Improved iron use efficiency in tomato using organically coated iron oxide nanoparticles as efficient bioavailable Fe sources

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Abstract

Background: Iron [Fe] deficiency is one of the nutritional issues of plants, especially in calcareous soils in which iron-fertilizers are used to solve this obstacle. Due to the pivotal role of iron, the introduction of efficient, cost-effective, and eco-friendly strategies is necessary to prevent its deficiency in plants. The nanoparticle-based formulations may provide efficient bioavailability, subsequently, reduce the amount of the required dosage of nutrients for extended periods, and decrease the environmental risks.

Results: In this study, the effects of different iron nanoparticles (NPs) including Fe₃O₄ nanoparticles (Fe₃O₄), citric acid coated Fe₃O₄ nanoparticles (Fe₃O₄@CA), humic acid coated Fe₃O₄ nanoparticles (Fe₃O₄@HA), and EDTA coated nanoparticles (Fe₃O₄@EDTA) were investigated as iron [Fe] sources on the vegetative growth and physiological parameters of tomato as a model plant in a soil system. The experimental results showed that the organically coated Fe₃O₄ NPs significantly increased the amount of [Fe] in the shoot and enhanced its growth. The highest and lowest amount of [Fe] was observed in the Fe₃O₄@HA NPs and control treatments, respectively. In addition, using organically coated Fe₃O₄ NPs, especially Fe₃O₄@HA increased plant growth and yield.

Conclusions: This study showed that using organically coated Fe₃O₄ NPs is promising for plant nutritional supplementation. In particular, the humic acid-coated Fe₃O₄ nanoparticles (Fe₃O₄@HA) were determined to be the most promising, due to more benefits for plant growth and yield compared to Fe₃O₄ NPs. Therefore, Fe₃O₄@HA nanofertilizer can be introduced as an inexpensive, effective, bioavailable, and biocompatible option to address [Fe] deficiency in the soil.

Highlights:

- Nanofertilizers may provide efficient bioavailability and reduce the required dosage of nutrients & environmental risks.
- The response of tomato plants to three organically coated Fe₃O₄ NPs as nanofertilizer were investigated.
- The behavior of citric acid, humic acid, and EDTA were investigated and compared as organic coating materials.
Background

Iron [Fe] is an essential nutrient for plants, acts as an enzyme cofactor in various physiological and metabolic processes, and is also involved in photosynthesis, nitrogen fixation, nitrate synthesis, respiration, hormone production, and DNA production [1]. The deficiency of micronutrients, including iron, is one of the nutritional problems of plants, especially in calcareous soils, due to the alkalinity, coarse texture, low organic matter, salinity stress, continuous drought, and high bicarbonate content of the soil. Improper use of fertilizers in semi-arid and desert areas and under irrigation leads to widespread nutrient deficiencies. Although iron is often high in the soil, much of it bonds to soil and exists as an insoluble form (Fe$^{3+}$). Iron solubility in calcareous soils is minimal, which explains the lack of iron in alkaline soils and, consequently, iron deficiency in plants in these areas [2, 3]. To obviate iron deficiency in plants, various mineral compounds, natural and artificial chelates are widely used. Common bulk fertilizers used to reduce iron deficiency are iron sulfate (FeSO$_4$·7H$_2$O) and iron chelate [4]. The consumption of iron sulfate by mixing it with the surface soil, spraying solution or injecting it into the tree trunk is considered one of the first ways to deal with iron deficiency [5]. In general, bulk iron fertilizers are divided into the following three categories. Inorganic Fe compounds such as iron (II) sulfate (FeSO$_4$), synthetic Fe chelates such as ethylene diamine-di-ortho-hydroxyphenyl acetic acid (EDDHA), and natural Fe complexes such as amino acids and humates [6, 7]. These fertilizers are often added, although they may occur in the availability of micronutrients to the plant is low, which can also be eliminated by producing nanofertilizer [8]. Due to the merits of nanoparticles, their use in the production of nanofertilizers coated with a variety of chelating agents increases their desirable properties. In addition, it increases the soluble [Fe] and its bioavailability for plants due to their unique properties such as small size and large surface area, which improves physiological properties and increases product yield [9].

Iron NPs have unique properties such as enhanced surface-to-volume ratio, inherent biocompatibility, quantum confinement, high surface energy, and several catalytic properties that are widely used in various fields, including environment, medicine, agriculture, and industry [10–14]. Compared to conventional fertilizers, nano-fertilizers are expected to significantly improve crop growth and yield, increase the efficiency of fertilizer application, reduce nutrient losses, and minimize adverse environmental effects. Nanofertilizers have been suggested as promising alternatives to tackle the drawbacks arising from traditional agrochemicals [8, 15].

- The organically coated Fe$_3$O$_4$ NPs, especially Fe$_3$O$_4$@HA improved the bioavailability and subsequently enhanced the amount of total iron determined in the shoot.
- Fe$_3$O$_4$@HA nanofertilizer has recognized as an effective, cost-effective, and biocompatible option to address Fe deficiency in soil.
- The plant height, dry weight, and the plant Fe content increased by 31%, 97%, and 247%, respectively.

Keywords: Humic acid, Iron nanofertilizer, Iron deficiency, Organically coated Fe$_3$O$_4$, Tomato

Graphical Abstract
However, it is important to note that the toxicity of NPs due to aggregation, agglomeration, and dissolution in the diffusion medium depends on the used dosages in a particular application [16]. Therefore, the use of nanomaterials in the optimal concentrations indicates the proper use of NPs-based nutrients in agricultural fertilizers, which reduces the negative effects and increases plant yields. The studies have reported both positive and negative effects of Fe$_3$O$_4$ NPs and γ-Fe$_2$O$_3$ NPs on plants [17–20]. For example, the effects of TiO$_2$ NPs and Fe$_3$O$_4$ NPs in different concentrations on soil mineral phosphorus on Lactuca sativa (lettuce) were recently investigated [19] and the results showed that the phosphorus accumulation in the shoots was higher with using Fe$_3$O$_4$ NPs. In addition, according to another effort, using 50 mg.L$^{-1}$ of γ-Fe$_2$O$_3$ NPs led to an increase in the chlorophyll content and root activity of Citrus maxima [11]. In addition, it was observed that at a concentration of 100 mg.L$^{-1}$ NPs, the MDA formation increased, whereas the root activity and chlorophyll content decreased. Rui et al. reported that Fe$_2$O$_3$ NPs could enhance plant growth, biomass, and antioxidant enzyme activity in Peanut [21]. Fe$_2$O$_3$ NPs can also regulate the phytohormone contents [22].

