



Nano-iron induces growth and nutrient accumulation on bean plants (*Phaseolus acutifolius* A. Gray) under tropical conditions

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Abstract

Purpose Iron deficiency in soils worldwide affects the growth and development of edible crops, exacerbated in agricultural lands with little or no sustainable management. In the last decade, interest has been shown in using nanoparticles (NPs) as nanofertilizers or biostimulants to promote morphological and biochemical parameters in Fabaceae-family crops and various edible crops.

Methods The effect of Fe NPs (Fe_2O_3 and Fe_3O_4) as soil-applied biostimulants was investigated on growth parameters and mineral uptake in roots, stems, and leaves of escumite bean (*Phaseolus acutifolius* A. Gray). The concentrations used were 100, 200, 300, and 400 mg Fe NPs kg^{-1} dry soil and a control treatment (no Fe NPs).

Results The results showed that low concentrations of Fe NPs (100, 200, and 300 mg kg^{-1}) as biostimulants improved plant growth, and fresh and dry biomass of stems and leaves. In contrast, high concentrations (400 mg kg^{-1}) of Fe NPs decreased most of the parameters evaluated. Moreover, Fe NPs promoted the uptake of P, Ca, Mg, Fe, Mn, and Zn in roots.

Conclusions The application to the soil at low concentrations of Fe NPs (100, 200, and 300 mg kg^{-1}) are effective in stimulating plant growth of beans and can be used to promote nutrient uptake from the soil to the roots and leaves. Although it is the first work that is tested in escumite bean plants, it is necessary to continue with the research work, specifically in the field, to evaluate physicochemical parameters, yield, and quality of the grain.

Keywords Agronanotechnology · Holistic approach · Iron deficiency · Nanosized fertilizer · Nutrients uptake

1 Introduction

Nanoscience and nanotechnology are technologies that comprise the control and understanding of matter at the nanoscale. Nanomaterials and nanometric-sized nanoparticles are defined as substances with at least one dimension

in the range of 1 and 100 nm (An et al. 2022). Specifically, iron NPs (Fe NPs) applied to the environment have been used for soil or water remediation (Jabbar et al. 2022; Roberto et al. 2020). However, it has also shown interest in the agricultural area since benefits were reported on morphological and biochemical parameters in various edible crops (Juárez-Maldonado et al. 2019; Landa 2021).

Iron microelement is essential for plants, participate in seed germination, growth, development, and nutrient supply, improve plant stress tolerance, acts as a cofactor of enzymes, and is involved in the process of photosynthesis (Liu et al. 2016; Schmidt et al. 2020; Zia-ur-Rehman et al. 2018). Nevertheless, despite the high total concentrations of iron in soils, it is an element that undergoes oxidation and precipitates into compounds of low solubility (ferrous and ferric states), limiting its availability for plants. Therefore, to meet the need for iron, the farmers use conventional fertilizers, which have caused losses due to runoff and leaching. Under this context, in the last 10 years, Fe NPs have

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been considered potential agents for agriculture as fertilizers or biostimulants, through efficient and controlled delivery (Kopittke et al. 2019). Authors suggest that its effectiveness results from its characteristics such as size, shape, high surface/volume ratio, and catalytic and magnetic properties (Huang et al. 2020; Juárez-Maldonado et al. 2019).

Biostimulation is described as a biological phenomenon in organisms in which their cells interact with external impulses or stimuli. Therefore, biostimulants based on metallic and non-metallic NPs have been proposed for agricultural production and improving abiotic stress tolerance (Juárez-Maldonado et al. 2019; Mannan et al. 2023). To a large extent, metallic NPs have been used to modify the nutraceutical composition and quality of plants and consequently promote the development of edible plants, yield, and fruit quality. In addition, these nanometric particles also favor the absorption of nutrients. However, biostimulation will be beneficial if the NMs are in an adequate range or at low concentrations (Juárez-Maldonado et al. 2019).

The stimulating effects of Fe NPs in various leguminous plants have been documented at low concentrations (1 to 300 mg kg⁻¹ of dry soil; Palmqvist et al. 2017; Raju et al. 2016). For instance, in soybean cultivation (*Glycine max* L.) Fe NPs at a concentration of 30 mg L⁻¹ improved growth, shoot and nodule biomass, and biochemical parameters compared to conventional fertilizers (Cao et al. 2022). Also, peanut plants (*Arachis hypogaea* L.) improved morphological, physiological, and biochemical traits and yield at 1000 mg kg⁻¹ of Fe₂O₃ NPs (Rui et al. 2016). In contrast, some reports have demonstrated that at high concentrations of nanometric Fe (500, 700, 1000 mg kg⁻¹ or higher), the effects are inhibitory (Pérez-Hernández et al. 2020), mainly on germination parameters and growth in several crops such as maize (*Zea mays* L.) or bean (*Phaseolus vulgaris* L.) (Wang et al. 2021; Sun et al. 2019). In bean crops under the natural soil, authors found that Fe NPs increased plant height, root size, and dry and fresh biomass compared to the control (El-Sayed et al. 2023). Another study under hydroponic conditions revealed that Fe NPs promoted stem and root elongation (Sun et al. 2019). However, researchers have discussed the forms of application and means of evaluation since the absorption of NPs, either by the leaves or roots, influences the effectiveness in plants. The plant species, varieties, evaluation time, culture media, and properties of the NPs are factors that determine the action potential of the NPs on edible plants (Juárez-Maldonado et al. 2019; Pérez-Hernández et al. 2020).

In other contexts, few works have evaluated the effect of Fe NPs on the absorption and accumulation of nutrients in roots, stems, and leaves. Feng et al. (2022) reported that wheat plants that grew in soil at concentrations of 200 and 500 mg L⁻¹ of Fe₃O₄ NPs improved growth and the content of leaves P, K, and Fe. Therefore, the authors conclude that supply of nanometric Fe is a viable option to improve

the photosynthetic process of plants and increase nutrient content. In this line, authors have suggested that Fe NPs in edible plants increase the growth and nutrition of crops. Even so, there are few studies evaluated in tropical conditions and with local plants using Fe NPs.

Beans (*Phaseolus* sp.) are one of Mexico's most important foods, economic, social, and cultural crops. Nevertheless, there is the species *P. acutifolius*, commonly known as escumite bean, a species with low economic demand for central and northern Mexico (Mwale et al. 2020). For the tropical zones of Chiapas and Oaxaca, it has economic and nutritional acceptance, being one of the most consumed varieties at the beginning of each year. Drought and low soil fertility have been identified as mean problems regarding the production of this bean, the latest a result of intensive agricultural activities. For this, we hypothesize that, compared to the control, concentrations between 100 and 400 mg of Fe NPs kg⁻¹ of dry soil positively affect morphological characteristics and cause changes in the concentration of other nutrients in stems, leaves, and roots in plants. Therefore, this research, for the first time in the Soconusco region, Chiapas, Mexico, is considering the application of Fe NP as a biostimulant for the growth of bean plants under field conditions. Under the previous context, the present research aimed to evaluate and understand how Fe NPs affect plant growth and content nutrients 35 days after the emergence of bean plants.

