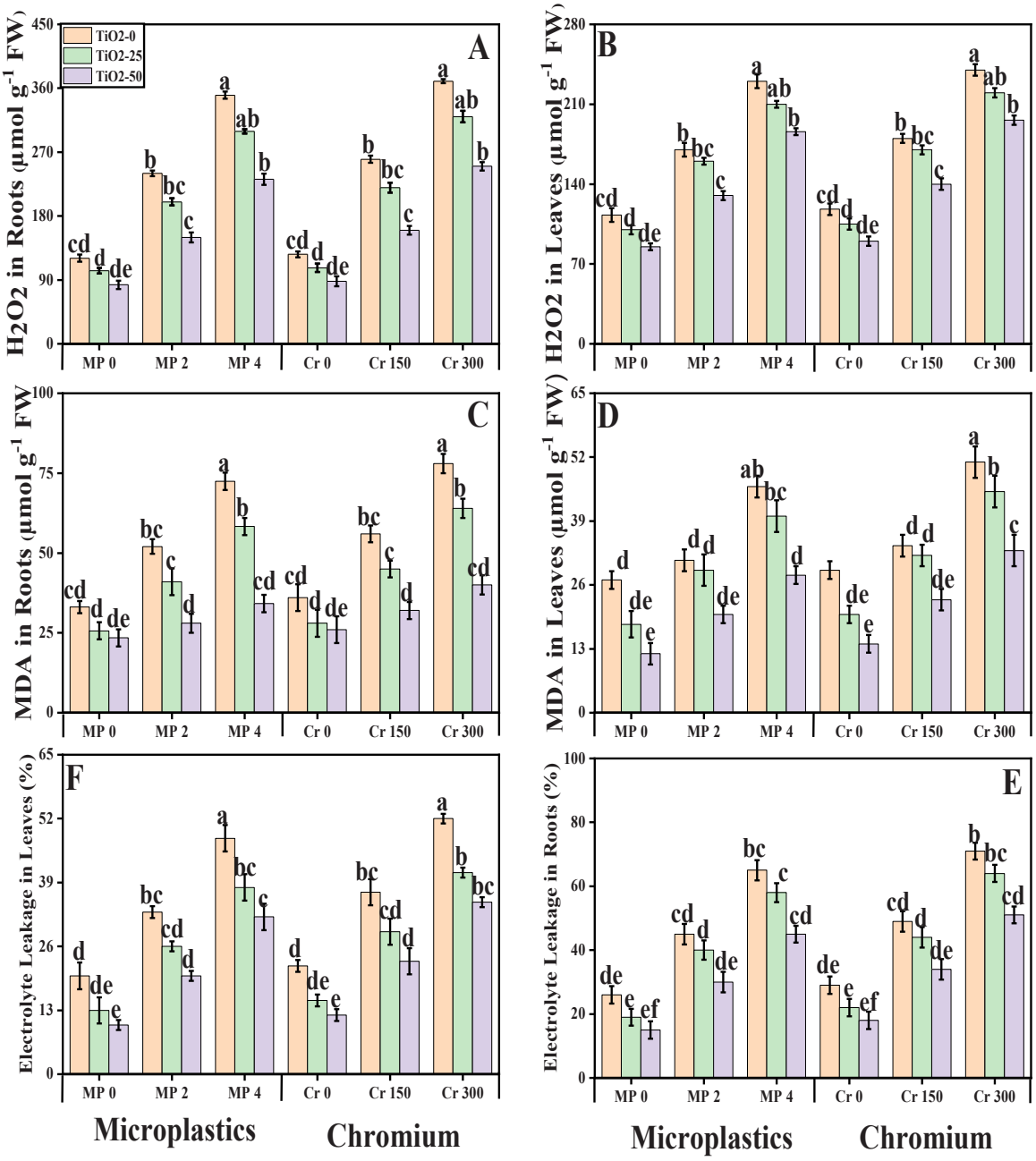

Fig. 3. Impact of PVC–microplastics (PVC–MPs), namely 0 (no PVC–MPs), 2, and 4 mg L^{-1}, along with chromium (Cr) levels of 0 (no Cr), 150, and 300 mg kg^{-1} in the soil on oxidative stress indicators, i.e., H_2O_2 contents in the roots (A), H_2O_2 contents in the leaves (B), malondialdehyde (MDA) contents in the roots (C), MDA contents in the leaves (D), electrolyte leakage (EL) percentage in the roots (E), and EL percentage in the leaves (F) is concurrently applying titanium dioxide–nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 µg mL^{-1} in *Trachyspermum ammi* L. Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

increased by 18% and 16% under PVC-MPs and Cr stress, with decreases of 8% and 10% after TiO_2−NPs application. EL in roots increased by 26% and 22% under PVC-MPs and Cr stress. TiO_2−NPs led to a decrease of 10% and 12%. In leaves, EL rose by 20% under both stresses, with TiO_2−NPs reducing it by 8% and 10%.

