Article

Effect of nano-biofortification with iron on yield and bioactive compounds in cucumber

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Abstract

Iron (Fe) is an indispensable micronutrient for living beings. However, and despite the fact that it is one of the most abundant metals in the earth's crust, there is low availability for crops, causing a deficit in the diet of around two million people in the world. Nano-biofortification can mitigate this deficiency since its application in crops improves the biosynthesis of bioactive compounds and promotes their bioaccumulation. The objective of this research was to evaluate the effect of foliar application of Fe nanoparticles (Fe_2O_3 NPs) on the yield and biosynthesis of bioactive compounds in cucumber fruits. Four treatments were applied via foliar: 0, 50, 75 and 100 mg L^{-1} of Fe_2O_3 NPs. Foliar spraying with Fe_2O_3 NPs improved the yield and biosynthesis of bioactive compounds in cucumber fruits, increasing the yield by 38.99%, the biosynthesis of compounds by 30.18% and an increase of 23.26% of Fe in fruits. Foliar spraying of Fe_2O_3 NPs is an alternative to increase agricultural production, decreasing Fe deficiency, while improving the biosynthesis of bioactive compounds in order to ensure food and nutrition security.

Keywords: *Cucumis sativus* L, bioactive compounds, nanoparticles.

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Introduction

Iron (Fe) is an indispensable micronutrient for living beings; in plants it participates in photosynthesis, respiration, enzymatic processes, conversion of the oxidation state, cell cycle, oxygen transport and storage, synthesis of hormones and DNA (Zhou and Tan, 2017). Although high concentrations of Fe are found in the earth's crust, there are deficiencies of this element in crops since it is not always in forms available to plants (Konate *et al*., 2018).

This causes that plant-based foods have low concentration of this trace element, generating health problems to two million people in the world, with anemia being the main nutritional pathology (43% children, 40% pregnant women and 33% non-pregnant women) (Lowry *et al*., 2019). The daily intake to prevent Fe deficiency is 0.27 to 10 mg day^{-1} for children and 11 to 27 mg day^{-1} for adults (Abbasifar *et al*., 2020). The alternatives used to mitigate this situation include the use of Fe supplements and fortified products, which have failed to reduce the deficit of this trace element, mainly due to the cost of the products (Tripathi and Mishra, 2020).

The use of metal nanoparticles has shown increases in germination, resistance to stress, increased absorption of nutrients and plant growth (Yusefi-Tanha *et al*., 2020). In addition, they reduce soil pollution and degradation caused by the excessive use of chemical fertilizers (Wang *et al*., 2018). Fe nanoparticles (Fe NPs) can increase crop production and quality (Hu *et al*., 2017) as they are highly effective when sprayed on plants, thus reducing the impact of chemicals on the environment (Sega *et al*., 2019).

Rui *et al*. (2016) states that Fe NPs can replace Fe fertilizers in *Arachis hypogaea* plants. Several studies show that iron oxide NPs (Fe_2O_3 NPs) increase biomass and yield up to 48% in *Glycine max* plants (Sheykhbaglou *et al*., 2010). As well as sugars, proteins and chlorophyll in *Cucumis melo* L (Wang *et al*., 2016); they also increase the level of phytohormones in *Oryza sativa* (Li *et al*., 2021). Some studies have reported the interesting participation of Fe in the generation of the hydroxyl (OH) radical through the Fenton reaction that causes damage in plants (Konate *et al*., 2018).

It also affects the content of Malondialdehyde (MDA), activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), as well as in other bioactive compounds in the plant (Moradbeygi *et al*., 2020). Currently, the positive effect of foliar spraying of Fe NPs on the growth, development and quality production of some crops has been reported; however, many metabolic pathways have not been fully understood (Xiong *et al*., 2017).

Therefore, further research is needed to understand the effect of NPs on agricultural production (Shakoor *et al*., 2022), because their effect depends on the chemical species used, the concentration, size of NPs, biosynthesis, the route and stage of application (Lu *et al*., 2020). On the other hand, the cultivation of cucumber (*Cucumis sativus* L.) occupies 10% of the total area of greenhouses in Mexico (Valencia *et al*., 2018). In addition, its fruits contain various organoleptic properties, which

are a source of minerals, fiber, vitamins and compounds with antioxidant activity (Preciado-Rangel *et al*., 2019). Therefore, the objective of this study was to evaluate the effect of foliar application of Fe_2O_3 NPs on yield and nutraceutical quality in cucumber cultivation.

