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Bio-Preparation of Iron Nano Fertilizers from Lemon Fruit Affected some of the Characters of Carrot plants (*Daucus carota* L.)

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Abstract

To study the biological preparation of iron nano fertilizers from lemon fruit extract and their effect on some quantitative traits of carrot (*Daucus carota* L.) plant. Experiments were conducted, a laboratory part and a field part, with two addition factors, foliar fertilization and soil fertilization, using two sources of iron. The first source was nano-iron at a concentration of 0.5, 1, 1.5, and 2 mg L^{-1}, and the second source was chelated iron at a concentration of 1.5 g L^{-1} and control treatment. The results showed the superiority of foliar fertilization in most of the studied traits such as iron concentration, root length, root diameter, fresh weight, dry weight, carotene and total dissolved solids (144.30 ppm, 17.10 cm, 2.1 cm, 137.98 g, 16.03 g, 1.62 mg g fresh $weight^{-1}$ and 8.79 %, respectively). Nano-iron spraying was superior than to chelated iron in the concentration of iron (Fe), root length, root diameter, fresh weight, dry weight, and total dissolved solids (1059.22 ppm, 20.82 cm, 2.29 cm, 168.97 g, 18.37 g, and 9.91 %, respectively). X-ray diffraction (XRD) was used to determine the size and purity of the nanoparticles, and scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX) were used to verify the composition. Iron oxide (Fe2O3) was in the form of α-Fe2O3 hematite with a size of 32.8 nm and Ỵ-Fe2O3 maghematite with a size of 44.6 nm. We conclude that the use of green synthesis method for nano-fertilizers has proven effective and efficient from an economic and environmental point of view.

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Introduction

Iron is one of the basic and essential micronutrients for the growth of plants, especially those of economic importance. It helps catalyze redox enzymes and also contributes to the transfer of electrons during respiration. Iron is also involved in vital compounds such as cytochromes, which are involved in the processes of photosynthesis, respiration, and active nutrient uptake. It also has a positive effect on improving RNA functions and has a positive effect on improving plant growth (Mengel and Kirkby, 2001). The most famous of these are hematite (ᶏ-Fe_2O_3), mega hematite (α-Fe_2O_3), which are the most stable forms of iron in nature (Pariona *et al.*, 2017). The amount of ready iron in agricultural soils is small. Very much compared to its total quantity in the same soil, and ready (dissolved) iron is present in the soil solution either in a mineral form Fe^{+2}, Fe^{+3} Fe $(OH)_2$, $FeOH^{+2}$) or in a dissolved organic form. The appearance of symptoms of iron deficiency in plants depends on the ready amount of iron, and iron deficiency is treated through the use of fertilizers. In general, treating iron deficiency is not an easy process, due to the low solubility of iron fertilizers, in addition to the fact that the efficiency of using these fertilizers is about 5 %. As a result, to compensate for the iron deficiency, iron-containing fertilizers are added frequently during one agricultural season (Ali and Al-Juthery, 2017).

The soils of arid and semi-arid regions, including Yemeni soils, suffer from a lack of availability and fixation of iron, and thus symptoms of deficiency appear in plants. The lack of iron availability in arid and semi-arid regions can be attributed to several reasons, including the high degree of soil interaction, pH, and the presence of calcium carbonate in a high amount. It should be noted here that 30 % of the mineral deficiency is found in calcareous soils and soils containing high levels of phosphorous, as a result of the deposition of iron in the form of iron phosphate (Ali and Majeed, 2016). To compensate for the lack of ready-made iron in the soil, the need to import large quantities of iron fertilizers, as the number of iron fertilizers allowed to enter Yemen for the year 2021 reached 13,363.7 tons of solid fertilizer and 33,000 liters of liquid fertilizer, which causes a huge financial burden on farms and the state (Statistics, 2022).

Nano-fertilizers are characterized by their ability to control the orientation process and increase the plant response due to their easy entry into the cells. They also provide a suitable mechanism for transporting nutritional compounds to the target places in the plant, whether leaves, roots, fruits, or other plant parts. Nano-fertilizers are prepared either by physical, chemical, or biological methods (Cicek and Nadaroglu, 2015). Nano-fertilizers provide more space for various metabolic reactions in the plant, which increase the rate of photosynthesis and produce more dry matter, thus increasing crop yield (Gahlawat *et al.*, 2016).

The biological methods used in the preparation of nanoparticles and using plant extracts that contain organic compounds such as flavonoids, amino and carboxylic acids, ketones, and phenols are among the most common methods for preparing nano fertilizers due to their ease and speed of preparation. In addition to being an environmentally safe method (Attia and Elsheery, 2020). What distinguishes biological methods in fertilizer preparation compared to other methods is that there is no need to use high pressures and temperatures during the preparation process, and the availability of raw materials used in preparation reduces the cost of fertilizer production significantly, in addition to those biological methods for preparing nano-fertilizers from safe methods, as their use does not result in the production of any toxic or environmentally harmful materials. In addition, it is possible to produce large quantities of fertilizers in relatively short periods due to the ease of this method and its lack of need for complex techniques in the preparation processes (Moslemi *et al.,* 2014).

FeO-NPs are one interesting metal oxide due to their many applications in advanced technologies, as it is widely used in many important medical and biological applications, including their use as fertilizer in the agricultural field. The measurement is very distributed within a narrow range of 1-100 nm Finding new ways to prepare nano-iron oxide that are inexpensive and give a product with a small and homogeneous measurement range with a large surface area is a requirement for the use of this oxide and given the importance of iron to plants. And the lack of studies on the use of nano-iron fertilizers in Agriculture. Moreover, the lack of nano-fertilizers in the local markets. The study aims to produce iron nano-fertilizer with an environmentally friendly and relatively inexpensive technology and with a large surface area employing biosynthesis using plant extracts (Hu *et al.*, 2005).

