

Elemental content response of broad bean (*Vicia faba* L.) seeds to metallic and nano-iron within Nineveh Governorate

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I. Abstract:

Broad beans are considered a strategically important legume crop globally due to their high nutritional value. However, their productivity in calcareous soils (such as those found in Iraq) is negatively affected by the reduced availability of micronutrients, particularly iron, and the high pH and calcium carbonate content, which fixes the element and prevents its absorption,

Iron chlorosis is the biggest challenge for growing broad beans in alkaline calcareous soils. The high pH and the presence of calcium carbonate convert iron from its available form to a non-absorbable form, hindering chlorophyll formation and causing yellowing of the leaves and reduced yield.

II. Introduction:

Broad beans (*Vicia faba* L.) are among the most important legume crops worldwide, possessing high nutritional value due to their high content of plant protein, amino acids, minerals, and vitamins. They are also economically important as a direct food crop for humans and animal fodder, in addition to being a strategic crop in sustainable agriculture thanks to their ability to fix atmospheric nitrogen and improve the physical and chemical properties of the soil (Abd El-Hady et al., 2023). However, broad bean productivity in calcareous soils, prevalent in many regions such as Iraq, is negatively affected by the reduced bioavailability of micronutrients, particularly iron. This is due to high pH levels and the presence of calcium carbonate, which fixes the element and prevents its absorption, resulting in yellowing symptoms and reduced growth and yield (Jassim et al., 2023)

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Iron (Fe) is a key micronutrient required by plants in small quantities, yet it plays essential roles in growth and development. It is a component of many enzymes and proteins responsible for oxidation-reduction reactions, such as cytochromes and ferritin, and indirectly contributes to chlorophyll formation. It also plays a crucial role in cellular respiration and energy production. Furthermore, it is a key factor in root nodule formation and promotes the activity of nitrogen-fixing Rhizobium bacteria in legumes, thus improving biofeedback efficiency and increasing soil fertility (Khan et al., 2022). Traditional mineral fertilization has been used to treat iron deficiency, with soil application of compounds such as ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) to increase the concentration of available iron in the soil and improve its uptake by roots. However, the effectiveness of this method may be negatively affected in calcareous soils due to the fixation of the element. Since it relies on foliar application as a complementary technique,



where iron is sprayed directly onto the leaves, this accelerates its absorption, improves chlorophyll content, and has a rapid effect on vegetative growth (Shirsat et al., 2024.)

With recent advancements in nanotechnology, nano-fertilization has emerged as an innovative strategy to enhance fertilizer use efficiency. Soil application of nano-iron contributes to improving the availability of the element in the soil and increasing its absorption by the roots, while foliar application has shown remarkable effectiveness in promoting vegetative growth (Mohamed et al., 2023; Al-Khafaji et al., 2024.)

Therefore, studying the effect of mineral and nano-iron fertilization through soil and foliar application on broad bean crops in calcareous soil conditions is a fundamental scientific step towards developing advanced fertilization programs that contribute to increased productivity and agricultural sustainability. Among its most important objectives are:

1-Evaluate the effect of both metallic and nano-iron, and their application methods, on the concentration and uptake of NPK (Fe) in broad bean plants.

2-Determine the optimal levels of nano- and metallic iron fertilizers, and the best combination of application methods, to achieve the highest nutrient concentration in broad bean plants.

Research and study objectives

This study aims to evaluate the efficiency of mineral and nano-iron fertilization via soil and foliar application to overcome the fixation problem and increase nutrient uptake.

III. Materials and working methods

The experiment was designed as a factorial experiment with a completely randomized design (CRD) with three replications in the greenhouse at the University of Mosul. It included 54 experimental units to study three factors

•Factor 1 (Soil Location): Two different locations in Nineveh Governorate (the Felfil S1 site with clay soil texture and the Rashidiya S2 site with clay loam soil texture.)

•Factor 2 (Metallic Iron - Ferrous Sulfate M): Three levels (comparison without fertilization M1, soil application of 15 kg ha⁻¹ M2, and foliar spraying of 100 mg L⁻¹ M3.)

