



# Green synthesis of Fe<sub>2</sub>O<sub>3</sub> nanoparticles using fruit extract of *Cornus mas* L. and its growth-promoting roles in Barley

Elham Rostamizadeh<sup>1</sup> · Alireza Iranbakhsh<sup>1</sup> · Ahmad Majd<sup>2</sup> · Sedigheh Arbabian<sup>2</sup> · Iraj Mehregan<sup>1</sup>

Received: 29 November 2019 / Accepted: 10 February 2020 / Published online: 17 February 2020  
© Islamic Azad University 2020

## Abstract

Iron oxide nanoparticle fabrications have gained increasing attention owing to their vital role in industrial application. Taking green synthesis of nano-products into account, this experiment was conducted to provide a simple, facile, economic, and fast procedure for the biosynthesis of Fe<sub>2</sub>O<sub>3</sub> nanoparticles using fruit extracts of Cornelian cherry. The FTIR spectroscopy revealed that hydroxyl and amino groups have the ability to perform dual functions of reduction and stabilization of iron oxide nanoparticles. XRD analysis manifested the crystal structure of the Fe<sub>2</sub>O<sub>3</sub> nanoparticle. The SEM image of synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticle demonstrated the morphology of nanoparticles is spherical. Transmission electron microscopy (TEM) images showed very fine spherical Fe<sub>2</sub>O<sub>3</sub> nanoparticles ranging from 20 to 40 nm. The EDXRF spectra displayed only iron and oxygen elements, which implies that the sample is highly pure Fe<sub>2</sub>O<sub>3</sub> nanoparticle. The Fe<sub>2</sub>O<sub>3</sub> nanoparticle and its bulk form at the concentrations ranging from 10 to 100 mg L<sup>-1</sup> showed statistically significant stimulation in both root and shoot biomass. However, Fe<sub>2</sub>O<sub>3</sub> nanoparticle was more effective than the bulk to stimulate barley growth. The findings may be functionalized in the production and formulations of nano-based fertilizers.

**Keywords** Eco-safety · *Cornus mas* · Green synthesis · Iron oxide · Nanoparticle

## Introduction

There are a large number of methods (physical, chemical, and biological) to synthesize various types of nanomaterials [1]. The exploitation of ecofriendly materials such as plant for fabrication of nanoparticles provides considerable benefits of compatibility and eco-friendliness for biomedical applications [1]. Using microorganisms or plant extract could be an alternative to chemical and physical procedures for the production of metal or metal oxide nanoparticles in an ecofriendly manner. Taking a biological synthesis of nano-products into account, plant different organs, including leaf, root, fruit, and seed may be utilized. Phytochemical compounds are proteins, vitamins, terpenoids, sugar, and the phenolic substances which act as capping, reducing, and stabilizing agents [2]. Nanomaterials are known as materials

with a particle size of 100 nm or less with the particular characteristics [3], determining their interactions with biomolecules [4, 5]. With the novel size and unique features, nanomaterials have been extensively investigated over a decade [6, 7]. These properties hold promises for remarkable performance in several crucial fields of application such as catalytic, magnetic, mechanical, and biological applications [8]. These materials have also high potential to affect cell differentiation in vitro cultures for diverse biological aims [9].

Iron (Fe) is a vital micronutrient for plants, which is mostly in the form of Fe<sup>3+</sup> in aerobic soils and high pH. Thus, these soils are mainly deficient in Fe<sup>2+</sup>, an available form of iron, leading to iron deficiency in plants [10]. Iron is a crucial element for a photosynthetic process, plant growth, activating various enzymes that are implicated in the reduction, nitrogen fixation, and lignin synthesis [10]. Many researchers have stated that nanomaterials should be utilized as a fertilizer to achieve sustainable agriculture [3–5, 11].

Several lines of reports indicated that Fe<sub>2</sub>O<sub>3</sub> nanoparticles at an optimized dose can promote growth rate and resistance against stress in various plant species, such as *Triticum aestivum* [12], *Brassica juncea* [13], and *Arachis hypogaea* [10].