Foliar feeding is of great importance in the provision of plant nutrients and is complementary to soil fertilization (Al-Eugabi, 2016). It reduces the risk of environmental pollution. Usually, the absorption of nutrients by the leaves on the lower surface is better than the upper surface and in the modern leaves faster than the old one (Ahad *et al.,* 2021).

The carrot plant (*Daucus carota* L.) is and belongs to the Umbelliferae family, and one of the important and major root vegetables used for different purposes in the daily human. It is used with other vegetables in the preparation of soup and is increasing importance as food, and fleshy roots are eaten as raw in salads, boiled or steamed in vegetable dishes. It is rich in beta-carotene, which enhances resistance to blood and eye diseases, and a large amount of carrots in the diet has a favorable effect on the nitrogen balance (Jönsson *et al.,* 2023). To study the biological preparation of iron nano fertilizers from lemon fruit extract and their effect on some quantitative traits of the carrot (*Daucus carota* L.) plant, experiments were conducted.

Materials and Methods

During the winter season 2021-2022 in the laboratory of Faculty of Agriculture, Sana'a University Nanoparticles (FeNOPs) iron fertilizers were prepared using Iron (III) nitrate (Fe $(NO_3)_2$ $.6H_2O$) (99.5 %, Fluka), Liquid ammonia) NH_4OH) (99.5 %) Yemeni lemon juice extracts (citric acid ($C_6H_8O_7$).

Synthesis of lemon Extract

Fresh lemon fruits were collected, washed, cut and squeezed to obtain its extract. The lemon extract was filtered by filter papers to get rid of impurities. The extract was collected in a glass beaker washed with distilled water. Thus, the extract is ready to prepare the fertilizer (Alaallah *et al.,* 2023).

Synthesis of Fe_2O_3 nano-composites using lemon Extract

The method of work, take 30 mL of lemon extract, put it in a 100 mL beaker after filtering it to get rid of impurities, and add 10 gm of iron nitrate Fe $(NO_3)_3$ $.6H_2O$ to the extract. The mixture was heated with a magnetic stirrer to dissolve the iron nitrate well, add drops of liquid ammonia until the pH became equal to (7). The temperature was raised to 90 degrees Celsius until the mixture turned into a viscous liquid Gel. The temperature was raised to 120 degrees Celsius until the mixture turned into a dry gel (Xerogel). The lemon extract was added as a fuel to increase the ignition process. The brick-red dry gel powder was left to cool. The resulting red powder was collected and placed in a ceramic crucible and placed in the incinerator at a temperature of 600 °C for two hours. The resulting powder was collected and ground in a ceramic manual mortar to conduct tests and measurements of X-ray diffraction (XRD) and an analysis scale (Sutka and Mezinskis, 2012).

Characterizations of FeO-NPs

Dispersive X-ray energies in the Geological Survey (Sana'a), to obtain structural information of iron nanoparticles and to identify the components of the sample according to (Ullrich *et al.,* 2019). Using a diffractometer (XD-2) (wavelength 0.154 nm, voltage 36 kv, current strength 20 AM, China), and calculate the particle size of the prepared sample using the Debye-Scherer equation $DSh=k\lambda / (\beta hkl \cos \theta)$ according to (Croteau *et al.,* 2000). Using Image j program to measure the diameters of the granules and then obtain the size of the sample granules according to what was mentioned by (Ahmed *et al.,* 2021).A scanning electron microscope diagnostic examination (SEM) at the Desert Research Center (Egyptian Center of Excellence for Water Desalination Research) Arab Republic of Egypt was used to identify the morphological surface of the sample surface and calculate the size of the constituent granules of the sample according to what was mentioned by (Aljawzi *et al.,* 2022). Examining the sample with an energy-dispersive spectrometer with X-rays (EDX) Energy-dispersive X-ray attached to the scanning microscope to identify the morphological shape of the surface of the nanoparticles and to know the proportions of the elements included in the composition of the sample and the degree of purity according to what was mentioned by (Mohamed and Selvakumar, 2012).

Agricultural experience

Preparing the pots

An experiment was carried out in plastic pots with a pot capacity of 12 kg, with 10 kg of soil for each pot of farm soil, in one of the nurseries of the College of Agriculture, University of Sana'a, in the winter agricultural season 2022-2023 AD, for 36 pots. Carrot seeds, a variety (Nelex), produced by Peugeot Company, and were planted. In pots filled with sifted soil mixed with organic fertilizer (peat moss), ten seeds per pot on 11/19/2021. Then direct watering was done with light surface irrigation to moisten the seeds. The irrigation process was repeated as often as necessary. After the seeds germinated within approximately a month from the date of planting, and after the leaves reached the stage of four true leaves, the plants were thinned, Phosphate fertilizer. 10% P superphosphate, was added to all treatments according to the fertilizer recommendation before planting, in one batch for all pots. Nitrogen fertilizer in the form of 21% N ammonium sulphate was added to all treatments. And according to the fertilizer recommendation in two batches, potassium fertilizer was added in the form of 50 % K potassium sulphate to all treatments, according to the fertilizer recommendation, also in two batches.