2 Materials and methods

2.1 Study site

The experiment was carried out for 35 days in the experimental field of the Autonomous University of Chiapas (UNACH), Faculty of Agricultural Sciences, Huehuetán, Chiapas, México. The photoperiod was 12 h from April to May 2022, with an average temperature of 35 ± 2.5 °C, maximum and minimum temperature of 33.06 ± 0.29 °C, 24.67 ± 0.09 °C in April, and 30.11 ± 0.30 °C, 24.61 ± 0.11 °C by May. The total precipitation was 101 mm and 116 mm in April and May, respectively. The geographical location of the study site is 15°00'30.05" N, 92°23'55.07" W), located 35 m above mean sea level.

2.2 Biologic material and nanoparticles

Seeds of *P. acutifolius* were provided by the UNACH. The soil was collected from the experimental zone of the Faculty of Agricultural Sciences of UNACH. It was dried at room temperature and sieved (2 mm) before being placed in black-nursery bags. The texture is a slit loamy (82.7%, silt; 11%, clay; 6.24%, sandy). The pH was 5.5, electrical conductivity

(EC) was 0.05 dS m^{-1} , and organic matter (OM) was 2.85%. In addition, we determined the content of N (0.13%), P (20.6 mg kg^{-1}), K ($0.26 \text{ meq } 100 \text{ g}^{-1}$), Ca ($26 \text{ meq } 100 \text{ g}^{-1}$), Mg ($1.86 \text{ meq } 100 \text{ g}^{-1}$), Fe (45 mg kg^{-1}), and Zn (3.45 mg kg^{-1}). The analyses were performed as proposed by the Official Mexican Standard methods.

Fe NPs were acquired from the industry ID-Nano, Mexico. The size and shape NPs were determined by FE-SEM. The size of iron NPs ranged between $65.6 \pm 18.5 \text{ nm}$ with a semi-spherical form (Fig. 1). Previously, the same nanomaterial was characterized in other studies (Pérez-Hernández et al. 2021).

2.3 Experimental design

Treatments consisted of Fe concentrations at 0 (no biostimulation), 100, 200, 300, and 400 mg kg^{-1} of dry soil ($n = 5$). The tested concentrations of Fe NPs were determined after searching for different effects on bean plants, which revealed that NPs caused positive effects at concentrations of 10 to 200 mg kg^{-1} dry soil. However, the literature found that

concentrations of 400 and 800 mg kg^{-1} did not cause effects and, in other cases, adverse effects were found (Yang et al. 2020). The studies consulted were carried out in different substrate conditions (laboratory, greenhouse, and field) with natural and artificial soil. Therefore, for this work, we used concentrations ranging between 100 and 400 mg kg^{-1} of dry soil under natural and tropical conditions.

The Fe NPs were added to the soil through a suspension with deionized water. For each replicate per treatment, NPs were placed in 10 mL capped glass tubes with deionized water and sonicated for 15 min. Next, the NPs were placed in a volumetric flask, deionized water was added to adjust the 1000 mL, and finally, it was added to each experimental unit (Pérez-Hernández et al. 2021).

2.4 Plant and soil sample collection

The experiment was carried out in the open field using plastic bags using the complete randomized design and five repetitions for each treatment. Each black plastic bag ($30 \text{ cm height} \times 25 \text{ cm diameter}$) was filled with 2.0 kg

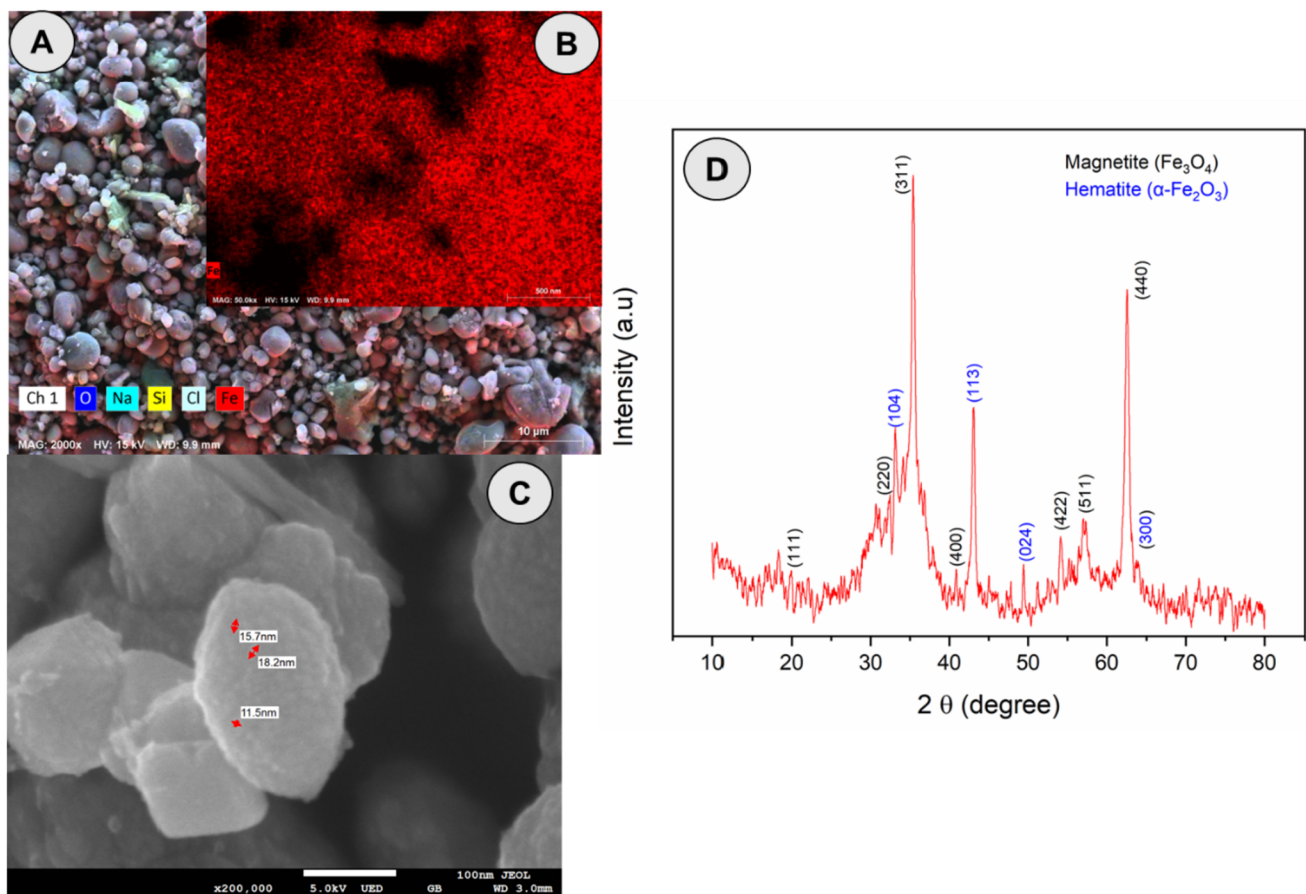


Fig. 1 Characterization of Fe NPs by field emission scanning electron microscopy (FE-SEM). (A) Corresponding elemental mapping images to $\times 2000$, $10 \mu\text{m}$ and (B) 50 kx , 50 nm , (C) average size, (D) elemental composition with EDX, and XRD spectrum

