Role of Rhizobium Inoculation and Addition of Zinc and Iron on Some Growth Indicators of Fava Beans in Gypsiferous Soil

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Abstract

A field experiment was conducted using a Randomized Complete Block Design (RCBD) at the University of Tikrit – College of Agriculture fields – Soil and Water Resources Department Station, during the autumn agricultural season of 2025 (starting on September 15, 2025 The The purpose of this study the effect of inoculation with Rhizobium bacteria at two levels (inoculated and non-inoculated), the addition of zinc and iron at three levels (0, 3, 6) along with control treatment no addition), and their interactions on some growth indicators of faba bean plants grown in gypsiferous soil. The triple interaction treatment (inoculation + Zn3 + Fe3) was superior to the control treatment, achieving increases in the number of root nodules, their fresh weight, dry weight, plant height, and leaf area by (88.33, 1.75, 44.69, 99.70, and 31.10), respectively. The results of sole inoculation also showed the superiority of the inoculated treatment over the non-inoculated one, with increases in the number of root nodules, fresh weight, dry weight, plant height, and leaf area by (73.85, 1.33, 38.73, 88.99, and 25.61), respectively. The results of zinc addition showed superiority over the control treatment, with the Zn3 level giving the highest values for all traits, achieving increases in the number of root nodules, fresh weight, dry weight, plant height, and leaf area by (63.56, 1.06, 40.20, 86.93, and 25.59), respectively. The results also showed the superiority of inoculation combined with zinc, where the Zn3 level gave the highest values, achieving increases in the number of root nodules, fresh weight, dry weight, plant height, and leaf area by (84.33, 1.68, 42.64, 95.45, and 27.53), respectively. The results indicated that iron addition was significantly superior to the control treatment, with the Fe3 level outperforming others, achieving increases in the number of root nodules, fresh weight, dry weight, plant height, and leaf area by (57.72, 0.92, 37.42, 87.50, and 25.88), respectively. The results of inoculation combined with iron also showed superiority over the non-inoculated treatment at the Fe3 level, achieving increases in the number of root nodules, fresh weight, dry weight, plant height, and leaf area by (76.66, 1.48, 40.81, 94.59, and 28.01), respectively. Furthermore, the binary interaction between zinc and iron was superior, with the level (Zn3+Fe3) recording the highest values compared to the non-addition treatment, achieving increases in the number of root nodules, fresh weight, dry weight, plant height, and leaf area by (66.50, 1.11, 41.97, 93.65, and 28.73), respectively.

Introduction

Biofertilizers are solid or liquid substances containing beneficial living microorganisms that are added to seeds, soil, or seedlings to improve plant growth or increase crop yield. They achieve this by supplying plants with nutrients, hormones, and chelating compounds that increase absorption efficiency or improve