Different enzymatic antioxidants, i.e., SOD, APX, POD, CAT, and non-enzymatic compounds, i.e., phenolic, anthocyanin, flavonoids, total free amino acid, ascorbic acid, proline, sugar content and also their relevant gene expression, i.e., SOD, POD, CAT, and APX were also measured from the leaves of *T. ammi*. The results regarding the enzymatic antioxidants are presented in Fig. 4, and the results regarding the gene expressions are presented in Table 1 and also the nonenzymatic compounds are presented in Fig. 5. In the conducted research on *T. ammi*, it was observed that environmental stressors affected the activity levels of several key antioxidant enzymes in both the roots and leaves of the plant. Specifically, the activity of SOD in the roots and leaves was noted to increase by approximately 50% when subjected to stress from PVC−MPs. Under Cr stress, there was a 45% increase in SOD activity. When TiO_2−NPs were applied, a 40% increase in SOD activity was recorded. Similarly, POD activity rose by 47% due to PVC−MPs stress, 42% due to Cr stress, and 37% with TiO_2−NPs treatment. Catalase activity also enhanced, showing a 44% increase under PVC−MPs, 39% under Cr stress, and 35% with the application of TiO2-NPs. Ascorbate Peroxidase followed this trend with a 41% increase in

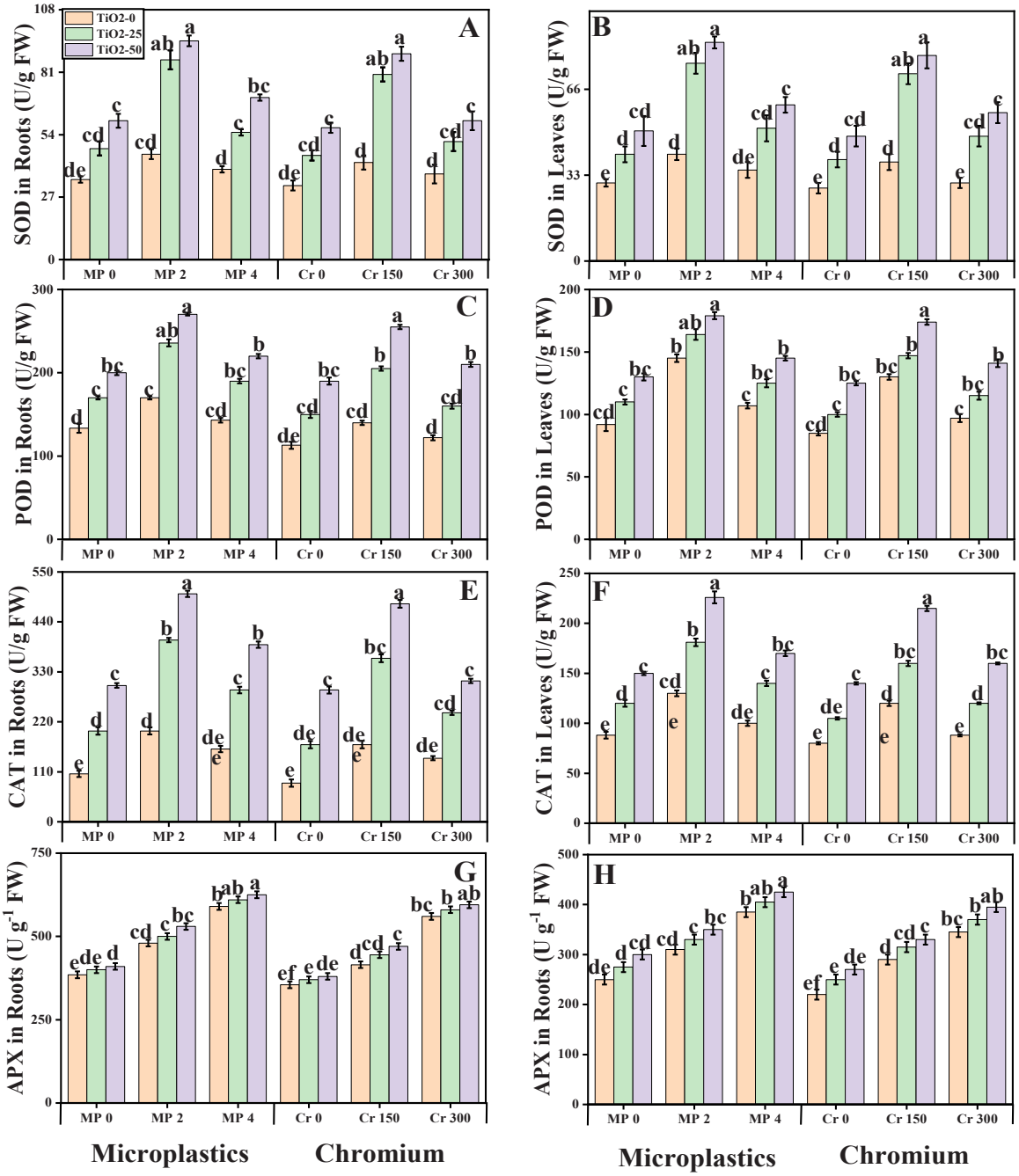

Fig. 4. Impact of PVC–microplastics (PVC–MPs), namely 0 (no PVC–MPs), 2, and 4 mg L^{-1}, along with chromium (Cr) levels of 0 (no Cr), 150, and 300 mg kg^{-1} in the soil on enzymatic antioxidants i.e., SOD activity in the roots (A), SOD activity in the leaves (B), POD activity in the roots (C), POD 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) in the leaves is concurrently applying titanium dioxide–nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 μg mL^{-1} in *Trachyspermum ammi* L. Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

activity in the presence of PVC−MPs, 36% with Cr, and 31% increase when TiO_2−NPs were applied.

