



Research article

Physiological and anti-oxidative response of biologically and chemically synthesized iron oxide: *Zea mays* a case study

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ABSTRACT

The synthesis methodology, particle size and shape, dose optimization, and toxicity studies of nano-fertilizers are vital prior to their field application. This study investigates the comparative response of chemically synthesized and biologically synthesized iron oxide nanorods (NRs) using *moringa oleifera* along with bulk FeCl₃ on summer maize (*Zea mays*). It is found that FeCl₃ salt and chemically synthesized iron oxides NRs caused growth retardation and impaired plant physiological and anti-oxidative activities at a concentration higher than 25 mg/L due to toxicity by over accumulation. While iron released from biologically synthesized NRs have shown significantly positive results even at 50 mg/L due to their low toxicity, an improved leaf area (13%), number of leaves per plant (26%), total chlorophyll content (80%) and nitrate content (6%) with biologically synthesized NRs are obtained. Moreover, the plant anti-oxidative activity also increased on treatment with biologically synthesized NRs because of their ability to form a complex with metal ions. These findings suggest that biologically synthesized iron oxides NRs are an efficient iron source and can last for a long time. Thus, proving that nanofertilizer are required to have specific surface chemistry to release the nutrient in an appropriate concentration for better plant growth.

1. Introduction

The nanoparticles are currently used for plant ailments, monitoring of growth rate and enhancement of food quality as well as for nanopesticides and nanofertilizers [1, 2]. The use of nanofertilizers is inexorable in the modern era to feed 9 billion people by 2050 [3, 4]. It is therefore imperative to inquire about the nexus between the nanoparticles and agriculture by adopting novel approaches for their synthesis and application to optimize their efficiency to boost crop yield [5].

Fertilizers serve as the major source of nutrition for the plants but a large amount is lost because of degradation, photolysis, decomposition, hydrolysis, fixation, leaching, and volatilization [6]. Nano size structure improve nutrient use efficiency through the slow release of nutrients, targeted application, higher plant uptake and avoid volatilization losses [7]. The nanoparticles can predate into plants and may become part of the food chain from herbivores to humans and thus leverage malnutrition through improvements in nutrients uptake [8]. The size and shape of the nanoparticles depend on the method used for preparation e.g. chemical

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[9], physical [10], and biological methods [10, 11]. Recently, *Moringa oleifera* leaf extract was as bioreducing agent for the synthesis of iron oxide nanorods and zinc-iron oxide ($ZnFe_2O_4$) nanocomposite [12]. Moreover, *Sageretia thea* was also used to synthesized iron oxide (Fe_2O_3) nanoparticles, NiO nanoparticles, zinc oxide nanoparticles, and Co_3O_4 nanoparticles [10, 13]. All these NPs were prepared by eco-friendly methods and have diverse applications including agriculture. However, instead of reactant substances to prepare nanoparticles, there are other parameters which help to tailor specific size, shape, and function of all these nanoparticles for their wide range of application such as photocatalytic response [14, 15], Industrial textile effluent treatment and antibacterial effectiveness, pharmacogenetic potential, biomedical [16, 17] and antimicrobial activities [18, 19].

The bio-efficiency of the nutrients increases with the decrease in the size of nanoparticles [20].

Iron is the fourth most abundant earth element but it is not readily available to crops as its solubility in the soil is controlled by soil pH [21]. It is a major determinant of the biological functions for different enzymes in the cells that are essential for plant metabolism, respiration and photosynthesis [22]. Iron deficiency shows multiple disorders, like chlorosis in plants that show the symptoms of poor growth, a smaller number of leaves, and decreased chlorophyll contents. When iron oxides nanorods (NRs) are absorbed by the plants they are distributed, accumulated, and used as fertilizer, which has attracted researchers' interest. Therefore, it is crucial to develop an environmentally less harmful and unique way to enhance the iron absorption in crops. At present focus is to understand the biochemical, physiological, and molecular mechanisms of plants in response to the nanoparticles [23]. Also, before large scale agricultural applications of nanomaterials, it is important to draw a comparison between biologically and chemically synthesized ones to investigate which can be favourable.