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the availability of nutrient elements, in addition to their role in enhancing plant resistance to unfavorable environmental conditions and pathogens, thus contributing to sustainable agriculture and a clean environment (1). These biofertilizers can be used to supply plants with essential nutrients such as nitrogen and phosphorus, as well as zinc- and iron-solubilizing bacteria, among other elements needed by the plant, by dissolving and stimulating their activity in the rhizosphere (the soil surrounding or adjacent to the roots) and making these nutrients available in a form ready for plants. The soils of arid and semi-arid regions, especially gypsiferous soils, are characterized by low clay and organic matter content and high concentrations of gypsum and calcium carbonate. This leads to a range of chemical, physical, fertility, and biological problems. Prominent among these problems are poor soil water-holding capacity, high pH, low content of essential nutrients (2), as well as low density of beneficial microorganisms in the rhizosphere, particularly plant growth-promoting rhizobacteria (PGPR), and specifically nitrogen-fixing Rhizobium bacteria (3) . Rhizobium bacteria are microorganisms capable of forming root nodules in a symbiotic relationship with leguminous plants to fix atmospheric nitrogen. They contribute to fixing an estimated 65% of the nitrogen used in agricultural production. This group comprises approximately 76 species distributed across 16 genera. Rhizobium bacteria are Gram-negative, ranging in length from 1.2 to 3 micrometers and in width from 0.5 to 0.9 micrometers. They are non-spore-forming, motile via peritrichous flagella, rod-shaped, and grow at temperatures between 20-30°C and a pH ranging from 6-8, with the ability to grow within a pH range of 5-9 (4,5) . Micronutrients in gypsiferous soils suffer from several problems, including the low availability of elements such as iron, zinc, manganese, and copper. This is due to the high pH of gypsiferous soils, which often exceeds 7.5, making them neutral to alkaline, leading to the precipitation of these elements as insoluble compounds, thereby reducing their availability in the soil solution for plants (6) . Additionally, calcium competes with or interferes with the absorption of micronutrients, especially divalent elements like Fe and Zn, at absorption sites due to the high concentrations of calcium ions in gypsiferous soils (7) . Furthermore, weak microbial activity in gypsiferous soil negatively affects the availability of micronutrients, as gypsum impacts the composition of microorganisms that contribute to the release of elements and the increase of organic matter (8) . Faba bean (Vicia faba) is one of the most important winter crops of the Fabaceae family, holding significant economic and high nutritional value due to its high protein content. It also has the ability to improve soil properties by fixing atmospheric nitrogen in symbiosis with root-nodule bacteria, thereby enhancing soil fertility and reducing reliance on chemical nitrogen fertilizers (9). (10) found that inoculating faba bean with Rhizobium led to a significant increase in plant characteristics. The single inoculation treatment with Rhizobium leguminosarum outperformed the non-inoculated treatment, with percentage increases in the number of root nodules and their fresh weight reaching (83.40% and 46.90%), respectively, as well as increases in plant height and shoot dry weight (3.00% and 16.65%), respectively.

This study aims to:

1. Investigate the relationship between micronutrient (iron and zinc) fertilization and Rhizobium inoculation on the activity of Rhizobium bacteria, root nodule formation, and nitrogen fixation.
2. Study the interaction between Rhizobium inoculation and iron and zinc fertilization on the growth of faba bean plants in gypsiferous soil.

Materials and Methods

Preparation of the Bioinoculant

A local bioinoculant containing Rhizobium sp. bacteria, prepared from a locally isolated and molecularly identified strain, was used. It was applied by loading it onto a peat moss carrier manufactured by a spain company and mixed with faba bean seeds. Gum arabic was used to fix the inoculant onto the seeds before planting them.

Experimental Procedure

Land plowing, leveling, and smoothing were carried out on September 5, 2025. The land was divided into three sectors (blocks). Each block was divided into eighteen plots. The area of each plot was (3 x 3) m², with a distance of 150 cm between blocks and 50 cm between plots. Soil samples were then taken from a depth of (0-30 cm) for, chemical, and biological analysis of the soil. Seeds were planted on September 15, 2025, in their designated plots three days after field disinfection. Seeds were sown in furrows at a distance of 60 cm between furrows and 40 cm between holes, at a depth of 2-3 cm, with 2 seeds per hole, later thinned to one plant per hole. Nitrogen was added at a rate of 80 kg N ha^{-1} using urea fertilizer (46% N) as a nitrogen source before planting. Phosphorus was added at a rate of 320 kg P ha^{-1} using triple superphosphate fertilizer (21% P), and potassium was added at a rate of 320 kg K ha^{-1} using potassium sulfate fertilizer (43% K) in a single application before planting (11) . The fertilizer quantities were mixed and added to the experiment in bands adjacent to the planting lines. Zinc and iron (0,3,6). were added 20 days after planting, based on each plant within the plot. Drip irrigation was used, and weeds were controlled manually.

Table 1. Pre-planting soil analysis

Fe $_{(PPm)}$	Zn $_{(PPm}$	K%	P%	N%	EC$_{(Ms)}$	pH
1.64	0.15	214.4	4.04	18	2.3	7.6

Studied Traits: Three plants (vegetative parts) were randomly uprooted from each experimental unit at the flowering stage to calculate the number of root nodules per plant, fresh weight of root nodules, plant height, dry weight of the vegetative part, and leaf area at this stage (12) . The measurements were as follows:

.1<u>Number of Root Nodules and Their Fresh Weight</u> (nodules $plant^{-1}$, g $plant^{-1}$)

Random plant samples were selected, and the area around the roots was carefully excavated to avoid damaging the nodules. The roots were placed in designated bags, and the data for each sample were recorded. The plants were then *transferred* to the laboratory, and the roots were washed under a gentle stream of water using fine sieves that allowed soil and suspended particles to pass through without losing the nodules. Afterwards, the number of nodules per plant was counted. The fresh weight of the nodules for each experimental unit was then determined using a sensitive electronic balance.

