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Ferric oxide nano-priming enhances photosynthetic and physicochemical properties of sunflower (*Helianthus annuus* L.) microgreens

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Abstract: In modern agriculture, nano-priming represents an innovative approach, harnessing the power of nanotechnology to enhance crop yields and nutrition. However, to effectively harness the potential of nanoparticles (NPs) for agriculture applications, understanding their mode of action and optimal application rates for positive effects on microgreen growth and physiology is critical. In this interdisciplinary study, we investigated the priming of sunflower seeds with a range of concentrations (25, 50, and 100 mg/L) of ferric oxide (Fe_2O_3) nanoparticles (FeNPs) and compared them with control samples. Our findings revealed a significant increase in plant biomass, leaf size, and photosynthetic activity in treated samples. The activities of photosystems I and II increased with higher FeNPs concentration. The treated samples exhibited elevated levels of total phenolics, anthocyanin, and antioxidant enzyme activity, along with increased macronutrients and micronutrients. These findings highlight the potential of FeNPs as a promising tool for enhancing plant growth and physiology in sunflower microgreens.

Keywords: seed priming; antioxidant; fluorescence activity; nutrients

Nanotechnology has emerged as a promising tool with virtually unlimited potential to enhance crop and food production. Nanoparticles (NPs) have garnered much attention because of their unique physical and

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chemical features that explicitly allow them to be synthesised to modulate a plant's physiological processes. NPs are defined as materials within the size range of 1 to 100 nm. They can be employed in many ways, including nano fertilisers, pesticides, and nanosensors (Sami et al. 2020). The activity of NPs depends on their physicochemical properties, concentration, timing, and application method. Research suggests NPs can enhance plant physiological parameters by improving photosynthetic efficiency, nitrogen assimilation, and carbon sequestration (Huang et al. 2016). The optimal concentration of NPs can boost seed germination, chlorophyll content, photosynthetic efficiency, and carbohydrate content and enhance the antioxidant defence system (Rani et al. 2016). Various modes, including foliar spray, soil treatments, nano priming, and hydroponics, have been used to deliver NPs to plants. Among these, nano priming is one of the most cost-effective and convenient approaches. Nano priming entails using traditional priming techniques but incorporating NPs, which is an excellent approach for modulating the expression of primary and secondary metabolites in plants (Farooq et al. 2022). For instance, fullerene NP priming has been shown to influence the concentration of phenolics and flavonoids in wheat in dose-dependent ways. Nano priming can effectively overcome seed dormancy in plants by modifying lipid metabolic pathways while also enhancing the production of photosynthetic pigments and the rate of photosynthesis, resulting in improved overall plant performance. Significant progress has also been made in utilising nano priming to alleviate various abiotic and biotic stresses. Some reports suggested that preliminary stresses caused by nano priming stimulated the formation of reactive oxygen species (ROS), which activated various signaling cascades that participate in stress tolerance and secondary metabolite production (Rai-Kalal et al. 2021). Despite the promising results of incorporating NPs in food production to increase quality, efficiency, and sustainability, this approach is still in its early stages and will require extensive investigation.

Studies on FeNPs have demonstrated promising potential for enhancing plant growth and production by increasing the rate of photosynthesis and promoting root development. They have also been shown to increase the biomass of various crops, such as rice, wheat, and soybean, by enhancing nutrient uptake and regulating plant hormones (Dola et al. 2022), improving crop yields and quality. Moreover, FeNPs can be used as a source of iron, an essential micronutrient required for various plant functions, including photosynthesis, respiration, and enzyme activity (El-Desouky et al. 2021). Although seed nano priming using FeNPs and other kinds of NPs has been carried out on various food crops to understand their effects on the plant system, areas such as microgreens have yet to be explored.

Microgreens are an emerging product and a new definition of greens in culinary trends due to their versatility in colour, rich flavours, texture, and high number of bioactive components. They are commonly used in salads, soups, sandwiches, and food dressings. Microgreens are generally cotyledon leaves and stems of edible vegetables, legumes, and herbs, which differ from sprouts as they are devoid of roots. Microgreens are considered functional foods because they have higher micronutrients, bioactive components, vitamins, and other essential nutrients than seeds and mature plants (Xiao et al. 2012). Numerous scientific studies have shown that certain plant species belonging to the families Asteraceae, Apiaceae, Amarillydaceae, Amaranthaceae, Brassicaceae, Cucurbitaceae, and Fabaceae are commonly used for producing microgreens due to their high concentrations of health-promoting phytochemicals (Benincasa et al. 2019). Of particular interest are the less studied microgreens of the Asteraceae family, such as sunflower, which are rich in fiber, carotenoids, proteins, and various bioactive substances. Sunflower microgreens are more digestible than sunflower seeds, which facilitates the absorption of nutrients.