✉ Alireza Iranbakhsh  
Iranbakhsh@iau.ac.ir

<sup>1</sup> Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

<sup>2</sup> Department of Biology, Tehran North Branch, Islamic Azad University, Tehran, Iran



In recent years, metal oxide nanoparticles have attracted considerable interest because of their unique physical and chemical properties [5, 14, 15]. Iron oxide nanoparticles are widely studied material, which is extensively used as photocatalysts, sensors, fine ceramics, medical applications, data storage materials, pigments, and photo electrochemical cells [16].

*Cornus mas* L. has extensively grown in some part of Europe and Asia including Iran. Cornelian cherry fruit provides a highly valuable supply of the highest ascorbic acid, anthocyanin, phenolic and antioxidant activity among many other fruits [17]. This plant, therefore, is suitable for the synthesis of nanoparticles due to outstanding phytochemistry of its fruits having anthocyanins, flavonoids, plenty of oxalic acids butylated hydroxyanisole, and butylated hydroxytoluene [18]. These non-toxic antioxidants can reduce the metal ion and protect them from aggregation. Various plant-derived iron nanoparticles have been synthesized by several researchers [2, 19, 20].

As highlighted above, the utilization of iron chemical fertilizers to optimize crop productivity is inevitable. It is important to note that the application of conventional chemical fertilizers can cause water and soil contamination. Considering green nanotechnology as a multidisciplinary field, diverse attempts have been made to introduce safe and ecofriendly nano-based fertilizer to reduce consumption of chemical fertilizers. However, there are knowledge gaps on the potential phytotoxicity of nano-compounds and possible variations in plant responses to bulk or nano-type of materials, especially  $\text{Fe}_2\text{O}_3$  nanoparticles. Taking green nanotechnology, sustainable agriculture, and environmental pollution into account, this study deals with the fabrication of iron oxide nanoparticles using the fruit extract of *C. mas*. Moreover, there are few scientific reports to compare the effects of nano- and bulk forms of  $\text{Fe}_2\text{O}_3$  on plant growth and biomass. Hence, the possible changes in barley (*Hordeum vulgare*) growth was also addressed in response to the application of the produced nano- or bulk forms of  $\text{Fe}_2\text{O}_3$  nanoparticles.

## Experimental

### Biosynthesis of $\alpha\text{-Fe}_2\text{O}_3$ nanoparticle

Fresh fruits of *C. mas* were prepared from the local green-grocery of Tehran, Iran.  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  (99.9%; Merck) was used to prepare the sample. Iron oxide nanoparticles were first synthesized by green synthesis method according to Ahmmad et al. [8] with a minor modification. Air-dried fruits were well grinded. Afterwards, 1.5 L of water was added to 20 g of fruit powder. After 48 h, the extract was prepared by maceration method. Then, 5 mL HCL solution

was injected into the mixture drop wise and stirred. The extract solution was stocked at 10 °C. 5 g of  $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  was dissolved in 30 mL of the fruit extract and then adjusted to 50 mL by distilled water. The solution was heated at temperature (190 °C) for 14 h under ambient pressure in autoclave. The solution was cooled to room temperature. The obtained nanoparticles were washed with water and then dried in 80 °C for 30 min.

### Physicochemical traits of the nanoparticles

Characterization of the obtained nanoparticles was determined using a FTIR spectrophotometer (Nexus 780, Thermo Nicolet, USA), XRD (3003 PTS, Seifert, Germany), transmission electron microscopy (TEM), scanning electron microscope (SEM), (TESCAN, VEGA 3, USA), and energy-dispersive X-ray fluorescence spectrometry (EDXRF).