The Fe-based materials stabilized with Lonardit HS and applied these polymers for easy delivery of the amorphous ferric ions into the leaves have been reported by Kulikova et al. [23]. They concluded that these nanofertilizers could replace synthetic chelates or be used in fertilizers containing (N, P, and K) [23]. Li et al., (2018) have reported that the use of γ-Fe$_2$O$_3$ NPs can increase the chlorophyll content and root activity of Citrus maxima (C. maxima) seedlings [24]. Siva and Benita showed that absorbed NPs by Ginger roots increase protein levels and the amount of chlorophyll, carotenoid, protein, and increase the iron of the rhizome [25]. The effect of Fe$_3$O$_4$ NPs on reducing the impact of arsenic toxicity on the Indian mustard plant (Brassica juncea var. Pusa Jagan-nath) was studied [26]. The results showed that the use of these NPs could increase plant growth and also enhance the amount of chlorophyll, carotenoid, protein, and also reduces the level of stress modulators and antioxidant enzymes. The effect of Fe$_2$O$_3$ nanoparticles on the absorption of cadmium by rice seedlings under hydroponic conditions was studied [27]. The results showed that foliar application of FeCl$_3$ and Fe$_2$O$_3$ nanoparticles reduced to some extent the DNA damage of cadmium to shoot. However, foliar application of Fe$_2$O$_3$ nanoparticles was more effective in reducing the toxic effects of cadmium than the application of FeCl$_3$ [27]. One of the effective ways to obviate iron chlorosis is to use natural organic chelates such as humic acid and synthetic iron chelates such as Fe-EDTA and Fe-EDDHA. The beneficial effects of these chelates make them commonly used to treat plant iron deficiency. However, the synthetic Fe chelates are not only expensive but also cause direct and indirect damages, such as the increased mobility of heavy metals [28–30] and increased uptake of radioactive metals [31]. Humic substances increase the bioavailability of metal ions such as Fe, Mn, Cu, and Zn due to their chelating properties and high affinity for metals [32].

In this study, efficient iron nanofertilizers containing organically coated iron nanoparticles were synthesized and applied as [Fe] nutrient resources for tomato plant using soil treatment during the growing season. The aim of this study was to introduce the more promising iron nanofertilizer that offers more efficiency and bioavailability of nutrient formulation together with a reduction of the required dosage and environmental concerns of agrochemicals. To this, the vegetative growth and physiological responses of the tomato plants were investigated in the presence of different organically coated Fe$_3$O$_4$ NPs including citric acid coated Fe$_3$O$_4$ nanoparticles (Fe$_3$O$_4$@CA), humic acid coated Fe$_3$O$_4$ nanoparticles (Fe$_3$O$_4$@HA), and EDTA coated nanoparticles (Fe$_3$O$_4$@EDTA) and compared with those of Fe$_2$O$_3$ nanoparticles.

**Experimental methods**

**Materials and characterization**

Tri-natrium citrate dihydrate (C$_3$H$_7$Na$_3$O$_7$·2H$_2$O), Ethylenediaminetetraacetic acid (EDTA), humic acid, polyvinylpyrrolidone (PVP), potassium phosphate diethylenetriaminepentaacetic acid (DTPA), and ferric & ferrous chloride were obtained from Sigma-Aldrich. The hydrodynamic particle size of the samples were determined using a dynamic light scattering instrument (DLS, Brookhaven, USA). The sample was prepared by dispersing the solid nanomaterials in deionized water by an ultrasonic bath for 15 min and was then used for size determination. The scanning electron microscopy (SEM, Hitachi S-4800 II, Japan) and transmission electron microscopy (TEM, Hitachi H-7650, 80 kV, Japan) were utilized to show the size and morphology of the samples. To this purpose, the TEM/SEM samples were prepared via the simply dispersing of NPs on the grids using a suitable solvent such as EtOH. Then, the prepared samples were dried and used for imaging. The topological characteristics of materials were observed using atomic force microscopy (AFM, DME-Ds95-50, Denmark) in ambient conditions at room temperature. To this, a sonicated solid nanomaterials mixture in deionized water was dispersed on the grids. The required spectrophotometric measurements were performed using a UV–Vis double-beam spectrophotometer (UV-3100 PC, Shimadzu).

**Synthesis of Fe$_3$O$_4$ nanoparticles (Fe$_3$O$_4$ NPs)**

To synthesize Fe$_3$O$_4$ NPs, the 50 mL of FeCl$_3$ (1 M) and 50 mL of FeCl$_3$ (2 M) solutions were prepared, and HCl
(37%) was added to form a transparent brick solution under an inert atmosphere. Then, NaOH (5 M) solution was gently added to the iron solution until pH adjusted to >10 and was homogenized at 40 °C for 2 h. Then, the later solution was refluxed for 2 h under an N₂ atmosphere at 90 °C. Then, the black solid was separated, washed, and dried at room temperature in a vacuum oven.

**Synthesis of citric acid coated Fe₃O₄ nanoparticles (Fe₃O₄@CA NPs)**

Fe₃O₄ NPs (5 g) were sonicated in deionized water (100 mL) for 30 min and then 125 mL of tri-natrium citrate dihydrate (1 M) was added and the mixture was refluxed at 90 °C for 2 h. The solid residue was centrifuged, washed, and dried at 30 °C using a vacuum oven to give the citric acid-coated Fe₃O₄ NPs (Fe₃O₄@CA NPs).