2. Plant Height (cm plant^{-1})

Plant height was measured using a measuring tape before uprooting, starting from the soil surface to the highest top of the plant.

3. Dry Weight of the Vegetative Part (g plant^{-1})

The plant samples were dried in a forced-air oven at a temperature of 65°C for 72 hours or until a constant weight was achieved. They were then weighed using a sensitive electronic balance for each experimental unit.

4. Leaf Area (cm^2 leaf^{-1})

Leaf area for faba bean plants was measured using the direct measurement method. Ten leaves were taken from different locations on each plant, and the length and width of each leaf were measured to calculate its area. The average per experimental unit was then derived (13).

Results and Discussion

1. Number of Root Nodules

Table 2 shows the effect of Rhizobium inoculation, zinc and iron addition, and their interactions on the number of root nodules of faba bean plants at the flowering stage. The results in the table indicate that the effect of inoculation was significant on the number of root nodules compared to the non-inoculated one, reaching an average of (73.85) nodules plant^{-1}, whereas it was (33.22) nodules plant^{-1} in the non-inoculated treatment. The reason for the increase in the number of root nodules in the inoculated treatments may be attributed to the efficiency of the prepared bioinoculant in forming a symbiotic relationship with the leguminous plant and fixing atmospheric nitrogen inside the root nodules, leading to improved plant growth and nodule formation. This agrees with what was mentioned by (14) . The results also showed that zinc addition led to a significant increase in the number of root nodules compared to the non-addition treatment, as the (Zn3) level gave the highest value (63.56) nodules plant^{-1}, while the (Zn2) level recorded an average value of (55.67) nodules plant^{-1}, and the (Zn1) level recorded the lowest value of (41.39) nodules plant^{-1}. The inoculated treatments with zinc showed significant differences, as the (Zn3) level excelled by giving (84.33) nodules plant^{-1}, the (Zn2) level gave an average of (75.22) nodules plant^{-1}, while the (Zn1) level gave the lowest value of (62.00) nodules plant^{-1}, compared to non-inoculated treatments. The effect of zinc may be due to its role in activating many essential enzymes for plant and rhizobial growth, in addition to

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its involvement in the formation of the amino acid tryptophan, which is the primary precursor for the formation of auxins that encourage root growth and nodule formation. This agrees with what was indicated by (15). The results also showed that iron addition had a significant effect on increasing the number of root nodules, as the (Fe3) level outperformed the other levels by giving the highest values compared to the non-addition treatment, reaching (57.72) nodules plant^{-1}, while the (Fe2) level gave an average value of (55.78) nodules plant^{-1}, and the (Fe1) level gave the lowest value of (47.11) nodules plant^{-1}. The table also shows that the effect of inoculation and iron addition resulted in significant differences in the number of root nodules, as the (Fe3) level gave the highest value (76.66) nodules plant^{-1}, the (Fe2) level gave an average of (74.78) nodules plant^{-1}, while the (Fe1) level recorded the lowest value (70.11) nodules plant^{-1}. The reason may be that inoculation increases the efficiency of root nodules in utilizing iron, which is necessary for the enzymes involved in the nitrogen fixation process within the root nodules. This agrees with what was mentioned by (16) . The table also shows significant differences in the interaction between zinc and iron, as the (Zn3+Fe3) level excelled over other levels, reaching an average of (66.50) nodules plant^{-1}, while the (Zn1+Fe1) level gave the lowest average of (28.67) nodules plant^{-1}. This difference may be attributed to the complementary interaction between the two elements in encouraging the formation of root nodules and increasing their efficiency (17) . The results of the triple interaction also showed that the addition of zinc led to a significant increase in the number of root nodules compared to the non-addition treatment, as the (Zn3) level gave the highest values when interacting with different iron levels under inoculation, reaching (78.67, 86.00, and 88.33) nodules plant^{-1}, while the (Zn2) level recorded average values of (74.33, 75.00, and 76.33) nodules plant^{-1}, and the (Zn1) level recorded the lowest values of (57.33, 63.33, and 65.33) nodules plant^{-1} in the inoculated treatments. This superiority may be attributed to the complementary and vital role of the inoculant and its interaction with appropriate levels of zinc and iron fertilization in significantly increasing the number of root nodules, enhancing their efficiency, and strengthening the symbiotic relationship. This agrees with the findings of (18,19).