The phenolic content increased by 49% under PVC−MPs stress, by 42% under Cr stress, and by 31% with the application of TiO_2−NPs. Anthocyanin content also rose, showing a 38% increase under PVC−MPs, 30% under Cr stress, and 21% with TiO_2−NPs. Flavonoid content went up by 31% with PVC−MPs, 24% with Cr, and 15% with TiO_2−NPs. Ascorbic acid content saw a 26% rise under PVC−MPs stress, 19% under Cr stress, and an 11% increase with TiO_2−NPs. Proline content increased by 22% with PVC−MPs, 15% with Cr, and 7% with TiO_2−NPs. Total free amino acids content increased by 16% under PVC−MPs stress, 9% under Cr stress, and showed a slight 1% increase with TiO_2−NPs. Sugar content in leaves increased by 10% under PVC−MPs stress, 3% under Cr stress, and by 0.5% with TiO_2−NPs application.

The study also measured the gene expression related to oxidative stress response in the leaves of *T. ammi*. The expression of the SOD gene saw an expected increase of 50% under PVC−MPs stress, 45% under Cr stress, and 40% with TiO_2−NPs. Peroxidase gene expression increased by approximately 47% under PVC−MPs, 42% under Cr stress, and 37%

Table 1
Effects of PVC–microplastics (PVC–MPs) at concentrations of 0 (no PVC–MPs), 2, and 4 mg L^{-1}, Cr at levels of 0 (no Cr), 150, and 300 mg kg^{-1} in soil, and the application of titanium dioxide nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 μg mL^{-1} on the relative gene expression in *Trachyspermum ammi* L.

MPs (mg L^{-1})	nTiO$_2$ (μg mL^{-1})	Fe-SOD	POD	CAT	APX
0	0	0.69 ±0.08 g	2.05 ±0.2 g	1.3±0.2 g	0.37 ±0.03 g
0	25	0.76 ±0.07fg	2.29±0.1 f	1.5±0.2 f	0.55 ±0.04fg
0	50	0.86 ±0.08 f	2.61 ±0.1ef	1.8±0.2e	0.64 ±0.05 f
2	0	1.96±0.1c	3.601 ±0.2c	2.8±0.3b	1.12 ±0.06bc
2	25	2.34 ±0.2b	4.19±0.3b	2.9±0.2ab	1.24 ±0.1ab
2	50	2.68±0.1a	4.38±0.3a	3.1±0.1a	1.34 ±0.09a
4	0	1.24±0.1e	2.89±0.2e	2.1±0.2d	0.89 ±0.04e
4	25	1.38 ±0.09de	3.28 ±0.1de	2.3 ±0.3 cd	0.96 ±0.06de
4	50	1.49 ±0.1d	3.4002 ±0.2 cd	2.4±0.2c	1.05 ±0.09 cd
Cr (mg kg^{-1})	**nTiO$_2$ (μg mL^{-1})**	**Fe-SOD**	**POD**	**CAT**	**APX**
0	0	0.37 ±0.04 g	1.34 ±0.07 g	0.86 ±0.08 g	0.31 ±0.03 g
0	25	0.53 ±0.03fg	1.64 ±0.08fg	0.93 ±0.07fg	0.35 ±0.03fg
0	50	0.61 ±0.05 f	1.86 ±0.09 f	1.08 ±0.08e	0.42 ±0.05 f
150	0	1.81 ±0.08bc	3.24±0.1c	2.26 ±0.2b	0.91 ±0.07b
150	25	2.09 ±0.1ab	3.43 ±0.2bc	2.46 ±0.3ab	1.01 ±0.08ab
150	50	2.24±0.1a	3.68±0.4a	2.69±0.2a	1.06 ±0.09a
300	0	1.35 ±0.08e	2.12±0.3e	1.54 ±0.1d	0.53 ±0.04e
300	25	1.48 ±0.09de	2.43 ±0.2de	1.700 ±0.2 cd	0.68 ±0.05d
300	50	1.68 ±0.07d	2.68±0.1d	1.86 ±0.08c	0.84 ±0.06bc

Values in the table indicate just one harvest. Mean±SD (n=4). One-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.

with TiO_2−NPs. Catalase gene expression was up by about 44% with PVC−MPs, 39% with Cr, and 35% with TiO_2−NPs. Ascorbate Peroxidase gene expression showed significant increases as well, with about 41% under PVC−MPs, 36% under Cr, and 31% with TiO_2−NPs application.

3.3. *Effects of PVC–MPs and Cr stress on organic acids, essential ions and Cr uptake in T. ammi under the application of TiO_2–NPs*

In the present study, the contents of essential minerals, i.e., iron (Fe^{2+}), calcium (Ca^{2+}) magnesium (Mg^{2+}), and phosphorus (P) were also determined from shoots of *T. ammi* seedlings grown in different application levels of TiO_2−NPs i.e., 0, 25 and 50 μg mL^{-1} under PVC−MPs (0, 2 and 4 mg L^{-1}) and Cr (0, 150, and 300 mg kg^{-1}) polluted soil. The contents of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P from the shoots of *T. ammi* seedlings are presented in Table 2. The current study's results depicted that the concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in the shoots of *T. ammi* seedlings were decreased with the increase in the PVC−MPs and Cr concentration in the soil, when compared with the plants grown without the addition of Cr and PVC−MPs in the soil in *T. ammi* seedlings (Table 2). We also noticed that the concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in the shoots of *T. ammi* seedlings could be increased under the toxic concentration of Cr and PVC−MPs in the soil by the exogenous application of TiO_2−NPs (Table 2). In addition, results also showing that exogenous application with TiO_2−NPs increased the concentrations of Fe^{2+}, Ca^{2+}, Mg^{2+}, and P in the shoots of *T. ammi* seedlings, compared to those plants, which were grown without the exogenous application with TiO_2−NPs.

The contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots of *T. ammi* seedlings grown under toxic levels of PVC−MPs and Cr in the soil, with or without the application of TiO_2−NPs are presented in Fig. 6. According to the given results, we have noticed that increasing the concentration of PVC−MPs and Cr induced a significant ($P < 0.05$) increased in the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots of *T. ammi* seedlings, compared to those plants which were grown without the addition of PVC−MPs and Cr in the soil. Results also illustrated that the application of TiO_2−NPs decreased the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid in the roots of *T. ammi* seedlings, compared with those plants, which were grown without the exogenous application with TiO_2−NPs. In addition, at all levels of PVC−MPs and Cr stresses, the contents of fumaric acid, formic acid, acetic acid, citric acid, malic acid, and oxalic acid decreased with the increasing levels of TiO_2−NPs, compared with those plants, which were grown without the application of TiO_2−NPs. We also manifested that the contents of Cr from the roots and shoots of *T. ammi* seedlings grown under the toxic levels of Cr in the soil, with or without the application of TiO_2−NPs are presented in Fig. 6.

Increasing levels of Cr in the soil induced a significant ($P < 0.05$) increase in the Cr concentration in the roots and shoots of *T. ammi* seedlings, compared to those plants which were grown in the control treatment. In addition, at all levels of Cr stress, the contents of Cr were decreased with the increasing levels of TiO_2−NPs, compared with those plants, which were grown without the application of TiO_2−NPs.

4. Discussion

Chromium is among the most toxic trace element present in agricultural soils and is being released through a variety of anthropogenic activities such as electroplating and leather tannin (Shahid et al., 2017; Alsafran et al., 2022). As a well-known toxic and heavy metal, Cr is harmful to the growth and development of plants (Ranieri et al., 2020; Ugwu and Agunwamba, 2020; Qureshi et al., 2024). 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 (Saleem et al., 2022; Zaheer et al., 2020b; Um e et al., 2021; Ma et al., 2022; Laila et al., 2023). 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 (Maqbool et al., 2018; Ali et al., 2023; Alwutayd et al., 2023). Chromium stress can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants (Hussain et al., 2018; Vishnupradeep et al., 2022), which promote ROS accumulation and membrane lipid peroxidation, and disrupt the structure and function of cell membrane system (Hussain et al., 2021; Ashraf et al., 2022). It is well documented that Cr toxicity directly caused oxidative injury in the plants through the Fetone and Haber–Weiss reactions which also helps in the generation of large amount of ROS which is toxic to the plant (Medda and Mondal, 2017; Noman et al., 2020). It is known that the accumulation of ROS in plants could be removed by a variety of antioxidant enzymes such as SOD, POD, CAT, and APX (Fig. 4); their specific gene expression (Table 1) and non-enzymatic antioxidant (Fig. 5) activities are highlighted in this study. Interestingly, the expression of antioxidative enzymes, such as SOD, POD, CAT, and APX under Cr stressed environment plays a significant role in reducing Cr toxicity, which was also reported in earlier studies for other species (Sallah-Ud-Din et al., 2017; Qadir et al., 2020; Ahmad et al., 2022). Essential mineral nutrients are required for the normal growth of