**Synthesis of humic acid coated Fe₃O₄ nanoparticles (Fe₃O₄@HA NPs)**

Humic acid (3.0 g) was dissolved in deionized water (50 mL) and pH was adjusted to 3 using HCl 37%. Then, Fe₃O₄ NPs (2.0 g) dispersed in deionized water (50 mL) using a sonicate bath, was added to the humic acid solution, and the mixture was then allowed to stir for 72 h at room temperature. The solid residue was centrifuged, washed, and dried at 30 °C under vacuum to give humic acid-coated Fe₃O₄ NPs (Fe₃O₄@HA NPs).

**Synthesis of EDTA coated nanoparticles (Fe₃O₄@EDTA NPs)**

To a mixture of Fe₃O₄ NPs (5.0 g) dispersed in deionized water (100 mL) via sonication, EDTA solution (10%) was added to the reaction mixture, and stirred for 24 h at room temperature. After that, the solid residue was separated, washed, and dried in a vacuum oven at 30 °C to have EDTA-coated Fe₃O₄ NPs (Fe₃O₄@EDTA NPs).

**Table 1** Physiochemical properties of the soil sample

| Parameter (unit) | Amount |
|------------------|--------|
| pH               | 7.3    |
| EC (dS/m)        | 2.38   |
| Organic matter (%) | 1.69  |
| N (ppm)          | 125    |
| P (ppm)          | 3.251  |
| K (ppm)          | 112    |
| Ca (ppm)         | 240    |
| Mg (ppm)         | 100    |
| Fe (ppm)         | 8.6    |
| Mn (ppm)         | 15.17  |
| Zn (ppm)         | 0.349  |
| Cu (ppm)         | 0.289  |
| CaSO₄ (%)        | 2.81   |
| Clay (%)         | 36.4   |
| Silt (%)         | 29.5   |
| Sand (%)         | 34.1   |

**Soil preparation and tomato cultivation**

**Physiochemical properties of soil**

A bulk soil sample from an agricultural field (Karaj, Iran) was collected, air-dried, and passed through a 2-mm sieve was carried Then physical and chemical properties of the soil sample were determined according to standard methods [33]. Soil pH was measured in soil saturation, and the electrical conductivity (EC) was measured in the saturated extract. The organic matter content was determined by Walkley and Black's method [34]. Clay, Silt, and sand percentages of the soil were determined by the hydrometer method [35]. Potassium (K) concentrations of the soil solution were measured using an emission absorption flame. The amounts of N and P were also determined by Kjeldahl digestion and molybdenum blue methods, respectively [36]. The [Fe] concentrations were determined using atomic absorption (AA) spectrophotometer. The physical and chemical properties of the soil samples are given in Table 1.

**Planting and harvesting tomatoes in the greenhouse**

Before growing tomatoes, a given amount of the prepared Fe₃O₄ NPs-based nanofertilizers were added to the pots in powder form and mixed with soil. The soil was mixed with Coco-Peat 50% by weight and transferred to 2 kg pots. Cherry tomato (*Solanum lycopersicum var. cerasiforme*) seeds were employed for the cultivation in the pots. Prior to the cultivation in pots, tomato seeds were placed on top of moist filter paper in a Petri dish for 24 h, afterward, three seeds were taken from the Petri dish and planted in each pot. After emergence and producing true leaves to achieve strong seedlings, the number of plants in each pot was thinned to one seedling. Greenhouse temperature was maintained at 24–18 °C for day–night cycles, 12–12 h light–dark cycles, and relative humidity of 40% were considered during the growing period. In addition, the irrigation was done whenever it was needed to maintain the soil moisture relatively stable (~80% of field capacity). At the time when completed the vegetative period, they were harvested. Therefore, to investigate the effect of the synthetic NPs on the plant bioavailable [Fe] in the soil, a factorial experiment was conducted in a completely randomized design. This included two sources of fertilizer (including the nanoscale and the balk versions), four types of nanofertilizer (Fe₃O₄ NPs, Fe₃O₄@CA NPs, Fe₃O₄@HA NPs, and Fe₃O₄@EDTA NPs) and four types of balk fertilizer (FeSO₄, iron citrate (C₆H₅FeO₇.H₂O), iron humate, and Fe-EDTA) at five concentrations of iron (0, 25, 50, 75, 100, and 200 mg per
Kg of soil), and three replications that performed in the greenhouse experiment.

**Estimation of chlorophyll content and biochemical parameters, total protein and catalase**

At the end of the growing period, the plants were harvested, and after weighting and rinsing with distilled water, the plant samples were dried at 65 °C until a constant weight. The dried specimens were weighed and powdered by milling and prepared for measuring the protein content [37] and the activity of catalase [38]. Chlorophyll contents were assayed using the Arnon’s method [39].

**Measurement of protein content**

To extract the total soluble protein, 0.5 g of abrasive wet tissue was placed in a porcelain mortar and then 50 mg of polyvinylpyrrolidone (PVP) was added. Then, 3 mL of potassium phosphate buffer (pH = 7) was added to it and the sample was ground and then poured into a 15 mL falcon and centrifuged for 15 min at 4° C at 15,000 rpm. The amount of total soluble protein was measured according to Bradford method [37]. The absorbance of the samples was measured at 595 nm using a spectrophotometer. Different concentrations of bovine serum albumin (BSA) protein were used to draw the standard curve.

**Measurement of activity of catalase**

Catalase activity was measured by the Aebi’s method [38] at a wavelength of 240 nm. The reaction mixture contained 250 μL of potassium phosphate buffer (100 mM, PH = 7), 250 μL of oxygenated water 70 mM dissolved in potassium phosphate buffer, 500 μL of sterile distilled water and 20 μL of enzyme extract.