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Table 2. Number of Root Nodules

Mean of Inoculation × Zinc	General Mean of Zinc	Mean (Zinc × Iron)		Inoculated		Non-inoculated		Iron	Zinc
62.00	41.39	28.67		57.33		0.00		Fe 1	Zn 1
		46.67		63.33		30.00		Fe 2	
		48.83		65.33		32.33		Fe 3	
75.22	55.67	53.50		74.33		32.67		Fe 1	Zn 2
		55.67		75.00		36.33		Fe 2	
		57.83		76.33		39.33		Fe 3	
84.33	63.56	59.17		78.67		39.67		Fe 1	Zn 3
		65.00		86.00		44.00		Fe 2	
		66.50		88.33		44.67		Fe 3	
		53.54		73.85		33.22		**Mean of Inoculation**	
		57.72	Fe3	55.78	Fe2	47.11	Fe1	**Mean of Iron**	
		76.66	Fe3	74.78	Fe2	70.11	Fe1	**Inoculation × Iron Mean**	
$LSD_{0.05\%}$									
	Zinc × Iron	**Inoculation × Iron**		**Inoculation × Zinc**		**Iron**		**Zinc**	**Inoculation**
	5.565	4.5438		4.5438		3.2129		3.2129	2.6234
7.8701						**Triple interaction**			

2. Fresh Weight of Root Nodules

Table 3 shows the effect of Rhizobium inoculation, zinc and iron addition, and their interactions on the fresh weight of root nodules of faba bean plants at the flowering stage. The inoculated treatment gave the highest value, reaching (1.33) g plant^{-1}, compared to the non-inoculated treatment (0.32) g plant^{-1}. This increase may be attributed to the bioinoculant leading to an increase in the number of bacteria infecting the roots of leguminous plants, which led to an increase in the number of root nodules and consequently led to an increase in the fresh weight of

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these nodules. This agrees with what was mentioned by . (19). The results also showed that zinc addition led to an increase in root nodule weights compared to the non-addition treatment, as the three levels of zinc gave values of (0.51, 0.89, and 1.06) g plant^{-1}, respectively, in the non-inoculated treatment. The results of the binary interaction between inoculation and zinc also showed significant differences in nodule weight compared to the non-inoculated treatment, reaching (0.87, 1.44, and 1.68) g plant^{-1}, respectively. The results also showed that iron addition led to an increase in root nodule weights, reaching (0.70, 0.85, and 0.92) g plant^{-1}, respectively, in the non-inoculated treatment. Meanwhile, the results of the interaction between inoculation and iron showed values reaching (1.16, 1.34, and 1.48) g plant^{-1}, respectively, compared to the non-inoculated treatment. This increase may be due to the vital role of bacteria and iron associated with activating the nitrogenase enzyme responsible for nitrogen fixation, as well as improving root nodules and increasing their number and weight in leguminous plants (20). The table also shows an effect of the interaction between zinc and iron levels on increasing nodule weights, as the interaction between the (Zn3) level and the (Fe3) level gave the highest value, reaching (1.11) g plant^{-1}, while the interaction between the (Zn1) level and the (Fe1) level recorded the lowest value, reaching (0.34) g plant^{-1} in the non-inoculated treatment (20). indicated that the interaction between zinc and iron improves the root environment and increases the efficiency of nutrient absorption, which is positively reflected in increasing root nodule weight. The results of the triple interaction also showed that the addition of zinc led to a significant increase in root nodule weights compared to the non-addition treatment, as the (Zn3) level gave the highest values when interacting with different iron levels under inoculation, reaching (1.63, 1.66, and 1.75) g plant^{-1}, while the (Zn2) level recorded average values of (1.18, 1.53, and 1.60) g plant^{-1}, and the (Zn1) level recorded the lowest values of (0.67, 0.83, and 1.10) g plant^{-1} in the inoculated treatments. This superiority is attributed to the fact that Rhizobium inoculation increases the number of root nodules, thereby increasing the weight and efficiency of these nodules in nitrogen fixation, while both zinc and iron contribute to enzyme activation as well as improving the root environment. These results agree with the findings of (21,22)