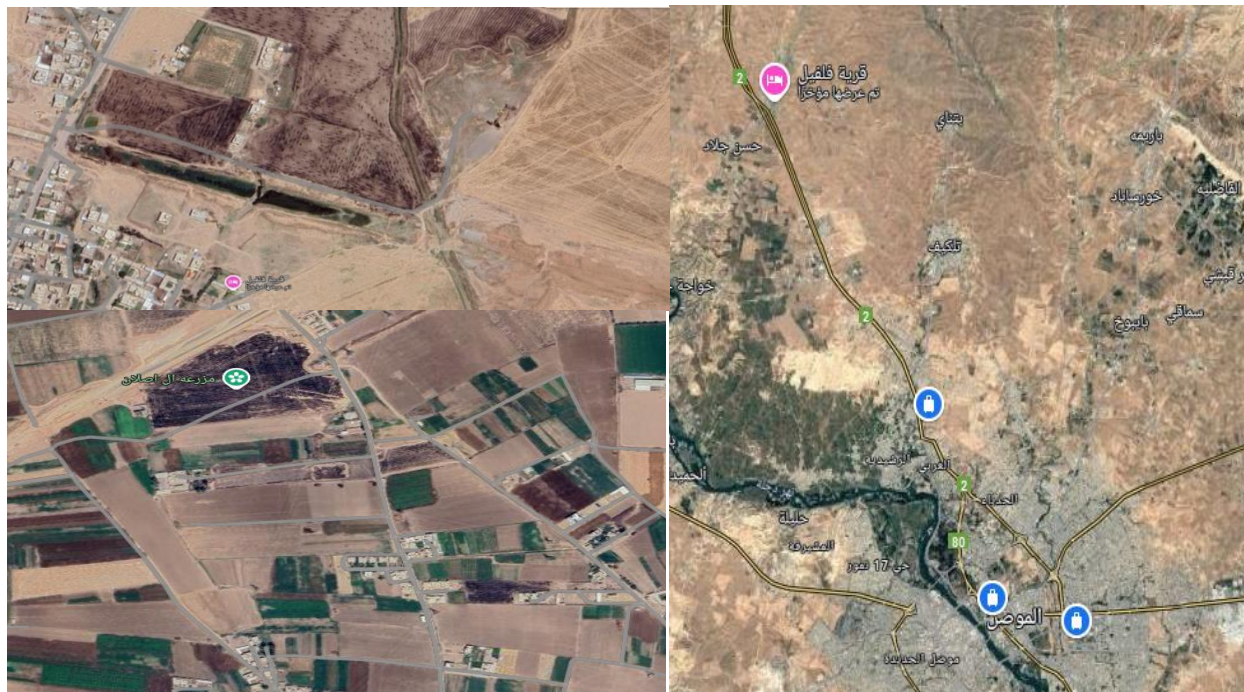
•Factor 3 (Nano-Iron N): Three levels (comparison without fertilization N1, soil application of 4 kg ha⁻¹ N2, and foliar spraying of 1.5 g (L⁻¹ N3.)

Soil Sample Collection and Preparation Sites for Study:

Two sites with different chemical and physical soil properties were selected in Nineveh Governorate to collect soil samples for broad bean cultivation. The first site is in the fields of the Rashidiya area, located at longitude 43.1167 and latitude 36.4333 North, in the northern outskirts of Mosul, about 10 km from the city center. The second site is in the fields of the Felfil district, located 60 to 70 km from the city center of Mosul at longitude 43.0343 East and latitude 36.5467 North. It should be noted that the soils are of different orders; the Felfil site belongs to the order Aridisols, while the Rashidiya site belongs to the order Andisols. The image below illustrates this,

Soil samples were collected from the surface layer at a depth of (0-30) cm on 10/11/2025. Samples were thoroughly mixed at each location to obtain a representative field soil sample. These samples were then air-dried, softened, ground, and passed through a 4 mm sieve for potting. 8 kg of air-dried soil were placed in each pot, which represents an experimental unit. A sample was taken from each soil type and passed through a 2 mm sieve. These samples were then placed in sealed plastic containers to estimate some of the physical and chemical properties of the two soils before planting.





Soil Analysis:

Analyses were conducted to estimate some of the physical and chemical properties of the two soils.