Experimental transactions and design

The experiment included two factors, the first factor representing the method of addition (foliar fertilization and land fertilization) and the second factor the concentrations, represented by levels of the prepared nano fertilizer: 0.5, 1, 1.5, and 2 mg L^{-1}, and the following symbols were given to it: L1, L2, L3, L4, and the recommended level of mineral iron fertilizer type (Fe 138 Secostrin) at a concentration of 1.5 g L^{-1} was given the symbol LL, in addition to the control without addition, and it was symbolized with the symbol L0, so that the number of concentrations used was 6 concentrations, distributed randomly among all the experimental units.

The experiment was designed according to completely randomized blocks (RCBD) with three replications, so that the number of experimental units was 2*6*3=36 experimental unit, the resulting data will be statistically analyzed test was used SPSS statistical analysis program according to what was stated by (Steel *et al.*, 1997).

Treatments preparation

The prepared fertilizer was added in two ways, the first was spraying on the plant. It was coded with the symbol A. Sprayers with a capacity of 1 liter were used for each level of addition, and an adhesive spreading material (Hygro Wet) was added to increase the spread and distribution of the fertilizer and increase its adhesion to the plant so that the plant can benefit from it for the longest possible period. It is washed with irrigation water, and the second is the addition to the soil and is symbolized by the symbol S, as the fertilizer produced was added at four levels and symbolized by the symbols L1, L2, L3, and L4. Adding the chelated iron fertilizer found in the local market Secostrin and at the rate of addition recommended by the manufacturer. It is symbolized by the symbol LL, the control without adding fertilizer, and symbolized by the symbol L0 (distilled water). The plants were sprayed early in the morning until complete wetness was obtained when the first drop landed on the soil, taking care to separate each replicate with pieces of cardboard during spraying to ensure that the spray did not spread between adjacent treatments, at a rate of three sprays during the growing season, where the first spray was three weeks after germination, after the appearance of four. True leaves and the second spraying was a month after the first spraying, and the last spraying was a month after the second spraying. The plants were irrigated with tap water whenever the need for irrigation arose and according to the conditions of the experiment. All agricultural operations were carried out in the production of this type of carrot plant, including hoeing, weeding, irrigation, fertilization, and preventive control against insects and diseases.

Measuring fruit growth indicators

Root Length (cm)

The length of carrot roots was measured with a measuring tape graduated from the base of the vegetative part from the area where the stem (crown) connects to the root to the end of the root.

Root Diameter (cm)

The average root diameter was calculated using a Chinese-made Vernier Caliper for all samples in each treatment. It was measured in the area above the root at a distance of 2 cm from the area of separation of the root from the stem for all treatments.

Dry weight of the root (g)

The roots were separated from the shoots of each experimental unit, then the roots were washed and cleaned of any dust stuck to them, and after taking the required measurements on the roots, they were weighed, then placed in perforated paper bags, then placed in an electric oven at 65 degrees Celsius until the weight was stable, and the weights were calculated using an electric sensitive balance.

Carotene (mg g plant^{-1})

The total carotenoid content of the roots was estimated by taking one gram of fresh roots and cutting them into small pieces so that they could be easily crushed in a ceramic bowl. Then adding of 10 mL of acetone (80 %). After separating the filtrate from the sediment, the visible density of the filtrate was measured using a spectrophotometer at a wavelength of 470 nm. By applying the equation below:

Total Carotenoid Content (mg g^{-1} tissue) =A. Y (ml) * 106 / A$^{1\%}$ 1 CM * 100

A = Reading the optical density at the wavelength (nm 470)

Y = Volume of solution that gives absorbance

A$^{1\%}$ 1CM = Absorption coefficient of cartons = (2592), (Lichtenthaler, 1987).

Percentage of dissolved solids TSS (%)

The percentage of dissolved solids was estimated using a refractometer after obtaining the concentrated plant root juice.

Estimating the concentration of nutrients in roots

Concentration of (Fe) (mg kg^{-1})

The content of digested root samples of iron was estimated using an atomic absorption spectrophotometer with a wavelength of 248.3 nm and calibrated with the standard curve (Cresser and Parsons, 1979).

Statistical analysis

The resulting data were analyzed statistically after being tabulated and arranged according to the Analysis of Variance (ANOVA) test using the statistical analysis program SPSS, and the averages of the coefficients were compared with the Revised Least Significant Difference (RLSD) test at a significance probability of 0.05, according to what was mentioned.

Results and Discussion

Characterization of iron oxide nanoparticles

X-ray diffraction (XRD) spectroscopy

The results of X-ray diffraction analysis using a diffractometer device for FeO-NPsformed by lemon extract (Figure 1a) showed clear peaks measuring 2ϴ at angles 33.5 and 36, respectively for the crystalline levels (104) and (119), and the results showed the formation of nanoparticles of iron oxide (NPS). (Fe_2O_3 in the form of Magnetite-Q, syn) with card number (PDF#25-1402) and Ỵ - Hematite, syn) with card number (PDF#33-0664) Figure 1b. and these results are consistent with the database Center for Diffraction Data (JCDD) INTERNATIONAL This is evidence of the formation of iron oxide nanoparticles. These results agreed with (Salman, 2018) who prepared nano-iron oxide from plant extracts and obtained two forms of iron oxide, hematite and mega hematite.