The current study aimed to assess the effects of FeNPs on sunflower microgreens. The primary focus was on elucidating the impact of nano priming with different FeNPs concentrations. These were evaluated by comparing physiological, biochemical, and inorganic parameters with the exact measurements on control plants. We proposed the hypothesis that applying FeNPs could effectively potentiate plant growth, leading to a substantial enhancement in the production of sunflower microgreens. This, in turn, could result in significant economic advantages for growers.

MATERIAL AND METHODS

Experimental setup and growth conditions. The experiments were conducted in a growth chamber under controlled environmental conditions (Photon System Instruments, Drasov, Czech Republic) using *Helianthus annuus* L. seeds obtained from a seed store in Prague, Czech Republic. The seeds were

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surface sterilised with a 20% commercial bleach solution (NaOCl) (*v*/*v*) for 5–6 min, followed by thorough washing with distilled water. Double-distilled water was used to prepare stocks of Fe_2O_3 NPs at 25, 50, and 100 mg/L (Sigma-Aldrich, nanopowder, < 50 nm particle size, BET, Missouri, USA). Sterilised seeds were soaked in the prepared FeNPs suspensions at a 1:4 (*w*/*v*) ratio for 8 h, while control seeds were soaked in double-distilled water (1:4; *w*/*v*) (Farooq et al. 2006). The seeds were stirred once an hour to facilitate FeNPs penetration. After 8 h of incubation, excess NPs were washed off with distilled water, and the seeds were allowed to dry at room temperature to a constant natural moisture content. The following day, the seeds were uniformly spread on a seedling tray (30 cm × 15 cm) containing a sterile modified peat substrate potting soil (Agro Profi-RS1, 's-Gravenzande, Noordlandseweg, Netherlands). The trays were placed in the growth chamber and maintained at 25 °C with a relative humidity of 65%. A total of 100 seeds/tray and three trays per treatment were used. To enhance germination, the seed beds were kept in complete darkness for the first two days and then exposed to 16 h of daylight and 8 h of darkness per day. To keep the soil moist, the seedbed was sprayed with distilled water once a day. After seven days of germination, the microgreens were harvested and immediately frozen at –80 °C for later analysis.

Analysis of nanoparticle structure. The morphology and structure of the Fe_2O_3 NPs were determined by high-resolution transmission electron microscopy (HRTEM, Massachusetts, USA) using an FEI Talos F200X microscope (Massachusetts, USA) operating at 200 kV. Specimens for TEM imaging were supported on a copper grid covered with a porous carbon film.

Growth parameters. Growth parameters were evaluated by randomly selecting ten leaves from each tray. Seedling and leaf size were measured using Image J software (IJ 1.46r, Maryland, USA), while the fresh weight of microgreens was measured using an electronic analytical balance (Adventurer AX224, Ohaus, USA).

Pigments. Non-invasive measurement of chlorophyll content was carried out using a SPAD-502 chlorophyll meter (Minolta Camera Co., Osaka, Japan). The meter was clamped over a section of leaf for 2 s to measure the transmittance at 650 nm (red) and 940 nm (near-infrared), where plants have peak absorbance and no light absorbance, respectively. The soil plant analysis development (SPAD) values were calculated using these two wavelengths to estimate the relative chlorophyll content per unit area. Twenty measurements were taken from the middle part of the leaf and averaged. In addition, the relative anthocyanin and flavonoid content were estimated using the Multiplex3® (Force-A, Orsay, France), a battery-powered instrument designed to operate in daylight in agricultural fields. The machine contains a multi-parametric fluorescence sensor with LED excitation at 375, 470, 516, and 635 nm and filtered photodiode detection (Rastogi et al. 2017). This experiment measured fluorescence at 735 nm (FRF) after excitation at 635, 516, and 375 nm.

Photosynthetic parameters. A portable PEA fluorometer (Hansatech Instruments Ltd, Norfolk, UK) was used to measure the effects of nano priming on PSII photochemistry, which provides readouts on the structural and functional photochemistry related to PSII. We randomly selected 20 seedlings from the tray, affixed the leaf clips from the instrument, and kept them in the dark for a 15-min adaptation period. A saturation pulse of photosynthetically active light with an intensity of 3 500/μmol^2/s was then applied for 1 s. Among the various calculated parameters, those that define fluorescence induction activity were chosen for analysis (Stirbet and Govindjee 2011): (1) F_v/F_m, the maximum quantum yield of PSII photochemistry; (2) RC/ABS, the number of active PSII reaction centers per unit of light absorbed; (3) ψ_{REo}, the probability of electron transfer from PSII to PSI; (4) PI_{abs}, the photochemical performance index; (5) δ_{REo}, the probability of an electron being transferred from Q_B to a PSI acceptor; and (6) Sm, the number of electron carriers per electron transport chain.

The MultispeQ device, in conjunction with the PhotosynQ mobile application, served as a photosynthesis meter capable of evaluating fluorescence- and absorbance-based parameters associated with photosystem I (PSI). To assess these parameters, the device was initially programmed with specific experimental conditions, then placed randomly on leaves from each treatment and allowed to stabilise before data was recorded using the mobile application. The following parameters, which measure energy flux, were selected for analysis: (1) gH^+, proton conductance of ATP synthase; (2) kP700, the rate constant of P700 electron transfer; and (3) NPQt, non-photochemical quenching calculated from data captured in light-exposed samples (Tietz et al. 2017).