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Table 3. Fresh Weight of Root Nodules

Mean of Inoculation × Zinc	General Mean of Zinc	Mean (Zinc × Iron)	Inoculated	Non-inoculated	Iron	Zinc
0.87	0.51	0.34	0.67	0.00	Fe 1	Zn 1
		0.54	0.83	0.24	Fe 2	
		0.67	1.10	0.24	Fe 3	
1.44	0.89	0.76	1.18	0.33	Fe 1	Zn 2
		0.95	1.53	0.36	Fe 2	
		0.98	1.60	0.36	Fe 3	
1.68	1.06	1.01	1.63	0.39	Fe 1	Zn 3
		1.06	1.66	0.45	Fe 2	
		1.11	1.75	0.47	Fe 3	
		0.82	1.33	0.32	Mean of Inoculation	

	0.92	Fe3	0.85	Fe2	0.70	Fe1	**Mean of Iron**
	1.48	Fe3	1.34	Fe2	1.16	Fe1	**Inoculation × Iron Mean**

LSD$_{0.05\%}$						
	Zinc × Iron	**Inoculation × Iron**	**Inoculation × Zinc**	**Iron**	**Zinc**	**Inoculation**
	0.0984	0.0803	0.0803	0.0568	0.0568	0.0464
0.1392				**Triple interaction**		

3. Dry Weight of the Vegetative Part

Table 4 shows the effect of Rhizobium inoculation, zinc and iron addition, and their interactions on the dry weight of the vegetative part of faba bean plants at the flowering stage. The inoculated treatment gave the highest value, reaching (38.37) g plant^{-1}, compared to the non-inoculated treatment, which gave (32.82) g plant^{-1}. This increase may be attributed to the prepared bioinoculant having a direct or indirect effect on promoting plant growth through increased nitrogen and nutrient uptake and the production of plant hormones. This agrees with what was

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mentioned by (23). The results showed that zinc addition led to an increase in the dry weight of the vegetative part compared to the non-addition treatment, as the three levels of zinc gave values of (31.96, 35.17, and 40.20) g plant^{-1}, respectively, in the non-inoculated treatment. The results of the binary interaction between inoculation and zinc also showed significant differences in dry weight, reaching (35.50, 38.05, and 42.64) g plant^{-1}, respectively. These results agree with those found by (24), who pointed to the role of bioinoculant in improving and forming organic compounds and proteins, as well as the important role of zinc in increasing the efficiency of nutrient uptake, which in turn increases the dry weight of the vegetative part in leguminous plants. The results also showed that iron addition led to an increase in the dry weight of the vegetative part, reaching (34.82, 35.08, and 37.42) g plant^{-1}, respectively, in the non-inoculated treatment. Meanwhile, the results of the interaction between inoculation and iron showed values reaching (37.80, 37.59, and 40.81) g plant^{-1}, respectively. This increase may be due to the role of iron, which is involved in the composition of enzymes associated with metabolic processes, and the availability of inoculation improves nutrient uptake, thereby increasing plant biomass, which is reflected in the dry weight of the vegetative part (25) . The table also shows an effect of the interaction between zinc and iron levels on increasing the dry weight of the vegetative part, as the interaction between the (Zn3) level and the (Fe3) level gave the highest value, reaching (41.97) g plant^{-1}, while the interaction between the (Zn1) level and the (Fe1) level recorded the lowest value, reaching (31.03) g plant^{-1}. This is attributed to the fact that zinc and iron jointly improve growth, possibly as a result of their shared role in reducing the antagonism of nutrients within the plant as well as improving nutrient transport between plant tissues. This agrees with what was found by (25). The triple interaction between the (Zn3) level with inoculation and different iron levels also gave the highest value, reaching (41.32, 41.92, and 44.69) g plant^{-1}, while the non-inoculated treatment for the same level recorded values of (37.27, 36.73, and 39.24) g plant^{-1}, respectively. The (Zn2) level gave values reaching (36.87, 36.52, and 40.75) g plant^{-1}, respectively, compared to the non-inoculated treatment, which gave (30.82, 32.86, and 33.20) g plant^{-1}, respectively. The (Zn1) level gave the lowest values (34.60, 34.93, and 36.98) g plant^{-1}, compared to the non-inoculated treatment, which gave (27.46, 28.14, and 29.64) g plant^{-1}. These results can be explained by the occurrence of functional integration among the three factors, leading to improved balance between nitrogen and carbon and energy production within the plant, as confirmed by (27).