Physical Analysis: Some physical soil analyses were performed, such as soil texture, field capacity, and bulk density.

-1 Estimation of the particle size distribution of soil components (soil texture):

Soil texture is determined using mechanical analysis and the hydrometer method (as described by Gee and Bauder, 1986), and the soil texture was determined using the texture triangle.

.2 Estimation of moisture content at field capacity.:

The moisture content of the soil samples was estimated using the gravimetric method (USDA, 2006) and the method described in Klute (1986).

3-Bulk Density: Bulk density was estimated using the paraffin wax method according to the methods described in Black (1965).

Chemical Analyses:

Chemical analyses were performed using the methods described below:

.1 pH: The pH of the soil was determined in the soil extract at a 1:1 ratio using the electrical potential method and a pH meter (as described in Rowell, 1996).

.2 Electrical Conductivity (EC): The electrical conductivity (EC) of the soil extract was determined at a 1:1 ratio at 25°C using an EC meter (as described in Rowell, 1996).

.3 Calcium Carbonate (CaCO₃): The calcium carbonate content in the soil was determined chemically using hydrochloric acid (HCl(1N)) in a quantity greater than required for the reaction. The excess acid was then titrated with sodium hydroxide (NaOH(1N)) in the presence of phenolphthalein (FAO, 1973).

4. Organic Matter (O.M.): The wet oxidation method was used to estimate organic matter using 95% concentrated sulfuric acid (H₂SO₄) with 1 M potassium dichromate (K₂Cr₂O₇) (for the purpose of oxidizing organic carbon). This



was followed by titration with ammonium ferrous sulfate (N_2O_4) and the presence of diphenylamine indicator to determine the excess dichromate. The organic carbon was then converted.

.5 Cation Exchange Capacity (CEC): The cation exchange capacity was estimated by saturating the soil with a sodium acetate solution (N_2O_4 , pH = 8.2) to replace all positive ions on the clay surfaces with sodium. An extraction process was then carried out with an ammonium acetate solution (N_2O_4), and the sodium concentration in the extract was determined using a flame photometer.

.6 Available nitrogen (N) in the soil: Available nitrogen in the soil was estimated using a potassium chloride solution ($KCl(2N)$) as a nitrogen extractant at a ratio of 20:1, then filtration, and the nitrogen in the filtrate was estimated by distillation using a micro-Kjeldahl apparatus and ammonia was received in boric acid in the presence of an indicator mixture according to the method of (Black, 1965).

.7 Available Phosphorus (P) in Soil: Available phosphorus was estimated from the soil extract using the ModOlsen method (as described in Page et al., 1982) with a 0.5 M sodium bicarbonate ($NaHCO_3$) solution at pH 8.5 as the phosphorus extractant at a 20:1 ratio. Phosphorus was determined colorimetrically using a spectrophotometer at a wavelength of 882 nm.

.8 Available Potassium (K) in Soil: Available potassium was estimated by extracting it with a 1 N neutral ammonium acetate solution at a 10:1 ratio at pH 7. The potassium in the filtrate was then determined using a flame photometer. 9. Available Iron (mg/kg) in Soil: Extraction with a 2:1 DTPA solution. Shake 10 g of the solution in 20 cm^3 of DTPA solution at pH 7.3. After shaking for two hours and filtration, the iron content in the solution was determined using an atomic absorption spectrophotometer.

Seeds Used in the Experiment: Broad bean seeds of the Mornach variety, produced by the Moroccan company Agroseme, were used in the experiment. The germination rate was 90%. Biological Experiment:

Experimental Design:

The best way to determine how plants can utilize added fertilizers is through cultivation. Therefore, a factorial experiment was conducted using a completely randomized design (CRD) in the greenhouse of the Department of Soil Science and Water Resources, College of Agriculture and Forestry, University of Mosul, during the 2024-2025 growing season. The experiment studied the effect of three factors with three replications:

Factor 1: Two locations with different soil textures and varying calcium carbonate and iron content.

Location 1: A- Felfil area, Nineveh Governorate.