Biochemical parameters. To estimate the total phenolic content (TPC) and malondialdehyde (MDA) concentration, an ethanolic extract was prepared by

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grinding 0.5 g of leaf sample in liquid nitrogen using a mortar and pestle until a homogeneous powder was obtained. The powder was added to 10.5 mL of 80% ethanol, incubated for 5 min at ambient temperature, and filtered through filter paper. The filtrate was utilised for subsequent analyses.

Phenolic content. To quantify the total phenolic content, an aliquot of ethanolic extract (125 µL) was mixed with a 10-fold diluted Folin-Ciocalteau reagent plus 7% sodium carbonate to prepare a reaction mixture. The volume was adjusted to 2.5 mL using ultrapure water (Singleton and Rossi 1965) and allowed to react for 90 min; then, the absorbance of the samples was measured at 765 nm against the blank using a UV/VIS spectrophotometer (Evolution 201, Thermo Scientific, Massachusetts, USA). The TPC was calculated as gallic acid equivalents (mg/g fresh weight (FW)).

Total malondialdehyde content. The total malondialdehyde content was quantified using the thiobarbituric acid (TBA) assay with slight modifications to the published protocol (Du and Bramlage 1992). Briefly, 700 µL of ethanolic extract was mixed with 700 µL of 0.6% TBA and 700 µL of 10% trichloroacetic acid (TCA). The mixture was then heated in a water bath at 95 °C for 25 min, cooled to room temperature, and centrifuged at 11 000 *g* for 1 min in a Hermle Z216 MK centrifuge (New Jersey, USA). The absorbance of the supernatant was measured at 600, 532, and 440 nm.

Antioxidant enzyme activity. Leaf samples weighing 0.2 g were homogenised on ice with 4 mL of 50 mmol phosphate buffer (pH 7.8) and centrifuged at 13 000 *g* for 20 min at 4 °C. To measure superoxide dismutase (SOD) activity, the supernatants were mixed with 175 µL of EDTA, 100 µL of methionine (13 mmol), 250 µL of (75 µmol) nitro blue tetrazolium chloride (NBT), 253 µL of sodium carbonate, and 97 µL of riboflavin (2 µmol). After exposure to white light for 15 min to initiate the reaction, the samples were kept in the dark for 10 min to stop the reaction. The absorbance of the reaction mixture was measured at 560 nm, and one unit of SOD activity was defined as the quantity of enzyme required to cause a 50% inhibition of NBT reduction. SOD activity was expressed as units per gram of biomass. This protocol was modified from (Heath and Packer 1968) with slight modifications. Guaiacol peroxidase (GOPX) activity was measured using the method (Pütter 1974) with slight modifications. The increase in absorbance was read at 436 nm, and GOPX activity was calculated using an extinction coefficient of 6.39 cm^2/µmol. The method (Nakano and Asada 1987) was used to estimate ascorbate peroxidase (APX) activity. The enzyme activity was measured by the rate of oxidation of ascorbic acid, which is directly proportional to the decrease in absorbance at 290 nm per minute.

ICP-MS analysis of micronutrients and macronutrients. The quantification of the macronutrients, calcium, phosphorous, and magnesium, and the micronutrients, zinc, manganese, iron, boron, copper, molybdenum, and nickel, across all treatments and the control was achieved using inductively coupled plasma mass spectrometry (ICP-MS) analysis. Prior to analysis, leaf samples were dried at 45 °C for 24 h and pulverised using a mortar and pestle. A 200-mg aliquot of plant biomass was placed in a quartz vessel and digested with 4 mL of cons HNO_3 (Analpure®, Analytika, Czech Republic) + 2 mL of H_2O_2 (Rotipuran®, Carl Roth, Germany). The closed-vessel microwave system (Discover SP-D, CEM Corp., Matthews, USA) was used to digest the samples at 180 °C for 20 min. The resulting solutions were transferred to 50 mL polypropylene tubes, filled up to a final volume of 45 mL with Milli-Q water (≥ 18.2 MΩ/cm; MilliQ system, Millipore, SAS, Cedex, France), and elemental concentrations were determined *via* ICP-MS (Agilent 7700x, Agilent Technologies Inc., Santa Clara, USA). For quality assurance, a certified reference material (peach leaves, SRM-1547, NIST) was used (Bharati et al. 2023).

Statistical analyses. Physiological parameters (n = 20), photosynthetic parameters (n = 20), biochemical parameters (n = 3), and ICP data (n = 3) were subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. The test results were considered significant at $P < 0.05$ using Microsoft Excel 2021 (Washington, USA). The graphs were plotted using GraphPad Prism (version 9.4.0, Massachusetts, USA).