of dry soil for plant growth. Three seeds were deposited per bag. When the plant emerged 100% uniform (day 5), one seedling was left per bag (replication). Subsequently, the evaluations were carried out on 7, 14, 21, 28, and 35 days after application (DAA) of NPs.

For each sampling, the plant height was measured with a ruler from the base of the stem on the soil surface to the tip of the last leaf. In field, Fresh weight was obtained after separating the stems and leaves from the roots. Later, the roots were washed and dried at room temperature (1 h). Root size was measured from the point of growth to the end point of the root (cm). Leaf area was measured with a leaf area integrator (Li-Cor 3000®), for this, four leaves were taken from three plants per treatment. Finally, all the samples (stems, leaves, and root) were dried in an oven at 80 °C for 72 h, when constant weight was obtained. The fresh and dry biomass of stems, leaves, and roots were measured with an analytical balance (g).

2.4.1 Chemical analysis

The acid digestion method was used to determine P, K, Ca, Mg, Fe, Mn, and Zn in plants (root, stems, and leaves). After drying the plant tissue, it was ground to obtain a fine powder. For digestion, 0.20 g of stems and leaves and 0.10 g of roots were used. A mixture of nitric acid (HNO₃), hydrogen peroxide (H₂O₂), and hydrofluoric acid (HF) was used (Pequerul et al. 1993). Fe concentrations and the elements (P, K, Ca, Mg, Mn, and Zn) were analyzed by inductively coupled plasma (ICP, using a Perkin Elmer Mod. Optima 8300 equipment).

2.5 Statistical analysis

An analysis of variance (ANOVA) was performed under a completely randomized design following the general linear model (GLM) procedure to evaluate the effect of the treatments on morphological characteristics and elements in plants. When significant differences were observed, a comparison of means was applied (Tukey's test; $p \leq 0.05$). For statistical analysis and principal component analysis (PCA), the Minitab Version 20.0 program was used, while the correlation analysis was performed with Past (4.09) software.

3 Results

3.1 Fe NPs in plant growth and biomass

For plant height, at 7 days of evaluation, there was no significant difference between treatments ($F_{C_{7DAA}} = 2.71$, $p = 0.063$; Fig. 2). At 14 days, no statistical differences were observed between the control treatments, 100 and 200 mg kg⁻¹. However, the concentration of 300 and 400 mg kg⁻¹ caused a higher plant height ($F_{C_{14DAA}} = 10.58$, $p = 0.000$; Fig. 2). In contrast, at 21, 28, and 35 days of evaluation, plant height at concentrations of 100, 200, 300, and 400 mg kg⁻¹ of dry NP Fe soil was higher compared to the control ($F_{C_{21DAA}} = 11.8$, $p = 0.000$; $F_{C_{28DAA}} = 2.58$, $p = 0.05$; $F_{C_{35DAA}} = 3.99$, $p = 0.018$; Fig. 2). In general, at least for the plant height variable, between 14 and 35 days of evaluation, bean plants responded positively to concentrations of 300 and 400 mg kg⁻¹ of dry soil of Fe NPs.

Fig. 2 Effect of iron NPs on plant height of *P. acutifolius*. Different letters on the bars indicate significant differences among treatments ($p \leq 0.05$; $n = 5$). Data are mean values \pm standard error. Numbers 7, 14, 21, 28, and 38 correspond to days after NPs application

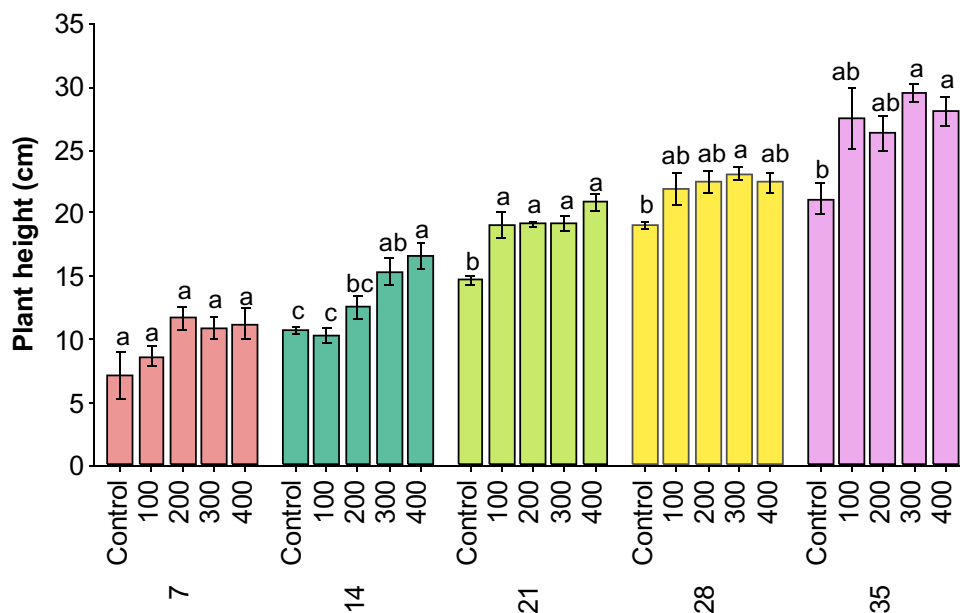
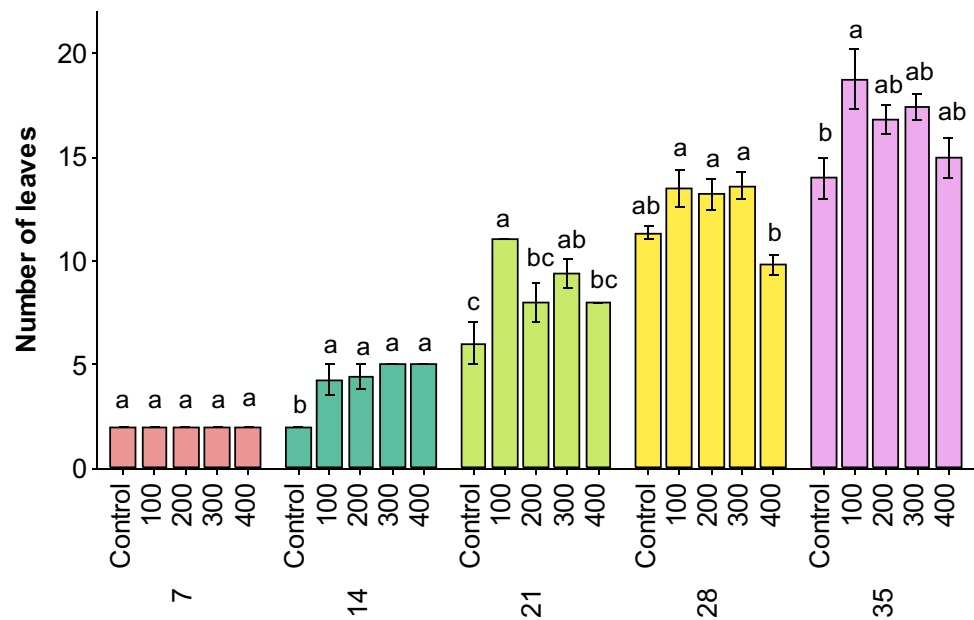