Therefore, the current work is designed to investigate the impact of iron oxides NRs synthesized by biological and chemical methods through co-precipitation on *Zea mays*' (*Z. mays*) growth, physiology, biochemical and anti-oxidative traits. It is found that biologically synthesized iron oxide is much more effective in the positive growth of *Z. mays* as shown in Figure 1. Moreover, the concentration of iron oxides NRs was also optimized for exogenous application without causing toxicity. The promising results will help in developing non-toxic iron oxide nanofertilizers that will help us to overcome iron deficiency and will improve the output of agricultural products.

2. Experimental methods

2.1. Synthesis of FeO NRs

The chemical and biological synthesis of FeO NRs were carried out using our previously reported method [15], the details are given in Supporting Information.

2.2. Hydroponic growth of *Z. mays* in response to FeO NRs

Maize (*Zea mays*) seeds (DK-6103) were procured from the local agriculture market in Bahawalpur, Pakistan. Initially, seeds were sown in a seedling tray consisting of organic compost as a growth medium (Figure S1). The seedlings tray was underlaid with water containing plastic pots ensuring sufficient moisture for germinating seeds. The seedling tray was placed in a sunny area with temperature ranging from 8.3-26.6 °C. After a week of germination, six uniform seedlings were transferred to each pot (Figure S2). A total of thirty pots were maintained and each pot was considered as an experimental unit. The pots were carefully placed in a separate plastic container which acts as a nutrient reservoir. The plastic container was filled with 1000 mL of Hoagland's solution. The level of the liquid medium was adjusted daily up to 1000 mL inside a plastic container to ensure that roots of the plants and solid hydroponic medium are always in contact with the liquid. Three concentrations (25, 50, and 100 mg/L) of each type of iron oxides NRs (biological and chemical) were prepared independently along with positive and negative control in deionized water. The pots with 0 mg/L iron oxides NRs and $FeCl_3$ (0.01 M) were maintained as the positive and negative control, respectively. Each concentration was prepared and applied in triplicates. The pots were exogenously sprayed with 12 mL of their respective iron oxides NRs solution daily. This practice initiated immediately after transplanting seedlings and continued for the next three weeks. The pots were maintained on 14 h light and 10 h dark cycle at 22 °C during the experiment. The pots were harvested four weeks after sowing. During the harvest, the plants were washed with Milli Q-water. Plant morphological characteristics such as shoot length, root length, fresh weight, dry weight, leaf area, and the number of leaves per plant were measured by following standard agronomic procedures. The fresh samples were immediately frozen at -80 °C for physiological, biochemical, and anti-oxidative analysis.

