Chemical and Biological
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RESEARCH

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 Fe_3O_4 nanoparticles (Fe_3O_4), citric acid coated Fe_3O_4 nanoparticles (Fe_3O_4@CA), humic acid coated Fe_3O_4 nanoparticles (Fe_3O_4@HA), and EDTA coated nanoparticles (Fe_3O_4@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 Fe_3O_4 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 Fe_3O_4@HA NPs and control treatments, respectively. In addition, using organically coated Fe_3O_4 NPs, especially Fe_3O_4@HA increased plant growth and yield.

Conclusions: This study showed that using organically coated Fe_3O_4 NPs is promising for plant nutritional supplementation. In particular, the humic acid-coated Fe_3O_4 nanoparticles (Fe_3O_4@HA) were determined to be the most promising, due to more benefits for plant growth and yield compared to Fe_3O_4 NPs. Therefore, Fe_3O_4@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 Fe_3O_4 NPs as nanofertilizer were investigated.
- The behavior of citric acid, humic acid, and EDTA were investigated and compared as organic coating materials.

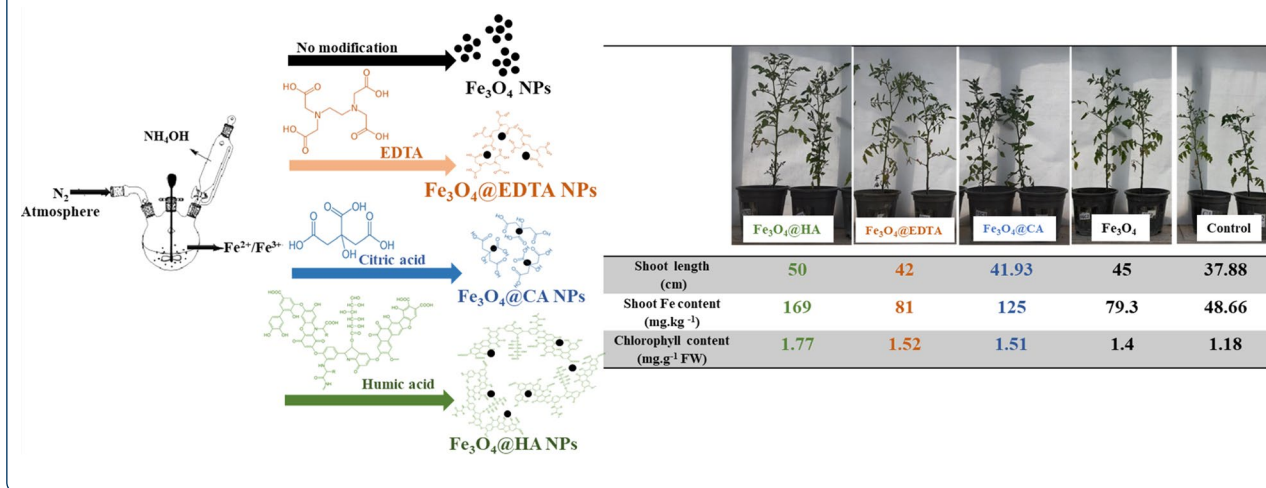*Correspondence: leila.mamani@abrii.ac.ir; m.chorom@scu.ac.ir

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- The organically coated Fe_3O_4 NPs, especially Fe_3O_4@HA improved the bioavailability and subsequently enhanced the amount of total iron determined in the shoot.
- Fe_3O_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_3O_4, Tomato

Graphical Abstract

	Fe₃O₄@HA	Fe₃O₄@EDTA	Fe₃O₄@CA	Fe₃O₄	Control
Shoot length (cm)	50	42	41.93	45	37.88
Shoot Fe content (mg.kg⁻¹)	169	81	125	79.3	48.66
Chlorophyll content (mg.g⁻¹ FW)	1.77	1.52	1.51	1.4	1.18

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 ($FeSO_4.7H_2O$) 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-hydroxy-phenyl 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].

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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_3O_4 NPs and γ-Fe_2O_3 NPs on plants [17–20]. For example, the effects of TiO_2 NPs and Fe_3O_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_3O_4 NPs. In addition, according to another effort, using 50 mg.L^{-1} of γ-Fe_2O_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_2O_3 NPs could enhance plant growth, biomass, and antioxidant enzyme activity in Peanut [21]. Fe_2O_3 NPs can also regulate the phytohormone contents [22].

The Fe-based materials stabilized with *Lonardite 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_2O_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 increase the iron of the rhizome [25]. The effect of Fe_3O_4 NPs on reducing the impact of arsenic toxicity on the Indian mustard plant (*Brassica juncea var. Pusa Jagannath*) 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_2O_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_2O_3 nanoparticles reduced to some extent the DNA damage of cadmium to shoot. However, foliar application of Fe_2O_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_3O_4 NPs including citric acid coated Fe_3O_4 nanoparticles (Fe_3O_4@CA), humic acid coated Fe_3O_4 nanoparticles (Fe_3O_4@HA), and EDTA coated nanoparticles (Fe_3O_4@EDTA) and compared with those of Fe_3O_4 nanoparticles.