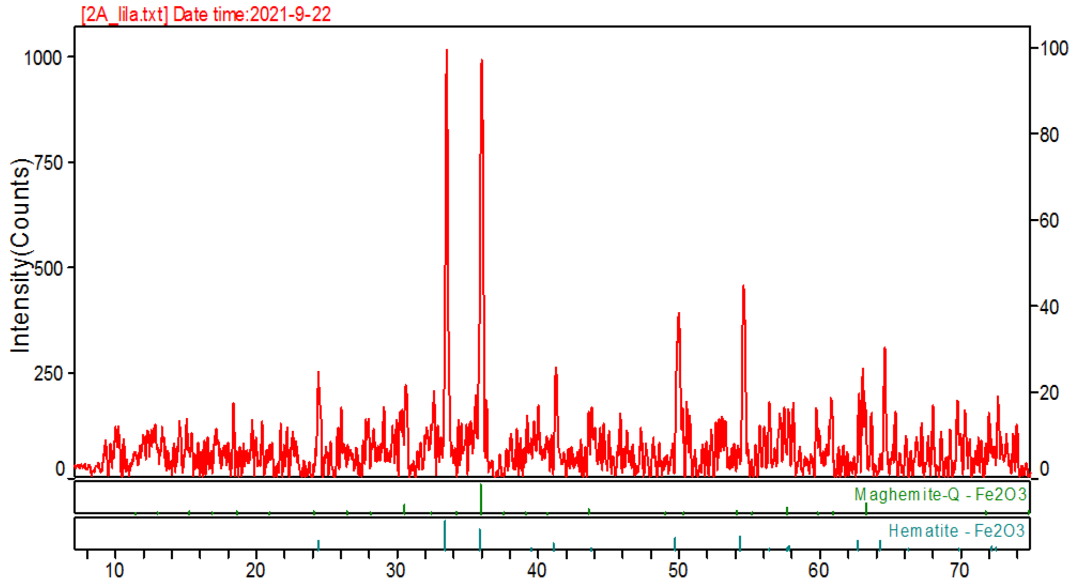


Figure. 1a. X-ray diffraction (XRD) spectrum of Fe_2O_3 nanoparticles

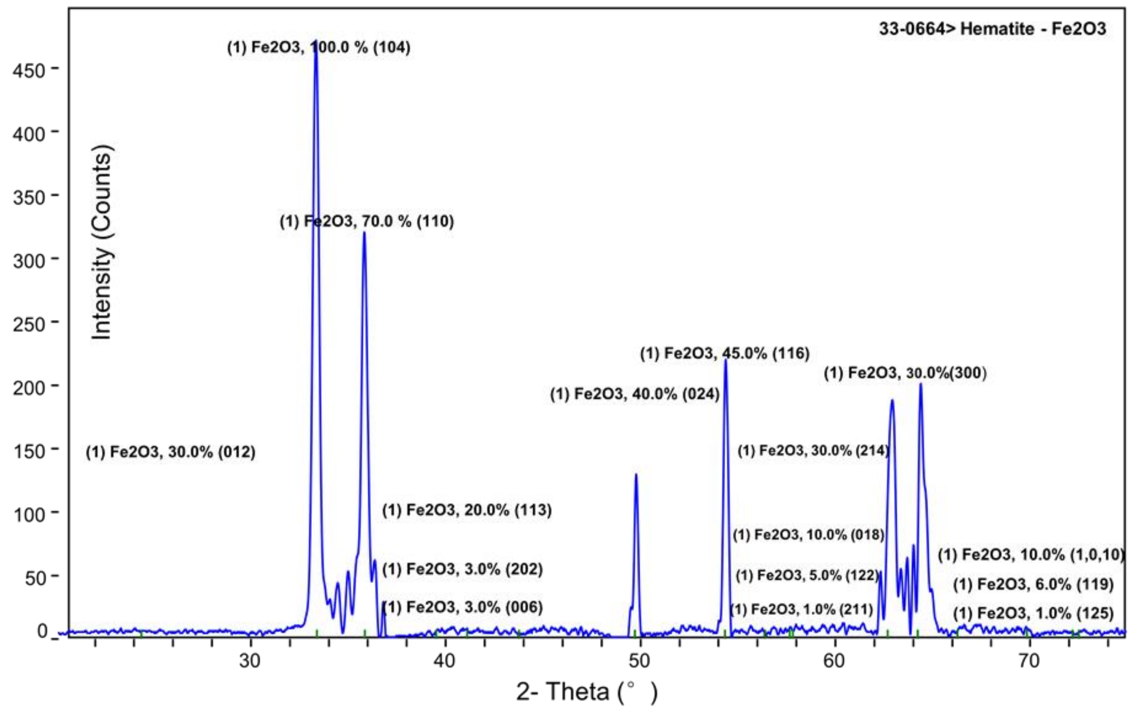


Figure. 1b. The X-ray diffraction (XRD) spectrum of Fe_2O_3 nanoparticles

From the X-ray diffraction (XRD) data, Figure 1a. the sizes of nanoparticles were calculated using the Debye-Scherr equation, according to Mohamed and Selvakumar, (2012) ; Sakura *et al.*, (2015) Table 2, which shows the formation of two phases of iron oxide, Maghemite-Q, syn) and Hematite with an angle of 33.501 2Ɵ and 36, respectively, and a maximum width of the FWHM 0.186 and 0.255, respectively. Celsius was converted into radians by multiplying by 0.0174 and applying the equation according to Mohamed and Selvakumar, (2012) ; Sakura *et al.* (2015), Table 1 which shows the formation of two iron oxide phases, Maghemite -Q, syn) and Hematite at an angle of 33.501 2Ɵ and 36, respectively.And a maximum width of the FWHM peak is 0.186 and 0.255, respectively. Celsius was converted to radians by multiplying by 0.0174 and applying the equation is a particle size function calculated from the Debye–Scherer equation $D\ Sh = k\ \lambda / (\beta hkl \cos \theta)$. λ is the wavelength of x-rays and is 0.15406 nm, βhkl is the maximum width of the mid-top, K is a constant called the form factor and is equal to 0.94, θ Brack angle.