Location 2: B- Rashidiya area, Nineveh Governorate

Factor 2: Fertilization with ferrous sulfate ($FeSO_4.7H_2O$) fertilizer with a mineral iron content of 19% (Fe), according to the recommended fertilizer application, using: a) Comparison, b) Soil application (15 kg Fe ha^{-1}), c) Foliar application (100 mg Fe L^{-1}).

Factor 3: Fertilization with an Iranian-made nano-fertilizer manufactured by Al-Khadra Company, containing 6% iron, according to the recommended fertilizer application, using: a) Comparison, b) Soil application (4 kg Fe ha^{-1}), c) Foliar application (1.5 g L^{-1}). The number of experimental units is 54 (2 soil sites x 3 levels of soil application x 3 levels of foliar application x 3 replicates = 54 experimental units). 27 experimental units per site

Experimental design:

S denotes the site (S1 Felfil, S2 Errachidia).

M denotes the type of iron fertilizer (M1 comparison, M2 soil application, M3 foliar application). N denotes nano-iron fertilizer (N1 comparison, N2 soil application, N3 foliar application).

Estimation of qualitative yield characteristics:

Digestion and preparation of plant samples for nutrient measurement: The seeds were digested according to Gupta (1999) by taking 0.4 g from all dry and ground plant samples and adding 10 ml of a 9:1 mixture of sulfuric acid (H_2SO_4) and perchloric acid ($HClO_4$). The mixture was left for 24 hours, then the digestion process was completed by heating on a hot plate. The digested sample was then quantitatively transferred to 50 ml standard flasks, and the volume was topped up with distilled water. The following were measured:



From the obtained concentrations of nitrogen, phosphorus, potassium, and iron, the total uptake of these elements in broad bean seeds was calculated according to the equation referred to by Tisdale et al. (1997) as follows:

- The amount of nitrogen, phosphorus, and potassium absorbed in the seeds $\text{mg plant}^{-1} = \text{Element concentration (\%)} \times \text{Seed dry weight in g} \times 10$

- Amount of iron absorbed in seeds $\mu\text{g plant}^{-1} = \text{Element concentration } \mu\text{g g}^{-1} \times \text{Seed dry weight in g}$

- Protein concentration in seeds %: Protein concentration is calculated after estimating the nitrogen content in the seeds using the equation below, according to the method of Bremner and Edwards (1965). Protein concentration in seeds % = % Nitrogen in seeds $\times 6.25$

Statistical Analysis: The results were statistically analyzed using a multiple-parameter test at a probability level of 0.05% and LSD values were calculated as described in Al-Rawi (2000) using the SAS system (2012.)

IV. Results and Discussion:

. The results showed a clear and significant advantage for the fertilized treatments compared to the control (non-fertilized) treatments, according to the following percentages

- Nitrogen uptake: Nano-foliar spray (N3) achieved an increase of 62.88%. Mineral foliar spray (M3) achieved an increase of 342.43%. With the complete overlap at the second site (M3N3S2), the increase reached 585.2%.

- Phosphorus uptake: Nano-foliar spray (N3) led to an increase of 55.35%, mineral foliar spray (M3) by 338.74%, and the overlap (M3N3S1) achieved the highest increase at 697.5%.

- Potassium uptake: The increase with nano-foliar spray was 45.76%, and with mineral foliar spray, it was 245.9%.

- Iron uptake: Nano-foliar spray led to an increase of 44.7%, and the metallic foliar spray by 223.8%, and the interaction (M3N3S2) achieved an increase of 389.9%.

- Protein concentration (%): The nano-foliar spray was superior, achieving an increase of 36.5%, while the metallic foliar spray achieved an increase of 167.8%, and when the combined interaction at the first site (M3N3S1) was recorded, the highest percentage increase in protein was recorded, reaching 244.7%, compared to the non-fertilized treatment,

The optimal fertilization combination for best growth and highest nutrient and protein uptake was recorded when foliar application of metallic iron and foliar application of nano-iron (M3N3) were combined. This combination achieved the highest uptake values for all elements (NPK Fe) and the highest protein concentration in broad bean seeds. This superiority is attributed to the high efficiency of the nanoparticles in rapidly penetrating cell membranes and leaf stomata, and their complementarity with the mineral fertilizer. This prevents iron fixation in calcareous soils and stimulates vital processes and protein synthesis within the plant,