**Measurement of chlorophyll a, chlorophyll b [39]**

Half a gram of the fresh tissues were poured into a porcelain mortar, then crushed using liquid nitrogen. 20 mL of 80% acetone was added to the samples and then centrifuged at 6000 rpm for 10 min. The upper isolated extracts from the centrifuge was transferred to a spectrophotometer cuvettes and then separately absorbance rates were read at wavelengths of 663 nm for chlorophyll a and 645 nm for chlorophyll b by a UV–Vis spectrophotometer. Finally, using the following formulas, the amount of chlorophyll a and b was obtained in terms of mg.g⁻¹ fresh weight of the samples [39]:

\[
\text{Chlorophyll a (mg.g}^{-1}\text{fresh weight)} = \frac{[19.3(A_{663}) - 0.86(A_{645})] \times V}{100W}
\]

\[
\text{Chlorophyll b (mg.g}^{-1}\text{fresh weight)} = \frac{[19.3(A_{645}) - 3.6(A_{663})] \times V}{100W}
\]

**Total chlorophyll = Chlorophyll a + Chlorophyll b**

where \(v\): volume of the extracted solution, \(A\): the absorption at 663 and 645 wavelengths, and \(w\): wet weight of the sample in grams.

**Element analysis**

For measuring total Fe, one gram of the plant samples was dried at 550 °C and, then 10 mL of HCl (2 N) was added to dissolve the sample then the dissolved sample was filtered using a Whitman 42 filter paper. The Fe element concentration was measured using atomic absorption (AA) spectrometry. In addition, the bioavailability of Fe in the soil was determined by DTPA (diethylene thiamine penta-acetic acid) method [40].

**Statistical analysis**

Statistical analyses were conducted using SPSS VERSION 16.0. The one-way analysis of variance (ANOVA) was conducted and the Duncan test (\(p > 0.05\)) was used to determine the differences between treatment means.

**Results**

**Preparation and characterization of Nanofertilizers**

Owing to the superiorities, iron-based nanomaterials are of great interest in different biological, chemical, agricultural, and pharmaceutical applications. We investigated the potent of different Fe₃O₄ NPs-based compounds as biocompatible and promising nanofertilizers. To this, for the achievement of the improved biocompatibility of iron nutrient without using large amounts of agrochemicals
and its consequent environmental & health concerns, the surface of Fe$_3$O$_4$ NPs was modified by different biodegradable organic compounds such as citric acid, humic acid, and EDTA. The behavior of Fe$_3$O$_4$, Fe$_3$O$_4$@CA, Fe$_3$O$_4$@HA, and Fe$_3$O$_4$@EDTA NPs were compared on the growth of the tomato plant as model plant (Fig. 1).

The nanomaterials were comprehensively characterized using different techniques such as XRD (X-ray diffraction), FT-IR (Fourier transforms infrared spectroscopy), TEM & SEM (transmission and scanning electron microscopy), DLS (dynamic light scattering), AFM (Atomic force microscopy), and zeta potential analysis. TEM and SEM were utilized to illustrate the particle size distribution and morphology of the synthesized nanofertilizers (Fig. 2). The DLS analysis was also performed to measure the size of hydrodynamic diameters of NPs. The uniformity and spherical shape of the NPs are shown by the SEM images (Fig. 2a, d, g, j).

The DLS analysis was also performed to measure the size of hydrodynamic diameters of NPs. The results of DLS showed that the average hydrodynamic diameter of samples including Fe$_3$O$_4$, Fe$_3$O$_4$@CA, Fe$_3$O$_4$@HA, and Fe$_3$O$_4$@EDTA NPs were about 54.6 nm, 74.2, 161.8, and 166.8, respectively. In addition, the TEM and AFM results revealed these particles were about 14.0, 29.4, 95.8, and 82.1 nm (Figs. 1i–1 and 2e–h, respectively). The particle diameters obtained by DLS were larger than those determined by TEM and AFM. This could be due to the fact that DLS measurement provides the average hydrodynamic diameter of the hydrated NPs, whereas TEM and AFM yield the size distribution of the dehydrated NPs.

**Evaluation of organically coated Fe$_3$O$_4$ NPs as Fe nanofertilizers**

**Effect of organically coated Fe$_3$O$_4$ NPs on tomato growth**

As presented in Fig. 3, the application of Fe$_3$O$_4$@HA NPs at a concentration of 50 mg.kg$^{-1}$ gave the highest significant values of plant height. On the contrary, Fe$_3$O$_4$@EDTA NPs at a concentration of 200 mg.kg$^{-1}$ gave the lowest values of plant height. As result, Fe$_3$O$_4$ NPs and Fe$_3$O$_4$@HA NPs treatments at a concentration of 50 mg.kg$^{-1}$ increased the plant height by 18% and 31%, respectively, compared to the control. It is noticeable that the best plant height enhancement using Fe$_3$O$_4$@CA NPs and Fe$_3$O$_4$@EDTA NPs have been determined by 27% and 26% at a concentration of 25 mg.kg$^{-1}$ compared to the control, respectively.

The effects of the Fe$_3$O$_4$ NPs and the organically coated Fe$_3$O$_4$ NPs were investigated in the plant biomass and the difference between the nanoscale and bulk iron fertilizers was comprised (Fig. 4a–d). Using Fe$_3$O$_4$ NPs at a concentration of 50 mg.kg$^{-1}$ the fresh shoot biomass has increased by 34% in comparison to control. As seen, in the case of ferrous sulfate fertilizer, higher concentrations of iron (such as 75, 100, 200 mg.kg$^{-1}$) has needed to achieve an efficiency as same as Fe$_3$O$_4$ NPs. Application of Fe$_3$O$_4$@CA NPs and Fe$_3$O$_4$@EDTA NPs at a concentration of 25 mg.kg$^{-1}$ were improved the fresh shoot biomasses by 26% and 43% compared to the corresponding bulk controls (Fig. 4b, d), respectively.

Noticeably, these improvements decreased in larger concentrations of Fe$_3$O$_4$@CA NPs and Fe$_3$O$_4$@EDTA NPs. However, Fe$_3$O$_4$@HA NPs at a concentration of 50 mg.kg$^{-1}$ increased the fresh shoot biomass by 68%. In addition, application of Fe$_3$O$_4$@HA NPs increased the plant growth compared to the bulk iron humate up to 75 mg.kg$^{-1}$. However, at higher concentrations (100 and
200 mg.kg⁻¹), the bulk version performed better than Fe₃O₄@HA NPs (Fig. 4c).