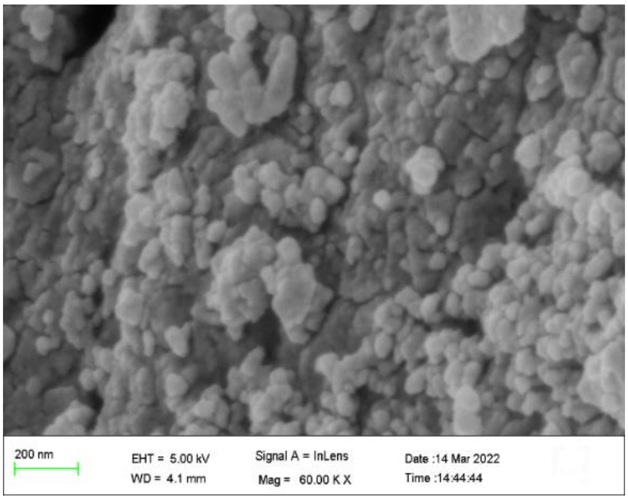
It was found that the size of the prepared particles is 32.76 nm (Hematite) and 44.61 nm (Maghemite-Q), and this is consistent with what was found by Ahmed *et al.* (2021), who obtained FeO-NPs from pomegranate extract with a size of 30.6 nm.

Table 1. Calculation of particle size using the Debye-Scherer equation

Sample	2-Theta (°)	FWHM (°)	Theta (rad)	FWHM (rad)	K	Lamda (nm)	crystallite size (nm)
(Maghemite-Q)	33.501	0.186	0.29	0.00325	0.9	0.154	44.7
Hematite	36	0.255	0.314	0.00445	0.9	0.154	32.8

Scanning electron microscopy (SEM) diagnosis

To find out the shapes of the nanoparticles of the prepared sample. The samples were examined using a scanning electron microscope (SEM), and from the results of the examination and diagnosis of the examination images of the FeO-NPs prepared biologically using the extract of lemon.



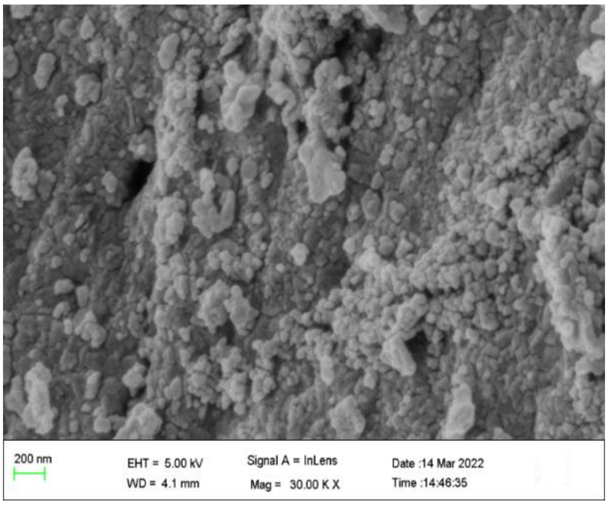


Figure 2. shapes of FeO-NPs using a scanning electron microscope (SEM)

Figure 2 (A and B), it was found that the shape of the nanoparticles is spherical, semi-spherical, and irregular shapes, and this is consistent with the findings of Salman, (2018), who found that by examining the SEM of the prepared nano-iron oxide sample. There are spherical and non-spherical shapes in irregular clusters belonging to the nano-iron oxide. From the examination images, the sizes of the granules were calculated using the ImageJ program, and the graph shows the average size of the granules in nanometers. Where it was found that most of the diameters of the granules centered in sizes between 0-28 nm. The result is consistent with (Askary *et al.*, 2017; Ahmed *et al.*, 2021).

Energy dispersive X-ray spectrometer (EDX)

To find out the quality and quantity of the elements included in the composition of the prepared sample, the sample was examined with the (EDX) device attached to the scanning electron microscope, as shown in (Figure 3), as it appears in the analysis that there are atoms of different elements, including the element Fe, with an atomic mass ratio of iron equal to 78.78 %, and when compared to the ratio The gravimetric arithmetic of the formula (Fe_2O_3) the mass was equal to 79.96 %.

Mass (%) = 55.88 * 2 / (55.88 * 2+3 * 15.9994) = 69.96 %

And when compared with the mass obtained from the analysis, we find that the difference is 8.82 %, and this is the result of some impurities present in the sample that have energies close to iron, such as (carbon). And the presence of oxygen O2 with an atomic mass of 16.78 %, and the final formula for nano iron oxide was 96.74 % (Fe_20_3).