Table (1) Soil Analysis Results Translation

Unit	Value		Property
g/Kg ⁻¹	200	160	Sand
	350	300	Silt
	450	540	Clay
)C.L()Clay(Soil Texture
Mg \c m ⁻³	1.35	1.35	Bulk Density
%	290	280	Field Capacity
Ds.m ⁻¹	1.4	0.9	(EC)
	7.4	7.1	(pH)
gkg/soil ⁻¹	8.2	7.9	(O.M)



gkg/soil ⁻¹	317	180	CaCO ₃
Cmol.gkg/soil ⁻¹	17	28.75	(CEC)
mgkg/soil ⁻¹	21	21	Available Nitrogen
	8.5	8.5	Available Phosphorus
	140	140	Available Potassium
	0.11	0.6	Available Iron

Table (1) indicates that the soil in the first study site, located in the Felfil area, has a clayey texture (54% clay, 30% silt, 16% sand), with a bulk density of 1.35 MG/m³ and a field capacity of 28%. These values indicate a good capacity for retaining moisture and nutrients. In contrast, the soil in the second study site, located in the Rashidiya area, has a clayey texture (45% clay, 35% silt, 20% sand), although the increased clay content may limit aeration. Zhang et al. (2023) explained that calcareous clay soils increase cation exchange capacity but reduce iron availability due to its strong adsorption onto clay minerals. The soil pH (pH = 7.4) (pH = 7.2) and high calcium carbonate content (180 g kg⁻¹ to 317 g kg⁻¹) reflect a calcareous nature that limits micronutrient availability. Li et al. (2022) showed that increasing carbonate above 150 g kg⁻¹ reduces iron solubility by 40–50%, while Hosseinpour et al. (2020) found that adding FeO to calcareous soil led to the precipitation of 67% of iron within 30 days, compared to only 42% when using the nano-form. The organic matter content was 1.9 g kg⁻¹, a very low percentage that reduces the formation of Fe-organic complexes. Khan et al. (2021) indicated that soils poor in organic matter (<2%) have a 25–30% reduction in iron absorption and exchange capacity. The cationicity (28.75 cmol/kg⁻¹) was 17 cmol/kg⁻¹, which is high and indicates good fertility. However, the predominance of calcium limits iron availability. Balci and Dağhan (2023) confirmed that high calcium reduces iron absorption by 31% in broad beans. The macronutrients showed variation: available nitrogen (21 mg/kg⁻¹) and available phosphorus (8.5 mg/kg⁻¹) were both below the critical threshold (10 mg/kg⁻¹). Sharma et al. (2022) showed that low phosphorus weakens growth, while excessive phosphorus inhibits iron absorption. Available potassium (140 mg/kg⁻¹) was also present. Soliman et al. (2024) confirmed that potassium stimulates the secretion of free radicals, which increases iron availability.

Available iron levels ranged from 0.6 mg/kg to 0.11 mg/kg, which is below the critical limit of 1–1.5 mg/kg. Rizwan et al. (2019) found that these generally low levels indicate soils with moderate nitrogen and water retention capacity, but are poor in phosphorus, potassium, and iron due to high pH and high carbonate content, along with very low organic matter content. This highlights the importance of iron nano-fertilization to overcome fixation problems and improve absorption in calcareous soils.





Table (2) Effect of fertilization with nano- and mineral iron and soil texture on nitrogen uptake (mg/plant) - 1

S	M	N3	N2	N1		
422	166 313 737	204	167	153	M1	S1
		414	317	259	M2	
		962	753	571	M3	
388	166 313 737	183	155	134	M1	S2
		363	294	229	M2	
		922	687	524	M3	
LSD M= 17.73 N= 17.73		508	396	312	N	
S= 14.477 MNS=43.43						