Materials and methods

Study site and vegetative material

The experiment was established in a greenhouse located at the Antonio Narro Autonomous Agrarian University, Laguna Unit. Cucumber seeds of the Poinsett 76 variety (Southhern Start Seeds), of indeterminate growth, were used. The crop was established by direct sowing in black polyethylene bags with a capacity of 10 L, the substrate used was sand and perlite in an 80:20 ratio. The crop was trained to a single stem, irrigation and nutrition was with the Steiner (1961) nutrient solution (SN), which had an electrical conductivity of 2 dS m^{-1} and a pH of 5.5.

Characterization of nanoparticles

The iron oxide nanoparticles (Fe_2O_3 NPs) were donated by the Mexican company Investigación y Desarrollo de Nanomateriales S.A. de C.V. The NPs used were synthesized by wet chemical method in the form of iron oxide nanoparticles (maghemite: γ-Fe_3O_3), the size of the nanoparticles has an average of less than 50 nm, a purity level of 99.7% and a density of 5.81 g cm^{-3} (Gutiérrez-Ruela *et al*., 2021).

Experimental design and treatments

The experimental design used was random blocks. The treatments consisted of the foliar application of Fe_2O_3 NPs (0, 50, 75 and 100 mg L^{-1}) with six repetitions. Foliar application of NPs was performed four times in the early morning hours every 15 days after planting. The harvest of the fruits was carried out from 70 days after planting. The yield, the commercial and nutraceutical quality were evaluated, in addition, the accumulation of Fe in the fruits was quantified.

Variables evaluated

The fruits of ten plants of each treatment were harvested, counting the weight, length and diameter of the fruits. Subsequently, eight fruits were randomly selected from each treatment for the analysis of commercial and nutraceutical quality.

Biomass, yield, total soluble solids and firmness

The yield (YI) was determined by harvesting the total fruits per plant, using a digital scale (VE-CB2000, VELAB), expressing the yield in kilograms per plant. The firmness of the fruit (FF) was measured using a penetrometer (FHT200, Extech instruments) with the plunger of eight mm in four fruits of each repetition, the fruits were placed on a flat and hard surface, three penetrations were made, which were averaged and recorded as maximum compressive force (N). The soluble solids

of the fruits (TSS) were quantified in three fruits per repetition to make a composite sample, three drops of the juice were placed in a refractometer (MASTER-53α, ATAGO®) to be averaged and the reading expressed as Brix was obtained.

Chlorophyll index

The relative chlorophyll content index was measured at 30 days after transplantation using the Minolta SPAD-502 equipment. Five fully expanded leaves (without physical damage and avoiding veins) were taken from each plant and four plants per treatment in the flowering and harvesting stages. The results were expressed in SPAD units.

Photosynthetic pigments

Photosynthetic pigments were determined according to the methodology reported by Palacio-Márquez *et al*. (2021). Foliar samples of seven mm in diameter were taken during flowering, avoiding veins, 0.125 g was weighed in test tubes. Afterwards, 10 ml of methanol was added and left to react for 24 h in the dark. Finally, it was read at an absorbance of 666, 653 and 470 nm on a UV-Vis spectrophotometer (VE-5100UV, VELAB). The results were expressed in mg g^{-1} of fresh weight (FW) and calculated with the following formulas: Chla= [15.65(A666) - 7.34(A653)]. Chlb = [27.05(A653) - 11.21(A666)].

Preparation of extracts for nutraceutical quality

The extraction of the samples for the determination of total Phenols, Flavonoids and antioxidant activity was carried out following the methodology reported by (Cardeño, 2007), 2 g of fresh sample was homogenized with 10 mL of 80% ethanol (v:v). The homogenized sample was poured in test tubes, and they were stirred for 30 s in a Vortex, left to stand for 24 h in a rotary shaker (HZ-300) at 70 rpm and at room temperature. Extractions were performed by treatment and each repetition in triplicate.

Bioactive compounds

Total phenols

The phenolic content was determined by a modification of the colorimetric method of Folin-Ciacalteau (Singleton *et al*., 1999). Fifty microliters were diluted in 3 ml of distilled H_2O, then 250 µl of Folin-Ciocalteau was added, a vortex was used for 30 s, and they were left to react for 3 min. Subsequently, 750 µl of Na_2CO3 (7.5%) and 950 µl of distilled H_2O were added. They were quantified in triplicate per repetition in the spectrophotometer (VE-5100UV, VELAB) with absorbance at 765 nm; a standard solution of gallic acid was prepared, the results were expressed in mg GA equivalents 100 g^{-1} of fresh weight (FW).