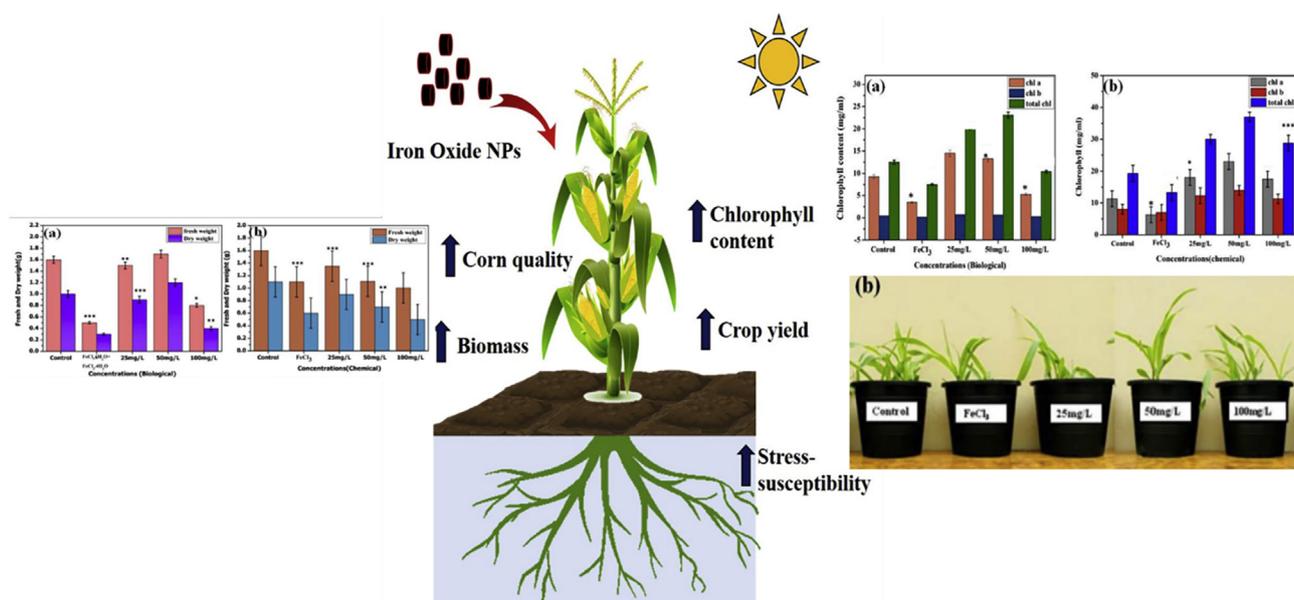


Figure 1. The schematic illustration of the effect of biologically and chemically synthesized FeO NRs on *Z. mays* growth parameters.

2.3. Maize physiological and biochemical analysis in response to FeO NRs

The chlorophyll content was estimated from 0.2 g leaf extract homogenized in 80% (v/v) ice-chilled acetone. The homogenate was refrigerated in the dark for 2 h followed by centrifugation for 15 min at 10,000 rpm. The optical density of green supernatant was recorded using a UV-vis spectrophotometer at wavelengths of 663 and 645 nm.

The soluble sugar content was measured by the Anthrone method keeping glucose as a standard. Soluble sugars were extracted with 3.5 aliquots of 80% ethanol. The homogenate was centrifuged for 15 min at 15,000 rpm. The optical density of supernatant was measured at a wavelength of 630 nm by using the Anthrone Colorimetric method.

To determine nitrate content, a 0.4 g fresh leaf sample was boiled in a sealed test tube for 100 min. The filtrate nitrate content was measured by using the sulfosalicylic acid method with KNO_3 as a standard. A UV-vis study was carried out at 410 nm.

2.4. Determination of anti-oxidative potential of FeO NRs

The antioxidant activity of maize in response to different types and levels of FeO NRs were determined from the preserved frozen fresh samples. Dimethyl sulfoxide (DMSO, 10 mg/mL) was used to prepare a suspension, after keeping for 48 h, the solution was centrifuged at 10,000 rpm for 10 min. The as-obtained supernatant was used to determine the activities of different antioxidants using DPPH (1,1 Diphenyl-2-picrylhydrazyl) assay. The capability of the sample to absorb DPPH ion was analyzed by the following formula:

$$\text{DPPH scavenging effect} = (\text{Control OD} - \text{Sample OD} / \text{Control OD}) \times 100$$

$$\text{Percent inhibition of the test sample} = \% \text{ scavenging activity} = (1 - \text{Ab}_s / \text{Ab}_c) \times 100$$

where Ab_s represents the absorbance of DPPH solution and Ab_c represents the absorbance of the negative control (having solvents and reagents only).

The total antioxidant capacity was expressed as the number of grams equivalent of ascorbic acid, details are provided in Supporting Information. The obtained total phenolic content was analyzed as microgram trihydroxy benzoic acid equivalent per mg FW ($\mu\text{g GAE}/\text{mg FW}$), details are provided in Supporting Information. Similarly, the AlCl_3 colorimetric method was used to estimate the total flavonoid content of the crude extract [24]. Absorbance was calculated using a microtiter plate at 415 nm after incubating the samples for 15 min.

2.5. Statistical analysis

The significance of the different concentrations of biologically and chemically synthesized NRs was determined using the least significant

difference (LSD) test; differences were determined to be statistically significant when p -value < 0.05.

3. Results and discussion

3.1. Effect of FeO NRs on *Z. mays* growth and biomass accumulation

The fabrication method of FeO NRs and their physiological characteristics significantly affected *Z. mays*' growth and biomass characteristics. For instance, FeO NRs significantly ($p \leq 0.05$) improved the shoot length and root length of *Z. mays* compared to control (Figure 2). The effect of NRs was prominent for shoot length growth (15%) as compared to root length (9%) with respect to control. The application of biologically synthesized NRs reported the maximum shoot length of 37 cm (Figure 2a) while the application of chemically synthesized NRs produced the maximum root length of 18 cm (Figure 2b), assuring the advantages of bioinspired growth of NRs. The effect of NRs was concentration-dependent and the plant response significantly changed with the increased dosage of iron oxides NRs [25, 26]. The biologically synthesized NRs promoted shoot length up to the concentration of 50 mg/L while the chemically synthesized NRs reported toxicity at this concentration. Both sources of iron oxides NRs reported toxic effects on root shoot length at 100 mg/L. The direct application of FeCl_3 also reported toxic effects as the obtained values for root shoot length were lower than control. Hence, proving that the biological synthesis of nanomaterials as fertilizer has a far positive impact on the growth of plant [27].