RESULTS AND DISCUSSION

Characterisation of Fe_2O_3 nanoparticles (FeNPs). FeNPs were characterised using TEM, and the micrographs (Figure 1) show that particles were shaped like cubes with sizes ranging from 10 nm to 100 nm. The calculated selected area electron diffraction (SAED) data correlated with reference points identified in X-ray diffraction (XRD) analysis is represented in Figure 1.

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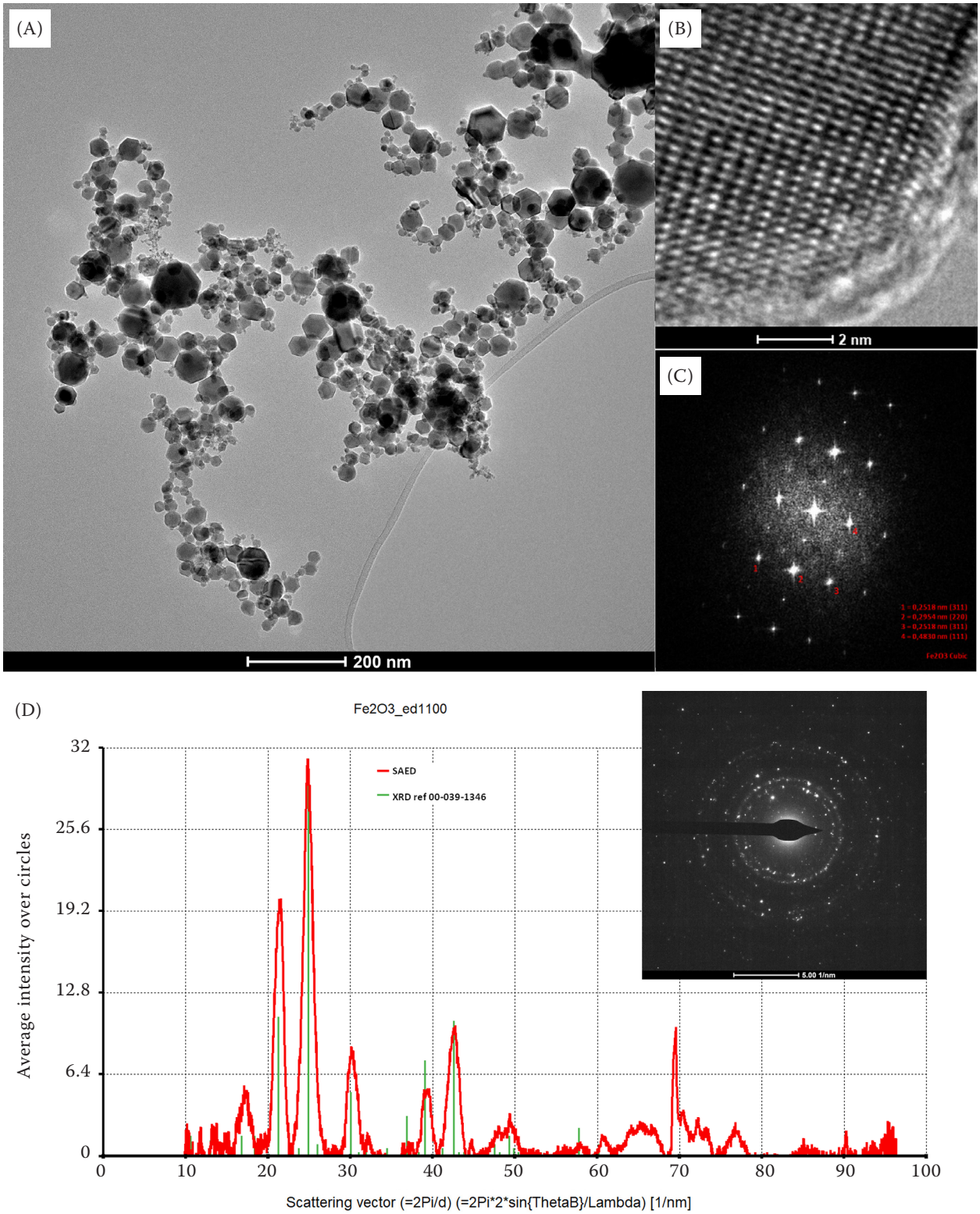

Figure 1. (A) Transmission electron microscope (TEM) micrographs of Fe_2O_3 nanoparticles show broad particle size distribution from 10 nm to 100 nm; (B) high-resolution transmission electron micrograph (HRTEM) of the cubic structure of Fe_2O_3; (C) FFT of HRTEM micrograph, most intensive reflections can be indexed to the (111), (220) and (311) planes of Fe_2O_3 cubic phase, and (D) the selected area electron diffraction (SAED) pattern, analysed using a specialised diffraction processing program, shows consistent results with the expected X-ray diffraction characteristics of the cubic phase of Fe_2O_3

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Table 1. Effect of Fe_2O_3 nano-priming on growth of sunflower microgreens