Table (2) shows the effect of fertilization with nano- and mineral iron and soil texture on nitrogen uptake (mg plant⁻¹). The table shows that fertilization with nano- and mineral fertilizers, using both soil and foliar application methods, affected the nitrogen uptake rate. The highest effect (508 mg plant⁻¹) was observed with nano-foliar spraying in the N3 fertilization treatment, which showed a significant increase of 62%. When nano-fertilizer was applied to the soil, the increase in N2 treatment, compared to the unfertilized treatment, was 26%. For mineral iron fertilization, the significant increase in foliar application in the M3 treatment was 343%, while in soil application it was 89%. A significant difference was found between the first and second sites, with the first site showing an 8.76% increase. Regarding the interaction between the application methods and fertilization with nano- and mineral iron and soil texture, the first site showed the highest increase. In M3N3S1, the nitrogen content increased by 529% compared to the unfertilized treatment, while in S2, the M3N3S2 treatment showed a significant increase of 588% compared to the unfertilized treatment. The increase in nitrogen content of broad bean seeds after fertilization with nano- and mineral Fe fertilizers is likely due to the fact that soil application of the fertilizer increased the available amount of nitrogen in the soil, thus increasing its uptake by the plant. Furthermore, foliar application of nano- and mineral fertilizers increased nitrogen uptake and concentration in the plant. This increase in uptake can also be attributed to the increased demand for this element resulting from improved plant growth. This improvement encourages the plant to increase nutrient uptake to achieve nutritional balance, thereby increasing the concentration and nitrogen content of the seeds. This result is consistent with the findings of Alzreejawi (2020), which indicated that the highest amount of nitrogen uptake occurred during the early stages of growth as a result of foliar application of nano-fertilizers, which complemented soil fertilization. This is attributed to the increased amount of Fe absorbed due to soil application, and also to the high efficiency of nano- and mineral fertilizers in their transfer across plant cell membranes, thus increasing vegetative growth due to the important role of these elements in cell division and photosynthesis processes, increasing the efficiency of enzyme action, in addition to increasing the action of biochemical reactions, which leads to an increase in cell divisions, as well as the role played by these particles in reducing the formation of free oxygen radicals and reducing their activity and oxidation, which delays aging and thus encourages vegetative growth, according to what was concluded by Muhammad (2022), who pointed to the role of nano- and mineral fertilizers in increasing the absorption of the element.



Table (3) Effect of fertilization with nano-iron and mineral and soil texture on phosphorus absorption (plant mg 1(-

S	M	N3	N2	N1		
61	25 52 108	29.73	26.18	15.88	M1	S1
		75.76	41.55	36.86	M2	
		126.65	106.36	90.93	M3	
62	25 52 108	35.83	21.01	18.46	M1	S2
		61.35	56.81	41.02	M2	
		126.11	107.83	87.46	M3	
LSD M= 1.192 N= 1.192 S= 0.9732 MNS= 2.9197		76	60	48	N	

Table (3) shows the effect of fertilization with nano- and mineral iron and soil texture on phosphorus uptake (mg/plant). It is observed from the table that fertilization with nano- and mineral fertilizers, using both soil and foliar application methods, had an effect on increasing phosphorus uptake by seeds (mg/plant). The effect of nano-foliar spraying was particularly noticeable, with the highest value observed in the N3 fertilization treatment (76 mg/plant), which significantly increased phosphorus uptake by 58.3%. When applying nano-fertilizer to the soil, the increase in N2 treatment compared to the unfertilized treatment was 25%. As for mineral iron fertilization, the significant increase in foliar application in treatment M3 was 332%, while when applying mineral iron to the soil, it was 108%. A significant difference was found between the first site, Felfil, and the second site, Rashidiya, where S2 exceeded S1 by 1.6%. Regarding the interaction between the application and fertilization methods using iron, the table also shows the effect of these two methods. The nano- and mineral fertilizers, along with the tissues, were superior in S1, reaching (126.65 mg plant-1) in the M3N3S1 treatment, representing a 697.5% increase compared to the unfertilized treatment. In S2, the M3N3S2 treatment showed a significant increase of (583.1%) compared to the unfertilized treatment. The nano- and mineral fertilizers improve the utilization of active light for photosynthesis, thus increasing the availability of substances that support the development of tiller primordia. This positively impacts the absorption of nutrients in the cultivated area due to increased concentrations of the nutrient Fe. The results are consistent with those of Al-Saidan (2019). Another reason is attributed to the plant's absorption of nutrients through the stomata, leading to increased plant growth and development (Sajadinia et al., 2021).