Figure 5 shows that the Fe₃O₄ NPs and Fe₃O₄@HA NPs treatments at a concentration of 50 mg.kg⁻¹ have increased the dry shoot biomass by 51% and 97%, respectively. In addition, the Fe₃O₄@CA NPs and Fe₃O₄@EDTA NPs treatments at a concentration of 25 mg.kg⁻¹ have enhanced the dry shoot biomass by 38% and 46%, respectively, compared to the corresponding bulk chemical fertilizers as control. As result using Fe₃O₄@HA NPs at a concentration of 50 mg.kg⁻¹ the highest significant values of dry shoot biomass has obtained.
Effect of nanofertilizers on the chlorophyll content, total protein, and catalase

To demonstrate the efficacy of the organically modified Fe$_3$O$_4$ NPs as nanofertilizer, their effect on the chlorophyll content (chlorophyll a and b), total protein, and catalase were examined and compared with their bulk controls. Based on the obtained results, the chlorophyll a and b contents between the bulk controls and the organically coated iron oxide nanoparticles-exposed plants showed significant differences. Total chlorophyll contents were significantly affected by Fe sources. Fe$_3$O$_4$ NPs, through enhancing the synthesis of the organic compounds such as proteins and chlorophyll leads to an increase in the absorption and transport of nutrient elements [41]. It was found that superparamagnetic iron oxide NPs (SPIONs) significantly could increase the Chl levels of soybean plants [42]. For example, the highest amount of chlorophyll was obtained using the Fe$_3$O$_4$@CA NPs at a concentration of 75 mg.kg$^{-1}$, which increased the amount of Chl content by 38% compared to iron citrate as the corresponding bulk control. In addition, in tomato shoots, the higher amount of the CAT activity and total protein were by 27% and 38% compared to the control that were achieved in the Fe$_3$O$_4$@CA NPs treatments at a concentration of 25 mg.kg$^{-1}$ and 75 mg. kg$^{-1}$, respectively.

In the case of Fe$_3$O$_4$@HA NPs as nanofertilizer, the higher amount of Chl was obtained from the Fe$_3$O$_4$@HA NPs treatment at a concentration of 50 mg.kg$^{-1}$, which increased the amount of Chl a by 49% and Chl b by 51% compared to iron humate as the corresponding bulk control. In addition, in tomato shoots the highest enhancement on CAT activity and total protein compared to the control were by 33% and 53%, respectively, that was related to the Fe$_3$O$_4$@HA NPs treatment at a concentration of 50 mg.kg$^{-1}$. Notably, using of Fe$_3$O$_4$@EDTA NPs has increased the total Chl contents and total protein compared to Fe-EDTA as the corresponding bulk control. The highest amount of Chl and total protein were obtained from the Fe$_3$O$_4$@EDTA NPs treatment at a concentration of 25 mg.kg$^{-1}$, which increased by 30% and 37%, respectively, compared to the control.

Table 2 shows a comparison between the organically coated iron oxide nanoparticles and Fe$_3$O$_4$ NPs as control.
on some of the important biochemical properties (chlorophyll content, total protein, and catalase in tomato plants). As shown in Table 2, the application of the organically coated iron oxide nanoparticles in particular Fe₃O₄@HA NPs have improved the total Chl, total protein, and catalase compared to Fe₃O₄ NPs as control. The plants grown in the presence of Fe₃O₄@HA NPs showed even higher total Chl content than those grown with Fe₃O₄@CA NPs and Fe₃O₄@EDTA NPs. As a result, the highest and lowest total Chl were observed in the case of Fe₃O₄@HA NPs (50 ppm) and Fe₃O₄ NPs (200 ppm), respectively.

**Impact of the organically coated Fe₃O₄ NPs on Fe content**

The total Fe content in the shoots of tomato plants significantly increased in the presence of all of the introduced nanofertilizers including Fe₃O₄@CA, Fe₃O₄@HA, and Fe₃O₄@EDTA in comparison with their bulk versions as controls (Fig. 6).

Exposure to the amount of 25 mg.kg⁻¹ Fe₃O₄@CA, 50 mg.kg⁻¹ Fe₃O₄@HA, and 25 mg.kg⁻¹ Fe₃O₄@EDTA resulted in 74% (P<0.05), 247% (P<0.05) and 82% (P<0.05) increase in the shoot Fe content, respectively. In addition, exposure to the amount of 200 mg.kg⁻¹ Fe₃O₄@CA resulted in 335% (P<0.05) increase in the shoot Fe content.

**Impact of the organically coated Fe₃O₄ NPs on tomato growth and Fe content**

Figure 7 shows a comparison of nanoparticles on the biomass, dry weight, and iron content of the tomato plants. The modified nanoparticles significantly increased the growth of the tomato plant compared to the control, and the Fe₃O₄@EDTA and Fe₃O₄@CA at a concentration of 25 mg.kg⁻¹ and Fe₃O₄@HA at a concentration of 50 mg.kg⁻¹ led to an increase in the growth of the tomato.
Based on the results of this study, different sources of Fe show different effects on the vegetative growth of the tomato plant. The found improvement in the growth of the tomato plant using the presented organically coated Fe₃O₄ NPs-based nanofertilizers at a concentration of 25, 50, 75, and 100 mg.kg⁻¹ can be explained by more efficient uptake, motility, and release of Fe because of their small size, large surface area, and bioavailability [9]. Various studies revealed that Fe₃O₄ NPs have a high potential to escalate the fresh and dry biomasses of various crops, including rice, wheat, tomato, peanut, soybean, and spinach [43]. The soil application of Fe₃O₄ NPs at a concentration of 20 mg.L⁻¹ increased tomato root growth [44]. Yan et al., also reported that using Fe₃O₄ NPs at a concentration of 50 mg.kg⁻¹ increased the corn root length by 25.3% [45]. They observed that using Fe₃O₄ NPs at a concentrations of 50 and 500 mg.kg⁻¹ increased the amount of Fe in the root by 209% and 271%, respectively.