Variant	Germination rate (%)	Average height (cm)	Average leaf size (cm^2)	Average wet mass of microgreens per 100 seedlings	Average dry mass of microgreens per 100 seedlings
Control	90.87 ± 1.4^a	8.48 ± 0.61^a	2.56 ± 0.31^c	53.36 ± 4.62^c	2.12 ± 0.19^c
25 mg/L	89.93 ± 2.03^a	8.20 ± 0.75^{ab}	2.44 ± 0.25^c	53.97 ± 5.03^c	2.41 ± 0.21^a
50 mg/L	89.72 ± 1.49^a	7.62 ± 0.81^b	3.12 ± 0.53^a	60.35 ± 1.95^b	2.49 ± 0.21^a
100 mg/L	87.15 ± 2.09^a	6.73 ± 0.54^b	3.43 ± 0.43^a	64.47 ± 2.56^a	2.69 ± 0.28^a

Different letters within the same column indicate significant differences according to Tukey's multiple comparison test, with $P < 0.05$. The data presented means ± standard deviation ($n = 20$)

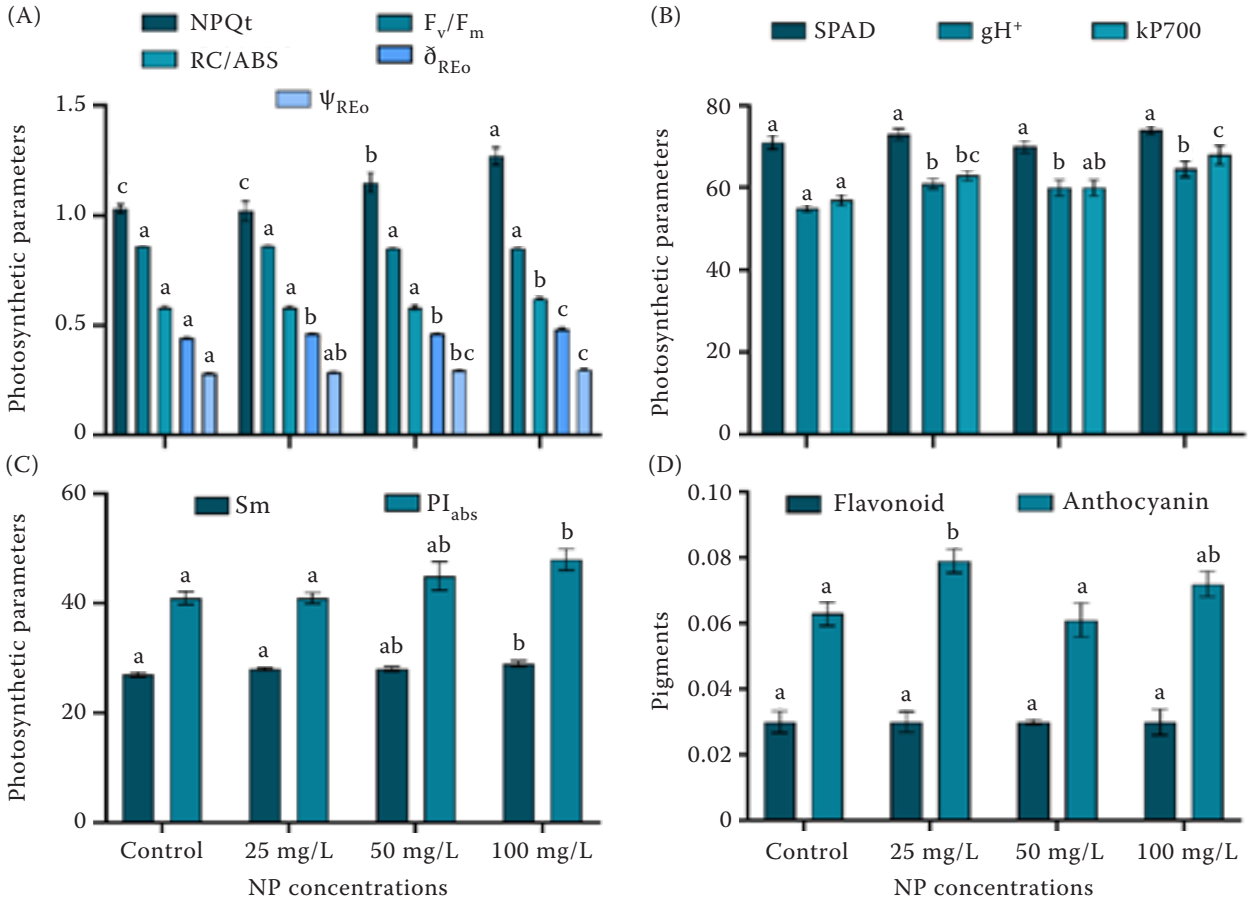

Figure 2. Effect of various Fe_2O_3 nanoparticles (NPs) priming concentrations on photosynthetic parameters (A) depicts the changes in non-photochemical quenching calculated from data captured in light-exposed samples (NPQt); the maximum quantum yield of PSII photochemistry (F_v/F_m); the number of active PSII reaction centers per unit of light absorbed (RC/ABS); the probability of electron transfer from PSII to PSI ($Ψ_{REo}$), and the probability of an electron being transferred from QB to a PSI acceptor ($ð_{REo}$); (B) shows variation in soil plant analysis development (SPAD) value; the rate constant of P700 electron transfer (kP700), and proton conductance of ATP synthase (gH^+); (C) the number of electron carriers per electron transport chain (Sm) and the photochemical performance index (PI_{abs}) is represented in (C), and (D) flavonoids and anthocyanin content. Letters show statistical significance (Tukey's multiple comparison tests, $P > 0.05$). The data presented means ± standard deviation ($n = 20$)