Total flavonoids

Total flavonoids were determined by a reported colorimetric method (Singleton *et al*., 1999). Two hundred fifty microliters of extract were taken, mixed with 1.25 ml of distilled water and 75 µl of $NaNO_2$ (5%). They were left to stand for 5 min, 150 µl of $AlCl_3$ (10%) was added (Sigma-Aldrich, St. Louis, MO, USA). Then 500 µl of NaOH 1M, 275 µl of distilled water were added and stirred vigorously. The samples were quantified in triplicate per repetition in the spectrophotometer (VELAB, VE-5100UV) with absorbance at 510 nm, a standard solution of quercetin was prepared. The results were expressed in mg QE g^{-1} FW.

Antioxidant activity

Antioxidant capacity was determined using the *in vitro* $DPPH^+$ (Aldrich) method according to Brand-Williams *et al*. (1995). A solution of $DPPH^+$ (Aldrich) in ethanol was prepared at a concentration of 0.025 mg ml^{-1}. Fifty microliters of the extract were mixed with 1.95 µl of the $DPPH^+$ solution, then they were left to stand for 30 min. They were quantified in triplicate per repetition in the spectrophotometer (VE-5100UV, VELAB) with absorbance at 517 nm. The results were expressed in µM Trolox equivalent 100 g^{-1} FW.

Vitamin C

Vitamin C (ascorbic acid) was determined by the method according to Hernández-Hernández *et al*. (2019). Ten grams of pulp were crushed with 10 ml of hydrochloric acid (2%), then filtered and measured with 100 ml of distilled H_2O. Ten milliliters of the filtered solution were taken and titrated using a solution of 2,6 dichlorophenolindophenol (1×10^{-3} N). The concentration is expressed in mg 100^{-1} FW.

Total proteins

For the determination of the total protein, 3 g of fresh sample was homogenized in a mortar placed on ice, adding 0.1 g of polyvinylpolypyrrolidone (PVP) and 3 ml of a sodium-potassium solution (100 mM, pH 7 and 0.1 mM of EDTA) as an extraction buffer, then centrifuged at 1200 rpm at 5 °C for 5 min. The supernatant was used to determine total protein by the Bradford (1976) method using bovine serum albumin as standard. The results were expressed in mg g^{-1} FW.

Leaf iron content

The content was determined by acid digestion. Zero point five grams of leaf tissue and 1 g of dried pulp were weighed in a porcelain crucible, then placed in a furnace muffle (Thermo Scientific, Thermolyne) for 4 h at 600 °C, which began to be counted from the temperature of 200 °C. At the end of the time, the samples were pre-calcined in a burner, finally 5 ml of HCl at a concentration of 20% was added and filtered using ultrafine filter paper (Whatman) in volumetric flasks of 50 ml, measuring with deionized water. Iron concentration was measured with an Inductively Coupled Plasma Optical Spectrophotometer (ICP-OES) series 700 Agilent Technologies. The results were expressed in mg kg^{-1} dry matter (DM).

Statistical analysis

Data on the response variables were analyzed by analysis of variance and comparison of means with the Tukey test ($p \leq 0.05$) using the statistical package Statistical Analysis System Institute (SAS) version 9.3.

Results and discussion

Yield

Foliar spraying of Fe_2O_3 NPs affected the yield and quality of cucumber fruits (Table 1). With the application of 75 mg L^{-1}, the greatest length of the fruit, TSS and YI were achieved, exceeding the control by 38.99%. Drostkar *et al*. (2016) applied Fe NPs in the cultivation of *Cicer arietinum*, as a result an increase in YI was observed compared to treatments without the application of NPs. The above results are explained by the size of the nanoparticle, which facilitates its absorption, as well as its adequate concentration for the metabolism of the plant (Kandpal *et al*., 2014).

Table 1. Yield, fruit length, firmness, total soluble solids, SPAD, chlorophyll and total protein in cucumber fruits, due to the foliar application of Fe_2O_3 NPs.