Experimental methods
Materials and characterization
Tri-natrium citrate dihydrate ($C_3H_5Na_3O_7.2H_2O$), Ethylenediaminetetraacetic acid (EDTA), humic acid, polyvinylpyrrolidine (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_3O_4 nanoparticles (Fe_3O_4 NPs)
To synthesize Fe_3O_4 NPs, the 50 mL of $FeCl_2$ (1 M) and 50 mL of $FeCl_3$ (2 M) solutions were prepared, and HCl

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(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 N_2 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 Fe_3O_4 nanoparticles (Fe_3O_4@ CA NPs)

Fe_3O_4 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 Fe_3O_4 NPs (Fe_3O_4@CA NPs).

Synthesis of humic acid coated Fe_3O_4 nanoparticles (Fe_3O_4@HA NPs)

Humic acid (3.0 g) was dissolved in deionized water (50 mL) and pH was adjusted to 3 using HCl 37%. Then, Fe_3O_4 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 Fe_3O_4 NPs (Fe_3O_4@HA NPs).

Synthesis of EDTA coated nanoparticles (Fe_3O_4@EDTA NPs)

To a mixture of Fe_3O_4 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 Fe_3O_4 NPs (Fe_3O_4@EDTA NPs).

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)

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
$CaSO_4$ (%)	2.81
Clay (%)	36.4
Silt (%)	29.5
Sand (%)	34.1

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 Fe_3O_4 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 (Fe_3O_4 NPs, Fe_3O_4@CA NPs, Fe_3O_4@HA NPs, and Fe_3O_4@EDTA NPs) and four types of balk fertilizer ($FeSO_4$, iron citrate ($C_6H_5FeO_7 \cdot H_2O$), iron humate, and Fe-EDTA) at five concentrations of iron (0, 25, 50, 75, 100, and 200 mg per

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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 con-

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

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

stant 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 polyvinylpyrrolidine (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]:

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 Fe_3O_4 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
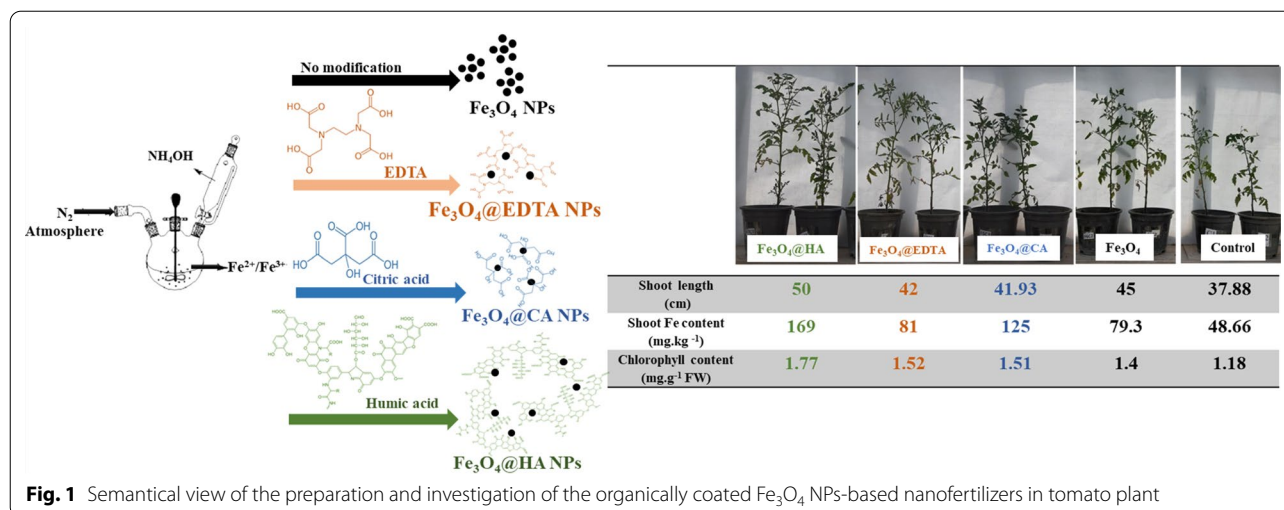
Fig. 1 Semantical view of the preparation and investigation of the organically coated Fe_3O_4 NPs-based nanofertilizers in tomato plant