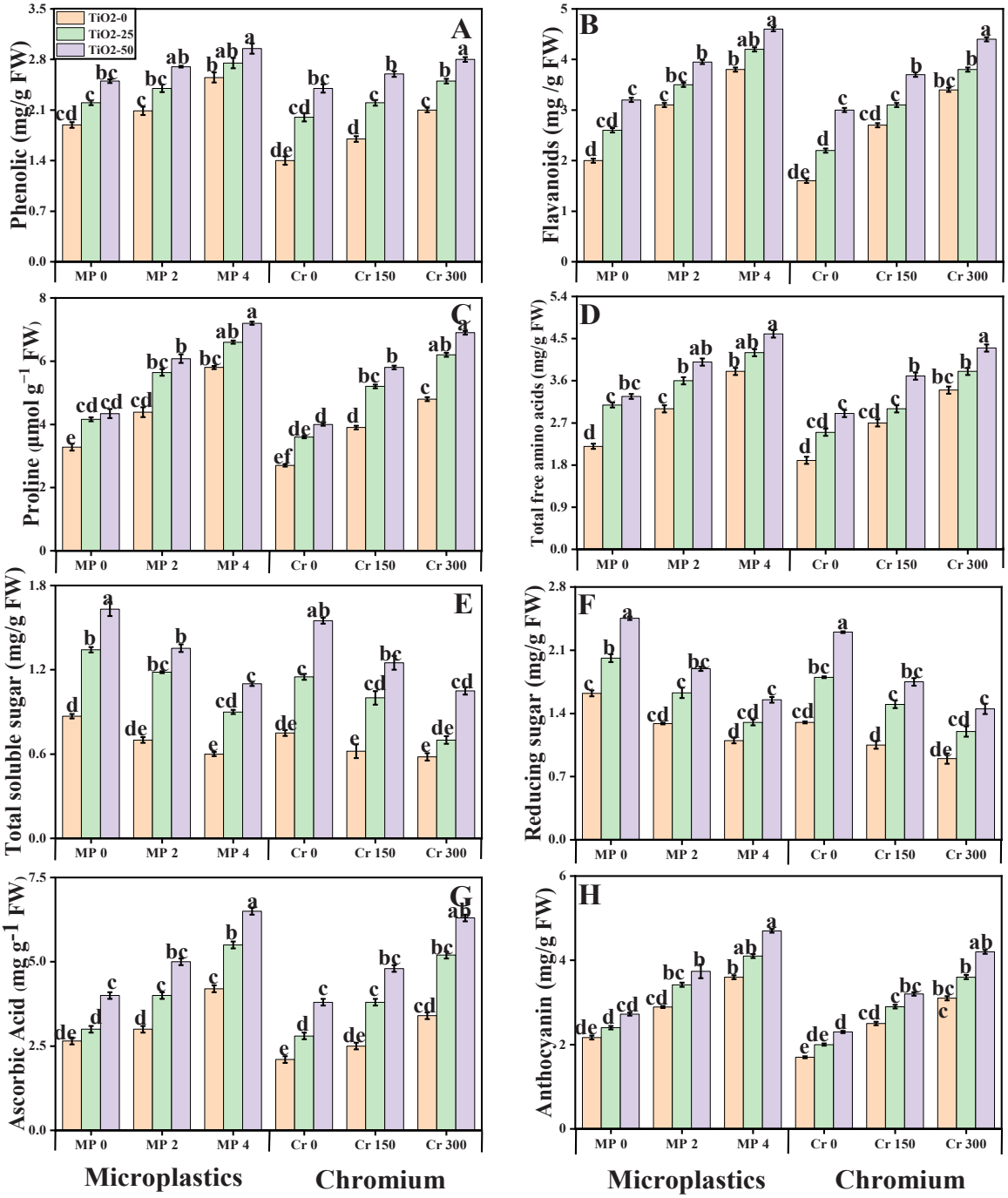

Fig. 5. Impact of PVC–microplastics (PVC–MPs), namely 0 (no PVC–MPs), 2, and 4 mg L^{-1}, along with chromium (Cr) levels of 0 (no Cr), 150, and 300 mg kg^{-1} in the soil on non-enzymatic antioxidants and sugars i.e., phenolic (A), flavonoid (B), proline (C), total free amino acid (D), total soluble sugar (E), reducing sugar (F), ascorbic acid (G) and anthocyanin (H) is concurrently applying titanium dioxide–nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 µg mL^{-1} in *Trachyspermum ammi* L. Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

plants, for example phosphorus (Shi et al., 2019). Numerous reports demonstrated that the uptake and translocation of essential elements in plants were restricted under Cr stress (Zaheer et al., 2020b; Ahmad et al., 2022; Ashraf et al., 2022). Excess Cr decreased the Fe^{2+}, Ca^{2+}, Mg^{2+}, and P contents from the shoots of the plants, which was also noticed in the present study (Table 2). It is well known that Cr toxicity in species was dependent on the bioavailability of Cr in soils and the concentration of elements, which can compete with Cr during plant uptake (Ma et al., 2022; Ulhassan et al., 2022). Acidification of mucilage after uptake of Cr is likely due to the release of protons when plant roots release more cations than anions in order to maintain their charge balance (Wen et al., 2018; Qureshi et al., 2020; Ma et al., 2022). The exudation of organic acids in the roots of *T. ammi* seedlings (Fig. 6), accelerating metal transport from roots to the aboveground parts, is possibly due to the formation of metal-chelated ions as suggested by (Javed et al., 2021), when they cultivated *Solanum lycopersicum* Mill. cultivars in Cr-polluted soil.

Through the present research, it was established that generally, the concentration of MPs is inversely related to plant growth. The extensive use of plastics in daily life leads to the accumulation of MPs in many food

Table 2
Effects of PVC–microplastics (PVC–MPs) at concentrations of 0 (no PVC–MPs), 2, and 4 mg L^{-1}, Cr at levels of 0 (no Cr), 150, and 300 mg kg^{-1} in soil, and the application of titanium dioxide nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 μg mL^{-1} on essential ions in *Trachyspermum ammi* L.

MPs (mg L^{-1})	TiO_2−NPs (μg mL^{-1})	Mg^{2+} (mg g^{-1} DW)	P (mg g^{-1} DW)	Fe^{2+} (mg g^{-1} DW)	Ca^{2+} (mg g^{-1} DW)
0	0	4.36±0.3c	31.1±3c	15.2±1.3c	40.2±4.3e
0	25	6.54±0.5fb	39.5±3.4fb	21.3±2.3bc	51.2±5.2fbc
0	50	7.95±0.9a	46.2±5a	29.5±3.5a	64.8±6.8a
2	0	2.61±0.3e	24.6±2.1d	11.3±1.4d	34.6±3.2e
2	25	3.35±0.3 cd	29.2±3.2c	15.3±1.4d	45.6±4.2d
2	50	4.24±0.5c	36.9±3.9bc	25.6±2.4b	59.5±5.4b
4	0	1.64±0.2 f	16.25±1.9e	9.6±1 f	31.2±3.1 f
4	25	2.16±0.2 f	23.35±2.8d	12.3±1.6ef	39.4±3.5e
4	50	2.99±0.3de	31.4±3.6c	20.8±2.2 cd	54.6±5.2bc
Cr (mg kg^{-1})	**TiO_2−NPs (μg mL^{-1})**	**Mg^{2+}**	**P**	**Fe^{2+}**	**Ca^{2+}**
0	0	3.96±0.4d	26.4±2.9c	14.2±1.6c	30.6±3.3fg
0	25	5.4±0.6fb	35.2±3.4fb	20.5±2.1c	42.6±4.6fd
0	50	6.2±0.5a	41.3±4.2a	25.6±2.8a	51.6±5.3a
150	0	3.24±0.2e	24.6±2.8d	12.2±1.5d	27.6±2.1 g
150	25	4.6±0.4c	30.2±3.3 cd	18.6±14 cd	37.6±3.2e
150	50	5.8±0.6ab	35.6±3.8b	22.5±2.6b	43.6±3.7b
300	0	2.89±0.3 f	22.9±2.5e	10.6±1.2e	24.6±3.0 h
300	25	4.2±0.4 cd	26.4±2.4d	15.6±1.6ef	33.8±3.4ef
300	50	4.9±0.5bc	32.4±3.6c	20.6±2.3c	37.6±3.9c

Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

crops, which adversely affects the growth and yield of plants and may potentially harm human health (Sun et al., 2023; Al-Huqail et al., 2024). As shown in Fig. 1, PVC–MPs adversely impacted the growth and biomass of *T. ammi* seedlings in this investigation. The presence of MPs inside the plant could hinder the uptake or translocation of water, thus affecting the growth (Pignattelli et al., 2020; Wang et al., 2020b). Reductions in the photosynthesis and stomatal conductance under PVC–MP treatments indicated the stressful situation which may have arisen due to MPs the *T. ammi* seedlings had to deal with it (An et al., 2020; Li et al., 2021b). The physiological and biochemical mechanisms involved in stress, caused by exposure to PVC–MPs, are not completely understood and clarified. Normally, phytotoxicity is related to the production of reactive oxygen species (ROS), such as hydrogen peroxide, superoxide anion, and hydroxyl radical (Hasanuzzaman et al., 2020; Sarraf et al., 2022). The ROS accumulation in plant cells leads to an impairment of plant growth, photosynthesis, and biochemical processes (Ali et al., 2020). Several studies revealed that the exposure of plants to abiotic stresses, such as MPs, affects the production of foliar chlorophylls and carotenoids by interfering with the synthesis of chlorophyll by the inhibition of enzymes involved in this process (Saleem et al., 2020; Yaseen et al., 2020). To ameliorate the negative effects associated with oxidative stress, plants have developed an antioxidant system that involves protective metabolities such as glutathione GSH, and ascorbic acid (Imran et al., 2021; Ahmad et al., 2022). The presence of environmental stressors like PVC–MPs could lead to an imbalance in oxidative stress, and this imbalance results in increased production of harmful ROS and a reduction in the activity of key antioxidant enzymes such as SOD, APX, POD, and CAT (Li et al., 2020; Ahmad and Guria, 2022). These antioxidants play a crucial role in detoxifying ROS, so their reduced activity under stress can significantly impact on crop yield and productivity (Li et al., 2021a; Bhat et al., 2022). Proline, a critical osmolyte in plants and its accumulation help plants maintain osmotic balance under stressful conditions (Stecker et al., 2022; Saleem et al., 2024b). Additionally, PVC–MPs may influence nutrient dynamics and increase and organic acid levels in plants through a couple of mechanisms. Firstly, these MPs can alter the soil's physical and chemical properties, affecting root growth and nutrient uptake (Saud et al., 2023; Wang et al., 2023). This disruption can lead to compensatory mechanisms in plants, such as increased secretion of organic acids to facilitate nutrient absorption (Thomas et al., 2020; Nguyen et al., 2021). The increase in MPs toxicity in plant roots can stimulate the stress responses, leading to an upregulation of metabolic pathways responsible for the synthesis of organic acids (Osman et al., 2023). These organic acids, including citric, malic, and oxalic acids, are secreted into the rhizosphere to chelate and mobilize nutrients, thus compensating for the impaired nutrient uptake caused by MP-induced stress (Chen et al., 2024). Furthermore, the secretion of these acids can also enhance the plant's ability to alleviate heavy metal toxicity, which is often exacerbated by the presence of MPs, by forming complexes with heavy metals and reducing their availability to plant roots (Saleem et al., 2022). Secondly, PVC–MPs can also adsorb and concentrate heavy metals like Cr from the soil, which may then be more readily taken up by the plant roots, leading to higher accumulation of these metals in plant tissues (Ge et al., 2021; Lwanga et al., 2022; Zhang et al., 2022). In addition, we would like to highlight that in the treatments involving only MPs or the controlled group, the amount of Cr accumulated by the plants was notably minimal. This observation can be attributed to the inherently low concentration of Cr present in the soil used for our experiments. The soil's natural Cr content is minimal, reflecting typical background levels that do not significantly contribute to Cr accumulation in plants under these specific treatment conditions.

Most of the metallic nanoparticles are unstable in water. Whereas, absorbent NPs may constitute carbon in their porous structure and are not a good choice to enhance plant growth (Bhatt et al., 2022; Wahab et al., 2023). On the other hand, the adjustable hydrophilicity, structural suitability, adequate constancy, appropriate photoactivity, and bio-compatibility are salient features which make TiO_2-NPs suitable for the growth of several plants (Irshad et al., 2021; Ma et al., 2023). We have observed that PVC–MPs and Cr stress in the soil decreased the plant growth and biomass while the seed priming with TiO_2-NPs showed better growth and physiological responses of the plants under PVC–MPs and Cr stress (Figs. 1 and 2). Our results are harmonious with the findings of (Hojjat et al., 2020) demonstrating increased germination, root growth, shoot growth, fresh weight, and biomass production of TiO_2-NPs treated *Lathyrus sativus* plants. (Cox et al., 2016) revealed that infiltration of NPs in seed coat enhances plant nutrition and the overall growth of the plant. Numerous elucidations have been anticipated with reference to the activities of Ti as an advantageous element in plant growth (Faraji et al., 2018; Sardar et al., 2022). These included the involvement of Ti in strengthening plant resilience through increasing uptake of nutrients (Alshegaihi et al., 2023; Ma et al., 2023; Al-Huqail et al., 2024). The surface of TiO_2-NPs is highly reactive; therefore, these particles increase the porosity of roots leading to the augmented uptake of water and nutrients under normal and stressed circumstances