Our results showed that all of the nanofertilizers included Fe₃O₄@CA, Fe₃O₄@HA, and Fe₃O₄@EDTA at a concentration of 200 mg.kg⁻¹ of Fe reduced the plant growth as compared to the control (Fig. 7). The possible reason for this decrease under the high level of NPs might be related to the generation of cytotoxic hydroxyl radicals because of the Fenton reaction in high levels of soluble and bioavailable Fe [11]. The Decreased leaf chlorophyll at 200 mg.kg⁻¹ of Fe can express in high concentrations due to iron stress. This is because nanoparticles at high levels can form clusters and tend to block the pathways of nutrition uptake [46]. High concentrations of iron cause the formation of free radicals, stimulate the oxidation of chlorophyll, and consequently decrease the chlorophyll content [47].

The results have shown that among three types of biodegradable organic chelating agents including EDTA, CA, and HA that were investigated for modifying Fe₃O₄ NPs as iron sources, the humic acid coated Fe₃O₄ NPs (Fe₃O₄@HA NPs) often gave us the best performance vs. the others. It has presented further improved vegetative growth and more bioavailable Fe for the plant. The highest values of plant biomasses were recorded in plants treated with Fe₃O₄@HA NPs. The humic-rich Fe₃O₄@HA NPs increased the growth of tomato plants.
compared to controls, including iron humate and iron sulfate as the bulk samples. For example, the effect of humic rich-iron fertilizer (Fe 4%, HA 68%) was investigated on wheat under hydroponic conditions that their results showed that small polymers are easily absorbed by plants, but the large ones remained on the root surfaces due to the size of the cell wall pores (5–20 nm) [23]. The iron nanoparticles increase the photosynthetic activity and the amount of bioavailable Fe to the plant. Similar results have been reported for Fe-NFs, which was tested for soybean plant grown under hydroponic conditions could supply the required iron for the plant and enhance the Fe uptake from root to plant stem [48]. There are some reports on comparing humic acid and synthetic chelators for the chelating the magnetic NPs. Owing to these reports, humic acid can cause rapid absorption, and transfer NPs from root to branches. It also can chelate Fe and play as an electron source for iron redox reaction in cells [47–50]. The application of Fe₃O₄ NPs-based nanofertilizers increased the bioavailability of Fe for plants (Fig. 7c). Therefore, all three organically coated Fe₃O₄ NPs improved the bioavailability of Fe as one of the critical micronutrients for plants, and Fe₃O₄@HA NPs at a concentration of 50 mg.kg⁻¹ represents a better impact in comparison with the other nanoscale and bulk iron fertilizers. This achievement is important because of the merits of humic acid as a biodegradable chelating agent. As previously reports, the presence of humic substances (HS) on iron oxides can improve its stability in soils, and thus may affect Fe mobilization and uptake by plants. HS leads to increase plant growth as well as enhance the nutrient uptake by the plant. HS reduces soil compaction, increases fertilizer efficiency, and also enhances the bioavailability of soluble hydroxides which leads to form the complexes with micronutrients and subsequently increase the plant growth [52, 53].

### Table 2  Comparison between Fe₃O₄ NPs and the organically coated Fe₃O₄ NPs in biochemical properties such as chlorophyll content, catalase, and total protein

| Source               | Concentration (mg.Kg⁻¹)a | ChlT (mg.g⁻¹ FW) | Catalase µmol H₂O₂ min⁻¹.mg⁻¹ | Total Protein (mg.g⁻¹ FW) |
|----------------------|--------------------------|-----------------|-------------------------------|---------------------------|
| Fe₃O₄ NPs           | 0                        | 1.18            | 0018bf                        | 1.89                      |
|                     | 25                       | 1.27ghi         | 00133(f)                      | 1.92g                     |
|                     | 50                       | 1.49            | 00136(f)                      | 2.04(g)                   |
|                     | 75                       | 1.48d           | 00139(f)                      | 2.38e                     |
|                     | 100                      | 1.35(n)         | 00129(f)                      | 2.38e                     |
|                     | 200                      | 1.19h           | 00120(f)                      | 2.03(g)                   |
| Fe₃O₄@CA NPs        | 0                        | 1.18            | 00118(f)                      | 1.89                      |
|                     | 25                       | 1.5c            | 00154(ad)                     | 2.43(c)                   |
|                     | 50                       | 1.51f           | 00132(f)                      | 2.39(e)                   |
|                     | 75                       | 1.62c           | 00129(fh)                     | 2.54(f)                   |
|                     | 100                      | 1.44f           | 00124(fh)                     | 2.24(f)                   |
|                     | 200                      | 1.21h           | 00124(fh)                     | 2.02(g)                   |
| Fe₃O₄@HA NPs        | 0                        | 1.18            | 00118(f)                      | 1.89                      |
|                     | 25                       | 1.67(ab)        | 00147(c)                      | 2.48(c)                   |
|                     | 50                       | 1.77a           | 00157(a)                      | 2.77a                     |
|                     | 75                       | 1.60bc          | 00151(ab)                     | 2.76(b)                   |
|                     | 100                      | 1.52(abc)       | 00141(ab)                     | 2.34(abc)                 |
|                     | 200                      | 1.27g           | 00121(hf)                     | 2.42(d)                   |
| Fe₃O₄@EDTA NPs      | 0                        | 1.18            | 00118(f)                      | 1.89                      |
|                     | 25                       | 1.53(ab)        | 00139(ab)                     | 2.48(c)                   |
|                     | 50                       | 1.52(c)         | 00141(c)                      | 2.44(d)                   |
|                     | 75                       | 1.47d           | 00134(ab)                     | 2.44(ab)                  |
|                     | 100                      | 1.39(abc)       | 00133(ab)                     | 2.36(ab)                  |
|                     | 200                      | 1.28g           | 00116(h)                      | 2.04(g)                   |

*The concentration of iron fertilizers has been reported based on mg of Fe per Kg of soil.*
CA causes to increase in the physiological availability of the Fe within the plant, which enhances the chlorophyll content (Table 2). Since CA, as a natural chelator increases iron motility. The chlorophyll increase can be explained by the direct effect of citrate ion on Fe plasma membrane of Fe-reductase [54, 55]. Since the active adsorption sites in the soil can be occupied by organic acid-based ligands and the fixation of heavy metals in the soil is reduced [56]. CA with three carboxyl groups can form stable five- or six-membered chelate rings and, therefore, help achieve an efficient [Fe] mobility [57].