### Complementary experiment

In a complementary experiment, the effects of the synthesized nanoparticle were evaluated on the growth of barley seedlings. The seeds were sterilized with 1% sodium hypochlorite, containing one drop liquid detergent for 10 min, and washed three times with sterile distilled water. The seeds were planted in pot containing vermiculite and perlite. Plants were watered with Hoagland solution containing different doses of Fe bulk or nano-forms, including 0, 10, 20, and 100  $\text{mg L}^{-1}$  for five times with a week interval. The treatment groups were called as follows:

C: control; BFe10: bulk  $\text{Fe}_2\text{O}_3$  of 10  $\text{mg L}^{-1}$ ; BFe20: bulk  $\text{Fe}_2\text{O}_3$  of 20  $\text{mg L}^{-1}$ ; BFe100: bulk  $\text{Fe}_2\text{O}_3$  of 100  $\text{mg L}^{-1}$ ; NFe10: nano- $\text{Fe}_2\text{O}_3$  of 10  $\text{mg L}^{-1}$ ; NFe20: nano- $\text{Fe}_2\text{O}_3$  of 20  $\text{mg L}^{-1}$ ; NFe100: nano- $\text{Fe}_2\text{O}_3$  of 100  $\text{mg L}^{-1}$ .

Root and shoot fresh biomass were measured for all the treatment groups.

### Statistical analysis

The results were statistically evaluated SPSS software. Data were represented in mean and standard error (SE) of three replications. Means were statistically separated using the Duncan test at  $p \leq 0.05$ .

## Results and discussion

### Physicochemical characteristics of the synthesized nanoparticles

The FTIR spectroscopy was utilized to detect the presence of the functional groups, which can act both as metal-reducing and capping agents, there by resulting in color change. The

FTIR spectra of the fruit extract is presented in Fig. 1a. The absorption peaks at 3363 and 1638  $\text{cm}^{-1}$  reflect the O–H and N–H stretching vibration in the phenolic compound and protein in the fruit extract. The bands at 2079  $\text{cm}^{-1}$  is assigned to C–H stretching in carbohydrates. The bands at 1071  $\text{cm}^{-1}$  is caused by the (C=O)  $\text{NH}_2$  stretching. FTIR spectra of synthesized iron oxide nanoparticles using extract of *C. mas* is shown in Fig. 1b. Band at 442–501  $\text{cm}^{-1}$  as characteristic peaks of  $\alpha\text{-Fe}_2\text{O}_3$  is observed [21]. Comparing Fig. 1a and b, The hydroxyl and amid groups at 3363 and 1638  $\text{cm}^{-1}$  in the fruit extract shift to 3408 and 1623  $\text{cm}^{-1}$  in the IR spectra of the synthesized iron oxide nanoparticle sample. From these IR spectra, it is observed that hydroxyl and amino groups of protein and carbohydrate have the ability to perform dual functions of reduction and stabilization of iron oxide nanoparticles.

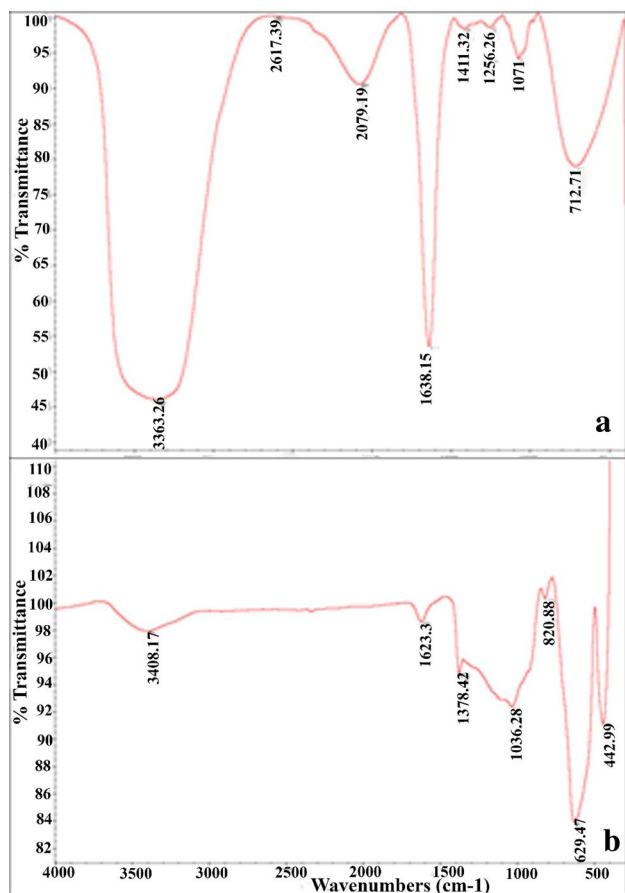
The crystal structure of  $\text{Fe}_2\text{O}_3$  nanoparticle was examined by XRD. The ranges of angles were 20°–60°. Figure 2a shows the XRD pattern of the synthesized  $\text{Fe}_2\text{O}_3$  nanoparticle. The pattern has been compared with reference to the standard XRD pattern of hematite (in accordance with the