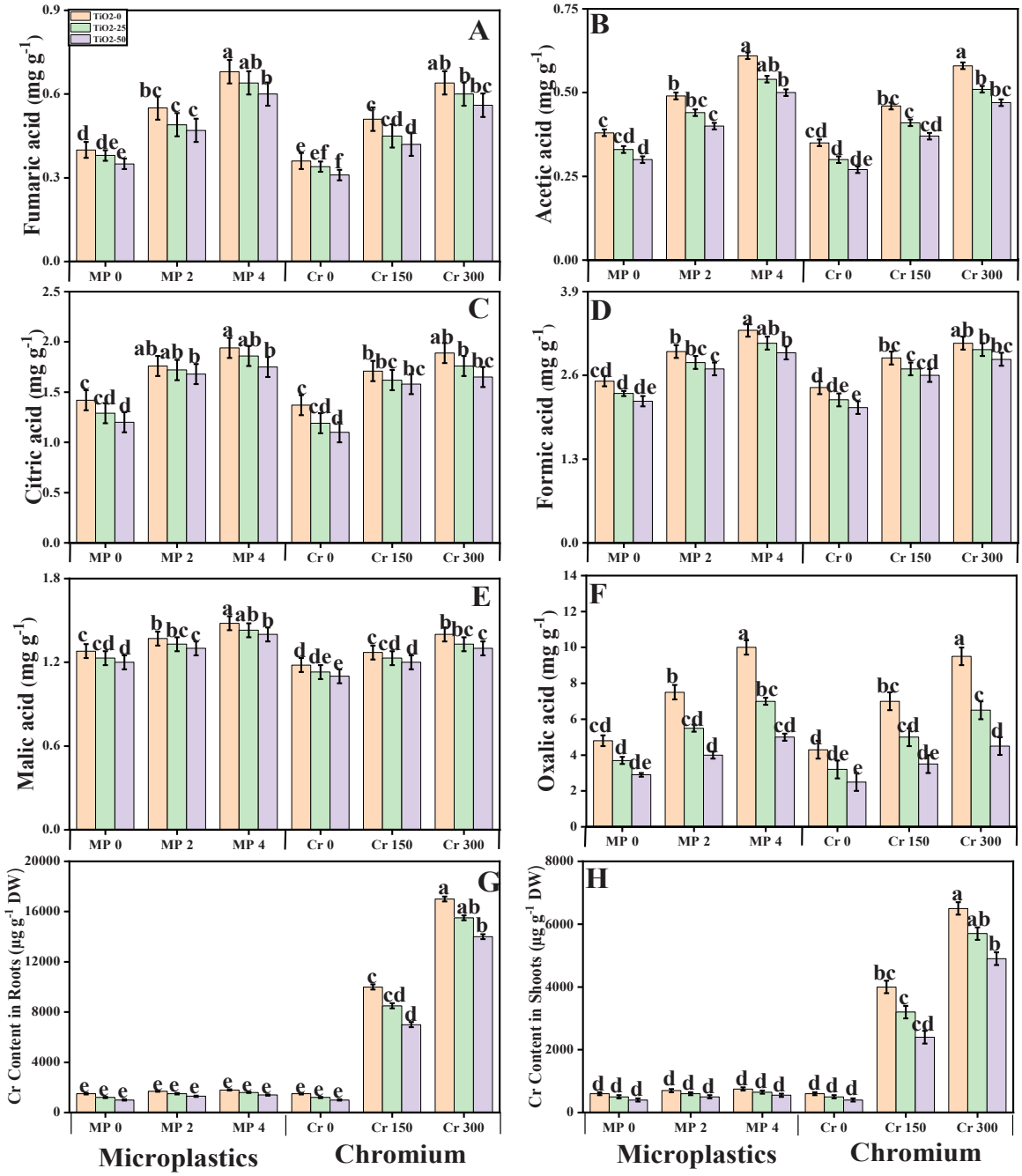

Fig. 6. Impact of PVC–microplastics (PVC–MPs), namely 0 (no PVC–MPs), 2, and 4 mg L^{-1}, along with chromium (Cr) levels of 0 (no Cr), 150, and 300 mg kg^{-1} in the soil on organic acids and Cr uptake i.e., fumaric acid contents (A), acetic acid contents (B), citric acid contents (C), formic acid contents (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) is concurrently applying titanium dioxide–nanoparticles (TiO_2–NPs) at 0 (no TiO_2–NPs), 25, and 50 µg mL^{-1} in *Trachyspermum ammi* L. Values in the figures indicate just one harvest. Mean±SD (n=4). One-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.

(Gohari et al., 2020; Irshad et al., 2021; Kiany et al., 2022). (Frazier et al., 2014) publicized that TiO_2-NPs alleviated environmental stresses by modulating the expression level of microRNAs. Interestingly, TiO_2-NPs, with their adjustable hydrophilicity, structural suitability, stability, photoactivity, and biocompatibility, were able to enhance plant growth and physiological attributes during abiotic stress environment by overcoming limitations posed by the instability of metallic NPs and the ineffectiveness of carbon-based absorbents; further restoring adequate nutrient uptake, reinforcing plant performance, and enhancing various stress response mechanisms. Fundamentally, TiO_2 operate as a two-pronged tool for plants: delivering a protective function from injurious metal contaminants; and also improving their capacity to uptake essential nutrients, and optimizing growth during unfavourable growth conditions (Fig. 7).