Figure S3 represents the data of leaf area and number of leaves per plant, the effect of FeO NRs source and concentrations were highly significant ($p \leq 0.01$) and positive up to the concentration of 50 mg/L for biologically synthesized (Figure S3a,d) and 25 mg/L for chemically synthesized NRs (Figure S3b,c). The application of biosynthesized NRs gradually improved the leaf area and the number of leaves per plant as compared to control and direct FeCl_3 . A dose of 50 mg/L of bio-synthesized FeO NRs not only improved the leaf area by 13% but also the number of leaves per plant up to 26%. While the chemically synthesized NRs negatively affected the leaf area (-37%) and the number of leaves per plant (-50%) at all concentrations except for 25 mg/L that reported a slightly positive effect. The application of 100 mg/L of chemically synthesized NRs proved to be 35% more toxic for leaf area as compared to the same concentration of biologically synthesized iron oxides NRs. Thus, once again proved that developing nanofertilizers through bioinspired methods will be highly productive towards enhancing the growth of crops [28, 29].

Similarly, the other growth traits like the fresh and dry weight accumulation of *Z. mays* were also influenced positively (Figure 3). Both types of NRs impaired fresh and dry biomass accumulation at all concentrations with an exception for biosynthesized NRs at 50 mg/L that showed a slight improvement in biomass accumulation (Figure 3b). On

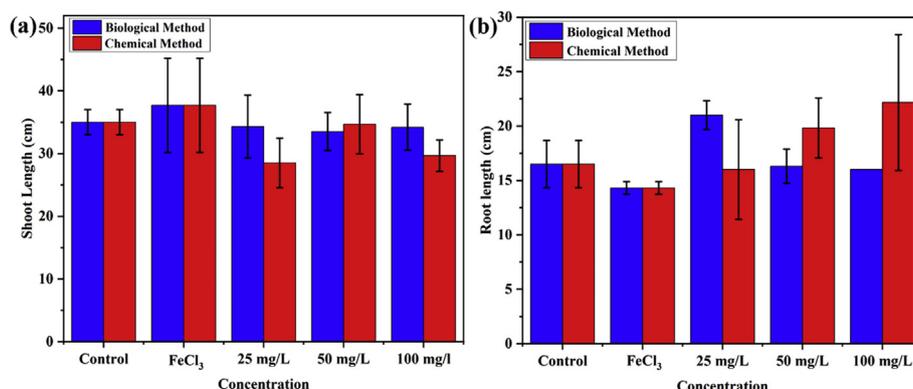


Figure 2. Root length and shoot length of *Z. mays* in response to FeO NRs synthesized via (a) biologically and (b) chemically.

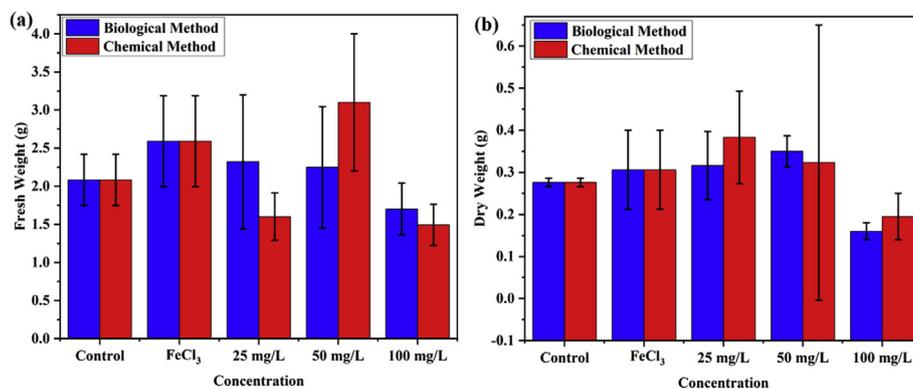


Figure 3. Impact of iron oxide NRs on (a) fresh weight and (b) dry weight of *Z. mays*.

the contrary, the chemically synthesized NRs have produced toxic effects even at the lowest concentration (25 mg/L) and the toxicity increased with the dosage. The effect of FeCl₃ was similar to that of chemically synthesized NRs (Figure 3a). Overall, the chemically synthesized iron oxides NRs severely decreased fresh weight (-37%) and dry weight (-54%) of *Z. mays* as compared to control.