standard card JCPDS No. 33-0664). All reflection peaks relate to the pure and single  $\alpha\text{-Fe}_2\text{O}_3$  phase. The average particle size ( $D$ ) of the  $\alpha\text{-Fe}_2\text{O}_3$  nanoparticles was estimated using Debye–Scherer equation (Eq. (1)) [22]:

$$D = \frac{k\lambda}{\beta \cos \theta}, \quad (1)$$

where,  $\lambda$  is the X-ray wavelength,  $K$  is a dimensionless shape factor, with a value close to unity. The shape factor has a typical value of about 0.9, but varies with the actual shape of the crystallite.  $\beta$  is the line broadening at half the maximum intensity (FWHM), after subtracting the instrumental line broadening, in radians. This quantity is also sometimes denoted as  $\Delta(2\theta)$ , and  $\theta$  is the diffraction angle. XRD analysis of the synthesized nano-product showed the average particle size of 20 nm.

The size and morphology of the  $\text{Fe}_2\text{O}_3$  nanoparticle were examined by TEM and SEM analyses. The SEM image of synthesized  $\text{Fe}_2\text{O}_3$  nanoparticle demonstrates the morphology of nanoparticles is spherical and spongy (Fig. 2b). It is clear that  $\text{Fe}_2\text{O}_3$  nanoparticles are mainly present as granules with small and big spherical-shaped particles and are well crystallize in nature. TEM images of the synthesized nanoparticles are shown in Fig. 2c. This image shows very fine spherical  $\text{Fe}_2\text{O}_3$  nanoparticles (20–40 nm) which are very close to XRD calculations. In the EDXRF spectra (Fig. 2d), the peaks around 0.8, 6.2, and 6.9 keV are related to the binding energies of Fe. The EDXRF spectra shows only iron and oxygen elements, which implies that the sample is highly pure  $\text{Fe}_2\text{O}_3$  nanoparticle. The remarkable potential of plant extracts (especially fruits) for application in the green synthesis of nano-products is due to the presence of various phytochemicals, such as flavones, polyphenols, and amides which can act as reducing and stabilizing agents [23].