Fig. 3 Effect of iron NPs on number of leaves of *P. acutifolios*. Different letters on the bars indicate significant differences among treatments ($p \leq 0.05$; $n = 5$). Data are mean values \pm standard error. Numbers 7, 14, 21, 28, and 38 correspond to days after NPs application



Regarding the number of leaves, after 7 days of evaluation, no significant difference was observed between treatments ($F_{C_{7DAA}} = 1.24$, $p = 0.33$; Fig. 3). The significant differences between treatments were evident at 14, 21, 28, and 35, with the concentrations of 100, 200, and 300 mg kg^{-1} of Fe NPs being the ones that promoted the greatest number of leaves ($F_{C_{14DAA}} = 6.34$, $p = 0.003$; $F_{C_{21DAA}} = 6.42$, $p = 0.002$; $F_{C_{28DAA}} = 6.45$, $p = 0.002$; $F_{C_{35DAA}} = 3.49$, $p = 0.03$; Fig. 3). Interestingly, there was no statistical difference in control and at the highest concentration (400 mg kg^{-1} of Fe NPs), i.e., both treatments caused a lower number of leaves per plant. In general, for this variable, the concentration of 100 mg kg^{-1} was the one that promoted a higher number of leaves as the days progressed (Fig. 3). In fact, in the image, it is observed that the concentration of 300 and 400 mg kg^{-1} , the Fe NPs promoted an increase in the width and length of leaves.

At the time of harvest, after 35 days of evaluation for root size, no significant differences were observed

between treatments ($F_c = 0.63$, $p = 0.648$). The values are not shown in the graphs.

At 35 days of evaluation, the analysis of variance for the rest of the physicochemical parameters indicated a significant difference between treatments (Table 1). However, compared with the control treatment and 400 mg kg^{-1} , the concentration of 300 mg kg^{-1} increased the fresh weight ($F_c = 3.98$, $p = 0.016$) and dry weight ($F_c = 2.86$, $p = 0.04$) of stems and leaves. Fe NPs at 100, 200, and 300 mg kg^{-1} significantly increased root fresh weight compared to the control and slightly compared to 400 mg kg^{-1} ($F_c = 4.31$, $p = 0.012$). On the contrary, the concentration of 300 mg kg^{-1} increased the dry weight of roots compared to the control treatment, 100 and 400 mg kg^{-1} of Fe NPs ($F_c = 6.16$, $p = 0.002$). Interestingly, the leaf area was higher at the concentration of 100 mg kg^{-1} compared to the rest of the treatments, including the control ($F_c = 15.53$, $p = 0.000$).

With PCA, we evaluate the variability of the data to confirm and further reveal the interactions between the

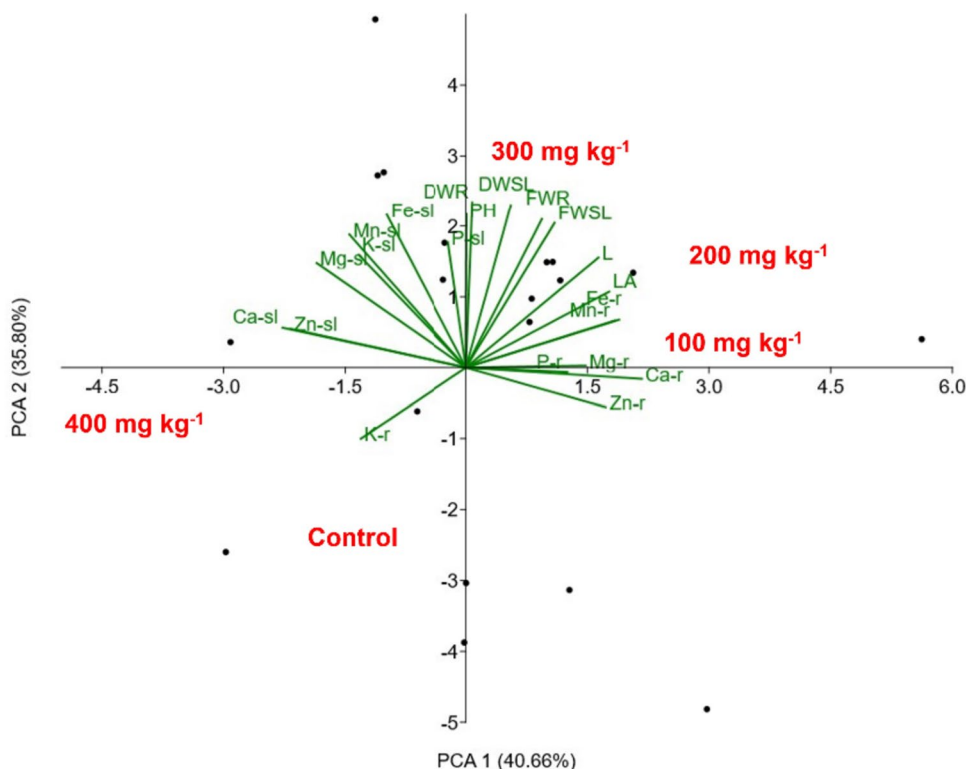
Table 1 Effect of iron NPs at 35 days on fresh weight and dry stems, leaves, roots, and leaf area of *P. acutifolios*