Fe_2O_3 NPs (mg L^{-1})	Yield (kg ha^{-1})	Fruit length (cm)	Firmness (N)	TSS (°Brix)	SPAD	Total chlorophyll (μg ml^{-1})	Total protein (mg ml^{-1})
0	2.18 c	16.76 c	0.21 c	3 c	29.93 c	30.32 c	0.023 c
50	2.55 b	20.11 a	0.38 a	4.16 ab	35.01 b	39.07 b	0.027 bc
75	3.03 a	20.66 a	0.3 b	4.68 bc	37.76 b	42.99 b	0.036 ac
100	2.3 b	18.71 b	0.25 b	3.83 abc	40.98 a	48.73 a	0.039 a

Values with equal letters in each column are statistically similar (Tukey $p \leq 0.05$).

Therefore, the YI and growth of the fruit could be affected by the NPs, altering its biological activities, causing the variations (Kanwar *et al*., 2019). Therefore, further studies are needed to explain the physiological effects of the use of metal NPs, at the same time, it is important to optimize the dose of Fe NPs considering the impact that this element has on the nutrition and development of the crop (Yuan *et al*., 2018; Shakoor *et al*., 2022).

Commercial quality of fruits

The largest size of the cucumber fruits was obtained with the foliar application of 75 mg L^{-1} of Fe_2O_3 NPs, exceeding the treatment without application of NPs by 23.26% (Table 1). Our results were similar within the ranges for commercialization. The adequate size of cucumber fruits for their commercialization fluctuates between 20 and 25 cm, it should not be less than 15 cm in length, while the equatorial diameter should be from 5 to 5.7, not exceeding 6 (Valencia *et al*., 2018).

On the other hand, with a higher dose of NPs (100 mg L^{-1}), the size of the fruit's decreases, this could be due to the bioavailability and high solubility of Fe_2O_3 NPs, which as long as the dose of NPs is higher, the absorption and ease of transport of nutrients in the plant could be lower (Moradbeygi *et al*., 2020). Regarding firmness and TSS, these were positively affected by foliar spraying of Fe_2O_3 NPs (Table 1), obtaining the greatest firmness in the fruit with 50 mg L^{-1} and in TSS with the dose of 100 mg L^{-1}. According to Valencia *et al*. (2018), the average of 4.76 °Brix is the appropriate standard for cucumber fruit quality.

The increase in TSS content, according to Rawat *et al*. (2017), is due to the foliar application of Fe_2O_3 NPs, which induces a better efficiency in the regulation of the Rubisco enzyme, causing an increase in the chlorophyll content, therefore, increasing the photoassimilation in the fruits due to the range of photosynthesis, obtaining a better growth and bioaccumulation.

SPAD values and total chlorophyll

Foliar spraying of Fe NPs significantly affected the chlorophyll index and total chlorophyll. With the application of 100 mg L^{-1}, the control treatment was exceeded by 35.24% in SPAD values and by 60.71% in total chlorophyll (Table 1). Rui *et al*. (2016) has reported an increase in SPAD units by increasing the dose of Fe_2O_3 NPs and Wang *et al*. (2019) has reported an increase in the total chlorophyll content with the application of Fe NPs, which means that, according to the dose applied, the deficiency of Fe in plants can be quickly corrected. Sharma *et al*. (2012) also reported an increase in the chlorophyll content in *B. juncea* treated with 100 mg L^{-1} of metal NPs.

It is evident that Fe has a direct influence on the concentration of chlorophyll, and this is affected depending on the concentration of NPs (Kanwar *et al*., 2019). However, it has been shown that the trend of chlorophyll content through the stages is in a constant decrease-increase-exchange (Wang *et al*., 2019). So, according to Rawat *et al*. (2017), depending on the concentration, stage of the plant and the time of application, the concentration of compounds such as chlorophyll content is promoted.

Total proteins

Leaf spraying of Fe_2O_3 NPs significantly affected the total protein content (Table 1). With the dose of 100 mg L^{-1}, the values obtained without the application of Fe_2O_3 NPs were exceeded by 69%. It has been reported in *Pisum sativum* with the application of Fe NPs, compared to control treatment (Gutiérrez-Ruelas *et al*., 2021). Total soluble proteins are produced by plants to adapt to the environment (Shang *et al*., 2013), in turn they are produced when there is environmental stress, increasing the concentration of proteins or antioxidants, the latter are key to eliminating free radicals (Wang *et al*., 2019).