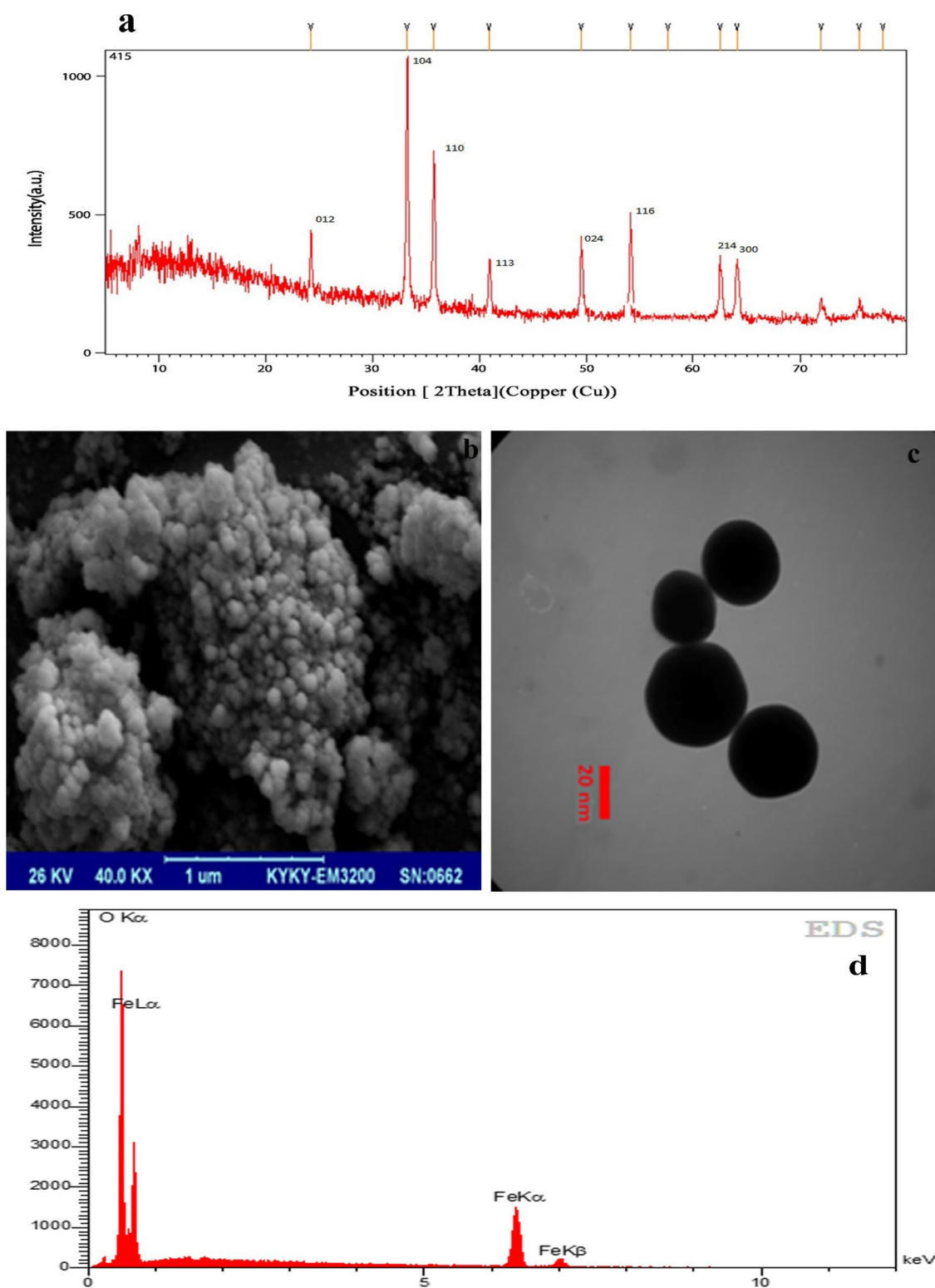
**Fig. 1** FTIR spectra of **a** *Cornus mas* L. fruit extract and **b** synthesized iron oxide nanoparticles

## The nano-product-mediated changes in plant early growth

With increase in concentration of nano- or bulk forms of  $\text{Fe}_2\text{O}_3$ , shoot fresh mass increased in all treated plants over the control. The highest fresh mass was observed at NFe100 group (Fig. 3a). The application of bulk form of  $\text{Fe}_2\text{O}_3$  increased shoot biomass by an average of 32.6%, whereas the synthesized nano-type enhanced this parameter by a mean of 68%, implying the higher efficiency of nano- $\text{Fe}_2\text{O}_3$  than the bulk to improve plant growth.