Treatments ^a	Fresh weight of stems and leaves (g)	Dry weight of stems and leaves (g)	Fresh weight of roots (g)	Dry weight of roots (g)	Leaf area (cm ²)
Control (0)	5.65 \pm 0.40b ^b	1.09 \pm 0.09b	0.57 \pm 0.02b	0.21 \pm 0.04b	6.26 \pm 0.49c
100	9.34 \pm 2.03ab	1.47 \pm 0.38ab	4.48 \pm 1.27a	0.45 \pm 0.10b	11.24 \pm 0.71a
200	8.26 \pm 1.16ab	1.61 \pm 0.25ab	4.56 \pm 0.57a	0.85 \pm 0.42ab	8.85 \pm 0.39b
300	11.96 \pm 1.07a	2.23 \pm 0.20a	4.74 \pm 0.68a	1.48 \pm 0.40a	8.67 \pm 0.34b
400	6.16 \pm 0.78b	1.29 \pm 0.19ab	3.08 \pm 0.77ab	0.36 \pm 0.03b	7.54 \pm 0.17bc

^amg Fe NPs kg^{-1} dry soil

^bData are mean values ($n = 5$) \pm standard error. The different letters along with the values show significant differences ($p \leq 0.05$)

Fig. 4 Principal component analysis (PCA) for the morphological variables and content nutrients based on the concentration of Fe NPs at 35 days of evaluation of *P. acutifoliosus*. PH, plant height; FWSL, fresh weight of stems and leaves; DWSL, dry weight of stems and leaves; FWR, fresh weight of roots; DWR, dry weight of roots; L, leaves; LA, leaf area. The initial of the minerals followed by the letters “-sl” means stems-leaves, while those followed by “-r” mean roots



morphological variables and the concentrations of Fe NPs. The PC1 (40.66%) and PC2 (35.80%) explained 76.46% of the general variability of the data (Fig. 4). For morphological variables, PCA 1 represented the variables fresh and dry weight of stems, leaves, and roots; number of leaves; and leaf area. The PCA 2 represented the variables plant height, fresh and dry weight of roots.

The effect of Fe NPs is appreciated by the location of the red letters in the biplot in relation to the green arrows and the distance between the two points that approximates their similarity. Briefly, the analysis showed that the 100, 200, and 300 mg kg⁻¹ Fe NPs treatments positively affected most variables. In contrast, the control treatment and 400 mg kg⁻¹ confirm that they negatively affected most variables.

3.2 Fe NPs in nutrient accumulation

Compared to the control, all concentrations (100, 200, 300, and 400 mg kg⁻¹) showed a statistical difference in the accumulation of P, Ca, and Mg in the root system ($F_{C_P} = 8.89$, $p = 0.001$; $F_{C_{Ca}} = 4.93$, $p = 0.009$; $F_{C_{Mg}} = 5.51$, $p = 0.006$; Table 2). However, compared to the control, the treatments with Fe NPs showed no significant effects on the accumulation of K ($F_C = 0.66$, $p = 0.627$). A statistical difference was observed between treatments, including the control, regarding

micronutrients. Only the concentrations of 200 and 300 mg kg⁻¹ of Fe NPs promoted a higher accumulation of Fe and Mn compared to the 100, 400 mg kg⁻¹, and control ($F_{C_{Fe}} = 29.34$, $p = 0.000$; $F_{C_{Mn}} = 23.7$, $p = 0.000$). The 200 mg kg⁻¹ treatment and slightly the control treatment promoted a higher accumulation of Zn in roots than the 100, 300, and 400 mg kg⁻¹ ($F_C = 5.87$, $p = 0.004$).

The treatments with Fe NPs have no significant impact on P, K, and Mg content in either leaf ($F_{C_P} = 4.0$, $p = 0.020$; $F_{C_K} = 1.39$, $p = 0.282$; $F_{C_{Mg}} = 2.57$, $p = 0.078$; Table 2). A statistical difference was noticeable between treatments for calcium content, which was higher in control compared to the rest of the treatments ($F_{C_{Ca}} = 4.13$, $p = 0.017$). Regarding the micronutrients in the leaves, in the accumulation of Mn and Zn, no significant differences were found between treatments, including the control ($F_{C_{Mn}} = 2.93$, $p = 0.054$; $F_{C_{Zn}} = 2.18$, $p = 0.118$). A statistical difference was found in the accumulation of Fe ($F_{C_{Fe}} = 4.76$, $p = 0.010$).

Finally, with the analysis of principal components (Fig. 4), it was shown that for the nutrient content in roots, PCA 1 represented the P, Ca, Mg, Mn, Fe, and Zn, while PCA 2 represented P, K, Ca, Mg, Mn, Fe, and Zn in stems and leaves. The K was represented in PCA 2 (stems, leaves, and roots). The Fe values were higher in 300 and 400 mg kg⁻¹ of Fe NPs and lower in 100 and 200 mg kg⁻¹ of Fe NPs. Interestingly, the control treatment was statistically similar to the concentration of 300 mg kg⁻¹.

Table 2 Effect of iron NPs at 35 days on content nutrients in roots or in stems and leaves of *P. acutifolius*

Treatments (mg of Fe NPs kg ⁻¹ of dry soil)	P mg kg ⁻¹ dry weight	K	Ca	Mg	Fe	Mn	Zn
Roots							
Control	1067 ± 302b ^a	20443 ± 2862a	1410 ± 1210b	1920 ± 767b	3740 ± 171b	86.7 ± 12b	176.7 ± 31.2ab
100	2120 ± 149a	23090 ± 2509a	8210 ± 1825a	3065 ± 281a	5570 ± 980b	145 ± 19.4b	122.5 ± 7.5b
200	1688 ± 69.8a	18968 ± 2597a	7056 ± 651a	2910 ± 118a	12656 ± 883a	226 ± 7.48a	418 ± 112a
300	1760 ± 61.7a	19344 ± 2499a	5602 ± 726ab	3016 ± 154a	12256 ± 725a	236 ± 10.3a	58 ± 3.74b
400	2135 ± 114a	22805 ± 739a	7520 ± 973a	3065 ± 168a	5083 ± 569b	142.5 ± 13.8b	77.5 ± 21b
Stems and leave							
Control	1037 ± 246a ^a	20173 ± 2862a	30820 ± 583a	4897 ± 414a	1127 ± 139ab	87 ± 7.0a	28 ± 5.8a
100	1264 ± 135a	17750 ± 2422a	22128 ± 2216ab	3582 ± 334a	754 ± 96.6b	68 ± 4.9a	18 ± 2.0a
200	1030 ± 303a	17728 ± 1804a	19165 ± 2431b	3405 ± 475a	805 ± 72.6b	65 ± 15a	17.5 ± 4.7a
300	1826 ± 199a	22498 ± 852a	24744 ± 1231ab	4362 ± 236a	1130 ± 103ab	94 ± 10.3a	22 ± 2.0a
400	2025 ± 258a	20763 ± 1298a	23463 ± 1892ab	4085 ± 329a	1890 ± 439a	102.5 ± 7.5a	27.5 ± 2.5a

^aData are mean values ($n = 5$) ± standard error. The different letters along with the values show significant differences between kinds of tissue ($p \leq 0.05$)

4 Discussion

4.1 Effect of iron NPs on morphological characteristics

The interactions of metal and metal oxides NPs with soil have been discussed. When NPs interact with the soil, the NPs can transform, such as homoaggregation, heteroaggregation, oxidation, dissolution, and precipitation. Consequently, they can cause biological (soil biota), physical (porosity, texture, apparent density), and chemical changes in the soil (pH, redox potential, electrical conductivity, and organic matter, among others). Furthermore, NPs also interact with soil minerals, which can affect availability in the soil solution. Due to the possible transformations or changes that NPs can cause, they influence their mobility and uptake by plants (Pérez-Hernández et al. 2020).