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Growth parameters. In the present investigation, seed priming with FeNPs did not significantly affect seed germination compared with the control treatment (Table 1). Similarly, a significant decrease in the average height of the seedlings was observed at 50 and 100 mg/L, but a notable increase in the leaf size by 21.87% and 33.98% was observed. A significant increase in the average fresh mass was observed by 13.00% and 20.82%, respectively, in higher concentration treatment (Table 1) – the present investigation aimed to evaluate the impact of FeNPs on sunflower microgreens. The results revealed that FeNPs priming induced alterations in sunflower microgreens' physiological and biochemical properties. For instance, an inverse relationship was observed between seedling height and cotyledon leaf size, whereby higher concentrations of NPs resulted in a significant increase in cotyledon leaf size but decreased seedling height. An increase in average cotyledon leaf size would be a desirable outcome for growers of microgreens, as leaves constitute the major component of this crop, and a significant increase in leaf size would increase productivity. We found that applying higher concentrations of FeNPs led to a 20.82% increase in biomass. This increase in biomass with the rising concentration of FeNPs might be attributed to the stimulation of water uptake by the FeNPs. The dry mass increase indicates that the microgreens are also nutrient-rich.

Pigments and photosynthetic parameters. Based on the SPAD values, there was no significant difference in relative chlorophyll content between the control and treated seedlings (Figure 2B). However, anthocyanin content increased significantly at 25 mg/L and remained unchanged at 50 and 100 mg/L (Figure 2D). The relative flavonoids were unaffected and were comparable in all the treatments (Figure 2D).

Measurements of dark-adapted leaf samples showed that FeNPs priming significantly influenced sunflower microgreens' photosynthetic efficiency and photochemistry. While the maximum quantum yield of PSII photochemistry (F_v/F_m) remained unchanged across all FeNPs concentrations, indicating that the photochemical efficiency and structural integrity of PSII were preserved, other critical parameters showed notable changes. Specifically, the number of active