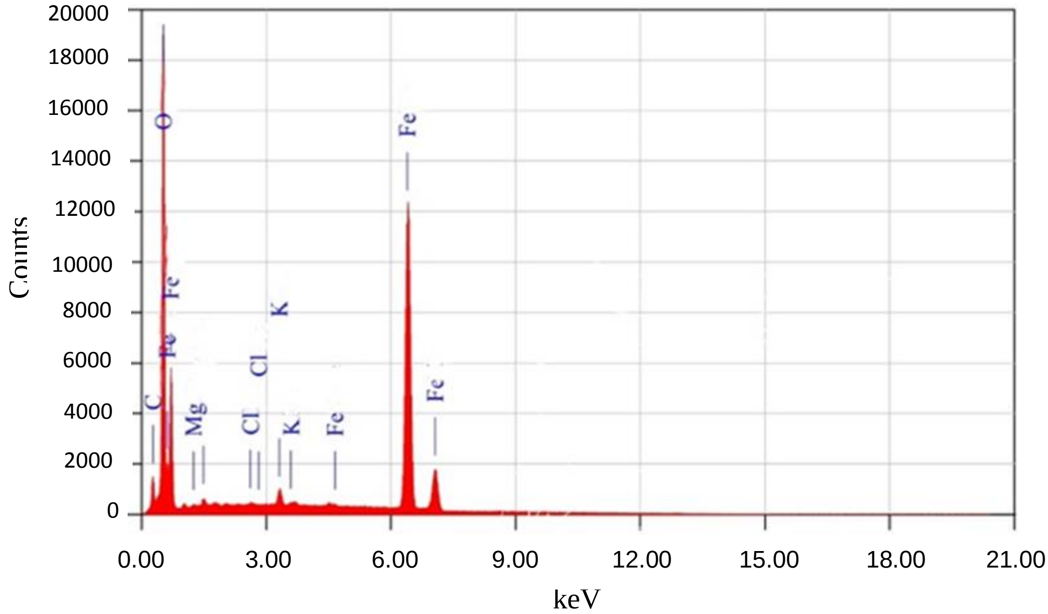


Figure 3. Energy dispersive X-ray EDX spectroscopy of iron oxide nanoparticle

Effect of Iron concentration in root (ppm)

Table 2 indicated that the amount of iron increased with the foliar fertilization method with an average of 1144.30 parts per million, significantly superior to the ground fertilization method with an average of 994.62 parts per million. The reason is that the soil bed has a reaction degree that tends to be basic. The amount of iron in the soil is 4.5 g kg $Soil^{-1}$ is reflected in the obstruction of iron absorption due to its formation of compounds that are not readily available to the plant in the soil, While iron is facilitated by the foliar spray method, it is the most appropriate method to compensate the plant in critical stages of growth in the event of soil problems (Alshaal and El-Ramady, 2017). The results showed a significant effect of fertilizer concentrations and the method of addition on the iron content in the fruit, which increased significantly with increasing concentrations in Table 2. The 2 mg nano fertilizer concentration gave the highest average of 1059.22 ppm; the difference was insignificant with the other nano concentrations. The concentration of the commercial chelated fertilizer, 1.5 mg, showed an average of 933.58 ppm, and it outperformed the control treatment, which gave the lowest average of 890.30 ppm. This is due to the effectiveness of the nano-fertilizers and the chelated ones, which contain the iron element in their composition, which facilitated and accelerated the process of its absorption in the leaves and the speed of its transfer from them. Which stimulated the formation of the chlorophyll pigment that is part of its composition (Khristozkova *et al.*, 2005). Thus, the rate of photosynthesis increases, which improves the state of vegetative growth of the plant, which is later reflected in good fruit growth, and the fruit contains essential nutrients, including iron (Strasser *et al.*, 1999). These particles exhibit properties of quantum mechanics, and quantum mechanical interactions become different from classical mechanical forces, allowing the propagation of unique physical and chemical properties due to the high surface area of the body and the presence of a high percentage of atoms on the surface. These interactions improve properties such as cation exchange capacity, diffusion, and ion adsorption. Due to the high surface area of nano fertilizers, they are able to retain a large amount of nutrient ions that are released in a slow manner similar to the crop requirements (Subramanian *et al*., 2015).

The double interference treatments showed significant superiority for this trait. The interaction treatment of concentration 2 mg * foliar fertilization gave the highest average, amounting to 1283.78 ppm, while the control treatment of interference * ground fertilization gave the lowest average, amounting to 831.67 ppm.

Table 2. Effect of biopreparation of nano - and chelated iron fertilizer and their interaction on the character of iron concentration (ppm)

Added concentrations (c)	Addition method (m)		Average concentrations (c)
	Ground fertilization	Fertilizing by spraying	
Control	831.67 d	812.67 d	890.3 c
Commercial 1.5 gm	901.20 c	1055.33 b	933.58a b
Nano 0.5 mg	1035.51 b	1196.65 a	1045.44a
Nawi 1 mg	1047.11 b	1252.89 a	1050.00 a
Nawi 1.5 mg	1051.56 b	1264.22 a	1057.89 a
nano 2 mg	1100.67 b	1283.78 a	1059.22 a
Average addition method (m)	994.62 b	1144.3 a	1017.37
LSD 0.05	LSD (m) 0.05 = 44.594		LSD 0.05
	LSD (c) 0.05 = 77.24		
	LSD (m*c) 0.05 = 109.233		

Root length (cm)

Table 3 shows significant differences for this trait in the application method, as the spray application method outperformed an average of 18.77 cm over the ground fertilization method, which gave an average of 17.10 cm. This is attributed to the fact that direct foliar feeding at the appropriate period of the growth stage helped increase the leaves' efficiency in absorbing the nutrients that contributed to division, expansion, and prolonging the duration of vegetative growth. The process of transferring the products of photosynthesis to the main root to continue its growth increased, which was reflected in an increase in the length of the root, which increased the surface area and abundance of root hairs, given that the effectiveness of the leaf is better than the root for absorption. Increasing the activity of meristematic tissues, cell division, and building amino acids such as tryptophan, which forms the basis for building the hormone auxin, which has the main role in cell division and expansion (Patil and Chetan, 2018). Table 3 shows a significant difference in root length with increasing concentrations of nano-fertilizer. The concentration of 2 mg gave the highest average of 20.82 cm. The difference was insignificant with the nano-concentration of 1.5 mg, which showed 20.31 cm, while the concentration of commercial fertilizer gave an average of 1.5 grams. It reached 16.05 cm compared to the comparison treatment, which gave the lowest average of 14.10 cm. Perhaps the reason for this is that providing sufficient food increases continuous longitudinal cell division due to the high effectiveness of the two fertilizer sources, which activate root growth by prolonging the growth period, stimulates the building of ATP, and facilitates the absorption process. The transfer of nutrients into the main root is reflected in an increase in root length. In addition, Apoplast roots are the main storehouse of nutrients in plants, especially iron, which facilitates the process of absorption and increased division, thus increasing root length (Strasser *et al.,* 1999).