Bioactive compounds

With the foliar application of Fe_2O_3 NPs, an increase in the biosynthesis of bioactive compounds was obtained (Figure 1). Phenolic compounds and flavonoids are recognized for their benefits on human health (Elkhatim *et al*., 2018). In the present study, the highest concentration of phenolic compounds was achieved with doses of 50 mg L^{-1}, exceeding the control by 27.12% (Figure 1a). While flavonoids obtained the highest concentration with 75 mg L^{-1}, exceeding the control by

30.18% (Figure 1b). Similar results are reported in studies by Mogazy *et al*. (2022) in *Fragaria ananassa* and *Cucumis melo* L (Wang *et al*., 2019). In *Arachis hypogaea*, a higher antioxidant activity is reported (Rui *et al*., 2016); however, high concentrations of Fe NPs reduce non-enzymatic antioxidants.

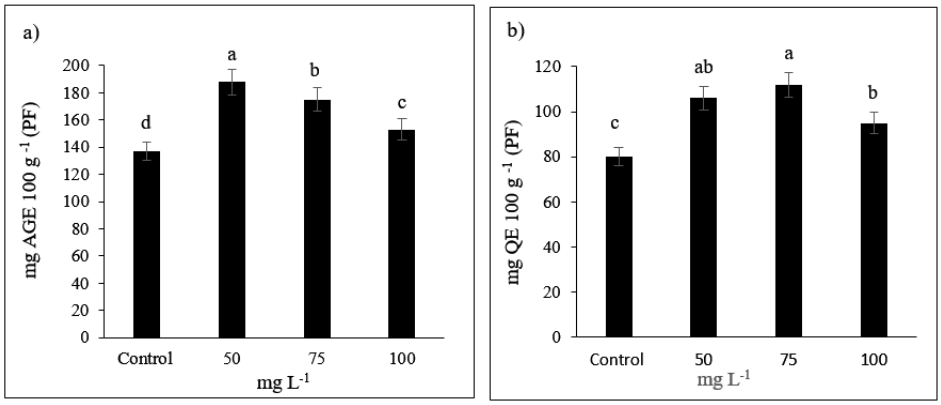

Figure 1. Effect of Fe_2O_3 NPs on the content of phenolic compounds (a), flavonoids (b), in cucumber fruits. Columns with different letters differ statistically from each other (Tukey $p \leq 0.05$).

The above results can be explained by the regulatory functioning of iron in: 1) catalyst of overproduction of ROS such as H_20_2 by Haber-Weiss, therefore, affecting the enzymatic line of defense (CAT, POD and SOD) and in the Fenton reaction (Mosa *et al*., 2018); 2) formation of MDA, lipid peroxidation (Konate *et al*., 2018); 3) interaction in cuticular wax due to foliar application of Fe NPs, as well as genes involved in intracellular wax synthesis and transport (Hu *et al*., 2017).

The response of crops to Fe NPs depends on the dose as this can affect various metabolic pathways (Yuan *et al*., 2018). Other reports indicate that the positive effect of Fe NPs is most evident when there is stress (Moradbeygi *et al*., 2020). Therefore, Fe is considered to be a dose-dependent element of regulation in crops, allowing metabolic and physiological adaptation mechanisms as long as it does not exceed its toxicity limit, since it functions as a response to oxidative stress caused by radicals under stress conditions (Hasanuzzaman *et al*., 2020).

Antioxidant capacity and vitamin C

Foliar spraying of Fe_2O_3 NPs positively affected antioxidant capacity and ascorbic acid (Figure 2). The highest concentration of antioxidant capacity was obtained with the application of 50 mg L^{-1} of Fe_2O_3 NPs, 39.18% higher than the control; with this same dose, the highest content of vitamin C was obtained, exceeding the content in the fruits of the control by 50%. Wang *et al*. (2019) reported a 46.95% increase in vitamin C content. Fe NPs produce oxidative stress, which activates a number of antioxidant mechanisms, stimulating the formation of ROS (Konate *et al*., 2018).