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Table 4. Dry Weight of the Vegetative Part

Mean of Inoculation × Zinc	General Mean of Zinc	Mean (Zinc × Iron)	Inoculated	Non-inoculated	Iron	Zinc
35.50	31.96	31.03	34.60	27.46	Fe 1	Zn 1
		31.54	34.93	28.14	Fe 2	
		33.31	36.98	29.64	Fe 3	
38.05	35.17	33.85	36.87	30.82	Fe 1	Zn 2
		34.69	36.52	32.86	Fe 2	
		36.98	40.75	33.20	Fe 3	
42.64	40.20	39.29	41.32	37.27	Fe 1	Zn 3
		39.33	41.92	36.73	Fe 2	
		41.97	44.69	39.24	Fe 3	
		35.77	38.73	32.82	Mean of Inoculation	
		37.42 Fe3	35.08 Fe2	34.82 Fe1	**Mean of Iron**	
		40.81 Fe3	37.59 Fe2	37.80 Fe1	**Inoculation × Iron Mean**	
$LSD_{0.05\%}$						
	Zinc × Iron	**Inoculation × Iron**	**Inoculation × Zinc**	**Iron**	**Zinc**	**Inoculation**
	4.6189	3.7713	3.7713	2.6667	2.6667	2.1774
6.5321				**Triple interaction**		

4. Plant Height

Table 5 shows the effect of Rhizobium inoculation, zinc and iron addition, and their interactions on the height of faba bean plants at the flowering stage. The inoculated treatment gave the highest value, reaching (88.99) cm $plant^{-1}$, compared to the non-inoculated treatment, which gave (71.49) cm $plant^{-1}$. The results demonstrate the stimulatory role of the prepared bacterial inoculant in improving growth traits, which is consistent with the hypothesis regarding the role of Plant Growth-Promoting Bacteria (PGPB). These isolates possess the ability to synthesize indole-3-acetic acid (IAA), which alters root architecture and stimulates their longitudinal growth, alongside their role in converting unavailable phosphorus into absorbable forms. This development in the root