Using Fe$_3$O$_4$@EDTA NPs at a concentration of 25 mg. kg$^{-1}$of soil increased the iron solubility for the plant (Fig. 6d). Non-organic forms of iron, including ferrous sulfate, are highly soluble at pH less than 5.5, but with increasing pH, their solubility decreases rapidly. In contrast, the organically coated forms of iron fertilizers can form stable five- or six-membered chelate rings and, therefore, help achieve an efficient [Fe] mobility [57].

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The impact of different sources of iron in soil (Fe-EDDHA, iron sulfate and iron nano-chelate) was studied on lettuce in a hydroponic culture medium. Their results showed that Fe-Chelate NPs significantly increased the amount of [Fe] in the plant and increased the vegetative growth of the plant compared to the other [Fe] sources [7]. The reduction of the fresh weight of shoots was reported the negative effects of EDTA on the plant growth and the activity of soil microorganisms due to the highly increased availability of functional elements in soil [58] and plant growth [59, 60]. The cause of EDTA toxicity is high stability and low biodegradability in the environment [61]. In addition, the use of iron chelate in high concentrations can cause the absorption of large amounts of iron and upset the nutritional balance of the plant, and also cause severe deficiency of copper, manganese, and
zinc in plants and leads to reduced leaf chlorophyll and reduced plant growth [62].

Iron oxide nanoparticles improved the vegetative traits of the tomato plant, including increasing the fresh and dry biomasses of the plant at appropriate concentrations of 20–50 mg.kg\(^{-1}\) which were consistent with a previous study [9]. The effect of zero-valent iron (ZVI), Fe\(_2\)O\(_4\) and Fe\(_2\)O\(_3\) nanoparticles on rice plant growth was studied [63]. The results showed that low dose of ZVI and Fe\(_2\)O\(_4\) nanoparticles improved plant growth under Fe deficient by alleviating oxidative stress and regulated rice plant phytohormones due to iron deficiency. Low dose of ZVI and Fe\(_2\)O\(_4\) nanoparticles can regulate iron accumulation in plants by regulating iron transport genes. However, high concentrations (500 mg.L\(^{-1}\)) caused phytotoxicity. At this concentration, a reduced in root volume and leaf biomass and an enhanced in oxidative stress were evident in the plant [63].

The application of nano-iron increased the amount of chlorophyll in the tomato plant. When different enzymatic processes are increased, chlorophyll needs iron for activity and the use of iron oxide NPs is a solution to this demand [9]. The existence of iron as a cofactor or structural component of this enzyme may be one of the main reasons for increasing the catalase activity in the presence of NPs-based fertilizers. With rapid release and increasing iron concentration in the plant, the activity of this enzyme subsequently increases [64]. Due to the small size and large surface area, the soluble iron component increased in the iron oxide NPs-based nano-fertilizers. Noticeably, the organically coating agent such as humic acid can facilitate the uptake and subsequently improve the nutrient efficiency in plants. This fact causes the improvement of physiological traits that leads to a greater performance of different products [9].

The results of this study showed that the application of coated nanoparticles including humic acid coated Fe\(_2\)O\(_4\) NPs (Fe\(_2\)O\(_4\)@HA), citric acid coated Fe\(_2\)O\(_4\) NPs (Fe\(_2\)O\(_4\)@CA), and EDTA coated NPs (Fe\(_2\)O\(_4\)@EDTA) at low concentrations of about 25 and 50 mg.Kg\(^{-1}\) compared to the bulk iron fertilizers increased plant growth. However, in high concentrations, nanoparticles reduce plant growth. Therefore, nanoparticles should be used in optimal concentrations for different plants. Fe\(_2\)O\(_4\)@HA showed the
better results than the other nanofertilizers and the use of these nanoparticles is recommended as an efficient, environmentally friendly, and nontoxic source of iron nutrient for plants.

Conclusions

The response of tomato plants to three organically coated iron oxide nanoparticles applied to the soil was investigated. In general, the difference in the coating material, the concentration of NPs, and soil properties are important factors in the iron solubility required by plants. Herein, due to the better uptake and bioavailability of these synthesized iron nanofertilizers, a higher Fe was provided to the tomato plants. In addition, the photosynthetic condition and better production of plant pigments were improved. The results showed that the use of humic acid coated Fe₃O₄ nanoparticles (Fe₃O₄@HA NPs) at a concentration of 50 mg.kg⁻¹ increases plant growth. The plant height, fresh shoot biomass, dry weight of the tomato plants, and the plant Fe content increased by 31%, 68%, 97%, and 247%, respectively, compared to the control. Fe₃O₄@HA NPs increased iron uptake and could incorporate iron into chelated complexes and so it maintains its availability to plants. Humic acid, due to having different functional groups such as phenolic, alcoholic, carboxylic acid, and hydroxyl, causes the plant to have more access to iron. At the second place, the citric acid coated Fe₃O₄ NPs (Fe₃O₄@CA NPs) and EDTA coated Fe₃O₄ NPs (Fe₃O₄@EDTA NPs) at a concentration of 25 mg.kg⁻¹ showed positive impacts on the plant growth. Due to the good and beneficial effects of humic substances on the absorption of Fe by plants and also the biocompatibility of this nanofertilizer, Fe₃O₄@HA NPs fertilizer is recommended as a suitable, eco-friendly, cost-effective, and more efficient nanofertilizer to eliminate the iron deficiency.

Abbreviations

AFM: Atomic force microscopy; Fe₃O₄@CA NPs: Citric acid coated Fe₃O₄ NPs; DLS: Dynamic light scattering; Fe₃O₄@EDTA NPs: EDTA coated NPs; FTIR: Fourier transform infrared; Fe₃O₄@HA NPs: Humic acid coated Fe₃O₄ NPs; HS: Humic substances; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; NPs: Nanoparticles; Fe₃O₄ NPs: Iron oxide nanoparticles.

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Author contributions

TRA prepared the nanomaterials, performed in vitro and greenhouse experiments, and helped in paper writing and editing. LM supervised and performed the conception and design of the study, and paper writing and editing. AM supervised and helped in paper writing and editing. All authors read and approved the final manuscript.