The double interaction appears when treating the nano fertilizer concentration of 2 mg * spray fertilization with the highest average of 20.96 cm. The difference was not significant with the concentrations of 1.5 and 1 mg of nano fertilizer * spray fertilization, which gave 20.59 and 20.47 cm, respectively, compared to the control treatment of the interference * ground fertilization, which gave less. The average was 14.20 cm.

Table 3. Effect of bio-preparation of nano- and chelated iron fertilizer and their interaction on root length (cm)

Added concentrations (c)	Addition method (m)		Average concentrations (c)
	Ground fertilization	Fertilizing by spraying	
Control	14.2 d	15 b c	14.1 d
Commercial 1.5 gm	15.87 c	17.6 b	16.05 c
Nano 0.5 mg	17.80 b	18 b	17.98 b
Nawi 1 mg	17.97 b	20.47 a	18.3 b
Nawi 1.5 mg	18. 2 b	20.59 a	20.31 a
nano 2 mg	18.54 b	20. 96 a	20.82 a
Average addition method (m)	17.1 b	18.77 a	17.93
LSD 0.05(c)	LSD (m) 0.05 = 0.78		LSD 0.05
	LSD (c) 0.05 = 1.36		
	LSD (m*c) 0.05 = 1.92		

Root diameter (cm)

The results of Table 4 showed a significant response in increasing root diameter with spray fertilization. The spray treatment gave the highest average of 2.1 cm compared to the ground fertilization treatment, which gave an average of 1.71 cm. The reason is that despite the direct fertilization of soil particles that are supposed to help provide the root hairs with the necessary nutrients needed for rapid growth of the main root, but the negative linear relationship between iron fertilization and the soil content of carbonate minerals greatly affects its readiness for the plant, which hinders its absorption (Sharma *et al.*, 2004).

The data in Table 4 shows that the root diameter characteristic took the path of root length in the presence of significant differences with increasing nano fertilization concentration. The nano fertilizer concentration of 2 mg gave the highest average of 2.29 cm, and without a significant difference with the concentration of 1.5, which gave an average of 2.09 cm. The commercial fertilizer treatment at a concentration of 1.5 grams averaged 1.71 cm, while the control treatment gave an average of 1.53 cm. The reason is the superiority of the root length treatment at the highest nano concentration which gave an opportunity to provide the necessary food to increase transverse division and expand the number of cells, and what is known as the dynamic structure of plant parts (Wang and Li, 2008).The binary intervention also showed significant superiority, as the 2 mg nano concentration treatment * spray fertilization outperformed with the highest average amounting to 2.62 cm compared to the control treatment of the intervention * ground fertilization which gave the lowest average amounting to 1.60 cm. Fertilization helped accelerate the transfer of photosynthesis products from the source to the downstream, which increased the expansion of the sieve tubes as a result of filling them with necessary and sufficient nutrients, which was reflected in an increase in the thickness of the stem (Loddo and Gooding, 2012).

Table 4. The effect of biopreparation of nano- and chelated iron fertilizer and their interaction on root diameter (cm)

Added concentrations (c)	Addition method (m)		Average concentrations (c)
	Ground fertilization	Fertilizing by spraying	
Control	1.60 c	1.61 c	1.53 bc
Commercial 1.5 gm	1.63 c	1.88 b	1.71 b
Nano 0.5 mg	1.85 b	1.90 b	1.87 b
Nawi 1 mg	1.83b c	2.10 b	2.02a b
Nawi 1.5 mg	1.68b c	2.51 a	2.09 a
nano 2 mg	1.67 bc	2.62 a	2.29 a
Average addition method (m)	1.71 b	2.1a	1.92
LSD 0.05 (c)	LSD (m) 0.05 = 0.12		LSD 0.05
	LSD (c) 0.05 = 0.20		
	LSD (m*c) 0.05 = 0.28		

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Weight dry of root (g)

The results of the statistical analysis of Table 5 indicated that there were significant differences in the dry weight characteristic in fertilizer concentrations. The nano fertilizer was superior at a concentration of 1 mg with the highest average of 18.37 grams, while the commercial fertilizer gave an average of 13.74 grams, while the control treatment gave the lowest average of 9.86 grams. The same table indicated that the spray fertilization method was significantly superior to the ground fertilization method, as it gave an average of 12.06 grams with an average of 16.03 grams. The reason may be that foliar spraying increased the activity of photosynthesis and thus increased the outputs of this process of carbohydrates, vitamins and fats and increased the efficiency of the plant in absorbing nutrients that are transferred to plant parts such as leaves and stems during the vegetative growth period, which improves the process of pollination and fertilization and reduces ovarian abortion and thus improves the quality of the roots in addition to the superiority of iron absorption (El-Saady and Rakha, 2018), (Table 2). The double interaction had a significant effect on the dry weight trait, as the interaction treatment of spray fertilization at a concentration of 1 mg nano had the highest average of 23.13 g, while the interaction treatment of spray fertilization with the control had the lowest average of 9.47 g.