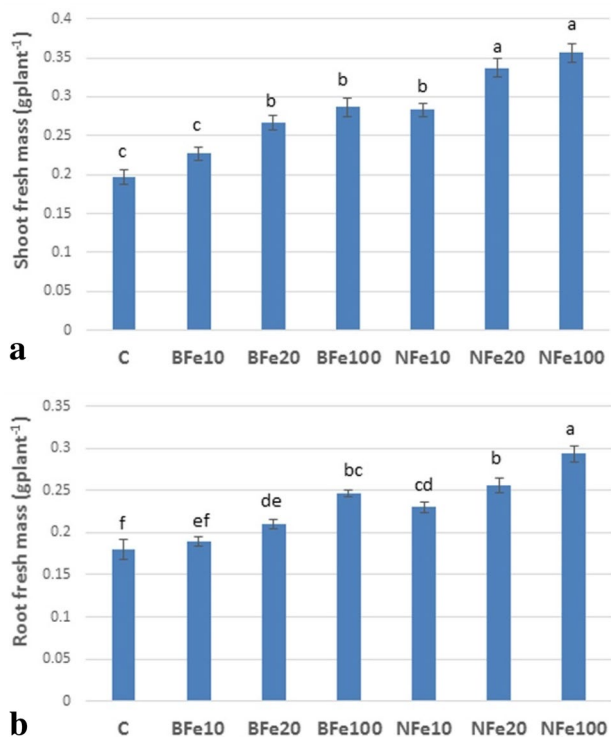
The  $\text{Fe}_2\text{O}_3$  nanoparticle and its bulk form at the concentrations of 10, 20, and 100  $\text{mg L}^{-1}$  showed statistically significant stimulation in root fresh mass of the treated plants (Fig. 3b). It was clearly observed that  $\text{Fe}_2\text{O}_3$  nanoparticle was more effective than the bulk to stimulate plant early growth. The nano-form stimulated root biomass by





**Fig. 2** XRD powder pattern of  $\alpha$ - $\text{Fe}_2\text{O}_3$  nanoparticle (a), SEM image (b), TEM (c) images of the synthesized  $\text{Fe}_2\text{O}_3$  nanoparticles, as well as EDXRF spectrum of  $\alpha$ - $\text{Fe}_2\text{O}_3$  nanoparticles





**Fig. 3** Changes in shoot (a) and root (b) fresh mass in response to the applications of bulk or nano-Fe supplements. C control, BFe10 bulk Fe<sub>2</sub>O<sub>3</sub> of 10 mg L<sup>-1</sup>, BFe20 bulk Fe<sub>2</sub>O<sub>3</sub> of 20 mg L<sup>-1</sup>, BFe100 bulk Fe<sub>2</sub>O<sub>3</sub> of 100 mg L<sup>-1</sup>, NFe10 nano-Fe<sub>2</sub>O<sub>3</sub> of 10 mg L<sup>-1</sup>, NFe20 nano-Fe<sub>2</sub>O<sub>3</sub> of 20 mg L<sup>-1</sup>, NFe100 nano-Fe<sub>2</sub>O<sub>3</sub> of 100 mg L<sup>-1</sup>

an average of 42%, while the bulk type increased this trait by a mean of 18%. The result of this research showed that biologically synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticles improved plant growth and biomass accumulation much higher than the corresponding doses of bulk type. Nanomaterials have specific features, including a more particular surface area and increased density of reactive areas, thereby easily penetrating the plant cell and affecting cellular biochemistry. In line with our results, several lines of evidence confirmed the differences between the bulk and nanomaterials [5, 6]. Iron oxide nanoparticles could be utilized as a reliable non-toxic substitution for other iron sources [2, 19]. The application of Fe<sub>2</sub>O<sub>3</sub> nanoparticles may associate with particular signaling, especially due to the changes in concentrations of reactive oxygen species leading to stimulation of plant growth. Rui et al., [10] have demonstrated that lower doses of Fe<sub>2</sub>O<sub>3</sub> nanoparticle improved the translocation of iron from the root to the shoot. Rui et al., [10] also found that the adsorption of Fe<sub>2</sub>O<sub>3</sub> nanoparticles to the soil particles can decrease nutrient loss and enhance their cost effectiveness as a fertilizer.