and its consequent environmental & health concerns, the surface of Fe_3O_4 NPs was modified by different biodegradable organic compounds such as citric acid, humic acid, and EDTA. The behavior of Fe_3O_4, $Fe_3O_4@CA$, $Fe_3O_4@HA$, and $Fe_3O_4@EDTA$ 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_3O_4, $Fe_3O_4@CA$, $Fe_3O_4@HA$, and $Fe_3O_4@EDTA$ 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–l & 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.
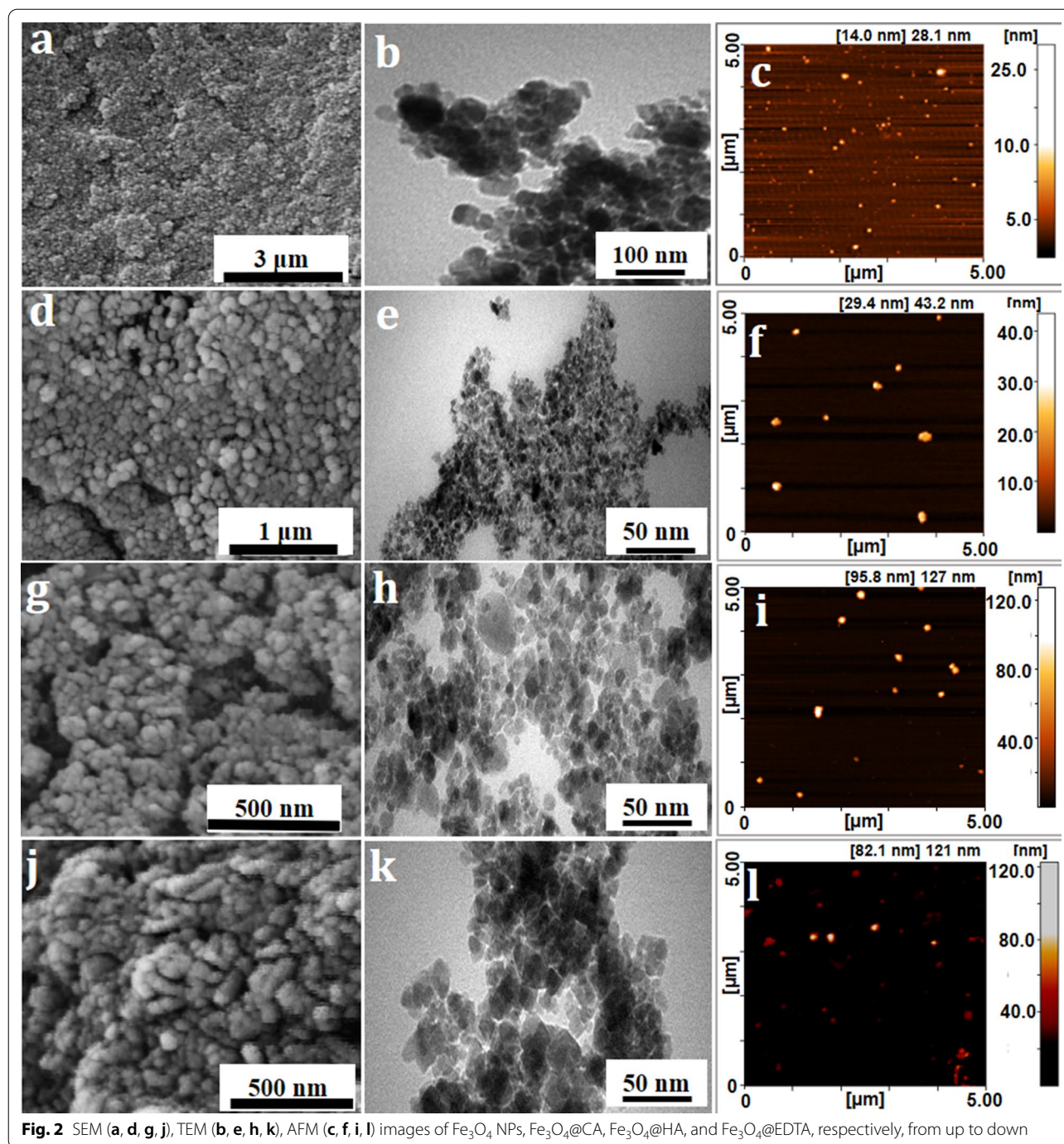
Evaluation of organically coated Fe_3O_4 NPs as Fe nanofertilizers

Effect of organically coated Fe3O4 NPs on tomato growth

As presented in Fig. 3, the application of $Fe_3O_4@HA$ NPs at a concentration of 50 mg.kg^{-1} gave the highest significant values of plant height. On the contrary, $Fe_3O_4@$ EDTA NPs at a concentration of 200 mg.kg^{-1} gave the lowest values of plant height. As result, *Fe_3O_4 NPs and $Fe_3O_4@HA$ 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 $Fe_3O_4@CA$ NPs and $Fe_3O_4@EDTA$ 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_3O_4 NPs and the organically coated Fe_3O_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_3O_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_3O_4 NPs. Application of $Fe_3O_4@CA$ NPs and $Fe_3O_4@EDTA$ 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 $Fe_3O_4@CA$ NPs and $Fe_3O_4@EDTA$ NPs. However, $Fe_3O_4@HA$ NPs at a concentration of 50 mg.kg^{-1} increased the fresh shoot biomass by 68%. In addition, application of $Fe_3O_4@HA$ NPs increased the plant growth compared to the bulk iron humate up to 75 mg.kg^{-1}. However, at higher concentrations (100 and