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Availability of data and materials

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Hell R, Stephan UW. Iron uptake, trafficking and homeostasis in plants. Planta. 2003;216(4):541–51.
2. Klätte M, Schuler M, Wirtz M, Fink-Stauble C, Hell R, Bauer P. The analysis of Arabidopsis nicotianamine synthase mutants reveals functions for nicotianamine in seed iron loading and iron deficiency responses. Plant Physiol. 2009;150(1):257–71.
3. Mimmo T, et al. Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. Eur J Soil Sci. 2014;65(5):629–42.
4. Askary M, Amirjani MR, Saberi T. Comparison of the effects of nano-iron fertilizer with iron-chelate on growth parameters and some biochemical properties of Catharanthus roseus. J Plant Nutr. 2017;40(7):974–82.
5. Cañasveras JC, Sánchez-Rodríguez AR, del Campillo MC, Barrón V, Torrent J. Lowering iron chlorosis of olive by soil application of iron sulfate or siderite. Agron Sustain Dev. 2014;34(3):677–84.
6. Abadía J, Vázquez S, Reilán-Alvarez R, El-Jendoubi H, Abadía A, Alvarez-Fernández A, López-Millán AF. Towards a knowledge-based correction of iron chlorosis. Plant Physiol Biochem. 2011;49(5):471–82.
7. Roosta HR, Jalali M, S. M. Ali Vakili Shahrbabaki, Effect of nano Fe-chelate, Fe-EDDHA and FeSO₄ on vegetative growth, physiological parameters and some nutrient elements concentrations of four varieties of lettuce (Lactuca sativa L) in NFT system. J Plant Nutr. 2015;38:2176–84.
8. Liu R, Lal R. Potentials of engineered nanoparticles as fertilizers for increasing agronomic productions. Sci Total Environ. 2015;514:131–9.
9. Zia-ur-Rehman M, Naeem A, Khalid H, Rizwan M, Ali S, Azhar M. Responses of plants to iron oxide nanoparticles. In: Tripathi DK, Ahmad P, Sharma S, Chauhan D, Dubey NK, editors. Nanomaterials in plants, algae, and microorganisms. Amsterdam: Elsevier; 2018. p. 221–38.
10. Sun C, Zhou R, Jianan E, Sun J, Ren H. Magnetic CuO@Fe₃O₄ nano-composite as a highly active heterogeneous catalyst of persulfate for 2, 4-dichlorophenol degradation in aqueous solution. Rsc Adv. 2015;5(70):57058–66.
11. Hu J, Guo H, Li J, Gan Q, Wang Y, Xing B. Comparative impacts of iron oxide nanoparticles and ferric ions on the growth of Citrus maxima. Environ Pollut. 2017;221:199–208.
12. Sharma P, Holliger N, Pfornmm PH, Liu B, Chikan V. Size-controlled synthesis of iron and iron oxide nanoparticles by the rapid inductive heating method. ACS Omega. 2020;5(31):19853–60.
13. Yang Y, Huang M, Qian J, Xing L. Tunable Fe3O4 nanorods for enhanced magnetic hyperthermia performance. Sci Rep. 2020;10(1):1–7.
14. Komarnytska K, Sujak A, Czernek G, Wiacek D. Effect of Fe₃O₄ nanoparticles on germination of seeds and concentration of elements in Helianthus annuus L. under constant magnetic field. Sci Rep. 2020;10(1):1–10.
15. Yoon HY, et al. Synergistic release of crop nutrients and stimulants from hydroxyapatite nanoparticles functionalized with humic substances toward a multifunctional nanofertilizer. ACS Omega. 2020;5(12):6598–610.
16. Kumar R, Ashfaq M, Verma N. Synthesis of novel PVA-starch formulation-supported GO-Zn nanoparticle carrying carbon nanofoamers as a nanofertilizer controlled release of micronutrients. J Mater Sci. 2018;53(10):7150–64.
17. Zhu H, Han J, Xiao JQ, Jin Y. Uptake, translocation, and accumulation of manufactured iron oxide nanoparticles by pumpkin plants. J Environ Monit. 2008;10(6):713–7.
18. Barrena R, Caralis E, Colón J, Font X, Sánchez A, Puntes V. Evaluation of the ecotoxicity of model nanoparticle. Chemosphere. 2009;75(7):850–7.
19. Zura Z, et al. Metallic nanoparticle (TiO₂ and Fe₃O₄) application modifies rhizosphere phosphorus availability and uptake by Lactuca sativa. J Agric Food Chem. 2015;63(11):6876–82.
20. López-Luna J, et al. Magnetite nanoparticle (NP) uptake by wheat plants and its effect on cadmium and chromium toxicological behavior. Sci Total Environ. 2016;565:941–50.
21. Rui M, et al. Iron oxide nanoparticles as a potential iron fertilizer for pea- (Arachis hypogaea). Front Plant Sci. 2016;7:815.
22. Bindraban PS, Dimkpa C, Nagarajan L, Roy A, Rabbinge R. Revisiting ferti- lizer NPK and Fertilizer use. J Environ Monit. 2010;12:1618–21.
23. López-Luna J, et al. Magnetite nanoparticle (NP) uptake by wheat plants and its effect on cadmium and chromium toxicological behavior. Sci Total Environ. 2016;565:941–50.
24. Walkley A, Black IA. An examination of the Degtjareff method for deter- mining organic carbon in soils. Soil Sci. 1934;37(1):29–38.
25. Lindsay WL, Norvell W. Development of a DTPA soil test for zinc, iron, manganese, and copper. Soil Sci Soc Am J. 1978;42(3):421–8.
26. Black CA. Methods of soil analysis: physical and mineralogical properties, and its effect on cadmium and chromium toxicological behavior. Sci Total Environ. 2016;565:941–50.
64. Rezaei S, Amiri ME, Bahari A, Razavi F, Aghdam MS. Influence of iron leaf nutrition on chlorophyll content and some antioxidant enzyme activities of strawberry fruit cv. Camarosa. Hortic Plants Nutrition. 2020;21(3):1–6.

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