Table (4) Effect of fertilization with nano- and mineral iron and soil texture on potassium absorption (plant mg 1(-

S	M	N3	N2	N1		
465	219 442 758	314	209	167	M1	S1
		526	455	382	M2	
		839	689	608	M3	
481	219 442 758	276	202	146	M1	S2
		496	435	363	M2	
		943	809	663	M3	
LSD M=7.5108 N= 7.5108 S= 6.1325 MNS=18.398		565	466	388	N	

Table (4) illustrates the effect of fertilization with nano- and mineral iron and soil texture on potassium uptake by seeds (mg/plant). The table shows that fertilization with nano- and mineral fertilizers, using both soil and foliar application methods, increased seed uptake (mg/plant). The effect of nano-foliar spraying was particularly noticeable, with the highest value observed in the N3 fertilization treatment (565 mg/plant), representing a significant increase of 45%. When applying nano-fertilizers to the soil, the increase in treatment N2, compared to the unfertilized treatment, was 20%. With mineral iron fertilization, the significant increase in foliar application in treatment M3 was 246%, while in soil application of mineral iron M2, it was 101%. A significant difference was found between the Felfil and Rashidiya sites, with S2 exceeding S1 by 3.4%. Regarding the interaction between the application and fertilization methods, the table also shows the effect of these methods. With nano- and mineral iron and tissue, the second site was superior, reaching (943 mg plant⁻¹) with treatment M3N3S2, an increase of 545% compared to the unfertilized treatment. At the first site, the M3N3S1 treatment showed a significant increase of (402%) compared to the unfertilized treatment. Furthermore, fertilization with nano- and mineral iron increased plant growth, which in turn increased potassium demand. These results are consistent with Al-Shammari's (2021) findings that fertilization with nano- and mineral iron increases the absorption and potassium content of broad beans. Another reason is that the plant's absorption of nutrients through the stomata led to increased plant growth and development (Sajadinia et al., 2021).



Table (5) Effect of fertilization with nano- and mineral iron and soil texture on iron absorption (micrograms per plant -1(

S	M	N3	N2	N1		
2220	1137 1938 3686	1305	1112	927	M1	S1
		2370	1926	1642	M2	
		4190	3559	2951	M3	
2288		1414	1127	942	M1	S2
		2195	1885	1612	M2	
		4618	3753	3046	M3	
LSD M=33.983 N= 33.983 S=27.747 MNS=83.24		2682	2227	1853	N	

Table (5) shows the effect of fertilization with nano- and mineral iron and soil texture on iron absorption in seeds ($\mu\text{g}/\text{plant-1}$). It is observed that fertilization with nano- and mineral fertilizers, using both soil and foliar application methods, had an effect on the iron absorption rate ($\mu\text{g}/\text{plant-1}$). The foliar application of nano-iron had a significant effect, with the highest value observed in the N3 fertilization treatment ($2682 \mu\text{g}/\text{plant-1}$), which was significantly higher with an increase of 44%. In contrast, with soil application of nano-iron, the increase in treatment N2, compared to the unfertilized treatment, was 20%. As for fertilization with mineral iron, the significant increase in foliar application in treatment M3 was 224%, while with soil application of mineral iron it was 70%. A significant difference was found between Felfil and Rashidiya, with S2 exceeding S1 by 3%. The results are observed regarding the interaction between the two methods of iron application and fertilization. The effects of nano- and mineral iron fertilization and soil texture were as follows: The second site showed superiority, with a 390% increase in iron uptake by seeds ($\mu\text{g}/\text{plant}$) in the M3N3S2 treatment compared to the unfertilized treatment. In the first site, the M3N3S1 treatment showed a significant increase of 351% compared to the unfertilized treatment. The increased iron uptake in the fertilized treatments may be attributed to increased uptake by the roots due to the soil application, which increased the available iron content in the soil. Furthermore, foliar feeding with nano- and mineral iron fertilizers enhanced the plant's ability to absorb more iron. Due to their small size, nanoparticles are readily absorbed and translocated from the leaf surface into the plant, thus increasing the seed iron content (Singh et al., 2017; Al-Haidari, 2021



Table (6) Effect of Nano- and Mineral Iron Fertilization and Soil Texture on Protein Concentration(%)