Table 5. The effect of biopreparation of nano- and chelated iron fertilizer and their interaction on dry weight (gm)

Added concentrations (c)	Addition method (m)		Average concentrations (c)
	Ground fertilization	Fertilizing by spraying	
Control	9.47 c	10.24 bc	9.86 c
Commercial 1.5 gm	12.91 b	14.57 b	13.74 b
Nano 0.5 mg	13.03 b	16.53 b	14.78 b

Nawi 1 mg	13.61 b	23.13 a	18.37 a
Nawi 1.5 mg	12.00 bc	16.02 b	14.01 b
nano 2 mg	11.36 bc	15.69 b	13.53 b
Average addition method (m)	12.06 b	16.03 a	14.05
LSD 0.05 (c)	LSD (m) 0.05 = 1.56		LSD 0.05
	LSD (c) 0.05 = 2.697		
	LSD (m*c) 0.05 = 3.81		

Carotene (mg g fresh weight^{-1})

The results showed in Table 6 that the spray fertilization method gave the highest average of 1.62 mg g fresh weight^{-1}, while the ground fertilization method gave 1.35 mg g fresh weight^{-1}. Perhaps the reason for this is that increasing carotene is linked to increasing the characteristics of dry weight. (Table 5) resulting from the effectiveness of iron fertilizer in stimulating the formation of proteins, cytochrome, auxin, and chlorophyll, and the activation of various enzymes, including nitrite and nitrate reductase, ferredoxin, cytochrome transfer enzyme, catalase, and peroxidase, and its formation of leguminous hemoglobin (Focus, 2003). The results (Table 6) showed no significant differences in the carotene pigment concentrations of the two fertilizer sources. The 0.5 mg nano fertilizer concentration gave the highest average of 1.72 mg g fresh weight^{-1}, while the commercial fertilizer concentration gave an average of 1.52 mg g fresh weight^{-1}. While the control treatment gave the lowest average of 1.20 mg g fresh weight^{-1}, and the reason is that iron increases the pigments present in the plant, such as chlorophyll, which is important for photosynthesis, and also increases the carotenoid pigments that protect the photosynthesis process from photo-oxidation, thermal stress, and free radicals (Gomathi and Rakkiyapan, 2011).

The effect of the binary intervention on this characteristic, as the intervention treatment of the nano concentration of 0.5 mg * spray fertilization excelled with the highest average of 1.79 mg g fresh weight^{-1}, while the intervention treatment of the control * ground fertilization gave the lowest average of 1.29 mg g fresh weight^{-1}.

Table 6. The effect of biopreparation of nano - and chelated iron fertilizer and their interaction on the character of carotene (mg g fresh weight^{-1})

Added concentrations (c)	Addition method (m)		Average concentrations (c)
	Ground fertilization	Fertilizing by spraying	
Control	1.29 a	1.31 a	1.20 a
Commercial 1.5 gm	1.43 a	1.65 a	1.52 a
Nano 0.5 mg	1.52 a	1.79 a	1.72 a
Nawi 1 mg	1.31 a	1.71 a	1.64 a
Nawi 1.5 mg	1.28 a	1.67 a	1.56 a
nano 2 mg	1.25 a	1.59 a	1.49 a
Average addition method (m)	1.35 a	1.62 a	1.52
LSD 0.05 (c)	LSD (m) 0.05 = 0.47		LSD 0.05
	LSD (c) 0.05 = 0.81		
	LSD (m * c) 0.05 = 1.14		

Percentage of dissolved solids TSS (%)

Table 7 indicated that spray fertilization was significantly superior to ground fertilization in this characteristic and gave an average of 8.79 %, while the ground fertilization treatment gave an average of 8.16 %, Table (7) shows that there is a significant difference in the percentage of dissolved solids at the nano concentration of 2 mg with an average of 9.91 %, while the commercial concentration of 1.5 mg gave an average of 8.17 %, and the control treatment gave the lowest average of 7.83 %. The reason is that fertilizers increase the absorption of major elements. The microelements necessary to build chlorophyll stimulate physiological processes and increase the activity of enzymes that decompose solids, thus increasing their concentration in their estuaries (Eskandari, 2011).

Table 7 also shows that the binary intervention gave significant differences in this characteristic, and the intervention treatment of nano concentration 2 mg * spray fertilization treatment outperformed the treatment with the highest average amounting to 10.40 %, while the comparison treatment of the interaction * ground fertilization and spraying gave the lowest average amounting to 7.00 % for the two treatments.

Table 7. The effect of biopreparation of nano- and chelated iron fertilizer and their interaction on the character of the percentage of dissolved solids (%)

Added concentrations (c)	Addition method (m)		Average concentrations (c)
	Ground fertilization	Fertilizing by spraying	
Control	7.00 c	7.00 c	7.83b
Commercial 1.5 gm	7.20 c	7. 23 c	8.17 b
Nano 0.5 mg	8.3b	8.61 b	9.33 a
Nawi 1 mg	8.5b	9.68 a	9.33 a
Nawi 1.5 mg	8.61b	9.81a	9.65 a
nano 2 mg	9.33 ab	10.4 a	9.91 a
Average addition method (m)	8.16 b	8.79 a	9.04
LSD 0.05 (c)	LSD (m) 0.05 = 0.43		LSD 0.05
	LSD (c) 0.05 = 0.75		
	LSD (m*c) 0.05 = 1.06		

Conclusions

FeO-NPs were prepared using the green synthesis method using lemon plant extract. It is a friendly method for the environment within green chemistry based on plant materials with chemicals It is non-toxic, inexpensive, takes place in one step and at a low temperature, and it produces excellent results. Iron oxide particles are abundant in homogeneous nano-sized dimensions and can therefore be used for commercial purposes.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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