3.2. Effect of FeO NRs on plant physiological and biochemical attributes

The measured chlorophyll contents (a, b, and total) of *Z. mays* are presented in Figure 4. Chlorophyll contents were significantly ($p \leq 0.05$) improved with the increase in the dose of NRs irrespective of their method of synthesis. The peaks were obtained at 25–50 mg/L for both methods for all types of chlorophylls except for the chlorophyll b for biosynthesized NRs that remained unchanged with or without the application of NRs (Figure 4a). The application of 100 mg/L produced a mild toxic effect on *Z. mays* chlorophyll contents. The effect of biologically synthesized NRs on carbohydrate contents was eloquent [30].

The FeO NRs at the dosage of 25 and 50 mg/L showed a significant ($p \leq 0.05$) increase in the carbohydrate concentration. At 25 mg/L concentration, a maximum positive increase (54%) in carbohydrate content over control was observed for biosynthesized NRs (Figure 5a). A significant decrease (-18%) was recorded at the highest dose of 100 mg/L over control. The FeCl₃ treatment showed the maximum significant decrease (-71%) in carbohydrate content when compared to control. For chemically synthesized FeO NRs the low and medium dose showed 40% and 20% increase in soluble sugar content over control (Figure 5b). The decline in *Z. mays* sugar content at high dose may be attributed to an increase in the toxicity of applied FeO NRs [31, 32].

A slight increase in nitrate content (4%) was noticed at the medium dose of FeO NRs. All other concentrations of biosynthesized NRs have shown significant reductions of -71%, -15%, and -60% in nitrate content

for 25, 50, and 100 mg/L, respectively (Figure 6a). The effect of chemically synthesized FeO NRs at the dose of 25 and 50 mg/L confirmed a significant positive effect over control. The highest tested concentration of FeO NRs significantly decreased (-12%) nitrate content over control similar to reported elsewhere [33]. Similarly, the FeCl₃ treatment revealed a significant decline of -70% in leaf nitrate content over control (Figure 6b).

3.3. Effect of FeO NRs on plant anti-oxidative activity

The plants' anti-oxidative activity i.e., 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), total flavonoid content (TFC), total antioxidant content (TAC) and total phenolic content (TPC) were measured in response to different types and concentrations of the FeO NRs (Figure 7). The DPPH free scavenging activity significantly ($p \leq 0.05$) increased at the 25–50 mg/L concentration for biologically synthesized FeO NRs (Figure 7a). The chemically synthesized NRs have presented a minor scavenging activity at 25 mg/L and it sharply declined with the increase in the dose of application. At 50 mg/L concentration, the biologically synthesized NRs showed 38% higher free radical scavenging activity over control and 28% to its counterpart chemically synthesized FeO NRs. Conclusively, the biologically synthesized NRs promoted free radical scavenging activity for all concentrations (25–100 mg/L) [34, 35].

The TFC content of the control group was higher than the negative control and both types of NRs (Figure 7b). The chemically synthesized NRs proved more toxic at the highest dose as compared to biosynthesized NRs. The plant TFC significantly ($p \leq 0.05$) decreased (-29%) with the increase in NRs concentration. There was a considerable variation in TAC in plants when they encountered with biologically and chemically synthesized FeO NRs (Figure 7c). The TAC increased significantly (12%) in the case of 25 mg/L of chemically synthesized NRs as compared to