Fig. 2 SEM (**a**, **d**, **g**, **j**), TEM (**b**, **e**, **h**, **k**), AFM (**c**, **f**, **i**, **l**) images of Fe_3O_4 NPs, Fe_3O_4@CA, Fe_3O_4@HA, and Fe_3O_4@EDTA, respectively, from up to down

200 mg.kg^{-1}), the bulk version performed better than Fe_3O_4@HA NPs (Fig. 4c).

Figure 5 shows that the Fe_3O_4 NPs and Fe_3O_4@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 Fe_3O_4@CA NPs and Fe_3O_4@ 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 Fe_3O_4@HA NPs at a concentration of 50 mg.kg^{-1} the highest significant values of dry shoot biomass has obtained.
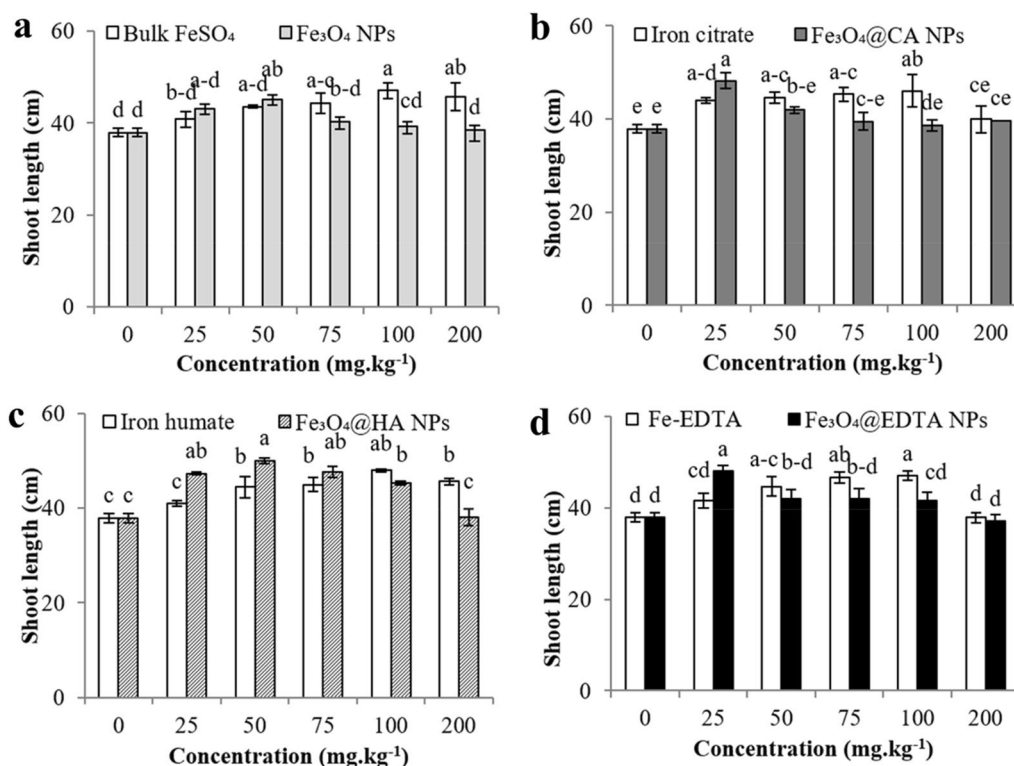
Fig. 3 Comparison of the plant height grown in the soil containing bulk vs. nanofertilizers including (**a**) FeSO$_4$ vs. Fe$_3$O$_4$ NPs, (**b**) iron citrate vs. Fe$_3$O$_4$@CA NPs, (**c**) iron humate vs. Fe$_3$O$_4$@HA NPs, and (**d**) Fe-EDTA vs. Fe$_3$O$_4$@EDTA NPs. The standard error is indicated by the error bars ($n = 3$). Different letters indicate differences between treatments ($p < 0.05$). The concentration of iron fertilizers has been reported based on mg of Fe per Kg of soil