## Conclusion

A simple, facile and ecofriendly method has been carried out for biosynthesizing Fe<sub>2</sub>O<sub>3</sub> nanoparticle using the fruit extract of *C. mas*. The characteristics of the biosynthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticles were examined by FTIR, XRD, TEM and SEM techniques confirming the production of nanoparticles with high quality and purity. This study would help to develop a new method for biosynthesis of nanoparticles with excellent properties. Moreover, comparative data were presented on the changes in biomass accumulations in root and shoot in response to the applications of the synthesized nano-compound or bulk material. The findings displayed a high efficiency of the biologically synthesized Fe<sub>2</sub>O<sub>3</sub> nanoparticle to improve growth of barley seedlings relative to the corresponding levels of bulk counterpart.

## References

1. Kanagasubbulakshmi, S., Kadirvelu, K.: Green synthesis of iron oxide nanoparticles using *Lagenariasisiceraria* and evaluation of its antimicrobial activity. *Def. Life Sci. J.* **2**, 422–427 (2017)
2. Demirezen, D.A., Yıldız, Y.Ş., Yılmaz, Ş., Yılmaz, D.D.: Green synthesis and characterization of iron oxide nanoparticles using *Ficus carica* (common fig) dried fruit extract. *J. Biosci. Bioeng.* **127**, 241–245 (2019)
3. Seddighinia, F., Iranbakhsh, A., Oraghi Ardebili, Z., Nejad Satari, T., Soleimanpour, S.: Seed priming with cold plasma and multi-walled carbon nanotubes modified growth, tissue differentiation, anatomy, and yield in bitter melon (*Momordica charantia*). *J. Plant Growth Regul.* (2019). <https://doi.org/10.1007/s00344-019-09965-2>
4. Safari, M., Ardebili, Z.O., Iranbakhsh, A.: Selenium nano-particle induced alterations in expression patterns of heat shock factor A4A (HSFA4A), and high molecular weight glutenin subunit 1Bx (Glu-1Bx) and enhanced nitrate reductase activity in wheat (*Triticumaestivum* L.). *Acta Physiol. Plant.* **40**, 117 (2018)
5. Babajani, A., Iranbakhsh, A., Ardebili, Z.O., Eslami, B.: Differential growth, nutrition, physiology, and gene expression in *Melissa officinalis* mediated by zinc oxide and elemental selenium nanoparticles. *Environ. Sci. Pollut. Res.* (2019). <https://doi.org/10.1007/s11356-019-05676-z>
6. Moghanloo, M., Iranbakhsh, A., Ebadi, M., Ardebili, Z.O.: Differential physiology and expression of phenylalanine ammonia lyase (PAL) and universal stress protein (USP) in the endangered species *Astragalusfridae* following seed priming with cold plasma and manipulation of culture medium with silica nanoparticles. *3 Biotech* **9**, 288 (2019)
7. Moghanloo, M., Iranbakhsh, A., Ebadi, M., Satari, T.N., Ardebili, Z.O.: Seed priming with cold plasma and supplementation of culture medium with silicon nanoparticle modified growth, physiology, and anatomy in *Astragalus fridae* as an endangered species. *Acta Physiol. Plant.* **41**, 54 (2019)
8. Ahmmad, B., Leonard, K., Shariful, M.D., Kurawaki, J., Muruganandham, M., Ohkubo, T., Kuroda, Y.: Green synthesis of mesoporous hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles and their photocatalytic activity. *Adv. Powder Technol.* **24**, 160–167 (2013)