In the present experiment, because of the application of nano-bioestimulants to soil, the height of escumite bean plants was observed to alleviate considerably compared to the plants in untreated soil conditions. Previous studies have reported that between 10 and 300 mg kg⁻¹ of dry soil of Fe NP increased the height of bean plants (Raju et al. 2016) and in other crops such as *Arachis hypogaea* L., *Zea Mays* L. *Capsicum annuum* L. (Rui et al. 2016; Wang et al. 2021). However, despite the positive results with 300 and 400 mg kg⁻¹ of Fe NP, these findings must be judged with caution since other investigations have shown that concentrations at 400 and 500 mg kg⁻¹ produce damage effects (Wang et al. 2021). For example, Fe NPs at 600 mg kg⁻¹ produced phytotoxicity in mung bean plants (Sun et al. 2019). In the study of Wang et al. (2021) they showed that at 500 mg kg⁻¹ in maize plants, Fe NPs affected antioxidants. Even

glutamic acid was reduced by 99%, highlighting that this amino acid is responsible for the synthesis of several amino acids in plants. Therefore, it is suggested that the effect of NPs at concentrations of 400 mg kg⁻¹ positively affects plant height. However, it cannot be concluded that the higher the concentration evaluated improves the rest of the parameters studied. Some research has found controversial effects. Studies affirm that while high concentrations of NPs favor parameters such as plant height and root size, parameters such as total biomass, fruit quality, and other biochemical parameters in plants are unfavorable (Yuan et al. 2018). Regarding the number of leaves in control and the highest concentration (400 mg kg⁻¹ of Fe NPs), no statistical difference was observed. Both treatments caused a lower number of leaves per plant. In general, for this variable, the concentration of 100 mg kg⁻¹ was the one that promoted a high number of leaves as the days passed. The results confirm the hypothesis that at least one of the concentrations positively affects the morphological characteristics of the plants. In the literature, it has been documented that high concentrations and the size of NPs allow them to accumulate in plants, alter the size of the leaves and affect their photosynthetic reactions by altering the composition of proteins, in the transport chain of electrons, the biosynthesis of chlorophyll, and the synthesis of carbohydrates (Jankovskis et al. 2022). Therefore, based on the results, it is suggested that the higher concentration of Fe NPs negatively affects the number of leaves; this may occur due to the harmful effects that NPs cause at the cellular level. The studies carried out by Afzal et al. (2022) demonstrated that applications of 500 mg kg⁻¹ of Fe NP applied to rice plants (*O. sativa* L.) caused a decrease in protein and total chlorophyll content compared to concentrations of 10 mg kg⁻¹ of soil. However, other experiments

showed that 500 mg L⁻¹ of iron oxide NP did not exert any toxic effect on pumpkins (*Cucurbita maxima* L.) that grew in a hydroponic medium (Zhu et al. 2008). We suggest that, for the present experiment, the concentration of 100 and 200 mg kg⁻¹ positively and constantly affected the number of leaves from 14 to 35 days of evaluation.

The latest research suggests that Fe NPs at low concentrations act as stimulators under different types of stress, such as salinity, drought, and humidity (Dola et al. 2022; Juárez-Maldonado et al. 2019). Reports have shown that even under stress conditions, plant responses substantially improve total fresh and dry weight (Dola et al. 2022). For the present experiment, the escumite bean was cultivated in the dry season since to its physiology, it requires little humidity. However, low soil fertility affects biomass production. Indeed, we observed that for control treatment, the fresh and dry biomass of stems, leaves, and roots was lower compared to plants that grew conditioned with Fe NPs between 100 and 300 mg kg⁻¹. Therefore, the stimulatory effect of Fe NPs is substantially effective in counteracting soil fertility problems (Zhao et al. 2020). Authors suggest that the stimulatory effect of NPs on plants is due to the surface charges of nanomaterials, which increase the internalization of NPs in cells, triggering changes in plant metabolism (Juárez-Maldonado et al. 2019). In this case, the beneficial effect was observed on biomass and parameters such as the height of the plant, the number of leaves, and the leaf area. In contrast, high concentrations of NPs cause cell damage, coming from the accumulation of NPs in the vacuoles and mitochondria (Ma et al. 2015), an effect that, for the present experiment, was observed at the concentration of 400 mg kg⁻¹ of dry soil, causing a marked decrease in leaf area. Authors revealed that high concentrations of Fe NPs cause damage in the formation of chloroplasts. In this line, the researchers suggest that low concentrations positively modified cell walls, resulting in higher elongation in *C. annuum* plants (Yuan et al. 2018). Indeed, it is important to comment that the positive effect of low concentrations (50–150 mg kg⁻¹) of Fe NPs on fresh, dry biomass and leaf area was documented for edible crops (Elizabeth et al. 2017; Iannone et al. 2021). For example, a study in carrots (*Daucus carota* L.) showed that, compared to the control, the application of 100 and 150 mg L⁻¹ of Fe NPs caused a beneficial effect on the leaf area (Elizabeth et al. 2017). In soybean (*Glycine max* L.) and alfalfa (*Medicago sativa* L.), the concentrations of 50 and 100 mg L⁻¹ of Fe NPs improve the length and weight of the root and shoot (Iannone et al. 2021). Also, in soybean (*Glycine max* L.), compared to the control, 30, and 60 mg/pot (pot of 2-kg dry soil), at 15 mg/pot there was greater, dry weight of root and shoots (Yang et al. 2020). Therefore, based on the results, we suggest that doses between 100 and 300 mg kg⁻¹ of Fe NPs may be a viable option to improve the quality of the morphological characteristics of beans

during the growth stage. The PCA (Fig. 4) confirmed that the concentrations of 100, 200, and 300 mg kg⁻¹ promoted high values on most of the morphological characteristics of the plants. In contrast, the control treatment and 400 mg kg⁻¹ caused low values for most of the variables. As mentioned above, NPs will only have a positive effect if they are in an optimal range for plants or at a low concentration since, in high concentrations, these cause damage. Therefore, it was evident that plants without the supply of NPs did not favor the improvement in all agronomic variables, thus suggesting that NPs have a stimulatory effect compared to the control (Juárez-Maldonado et al. 2019).