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
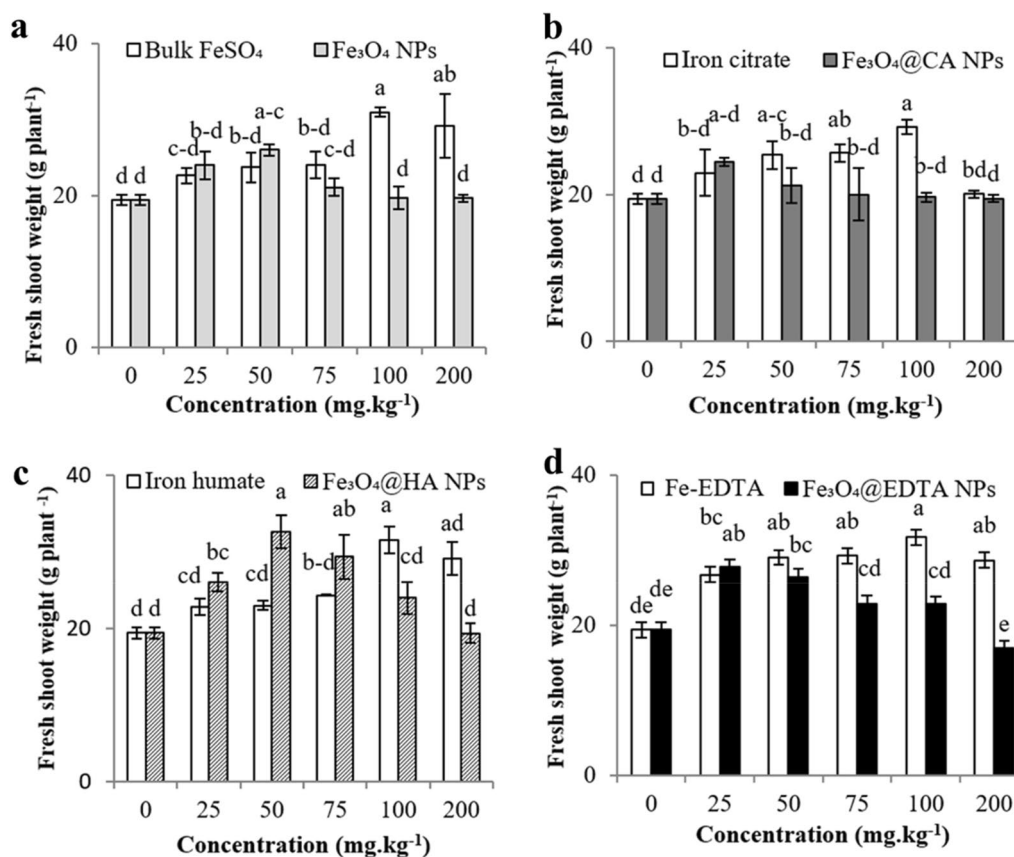
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Fig. 4 Comparison of the fresh biomasses of the tomato plants grown in soil containing bulk vs. nanoscale fertilizers including (**a**) bulk FeSO$_4$ vs. Fe$_3$O$_4$ NPs, (**b**) iron citrate vs. Fe$_3$O$_4$@CA NPs, (**c**) iron humate vs. Fe$_3$O$_4$@HA NPs, and (**d**) Fe-EDTA vs. Fe$_3$O$_4$@EDTA NPs. The standard error is indicated by the error bars (*n* = 3). Different letters indicate differences between treatments (*p* < 0.05). The concentration of iron fertilizers has been reported based on mg of Fe per Kg of soil

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 Fe$_3$O$_4$@HA NPs have improved the total Chl, total protein, and catalase compared to Fe$_3$O$_4$ NPs as control. The plants grown in the presence of Fe$_3$O$_4$@HA NPs showed even higher total Chl content than those grown with Fe$_3$O$_4$@CA NPs and Fe$_3$O$_4$@EDTA NPs. As a result, the highest and lowest total Chl were observed in the case of Fe$_3$O$_4$@HA NPs (50 ppm) and Fe$_3$O$_4$ NPs (200 ppm), respectively.

Impact of the organically coated Fe$_3$O$_4$ 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 Fe$_3$O$_4$@CA, Fe$_3$O$_4$@HA, and Fe$_3$O$_4$@EDTA in comparison with their bulk versions as controls (Fig. 6).

Exposure to the amount of 25 mg.kg^{-1} Fe$_3$O$_4$@CA, 50 mg.kg^{-1} Fe$_3$O$_4$@HA, and 25 mg.kg^{-1} Fe$_3$O$_4$@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} Fe$_3$O$_4$@CA resulted in 335% (*P* < 0.05) increase in the shoot Fe content.

Impact of the organically coated Fe$_3$O$_4$ 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 Fe$_3$O$_4$@EDTA and Fe$_3$O$_4$@CA at a concentration of 25 mg.kg^{-1} and Fe$_3$O$_4$@HA at a concentration of 50 mg.kg^{-1} led to an increase in the growth of the tomato.