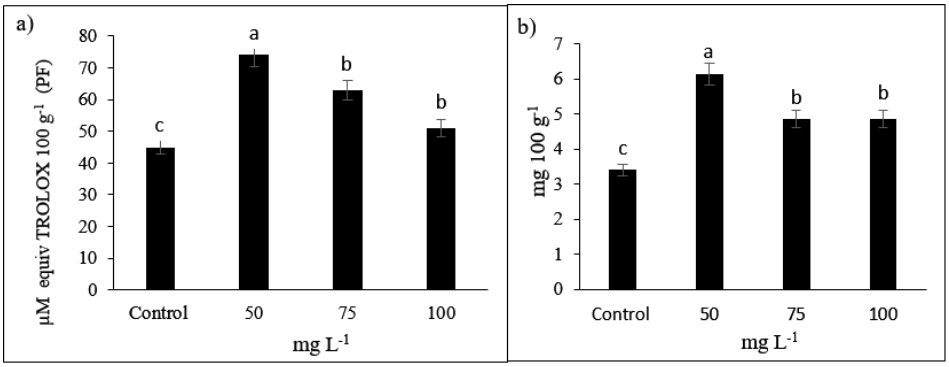

Figure 2. Effect of Fe_2O_3 NPs on antioxidant activity (a) and ascorbic acid content (b), in cucumber fruits. Columns with different letters differ statistically from each other (Tukey *P*≤ 0.05).

Fe content

Foliar spraying of Fe_2O_3 NPs significantly increased the foliar content of Fe in the cucumber (Figure 3). The highest concentration of Fe in leaves and fruit was found with the dose of 100 mg L^{-1}, exceeding the control by 52.3% and 71.37%. Foliar spraying of 100 mg L^{-1} Fe_2O_3 NPs in *Citrus maxima* is reported to have increased malondialdehyde content by 44.7% (Hu *et al*., 2017), Fe content by 103% in *Raphanus sativus* (Shakoor *et al*., 2022).

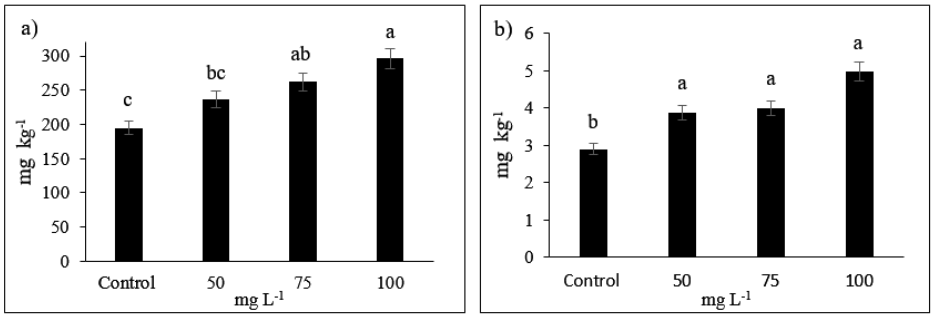

Figure 3. Mineral content of iron in leaf (a) and fruit (b) with the application of Fe_2O_3 NPs. Columns with different letters differ statistically from each other (Tukey *P*≤ 0.05).

Therefore, the above results are due to the fact that most NPs are trapped in the surface wax, promoting the formation of bunches and agglomerates (Hu *et al*., 2017). Therefore, the spraying of Fe_2O_3 NPs affects the development of the plant, these impacts are associated with the application of the dose and its integration into its foliar system (Lu *et al*., 2020). The nano-biofortification of cucumber is an alternative to complement human nutrition by increasing the Fe content in the fruits, which can be used by humans for energy production, oxygen utilization/transport and cell proliferation (Lynch *et al*., 2018).

The doses used could help meet the daily requirements of 7.8-10 mg day^{-1} for infants, adolescents of 11-15 mg day^{-1} and adults of 10-20 mg day^{-1} (Blanco-Rojo and Vaquero, 2018). Avoiding cardiovascular risks, chronic diseases, reducing cases of anemia and mortality (Blanco-Rojo and Vaquero, 2018).

Conclusions

Foliar application with Fe_2O_3 NPs improved the yield, the content of bioactive compounds and their bioaccumulation, as well as the content of Fe in cucumber fruits. The highest yield and biosynthesis of bioactive compounds was achieved with the treatments of 50 and 75 mg L^{-1} of Fe_2O_3 NPs 50, while the highest concentration of Fe in leaves and fruits was achieved with 100 mg L^{-1}. The foliar spraying of Fe_2O_3 NPs is an alternative to increase agricultural production and biosynthesis of bioactive compounds, in addition to promoting their bioaccumulation of Fe in cucumber fruits.

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