It is prudent to argue that NPs can improve the morphological characteristics. However, although we did not evaluate this last parameter, the literature shows evidence that agronomic management of the crop, based on organic agriculture and the application of NPs at low concentrations, can achieve quality products.

4.2 Effect of iron NPs on nutrient concentration

The effect of different concentrations of nanomaterials has been evaluated in many edible crops but not in escumite beans, specifically, Fe NPs on the accumulation of nutrients in plant tissue (roots, stems, and leaves). Although there are few reports, researchers show evidence that supplementing Fe NPs to the soil promotes the absorption of other essential elements in plants. Therefore, iron supplementation in nanometric form is a viable option to mitigate Fe deficiency in soil and plants (Rizwan et al. 2019).

As mentioned in the literature, even though most soils are rich in Fe, up to 35% of Fe is plant limited. Therefore, the presenting high surface charge by Fe NPs allows physicochemical and electrical changes in the surrounding environment. These properties of Fe NPs act as nanometric fertilizer additives, thus allowing greater availability to plants compared to conventional fertilizers, consequently being lost by leaching, fixation, or volatilization (Le Wee et al. 2022). So, for our results suggest that for the escumite bean crop, the nano iron at concentrations of 100 and 300 mg kg⁻¹ promotes the absorption and accumulation of macronutrients in the root system, at least for Ca, P, and Mg. Nevertheless, in the treatments with NPs, there was a higher accumulation of P and K in the root than in stems and leaves, but not with Ca, which was 3 to 4 times higher in stems and leaves than in roots.

The differences in the accumulation of nutrients in the aerial part and the roots of the plants due to the effect of the Fe NPs is still a questionable issue since, regardless of the unique properties of the NPs, soil factors play a role important in the process of absorption of the nutrients. Ahmed et al. (2021) reported in an experiment with rice plants (*O. sativa*) that 100 mg kg⁻¹ of Fe NPs stimulated the uptake

of N, P, K, Ca, and Mg, even when the plants grew under Cd stress. Similarly, Banerjee and Roychoudhury (2021) reported that the γ Fe₂O₃ NPs in rice plants accelerated the uptake of K, Zn, Cu, and Mn.

A study that evaluated the effect of Fe NPs in bean crops revealed that, compared to the control, concentrations of 1000 and 2000 mg kg⁻¹ caused a greater uptake of total P in plants (De Souza et al. 2019). To the best of our knowledge, most of the phosphorus in the soil is immobile and insoluble (Abd-Alla 1994). Coinciding with De Souza et al. (2019), this suggests that, for the present experiment, the treatments with Fe NPs facilitated the conversion of insoluble phosphorus to soluble phosphorus, which enabled the accumulation of this nutrient in roots, stems, and leaves, which in turn promoted an effect on plant growth and movement of nutrients in plants. The reasons for the availability of phosphorus and its accumulation in plants by the action of Fe NPs continue in debate. Some authors suggest that Fe₃O₄ NPs increased phosphorus uptake in calcareous soil (Moharami and Jalali 2015), while others indicate that phosphorus absorption decreases with increasing Fe NPs (Koopmans et al. 2020).

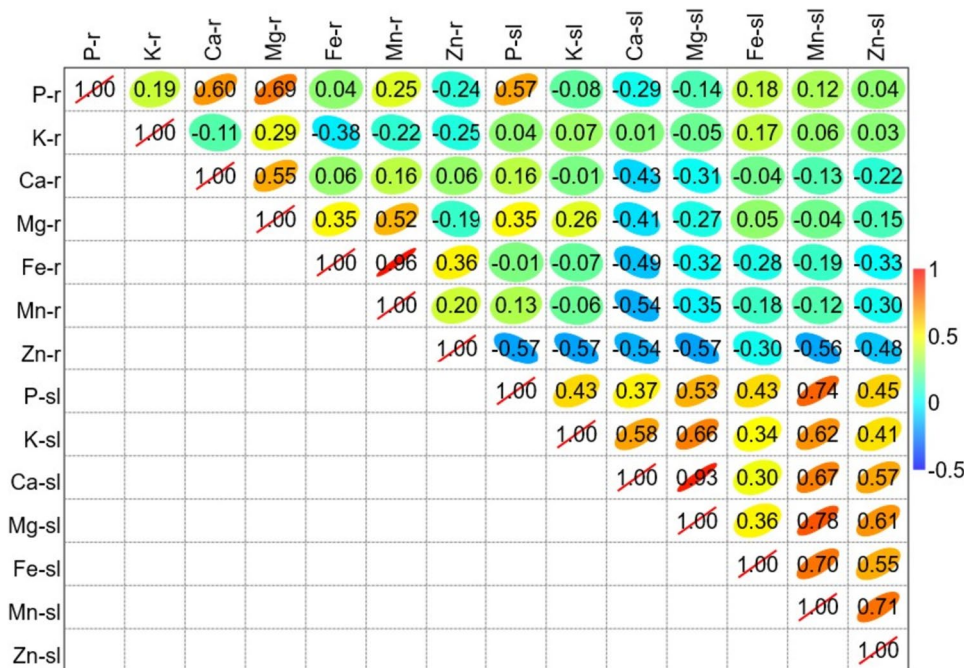
In the experiment, possibly the Fe NPs joined the phosphorus ions, forming Fe-phosphate (Feng et al. 2022), which could have had a beneficial effect on the plant since a height plant difference was observed between the treatments with NPs and the control. The correlation analysis of the nutrients in the leaf can confirm this hypothesis, since the value of Pearson’s *r* is moderate in the Fe-P relationship ($r = 0.43, p = 0.05$; Fig. 5). However, although no significant relationship was observed between Fe and K, Ca and Mg ($r = 0.34, p = 0.137; r = 0.30, p = 0.18; r = 0.36, p =$

0.111, respectively; Fig. 5), the amount of macronutrients in stems and leaves was sufficient for the plant. In addition, since the Mg content was higher in all treatments with NPs, this allowed a higher fresh and dry weight of stems, leaves, and the number of leaves. Indeed, in the literature, it is reported that Mg plays an essential role in photosynthesis (Wolf et al. 2019).

Little has been reported in the literature on the effect of Fe NPs on calcium availability and accumulation in the roots, stems, and leaves of bean plants. In the present experiment, Ca content was higher in stems and leaves and lower in roots, but, compared to the control, Fe NPs treatments caused higher Ca accumulation independent of tissue (Tables 1 and 2). White (2003) suggest that when the Ca abundant is present in the xylem sap, there is a close relationship between the distribution of Ca to the shoot and transpiration, in which Ca will be lodged either in the mesophyll, epidermal cells or trichomes, depending on the plant species. On the other hand, interactions between Ca and other nutrients have been reported. In this case, in the leaves, the relationship between Ca and Mg, Mn and Zn were positive but negative in the root system. In fact, the relationship between Ca and Fe for both the root and stems and leaves were low to null (Fig. 5). Although the Fe NPs caused a difference in Ca accumulation, possibly other factors may respond to the differences in Ca concentration in escumite beans.