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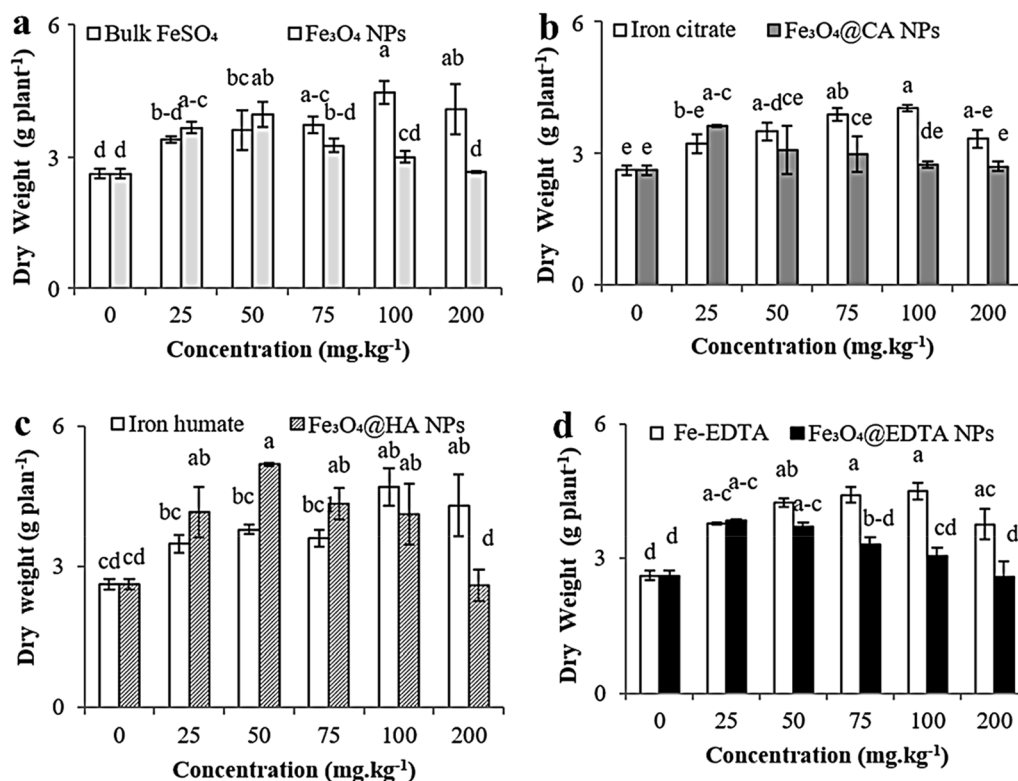
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Fig. 5 Comparison of the dry weight of the tomato plants grown in soil containing bulk vs. nanofertilizer including (**a**) bulk FeSO₄ and Fe₃O₄ NPs, (**b**) iron citrate vs. Fe₃O₄@CA NPs (**c**), iron humate vs. Fe₃O₄@HA NPs, and (**d**) Fe-EDTA vs. Fe₃O4@EDTA NPs. The standard error is indicated by the error bars ($n = 3$). Different letters indicate differences between treatments ($p < 0.05$). The concentration of iron fertilizers has been reported based on mg of Fe per Kg of soil

Discussion

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 Fe_3O_4 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 Fe_3O_4 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 Fe_3O_4 NPs at a concentration of 20 mg.L^{-1} increased tomato root growth [44]. *Yan* et al., also reported that using Fe_3O_4 NPs at a concentration of 50 mg.kg^{-1} increased the corn root length by 25.3% [45]. They observed that using Fe_3O_4 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 Fe_3O_4@CA, Fe_3O_4@HA, and Fe_3O_4@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 Fe_3O_4 NPs as iron sources, the humic acid coated Fe_3O_4 NPs (Fe_3O_4@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 Fe_3O_4@HA NPs. The humic-rich Fe_3O_4@HA NPs increased the growth of tomato plants

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Table 2 Comparison between Fe_3O_4 NPs and the organically coated Fe_3O_4 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 H_2O_2 min^{-1}.mg^{-1} protein	Total Protein (mg.g^{-1} FW)
Fe_3O_4 NPs	0	1.18[i]	0.018[gh]	1.8 [g]
	25	1.27[ghi]	0.0133[cg]	1.92 [fg]
	50	1.4[eg]	0.0136[bf]	2.04[dg]
	75	1.48[cf]	0.013[dh]	2.38[be]
	100	1.35[fh]	0.0139[be]	2.38[be]
	200	1.19[hi]	0.0120[gh]	2.03[eg]
Fe_3O_4@CA NPs	0	1.18[i]	0.0118[gh]	1.8 [g]
	25	1.5[cf]	0.015[ad]	2.43[ac]
	50	1.51[bf]	0.0132[ch]	2.39[be]
	75	1.62[ac]	0.0129[dh]	2.5[ac]
	100	1.44[df]	0.0124[eh]	2.24[cf]
	200	1.21[hi]	0.0124[eh]	2.02[eg]
Fe_3O_4@HA NPs	0	1.18[i]	0.0118[gh]	1.8 [g]
	25	1.67[ab]	0.0147[ac]	2.48[ac]
	50	1.77[a]	0.0157[a]	2.77[a]
	75	1.6[bd]	0.0151[ab]	2.76[ab]
	100	1.52[be]	0.0141[be]	2.34[ce]
	200	1.27[gi]	0.0121[fh]	2.42[ad]
Fe_3O_4@EDTA NPs	0	1.18[i]	0.0118[gh]	1.8 [g]
	25	1.55[be]	0.0139[be]	2.48[ac]
	50	1.52[bf]	0.0141[bd]	2.4[ae]
	75	1.47[cf]	0.0134[cg]	2.4[ae]
	100	1.39[eg]	0.0133[cg]	2.36[ce]
	200	1.28[gi]	0.0116 [h]	2.04[dg]