9. Ghasempour, M., Iranbakhsh, A., Ebadi, M., Ardebili, Z.O.: Multi-walled carbon nanotubes improved growth, anatomy, physiology, secondary metabolism, and callus performance in *Catharanthus roseus*: an in vitro study. *3 Biotech* **9**, 404 (2019)
10. Rui, M., Ma, C., Hao, Y., Guo, J., Rui, Y., Tang, X., Zhao, Q., Fan, X., Zhang, Z., Hou, T., Zhu, S.: Iron oxide nanoparticles as a potential iron fertilizer for peanut (*Arachis hypogaea*). *Front. Plant Sci.* **9**, 815 (2016)
11. Nazerieh, H., Ardebili, Z.O., Iranbakhsh, A.: Potential benefits and toxicity of nanoselenium and nitric oxide in peppermint. *Acta Agric. Slov.* **111**, 357–368 (2018)
12. Rizwan, M., Ali, S., Ali, B., Adrees, M., Arshad, M., Hussain, A., ur Rehman, M.Z., Waris, A.A.: Zinc and iron oxide nanoparticles improved the plant growth and reduced the oxidative stress and cadmium concentration in wheat. *Chemosphere* **214**, 269–277 (2019)
13. Praveen, A., Khan, E., Perwez, M., Sardar, M., Gupta, M.: Iron oxide nanoparticles as nano-adsorbents: a possible way to reduce arsenic phytotoxicity in Indian mustard plant (*Brassicajuncea* L.). *J. Plant Growth Regul.* **37**, 612–624 (2018)
14. Iranbakhsh, A., Ardebili, Z.O., Ardebili, N.O., Ghoranneviss, M., Safari, N.: Cold plasma relieved toxicity signs of nano zinc oxide in *Capsicum annuum* cayenne via modifying growth, differentiation, and physiology. *Acta Physiol. Plant.* **40**, 154 (2018)
15. Babajani, A., Iranbakhsh, A., Ardebili, Z.O., Eslami, B.: Seed priming with non-thermal plasma modified plant reactions to selenium or zinc oxide nanoparticles: cold plasma as a novel emerging tool for plant science. *Plasma Chem. Plasma Process.* **39**, 21–34 (2019)
16. Rafi, M.M., Ahmed, K.S., Nazeer, K.P., Kumar, D.S., Thamilselvan, M.: Synthesis, characterization and magnetic properties of hematite ( $\alpha$ -Fe<sub>2</sub>O<sub>3</sub>) nanoparticles on polysaccharide templates and their antibacterial activity. *Appl. Nanosci.* **1**, 515–520 (2015)
17. Pantelidis, G.E., Vasilakakis, M., Manganaris, G.A., Diamantidis, G.: Antioxidant capacity, phenol, anthocyanin and ascorbic acid contents in raspberries, blackberries, red currants, gooseberries and cornelian cherries. *Food Chem.* **102**, 777–783 (2007)
18. Narimani-Rad, M., Zendehtdel, M., Abbasi, M.M., Abdollahi, B., Lotfi, A.: Cornelian chery (*Cornusmas* L.) extract affects glycemic status in wistar rats. *Bull. Environ. Pharmacol. Life Sci.* **2**, 48–50 (2013)
19. Rosli, I.R., Zulhaimi, H.I., Ibrahim, S.K.M., Gopinath, S.C.B., Kasim, K.F., Akmal, H.M., Nuradibah, M.A., Sam, T.S.: Phytosynthesis of iron nanoparticle from *Averrhoabilimbi* Linn. *IOP Conf. Ser. Mat. Sci. Eng.* **318**, 012012 (2018)
20. Kumar, V., Yadav, S.K.: Synthesis of different-sized silver nanoparticles by simply varying reaction conditions with leaf extracts of *Bauhinia variegata* L. *J. Chem. Technol. Biotechnol.* **84**, 151–157 (2009)
21. Zhou, W., He, W., Ma, J., Wang, M., Zhang, X., Yan, S., Tian, X., Sun, X., Han, X.: Biosynthesis of mesoporous organic-inorganic hybrid Fe<sub>2</sub>O<sub>3</sub> with high photocatalytic activity. *Mat. Sci. Eng. C* **29**, 1893–1896 (2009)
22. Sadegh, H., Helmi, H., Hamdy, A.S., Masjedi, A., Dastjerdi, M.J.H., Shahryari-ghoshekandi, R.: A developed simple, facile, economic and fast technique for preparation of high quality Fe<sub>3</sub>O<sub>4</sub> magnetic nanoparticles. *Chem. Adv. Mater.* **1**, 1 (2016)
23. Sharma, G., Pandey, S., Ghatak, S., Watal, G., Rai, P.: Potential of spectroscopic techniques in the characterization of “green nanomaterials”. In: *Nanomaterials in Plants, Algae, and Microorganisms*, pp. 59–77. Academic Press (2018)

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.