S	M	N3	N2	N1		
11.62	6.36 9.69 17.06	7.17	6.30	6.24	M1	S1
		11.54	9.86	8.32	M2	
		21.51	18.59	15.01	M3	
10.46	6.36 9.69 17.06	6.53	6.19	5.77	M1	S2
		11.29	9.42	7.72	M2	
		18.59	15.57	13.08	M3	
LSD M= 0.3363 N=0.3363 S= 0.2746 MNS= 0.8238		12.77	10.99	9.36	N	

Table (6) shows the effect of fertilization with nano- and mineral iron and soil texture on the protein concentration in seeds (%). The table shows that fertilization with nano- and mineral fertilizers using both soil and foliar application methods had an effect on the percentage of protein concentration. The effect of nano-foliar spraying was most noticeable, with the highest value observed in the N3 fertilization treatment, reaching 12.77%, which significantly exceeded the previous value by an increase of 36.4%. When applying nano-fertilizers to the soil, the increase in treatment N2, compared to the unfertilized treatment, was 17.4%. As for fertilization with mineral iron, the significant increase in foliar application in treatment M3 was 168%, while in soil application it was 52%. A significant difference was found between the Felfil and Rashidiya areas, with S1 exceeding S2 by an increase of 11%. Regarding the interaction between the application methods and fertilization with nano- and mineral iron and soil texture, the Felfil area exhibited the highest percentage. The protein concentration in the seeds (%) increased by 244% with treatment M3N3S1 compared to the unfertilized treatment. In the Rashidiya region, the increase was significant (222%) with treatment M3N3S2 compared to the unfertilized treatment. The increase in protein percentage in broad bean seeds is closely related to the increase in nitrogen percentage and concentration in the seeds. Nitrogen plays a crucial role in protein synthesis in the leaves, which is subsequently transported and stored in the seeds. Phosphorus also plays a vital role in the formation and production of energy compounds (ATP), which are essential for photosynthesis, carbohydrate formation, and amino acid synthesis, thus increasing protein percentage. Furthermore, phosphorus participates in protein synthesis through its crucial role in the formation of nucleic acids (DNA and RNA), which are essential for protein synthesis (Minkel and Kirby, 1982). These results are consistent with those of Isho (2020) and Zyada et al. (2020), who found increased concentrations of the nutrient NPK in the seeds. Broad beans benefit from the addition of soil and foliar nano-fertilizers, which leads to an increase in the protein content of the seeds

Conclusion

Iron (Fe) is a key micronutrient required by plants in small quantities, yet it plays essential roles in growth and development. It is a component of many enzymes and proteins responsible for oxidation-reduction reactions, such as cytochromes and ferritin, and indirectly contributes to chlorophyll formation. It also plays a crucial role in cellular respiration and energy production. Furthermore, it is a key factor in root nodule formation and promotes the activity of nitrogen-fixing Rhizobium bacteria in legumes, thus improving biofeedback efficiency and increasing soil fertility (Khan et al., 2022). Traditional mineral fertilization has been used to treat iron deficiency, with soil application of compounds such as ferrous sulfate ($FeSO_4 \cdot 7H_2O$) to increase the concentration of available iron in the



soil and improve its uptake by roots. However, the effectiveness of this method may be negatively affected in calcareous soils due to the fixation of the element. Since it relies on foliar application as a complementary technique, where iron is sprayed directly onto the leaves, this accelerates its absorption, improves chlorophyll content, and has a rapid effect on vegetative growth (Shirsat et al., 2024).

With recent advancements in nanotechnology, nano-fertilization has emerged as an innovative strategy to enhance fertilizer use efficiency. Soil application of nano-iron contributes to improving the availability of the element in the soil and increasing its absorption by the roots, while foliar application has shown remarkable effectiveness in promoting vegetative growth (Mohamed et al., 2023; Al-Khafaji et al., 2024).

Therefore, studying the effect of mineral and nano-iron fertilization through soil and foliar application on broad bean crops in calcareous soil conditions is a fundamental scientific step towards developing advanced fertilization programs that contribute to increased productivity and agricultural sustainability. Among its most important objectives

V. References

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