

An environmentally friendly method to support broad bean plant in reducing salinity affecting root nodule growth and nitrogenase enzyme activators

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Abstract

Salinity can impair root nodulation and biological nitrogen fixation in faba bean (*Vicia faba* L.), potentially limiting plant development. This study investigated the potential of magnetically treated water and water stored in clay pots to mitigate the effects of irrigation water salinity on root nodulation, nitrogen content, and nitrogenase activity in faba bean plants. The experiment was conducted using a randomized complete block design with five irrigation treatments: distilled water (control), saline water (7 dS m⁻¹), water stored in clay pots for 24 h, magnetically treated water (135 mT), and water subjected to both clay pot storage and magnetic treatment. Magnetic treatment was applied three times for one minute each. Root nodulation, nitrogen content, and nitrogenase activity were evaluated to determine plant responses to the different irrigation treatments. The combined clay pot and magnetic water treatment was associated with higher nitrogen content (25.44 mg plant⁻¹) than saline irrigation (8.65 mg plant⁻¹). Similarly, nitrogenase activity reached 36.87 µg under the combined treatment, compared with 8.77 µg under saline irrigation. The reported findings also indicated favorable responses in root nodule development following water treatments. Overall, the combined treatment showed potential to improve nitrogen-related physiological responses in faba bean plants compared with saline irrigation, although further methodological clarification is needed to establish its effectiveness in mitigating salinity stress.

Keywords: biological nitrogen fixation, faba bean, irrigation water, magnetic treatment, root nodulation

Introduction

One of the major problems that plants are exposed to is salinity. Environmental and climatic changes, in addition to the policies of some countries, have led to a shortage of fresh water suitable for irrigation. This is related to the food security of the population, as salinity causes root damage due to salt elements such as Na⁺, Cl⁻, Ca²⁺, Mg²⁺ which would interfere with the plants nutrients and prevent the plant from benefiting from them and growing and developing, because of the osmotic change that occurs in the environment of the plant roots, the proposed treatments have become, to save the plant from stunted growth and damage. And use it, the researchers used different techniques, for example, use organic materials, and mixing them into the soil, or use of certain chemical compounds, such as salicylic acid in research was conducted by Mana & Al-kinani (2025) to reduce negative effect of salinity, other study was

conducted by Hamza & Saeed (2025) on *Vicia faba* were using physical water to reduce salinity effect. Promoting plant growth and stimulating the activity of antioxidant enzymes. Researchers have also employed physical methods, such as the use of water stored in ceramic containers and the exposure of plants to magnetic fields, to enhance plant physiological activity. These techniques may improve nutrient transport and uptake, thereby contributing to plant growth and development, particularly under environmental stress conditions. In this context, Samarah et al. (2021) conducted a study on tomato plants (*Solanum lycopersicum* L.), investigating strategies to mitigate the adverse effects of salinity stress.

Plant of *Vicia faba* L. one of the important crops worldwide, it belongs to the Fabaceae family, and is an annual plant, its height reaches 2 m with white flowers contain black leach, the fruits like pod, the original habitat of this plant is China, then Ethiopia, the importance of

this plant is further enhanced by its seed, which contain high concentration of protein, reaching up to 35%, it's also contains sugars and starches, and some vitamins (El-Beltagi, et al., 2025).

The technique of storing water in pottery help to activation processes and adding some minerals from leaching wall of pottery, which help the plants to recover and improve the absorption of nutrients, thus helping the plant to grow and resist stresses to an acceptable degree, there are some studies that have focused on using water stored in pottery to irrigate plants and have concluded very satisfactory results, such as the study of Rahayu, et al., (2022) on *Capsicum* plant and Hamza & Saeed, (2025); on *vicia faba* plant Hossain, (2023).

The process of water magnetization is a process that has been used a lot recently to improve plant growth and increase its resistance to stresses , it occurs when water is transformed from its inert form to an active and vital form through a change in the structure of water ions dissolved in inorganic salts and a reduction in water viscosity as well as the surface condition, thereby reducing soil it facilitates the dissolution, elevation, binding, and transport of nutrients within the plant, thereby improving growth and plant water magnetization is a process that has been widely used recently to improve plant growth and increase its resistance to stress, it occurs when water is transformed from its inert form to an active, vital form through a change in the structure of water ions dissolved in inorganic salts , reducing water viscosity and surface tension , this, in turn ,reduces soil alkalinity and facilitates the dissolution uplift ,and binding of nutrients to the water ,thus improving their transport within the plant and ultimately enhancing its overall plant growth, the researchers noted the plants success in resisting stress and growing normally when treated with magnetized water compared to ordinary water such as study of (Ben Hassen, et al., 2020) on wheat plant ,they resulted increase in antioxidant activity and improved in nitrogen absorption, also (Alattar, et al., 2021) ,they noted improved in all growth parameters of Corn plant with treated by magnetic water ,and (Ehtaiwesh, et al., 2021) ,they mentioned improve in germination and growth parameters when irrigation with magnetize water. The current study was conducted to clarify the extent of the effect of the interaction between pottery water and magnetite water on the properties of root nodules, nitrogen content and activity of nitrogenase enzyme in *vicia faba* L. plant, to reduce negative effect of salinity stress, and modulate growth of *Vicia faba* plant L.