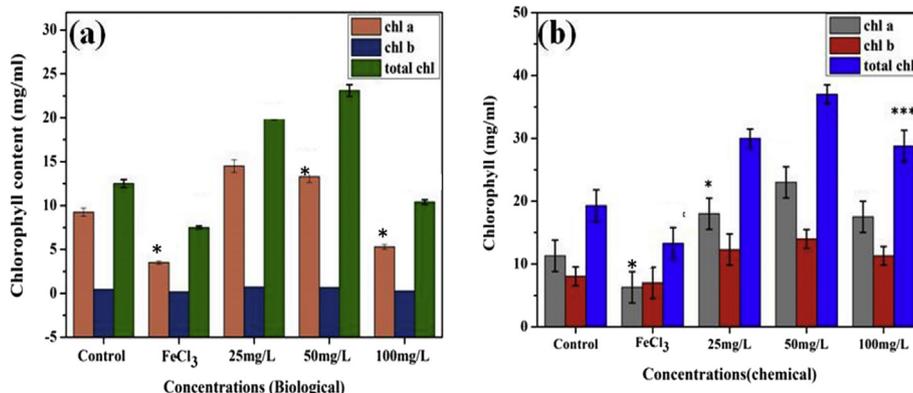


Figure 4. Chlorophyll contents of *Z. mays* in response to (a) biologically and (b) chemically synthesized FeO NRs.

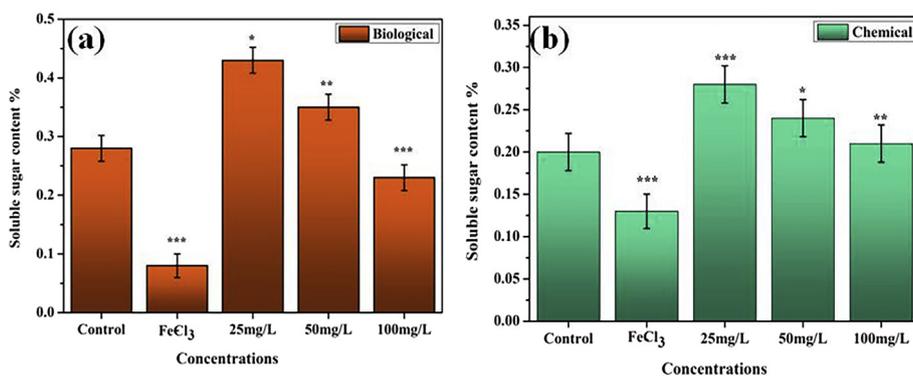


Figure 5. Soluble Sugar contents of *Z. mays* in response to (a) biologically and (b) chemically synthesized FeO NRs.

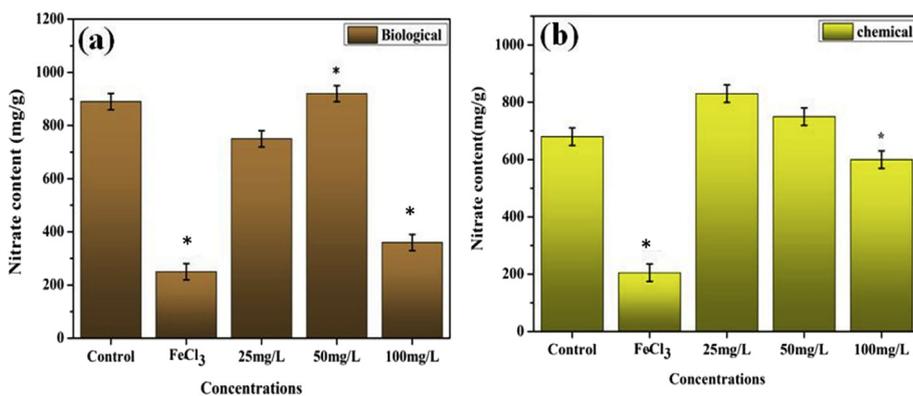


Figure 6. Nitrate contents of *Z. mays* in response to (a) biologically and (b) chemically synthesized FeO NRs.