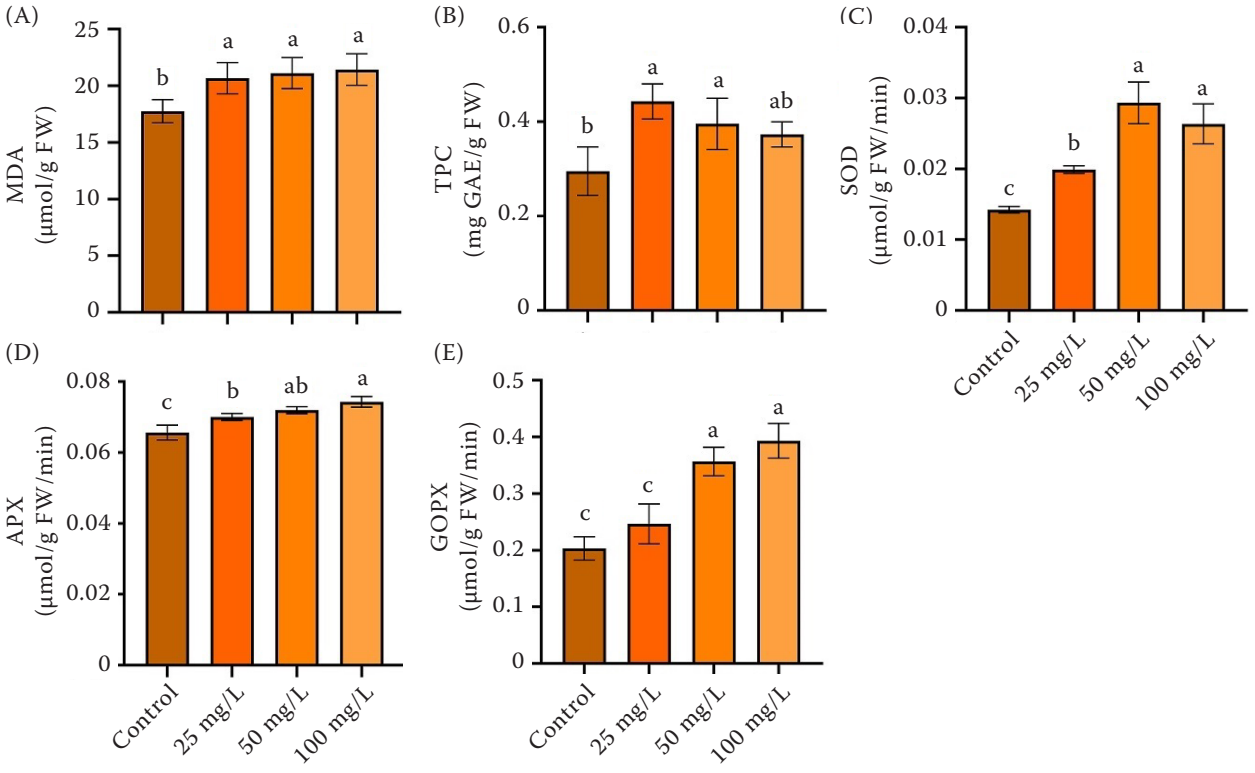

Figure 3. Effect of Fe_2O_3 nanoparticles (NPs) on biochemical parameters of fresh weight (FW) of (A) malondialdehyde (MDA) content; (B) total phenolic content (TPC); (C) superoxide dismutase (SOD) content; (D) ascorbate peroxidase (APX) content, and (E) guaiacol peroxidase (GOPX) content. Letters show statistical significance (Tukey's multiple comparison tests, $P > 0.05$). The data presented mean ± standard deviation ($n = 3$)

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PSII reaction centers (RC/ABS) increased significantly at the highest FeNPs concentration (100 mg/L), suggesting enhanced light absorption and energy transfer within PSII (Figure 2A). Additionally, the probability of electron transfer from PSII to Q_A (ψREo) and from Q_B to PSI acceptors (ðREo) significantly increased with higher FeNPs concentrations (Figure 2A), reflecting an improved efficiency in the electron transport chain (Rai-Kalal and Jajoo 2021). This enhancement likely contributed to increased ATP synthesis and NADPH production, essential for the Calvin cycle.

Furthermore, the photochemical performance index (PI_{abs}), proton conductance (gH^+), and non-photochemical quenching (NPQt), indicators of overall PSII efficiency and photoprotection, respectively, were significantly elevated at FeNPs concentrations above 25 mg/L (Figure 2A, B). These findings suggest that FeNPs priming optimises energy conversion processes and improves the protective mechanisms within the chloroplasts, enabling the sunflower microgreens to manage light stress more effectively. The increase in Sm indicates a higher number of electron carriers per transport chain (Figure 2C), further supporting the conclusion that FeNPs priming enhances photosynthetic activity. This enhanced efficiency may be linked to the increased phosphorus availability observed in FeNPs-treated plants, as phosphorus is crucial for ATP synthesis and the functioning of key Calvin cycle enzymes (Stirbet and Govindjee 2011). These results suggest that FeNPs priming improves photosynthetic efficiency and stress tolerance in sunflower microgreens, offering potential benefits for their growth and productivity.

Biochemical parameters. In this study, the measurement of MDA content to assess cellular membrane damage due to lipid peroxidation showed a significant increase in treated samples compared to control (Figure 3A). Still, no significant difference was found among different FeNPs concentrations. However, the total phenolic compounds increased significantly in the treated samples (Figure 3B). The SOD activity was found to increase gradually with increasing FeNPs concentration (Figure 3C). Similarly, APX and GOPX increased substantially with increasing concentrations of NPs (Figure 3D, E). As a part of some biochemical processes, plants produce ROS, and abiotic and biotic stresses can further stimulate ROS production, leading to plant cell damage. FeNPs have been found to either downregulate ROS production or upregulate plant repair mechanisms. The degree of lipid peroxidation is often used as an oxidative stress marker in physiological assays, and some studies suggested that FeNPs could promote lipid peroxidation and accumulation of MDA in cell membranes. However, in this study, a relatively slight increase in lipid peroxidation was observed, confirming the activity of FeNPs in sunflower microgreens. Non-enzymatic antioxidants, such as total phenolic content, were studied to assess the repair systems. The results confirmed increased non-enzymatic antioxidants in sunflower microgreens in the presence of FeNPs.

Additionally, the enzymatic antioxidant SOD, APX, and GOPX activity was found to increase with increasing FeNPs concentration gradually. This increase in antioxidant enzyme activity is consistent with our hypothesis that priming leads to stress mitigation, as studies have suggested that antioxidative enzyme requires Fe for activation (Feng et al. 2022). Based on the observations made in sunflower microgreens, it can be inferred that the relationship between malondialdehyde and antioxidative enzyme exhibits a dual property that depends on the concentration of MDA and the stress level within the cell. Cellular damage is precisely the result when MDA concentration is increased without a corresponding increase in the antioxidative enzyme. However, when MDA concentration is increased in conjunction with higher antioxidative enzyme activity, a protective response is induced in the cells. Therefore, it can be concluded that in sunflower microgreens, the increased antioxidative enzyme activity with a concurrent rise in MDA suggests that MDA is signaling activation of a protective response by stimulating antioxidative enzyme activity in the seedlings (Morales and Munné-Bosch 2019).