5. Conclusion

Seed priming with TiO_2-NPs can mitigate the negative effects of PVC–MPs and Cr stress in *T. ammi* seedlings. This study demonstrated that toxic levels of PVC–MPs and Cr toxicity in soils caused a significant decline in the growth, gas exchange attributes, sugars, photosynthetic pigments, and essential minerals in *T. ammi* seedlings. Interestingly,

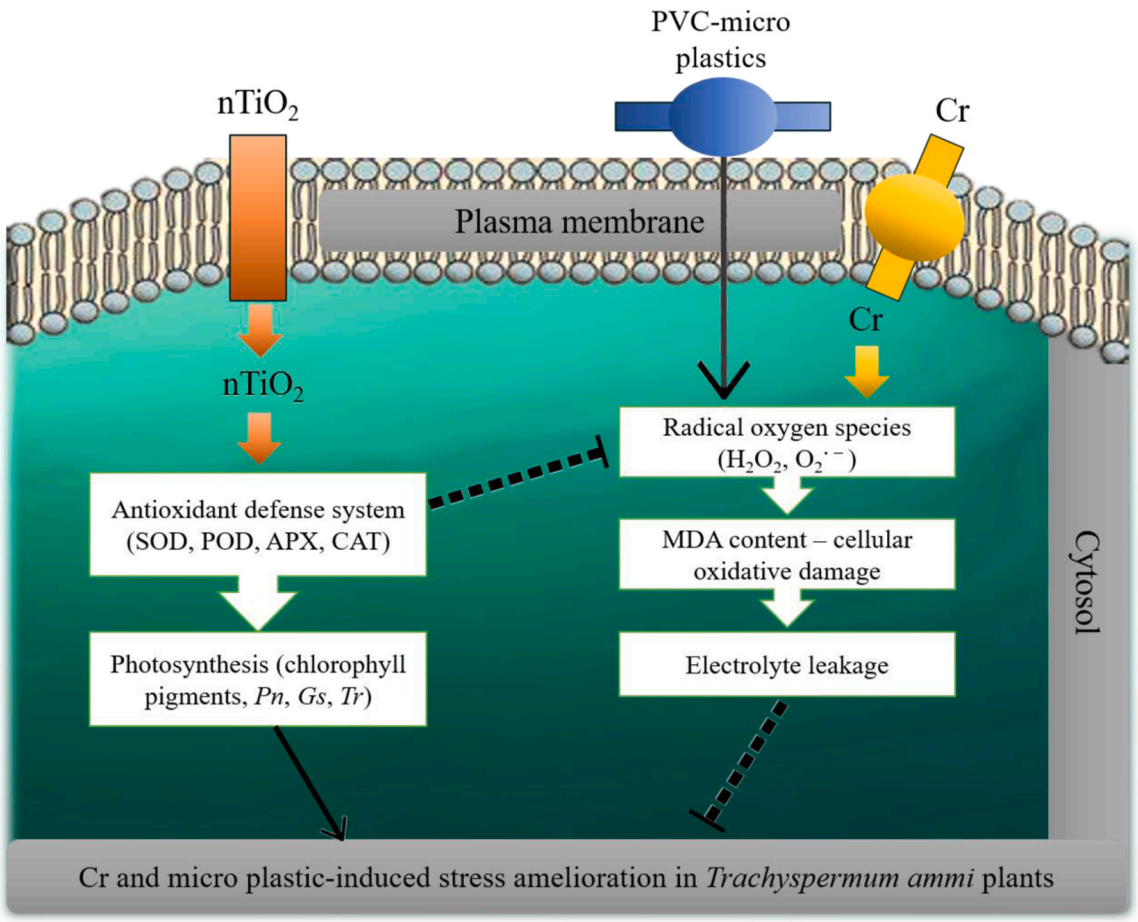

Fig. 7. A proposed mechanism to demonstrate the mitigative potential of TiO_2-NPs application against PVC-MPs and Cr toxicity. Straight lines/arrows indicate promotion and dashed ones denote inhibition.

PVC–MPs and Cr toxicity significantly increased the oxidative stress biomarkers, enzymatic and non-enzymatic antioxidants (including their gene expression) in *T. ammi* seedlings. Seed priming with TiO_2-NPs decreased the oxidative stress in *T. ammi* seedlings undergoing PVC–MPs and Cr stress. This strategy might safeguard the cell wall compartment and reducing plausible oxidative stress with concomitant improvements in nutrients balance of plant tissues. We observed that TiO_2-NPs would increase plant resilience against PVC–MPs and Cr toxicity plausibly through different mechanisms. As this research represented an initial investigation into the effects of nanoparticles on plant stress responses, further studies are needed to explore the optimal concentrations and types of nanoparticles that maximize protective benefits while minimizing the negative effects on tissues. Such research should also be extended to other species to ensure a comprehensive understanding of nanoparticle interactions in diverse agricultural contexts, thereby promoting the use of nanoparticles as soil amendments to strengthen plant resilience.

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CRediT authorship contribution statement

Faisal Zulfiqar: Writing – review & editing, Software, Methodology. **Muhammad Hamzah Saleem:** Writing – review & editing, Software. **Yanfeng Zhu:** Visualization, Software, Methodology, Data curation. **Ziyi Hua:** Writing – original draft, Methodology, Formal analysis. **Mohamed A. El-Sheikh:** Writing – review & editing, Software, Funding acquisition. **Touqeer Abbas:** Writing – review & editing, Software. **Fu Chen:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Jing Ma:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Muhammad Faheem Adil:** Writing – review & editing, Visualization, Validation, Funding acquisition, Conceptualization. **Jean Wan Hong Yong:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of Competing Interest

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

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116181.

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