[a] The concentration of iron fertilizers has been reported based on mg of Fe per Kg of soil

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 Fe_3O_4 NPs-based nanofertilizers increased the bioavailability of Fe for plants (Fig. 7c). Therefore, all three organically coated

Fe_3O_4 NPs improved the bioavailability of Fe as one of the critical micronutrients for plants, and Fe_3O_4@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 by preventing precipitation can enhance the Fe availability to plants. These findings provide new insights on the role of soil organic matter on plant Fe nutrition [51, 52]. 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]. As a result, using Fe_3O_4@CA NPs in a concentration of 25 mg.kg^{-1} of soil increased the vegetative growth of tomatoes (Figs. 5b, 6b). Based on our results, the acidification of soil using
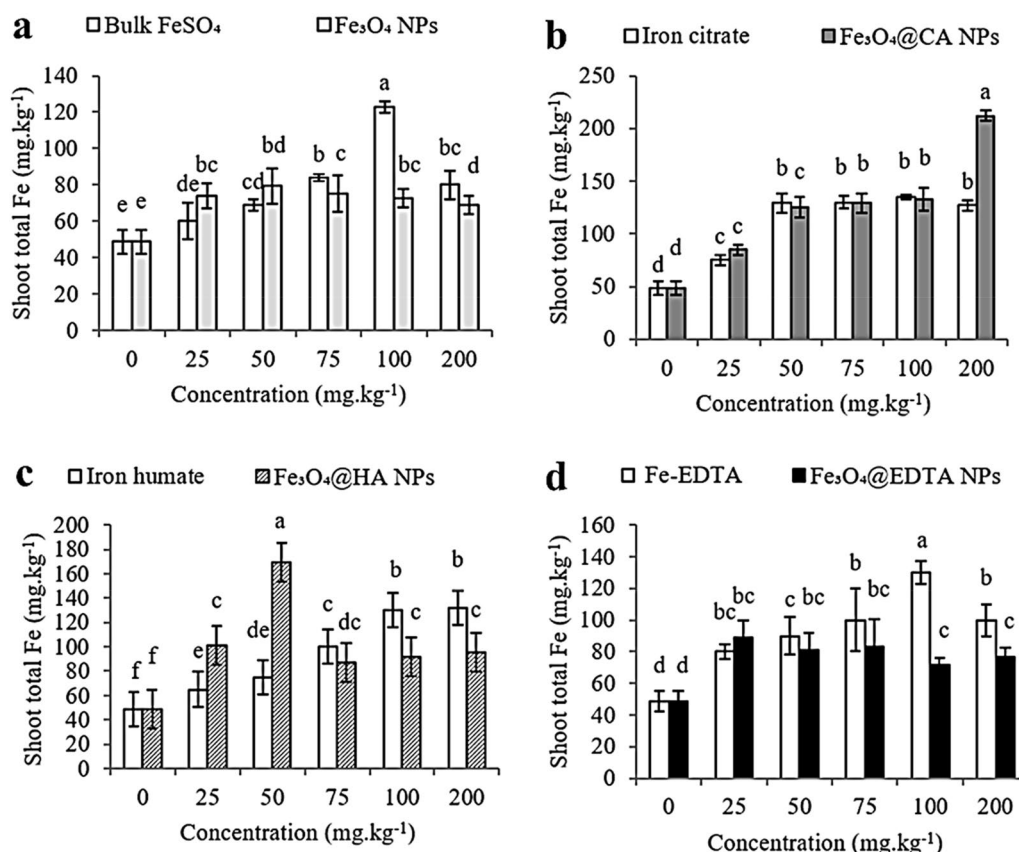
Fig. 6 Shoot total Fe of (**a**) bulk FeSO$_4$ vs. Fe$_3$O$_4$ NPs, (**b**) iron citrate vs. Fe$_3$O4@CA, (**c**) iron humate vs. Fe$_3$O$_4$@HA, and (**d**) Fe-EDTA vs. Fe$_3$O$_4$@EDTA NPs. The standard error is indicated by the error bars ($n = 3$). Different letters indicate differences between treatments ($p < 0.05$). The shoot total Fe has been reported based on mg of Fe per Kg of shoot dry mass. 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 at pH more than 5.5 retains soluble, since their application in the soil increases the dissolution of metal ions and affects the usability of the microelements required

by plants [40]. Therefore, iron nano-chelates can act as a rich source of iron for plants due to their high stability and also for the slow-release of iron in various pH in the soil [7].