In the case of micronutrients, the concentration of Fe, Mn, and Zn was higher in the roots than in the aerial part. Authors have reported that the efficiency in the absorption of Fe and other nutrients by the roots is mediated by the regulation of iron transporter genes in plants with iron

Fig. 5 Effect of iron NPs at 35 days on correlation nutrients in roots and leaves of *P. acutifolius*. The initial of the minerals followed by the letters “-sl” means stems-leaves, while those followed by “-r” mean roots. The color of the ellipses denotes high correlation (deep red), low correlation (light green), and negative correlation (blue intense). Furthermore, the flat-tened ellipses indicate values close to 1



deficiency, verified in crops such as *Oryza sativa* L. (Li et al. 2016), *Citrus maxima* L. (Hu et al. 2017). The absorption and accumulation of macronutrients and Fe occur through the apoplastic pathway in the root (Rai et al. 2022; Rui et al. 2016). However, the absorption depends on the concentration supplied, the size, the shape of the NP, and the plant phenotype (Dimkpa 2018). For the present experiment, the minimum size of the Fe NPs ranged between 11.5 and 15.7 nm, which allowed them to easily penetrate the cell wall, which in plants ranges in size from 1 to 100 μm (Rai et al. 2022). Therefore, nano-biostimulants, through their physicochemical properties, guarantee high reactivity within the cells, resulting in greater availability and efficiency of plant nutrients (Khan et al. 2019). In fact, in leaves, 80% of the Fe is found in the chloroplast. So that a drop in the content reduces the photosynthetic activity and, consequently, plant growth (Kim et al. 2014).

According to the correlation analysis in roots, no significant differences were observed between Fe versus Zn ($r = 0.36$, $p = 0.106$; Fig. 5), but not in the relationship between Fe and Mn, which was significant ($r = 0.96$, $p = 0.000$; Fig. 5). Although the accumulation of micronutrients for Mn and Zn in roots was dose-dependent (Table 2), doubts remain as no correlation effects were observed between Fe and Zn. As other authors have indicated, the supplementation of NPs to the soil and the effect that nanomaterials as fertilizers have not been fully investigated (Martínez-Fernández et al. 2015). The reports by Yoon et al. (2019) suggest that an increasing concentration of nZVI NPs can suppress the uptake of Mn and Zn in the leaves, as occurred in the present for the treatments with NPs. Also, the authors discussed the antagonistic effect between soil nutrients and the low Zn absorption by plants. The high content of P and Ca in the soil decreases the absorption capacity of Zn in different crops, such as corn, beans, peanuts, and potatoes, among others (Prasad et al. 2016), a situation that could have occurred in our experiment. According to Figure 5, a negative or null correlation of Zn versus P and Ca is observed ($r = -0.24$, $p = 0.305$; $r = 0.06$, $p = 0.792$), as well as with other nutrients. However, we suggest that Fe NPs influenced on Zn accumulation in roots. Despite our interesting findings, many questions remain to be resolved. Although we found higher values of accumulation of macro- and micronutrients in the roots than in the leaves, when performing the correlation analysis of Fe NPs and micronutrients in the leaves, Pearson's r values were moderate to high for the Zn and Mn ($r = 0.55$, $p = 0.00$; $r = 0.70$, $p = 0.18$, respectively; Fig. 5). The reason for the relationship is that the Mn and Zn interact due to the chemical similarity between their divalent cations and the lack of specificity of Fe transporters in certain plant species (Sinclair and Krämer 2012; Yoon et al. 2019). On the other hand, studies have shown that the ability of the plant to accumulate nutrients in the root depends on the species and

soil factors (Rastogi et al. 2017). The research by González-MoscOSO et al. (2021) demonstrated that 250 and 1000 mg L^{-1} NPs of SiO_2 in tomato plants under greenhouse conditions caused a greater absorption of Cu and Zn in the roots but not in the leaves. These results agree with our findings since bean plants concentrate higher macronutrient (P, Ca, Mg) and micronutrient (Fe, Mn, and Zn) content in roots than in stems and leaves. These differences in accumulation could have occurred due to an accumulation of Fe NPs in the epithelial cells of the root surface (Martínez-Fernández et al. 2016). On the other hand, the high specific surface area and the reactivity of the Fe NPs could block pores and therefore decrease the absorption of water and nutrients from the root to the xylem (Martínez-Fernández et al. 2016). In addition, other studies suggest that during the uptake of Fe NPs in the root, mediated by transporter genes; they can interact with proteins and, at the same time, cause a blockage with the uptake channels, which prevents the accumulation of Fe in stems and leaves (Dietz and Herth 2011). Finally, in the experiment, both Mg and Zn correlated with the rest of the nutrients in the leaves (Fig. 5).

On the other hand, interesting results were found in the relationship between nutrients in stems and leaves vs roots. For example, Fe in the root presented a negative correlation with most nutrients in stems and leaves. However, the effect was not significant ($p \leq 0.05$). Regarding Zn in the root, it was negatively correlated with the rest of the nutrients in the leaves (P, K, Ca, Mg, Mn, and Zn; $p \leq 0.05$, Figs. 4 and 5). In the case of Ca in stems and leaves, it was negatively related to most nutrients in the roots but was only significant with Fe, Mn, and Zn ($p \leq 0.05$; Figs. 4 and 5). Other but non-significant relationships are shown in Figure 5.

As reported in the literature, the soil matrix is complex due to the physicochemical and biological factors of the soil. Soil texture, pH, electrical conductivity, organic matter, and enzyme secretion in the rhizosphere appear to be factors in the availability and mobility of nutrients to the plant (Suazo-Hernández et al. 2023).

5 Conclusions

This study investigates the biostimulation effect of Fe NPs on morphological parameters during the growth stage of *P. acutifolius* beans. Additionally, the absorption of macronutrients and micronutrients in stems-leaves and roots was studied. We found that the concentrations of 100, 200, and 300 mg Fe NPs kg of dry soil positively affect the fresh and dry biomass of stems-leaves and roots. At the same time, they promote a higher accumulation of P, Ca, Mg, Fe, Mn, and Zn in roots and less in stems and leaves. We suggested that the Fe NPs at low concentrations may increase the morphological characteristics of Fe-deficient bean plants under

the soil. For future research, it is opportune to evaluate the effect of NPs on production parameters and grain quality. Also, it is important to study with a holistic approach in which evaluations of the soil biota are included to avoid damage to the environment.

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Declarations

Conflict of interest The authors declare no competing interests.

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