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system allowed the plant to utilize fixed nitrogen in carbon assimilation processes and amino acid synthesis, ultimately leading to a significant superiority in plant height. This agrees with what was mentioned by (15). The results showed that zinc addition led to an increase in plant height, as the three levels of zinc gave values of (75.87, 77.93, and 86.93) cm plant^{-1}, respectively. The results of the binary interaction between inoculation and zinc also showed significant differences compared to the non-inoculated treatment, reaching (85.66, 85.87, and 95.45) cm plant^{-1}, respectively. The reason for this increase may be attributed to the role of zinc in increasing cell division and improving growth through hormone regulation, and the effective role of the inoculant in increasing and improving nutrient uptake. This agrees with what was mentioned by (27). The results also showed that iron addition led to an increase in plant height, reaching (72.64, 80.60, and 87.50) cm plant^{-1}, respectively. Meanwhile, the results of the interaction between inoculation and iron showed values reaching (82.96, 89.43, and 94.59) cm plant^{-1}, respectively. This increase may be due to the vital role of iron in enhancing photosynthetic efficiency and the building of proteins and enzymes, thereby providing the necessary energy for cell division and elongation processes in growing tips, as inoculation synergizes with iron in this process, as indicated by (28,29) . The table also shows an effect of the interaction between zinc and iron levels on increasing plant height, as the interaction between the (Zn3) level and the (Fe3) level gave the highest value, reaching (93.65) cm plant^{-1}, while the interaction between the (Zn1) level and the (Fe1) level recorded the lowest value, reaching (66.52) cm plant^{-1}. This increase may be attributed to the roles of zinc and iron and the complementarity between them through their contribution to a range of physiological processes in which zinc and iron play an important role in increasing plant height. This agrees with what was found by (25). The interaction between inoculation, the (Zn3) level, and different iron levels gave the highest values, reaching (98.33, 88.33, and 99.70) cm plant^{-1}, while the non-inoculated treatment for the same level recorded values of (68.60, 79.00, and 87.60) cm plant^{-1}, respectively. The (Zn2) level gave values reaching (71.96, 92.67, and 92.97) cm plant^{-1}, respectively, compared to the non-inoculated treatment, which gave (63.90, 65.09, and 81.01) cm plant^{-1}, respectively. The (Zn1) level gave the lowest values (78.59, 87.29, and 91.11) cm plant^{-1}, respectively, compared to the non-inoculated treatment, which gave (54.45, 71.21, and 72.58) cm plant^{-1}. The positive response in plant height under the triple interaction is attributed to the integrated role of these factors in improving the efficiency of nutrient uptake and utilization. Zinc acts as a structural activator of enzymes, enhancing nucleic acid synthesis and cell division in apical meristematic tissues. This synergizes with the role of iron in activating redox enzymes that provide stem cells with the necessary energy for elongation, in addition to the vital role of inoculation in secreting siderophores and growth-stimulating substances that increase cell membrane permeability and facilitate the

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movement of micronutrients within the conductive vessels (26,27).

Table 5. Plant Height

Mean of Inoculation × Zinc	General Mean of Zinc	Mean (Zinc × Iron)	Inoculated	Non-inoculated	Iron	Zinc
85.66	75.87	66.52	78.59	54.45	Fe 1	Zn 1
		79.25	87.29	71.21	Fe 2	
		81.85	91.11	72.58	Fe 3	
85.87	77.93	67.93	71.96	63.90	Fe 1	Zn 2
		78.88	92.67	65.09	Fe 2	
		86.99	92.97	81.01	Fe 3	
95.45	86.93	83.47	98.33	68.60	Fe 1	Zn 3
		83.67	88.33	79.00	Fe 2	
		93.65	99.70	87.60	Fe 3	
		80.24	88.99	71.49	Mean of Inoculation	
		87.50 Fe3	80.60 Fe2	72.64 Fe1	**Mean of Iron**	
		94.59 Fe3	89.43 Fe2	82.96 Fe1	**Inoculation × Iron Mean**	
LSD$_{0.05\%}$						
	Zinc × Iron	**Inoculation × Iron**	**Inoculation × Zinc**	**Iron**	**Zinc**	**Inoculation**
	4.2766	3.4918	3.4918	2.4691	2.4691	2.016
6.048				**Triple interaction**		

5. Leaf Area

Table 6 shows the effect of Rhizobium inoculation, zinc and iron addition, and their interactions on the leaf area of faba bean plants at the flowering stage. The inoculated treatment gave the highest value, reaching (25.61) cm² plant^{-1}, compared to the non-inoculated treatment, which gave (21.24) cm² plant^{-1}. This increase may be attributed to the role of inoculation in increasing leaf area due to the ability of Rhizobium bacteria to secrete internal growth regulators, most importantly cytokinins. These hormones stimulate cell division in the leaf blade and