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
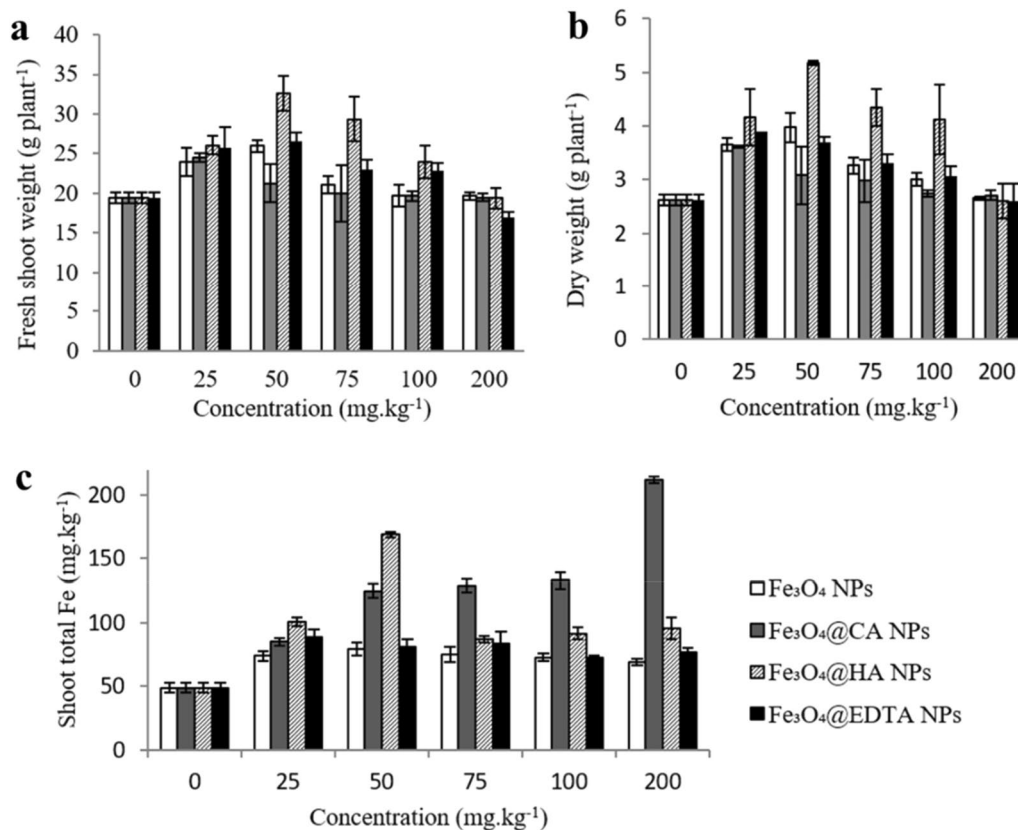
Fig. 7 Comparison between the organically coated iron oxide nanoparticles and Fe_3O_4 NPs in (**a**) fresh biomass of the tomato plants, (**b**) dry weight of the tomato plants, and (**c**) Shoot total Fe. Error bars represent a standard error ($n = 3$). The shoot total Fe has been reported based on mg of Fe per Kg of shoot dry mass). The concentration of iron fertilizers has been reported based on mg of Fe per Kg of soil

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_3O_4 and Fe_2O_3 nanoparticles on rice plant growth was studied [63]. The results showed that low dose of ZVI and Fe_3O_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_3O_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 nanofertilizers. 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_3O_4 NPs) (Fe_3O_4@HA), citric acid coated Fe_3O_4 NPs (Fe_3O_4@CA), and EDTA coated NPs (Fe_3O_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_3O_4@HA showed the

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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 Fe_3O_4 nanoparticles ($Fe_3O_4@$ 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. $Fe_3O_4@$ 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 Fe_3O_4 NPs ($Fe_3O_4@CA$ NPs) and EDTA coated Fe_3O_4 NPs ($Fe_3O_4@EDTA$ 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, $Fe_3O_4@HA$ 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; $Fe_3O_4@CA$ NPs: Citric acid coated Fe_3O_4 NPs; DLS: Dynamic light scattering; $Fe_3O_4@EDTA$ NPs: EDTA coated NPs; FTIR: Fourier transform infrared; $Fe_3O_4@HA$ NPs: Humic acid coated Fe_3O_4 NPs; HS: Humic substances; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; NPs: Nanoparticles; Fe_3O_4 NPs: Iron oxide nanoparticles.

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. MC supervised and helped in paper writing and editing. AM advised in interpreting data. All authors read and approved the final manuscript.

Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Declarations

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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