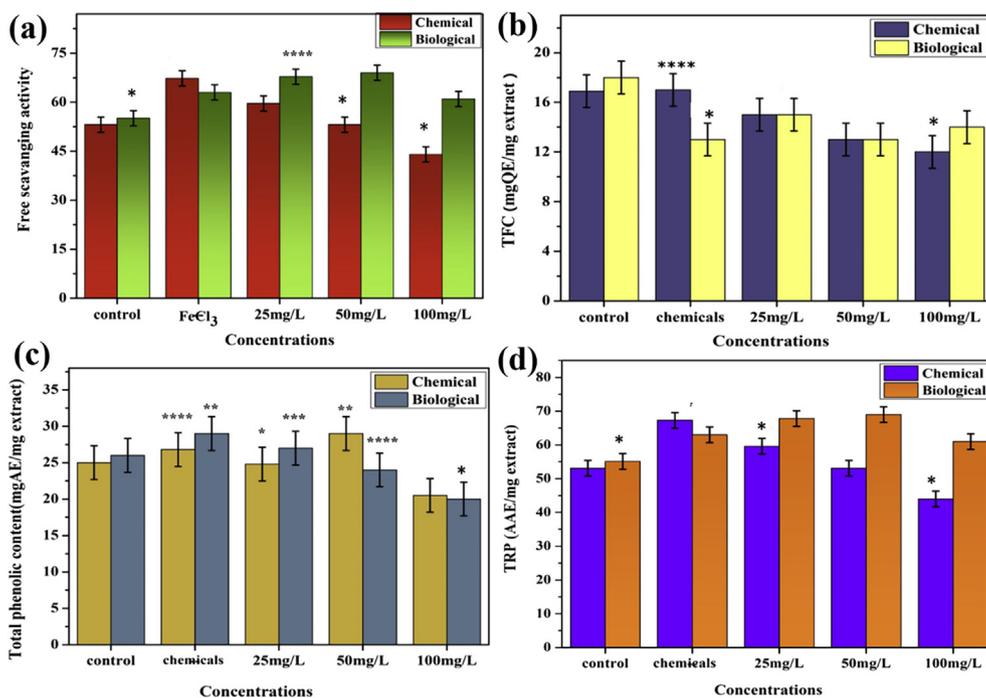


Figure 7. Biochemical response of *Z. mays* in response to biologically and chemically synthesized iron oxide NRs for radical scavenging activity (DPPH) (a) total flavonoid content (TFC) (b), Total phenolic content (TPC) (c) total antioxidant content (TRP) (d).

control. Among biologically synthesized NRs, all concentrations produced a significant ($p \leq 0.05$) decline in TAC as compared to positive and negative control [36, 37]. The TPC showed a minimum variation between the two sources of NRs, however, the effect of dose was significant (Figure 7d). The TPC decreased at the low concentration, increased at medium concentration, and again decreased at the highest concentration of chemically synthesized NRs. On the contrary, the increase in biologically synthesized NRs concentration has gradually declined the plant TPC.

Hence, bioinspired synthesis of nanofertilizers will be a key not only in decreasing the cost of production and making the production safe, but it also influences the growth at a much higher pace. The reason behind their biocompatibility which has no toxic impacts on plants and slow-release with the passage of time which allows the plants to utilize them properly [38, 39]. However, the negative influence at higher concentration might be due to the accumulation of NRs around the shoots or extensive release of iron which negatively impact the growth like iron salt [40, 41]. In short, our results open a pathway towards bioinspired fertilizers for faster and healthier growth of crops to increase their production to fulfill the demands of a quickly growing population.

4. Conclusions

In this study, we investigated the impact of FeO NRs synthesized by chemical and biological methods on the morphological and biochemical parameters of *Zea mays*. The biologically synthesized NRs promoted plant growth and physiological attributes at low to medium concentrations while the chemically synthesized nanoparticles reduced plant growth at medium dose. This decline in plant traits may be due to over-accumulation at a lower concentration of chemically synthesized nanoparticles that produced toxicity. Biologically synthesized FeO NRs are not only eco-friendly but also more effective for plant growth. The study encourages the use of green synthesized FeO NRs for not managing iron deficiency but also for multiple uses in agriculture in the future. Thus, biologically synthesized FeO NRs interaction on the morphological, physiological, and anti-oxidative activities of *Z. mays* is important to manage iron nutrition in crops and to fulfill the needs of food in the future.

Declarations

Author contribution statement

Murtaza Hasan, Saira Rafique: Performed the experiments; Wrote the paper.

Ayesha Zafar, Rida Khan, Shahbaz Gul Hassan, Sadaf Zahra, Ghazala Mustafa, Xugang Shu, Zahid Ihsan: Analyzed and interpreted the data.

Suraj Loomba: Analyzed and interpreted the data; Wrote the paper.

Muhammad Waqas Khan, Muhammad Zia: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Nasir Mahmood: Conceived and designed the experiments; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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