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increase its diameter, as well as playing a role in delaying chlorophyll degradation, which keeps leaves physiologically active for a longer period and increases their lateral expansion (30). The results showed that zinc addition led to an increase in plant leaf area, as the three levels of zinc gave values of (21.74, 22.94, and 25.59) cm² plant⁻¹, respectively. The results of the binary interaction between inoculation and zinc also showed significant differences compared to the non-inoculated treatment, reaching (23.82, 25.47, and 27.53) cm² plant⁻¹, respectively. The reason for this increase may be attributed to the vital role of zinc in increasing leaf area as it is the primary element responsible for building the amino acid tryptophan, which is the direct source for building auxin (IAA). Auxins are responsible for cell wall elongation and the expansion of leaf cells. Zinc also maintains the integrity of cell membranes under stress conditions, ensuring the flow of water and nutrients necessary for leaf blade expansion . The results also showed that iron addition led to an increase in leaf area, reaching (21.33, 23.06, and 25.88) cm² plant⁻¹, respectively. Meanwhile, the results of the interaction between inoculation and iron showed values reaching (23.48, 35.33, and 28.01) cm² plant⁻¹, respectively. The effect of iron on leaf area may be linked to its direct role in chloroplast efficiency. Iron is involved in the composition of cytochrome and ferredoxin proteins, which are essential components of electron transport, thereby raising the net photosynthetic rate. This surplus of synthesized chemical energy (ATP) is directed towards building new leaf tissues and increasing the thickness and area of the mesophyll cell layer in the leaf (27). The table also shows an effect of the interaction between zinc and iron levels on increasing leaf area, as the interaction between the (Zn3) level and the (Fe3) level gave the highest value, reaching (28.73) cm² plant⁻¹, while the interaction between the (Zn1) level and the (Fe1) level recorded the lowest value, reaching (20.18) cm² plant⁻¹. The interaction between inoculation, the (Zn3) level, and different iron levels gave the highest values, reaching (24.97, 26.53, and 31.10) cm² plant⁻¹, while the non-inoculated treatment for the same level recorded values of (21.93, 22.67, and 26.35) cm² plant⁻¹, respectively. The (Zn2) level gave values reaching (22.58, 25.30, and 28.53) cm² plant⁻¹, respectively, compared to the non-inoculated treatment, which gave (18.13, 19.37, and 23.73) cm² plant⁻¹, respectively. The (Zn1) level gave the lowest values (22.89, 24.17, and 24.40) cm² plant⁻¹, respectively, compared to the non-inoculated treatment, which gave (17.47, 20.34, and 21.17) cm² plant⁻¹. This increase in leaf area may be attributed to the improvement of the plant's hormonal and water status. Bacterial inoculation stimulates the production of cytokinins, which play a key role in activating mesophyll cell division and increasing the leaf blade diameter, while zinc contributes to maintaining cell turgor pressure through its role in stabilizing cell membranes, allowing cell wall expansion and increasing leaf blade area. This integrates with the role of iron in activating photosynthetic enzymes, which raises the efficiency of the carbon budget within the plant, providing the necessary structural materials for building wider leaf tissues, in addition to the role of this interaction in reducing the leaf senescence rate by inhibiting ethylene production, thus maintaining leaf vitality and sustaining growth for a longer period. This agrees with what was found by (28).

Table 6. Leaf Area

Mean of Inoculation × Zinc	General Mean of Zinc	Mean (Zinc × Iron)	Inoculated	Non-inoculated	Iron	Zinc
23.82	21.74	20.18	22.89	17.47	Fe 1	Zn 1
		22.26	24.17	20.34	Fe 2	
		22.79	24.40	21.17	Fe 3	
25.47	22.94	20.36	22.58	18.13	Fe 1	Zn 2
		22.34	25.30	19.37	Fe 2	
		26.13	28.53	23.73	Fe 3	
27.53	25.59	23.45	24.97	21.93	Fe 1	Zn 3
		24.60	26.53	22.67	Fe 2	
		28.73	31.10	26.35	Fe 3	
		23.42	25.61	21.24	Mean of Inoculation	
		25.88 Fe3	23.06 Fe2	21.33 Fe1	**Mean of Iron**	
		28.01 Fe3	25.33 Fe2	23.48 Fe1	**Inoculation × Iron Mean**	

قيمLSD$_{0.05\%}$

	Zinc × Iron	Inoculation × Iron	Inoculation × Zinc	Iron	Zinc	Inoculation
	2.6814	2.1893	2.1893	1.5481	1.5481	1.264
3.792				**Triple interaction**		

Conclusion

From this study, we conclude that the triple interaction (inoculation + zinc + iron) achieved a qualitative leap in the efficiency of the faba bean plant. The integration between biological nitrogen fixation through increased root nodules and the stimulatory role of chelated zinc and iron in photosynthesis and hormonal regulation led to the maximization of all vegetative growth indicators. This proves that the availability of an integrated nutritional environment (biological and mineral) is the key to achieving the best plant growth vigor.

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