Macronutrient and micronutrient content. This study investigated the impact of FeNPs priming on the uptake of macro- and micronutrients in sunflower microgreens using inductively coupled plasma mass spectrometry (ICP-MS). FeNPs priming significantly influenced the nutrient composition of sunflower microgreens, enhancing the uptake of several essential elements compared to the control (Table 2). Among macronutrients, calcium (Ca) content increased significantly with all FeNPs treatments, peaking at 50 mg/L (99.88 mg/g) compared to the control (91.1 mg/g), indicating a dose-dependent response and suggesting that FeNPs priming enhances Ca uptake, potentially improving cellular signaling and membrane integrity. Phosphorus (P) content showed the most significant increase at 25 mg/L (96.71 mg/g), significantly higher than the control (76.81 mg/g), though it plateaued

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Table 2. Effect of Fe_2O_3 nano-priming on the content of macronutrients and micronutrients sunflower microgreens

	Control	25 mg/L	50 mg/L	100 mg/L
Macronutrient (mg/g)				
Ca	91.1 ± 1.59^{b}	98.01 ± 0.54^{a}	99.88 ± 1.01^{a}	98.68 ± 1.97^{a}
P	76.81 ± 1.37^{c}	96.71 ± 1.96^{a}	83.62 ± 3.81^{b}	83.61 ± 1.8^{b}
Mg	42.58 ± 3.37^{c}	50.66 ± 1.52^{a}	46.61 ± 4.98^{b}	44.65 ± 2.62^{bc}
Micronutrient (µg/g)				
Zn	196.53 ± 1.52^{b}	198.27 ± 1.1^{a}	199.13 ± 1.25^{a}	200.77 ± 1.47^{a}
Mn	156.47 ± 0.87^{c}	166.4 ± 1.8^{b}	174.77 ± 1.8^{a}	177.23 ± 0.9^{a}
Fe	96.49 ± 0.56^{b}	98.31 ± 0.67^{b}	120.16 ± 1.57^{a}	121.29 ± 0.66^{a}
B	51.22 ± 1.1^{a}	47.97 ± 0.3^{b}	47.27 ± 0.48^{b}	47.43 ± 0.49^{b}
Cu	20.23 ± 0.35^{b}	22.22 ± 0.29^{a}	21.17 ± 0.48^{b}	20.87 ± 0.67^{b}
Mo	1.15 ± 0.02^{c}	1.18 ± 0.01^{bc}	1.21 ± 0.02^{b}	1.42 ± 0.007^{a}
Ni	1.04 ± 0.05^{c}	1.26 ± 0.02^{b}	1.37 ± 0.03^{a}	1.38 ± 0.06^{a}

Different letters within the same row differ significantly according to Tukey's multiple comparison test, with $P < 0.05$). The data presented means ± standard deviation ($n = 6$)

at higher concentrations. This suggests that FeNPs priming at lower concentrations optimises P uptake, potentially enhancing energy transfer and metabolic processes. Magnesium (Mg) content was highest at 25 mg/L (50.66 mg/g), with less pronounced increases at higher concentrations, indicating that FeNPs priming at lower concentrations can boost Mg uptake, essential for chlorophyll production and photosynthesis. Among micronutrients, zinc (Zn) content consistently increased with rising FeNPs concentrations, peaking at 100 mg/L (200.77 µg/g) compared to the control (196.53 µg/g), suggesting that FeNPs promote Zn uptake, enhancing enzymatic activities and stress responses. Manganese (Mn) content also increased significantly at 100 mg/L (177.23 µg/g), further supporting the role of FeNPs in promoting Mn accumulation, which is vital for plant development and stress tolerance. Iron (Fe) content saw the most significant increase among micronutrients, particularly at 50 mg/L (120.16 µg/g) and 100 mg/L (121.29 µg/g), compared to the control (96.49 µg/g), reflecting efficient FeNPs absorption and translocation within the plant, enhancing Fe availability for crucial biochemical processes like photosynthesis and respiration. Copper (Cu) content increased significantly at the lowest FeNPs concentration (25 mg/L, 22.22 µg/g) but decreased slightly at higher concentrations, indicating a potential threshold effect in Cu uptake. Interestingly, boron (B) content was highest in the control group and decreased with FeNPs treatment, suggesting a possible antagonistic interaction between FeNPs and boron uptake mechanisms, warranting further investigation. Molybdenum (Mo) and nickel (Ni) contents steadily increased with rising FeNPs concentrations, peaking at 100 mg/L (1.42 µg/g and 1.38 µg/g, respectively), highlighting FeNPs' role in enhancing the uptake of these micronutrients (Ayala-Silva and Beyl 2005, Al-Salama 2022).

Overall, these findings highlight the potential of FeNPs priming to enhance the nutrient profile of sunflower microgreens by significantly increasing the content of essential macro- and micronutrients, offering a promising strategy for improving crop yield and resilience. However, further research is necessary to explore the long-term effects of FeNPs on plant health and their potential implications for human consumption and environmental sustainability.

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