Materials and Methods

Uniformly sized uncontaminated seeds were selected (the seeds were brought from the crops department at the college of agriculture, and the plant growth rate was selected and was 100%), the seeds washed several times and sterilized with a sodium hypochlorite 3 %. Then were germinated in petri dishes for five days under room temperature (27-32 °C), the seeds were considered successfully germinated if with radical 2mm.

The germinated seeds transports to pots (15×30 cm), which were filled with 1kg mixed soil (peat moss 50 % with agriculture soil 50 %). The chemical and physical composition of the soil is explained in Table 2. The pots were left in growth chambers. (27-32 °C) and (light 16h and dark 8h + humidity 85%) for 4 weeks. The pots were watered with 500ml with types of treatments in twice a week.

Preparation of type water treatment

Irrigation water as treatments of experiment was prepared as following and the symbols were used as abbreviations to denote the type of water treatment, see **Table 1**.

Preparation of pottery water

Five liters of distilled water were placed in pottery pots obtained from Al Fares nursery. The pottery pots were composed of 85% clay, 10% silt, and 5% sand. The water was left in the pottery pots for 24 h, after which the water dripping from the bottom of the pots was collected.

Preparation of magnetized water

Magnetized water was prepared by placing four magnets (5 cm diameter + 2 cm thick), were placed around the water source (plastic pipe gate out of the Tank 500 L) for one min and repeated three times, the intensity of the magnetism (135 mT) was measured by magnetometer German made.

Preparation of mixed water

The water was dripping from pottery pots were collected after 24 h and exposed it to a magnetic intensity (135 mT) in the same previous method.

Saline water (dsm⁻¹)

Saline water prepared by adding amount of NaCl into distils water as following formula:

$M = Wt \cdot 1000 \cdot Mwt^{-1} \cdot V^{-1}$. Chemical composition of water in **Table 3**.

Table 1 - Symbols used in preparing water treatments for the experiment.

Control (0.0 dsm ⁻¹)	Salinity (7dsm ⁻¹)	Pottory water (5L in clay pots. For 24h)	Magnetic water (135mT One min× 3 times)	Mixed pottory water + Magnetic water
C	S	P	M	PM

Table 2 - The chemical composition of the soil under investigation.

PH [*] (1:2.5)	EC ^{**} (dsm ⁻¹)	Soluble cation (mg/L ⁻¹)				Soluble Anions(mg/L ⁻¹)			
6.78	1.28	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	CO ₃ ⁻²	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻²
		6.12	5.17	1.32	2.12	6.15	5.21	2.33	3.87
Soil texture (%)				Plant nutrition (mg kg ⁻¹)					
Coarse sand	Fine sand	Silt	Clay	OM	P	K	B	Zn	Cu
22.30	30.34	13.22	24.10	17.42	3.21	66.23	18.50	0.77	3.89

* Soil water suspension; ** soil paste.

Table 3 - Composition of the water treatment used in experiment.

Type of water	Soluble cation (mgL ⁻¹)				Soluble Anion (mgL ⁻¹)			
	Ca	Mg	Na	K	CO ₃ ⁻²	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻²
Control water	3.25	4.11	1.02	2.12	5.23	5.5	3.07	3.87
Magnetic water	3.02	4.03	1.2	2.24	5.2	5.1	2.02	3.62
pottory water	3.68	4.88	4.99	2.99	5.02	4.27	2.02	3.43
Mixed water	3.2	3.27	3.03	3.98	4.22	4.11	2.03	2.37
Type of water	Water Nutrient (mg L ⁻¹)							
	NH ₄ ⁺	NO ₃ ⁻	P	B	Zn	Cu		
Control water	3.15	13.94	0.04	0.25	0.02	0.01		
Magnetic water	2.33	14.22	0.04	0.33	0.03	0.03		
pottory water	2.59	17.65	0.05	0.33	0.04	0.04		
Mixed water	2.64	17.89	0.05	0.56	0.05	0.04		

Collect of data

After four weeks the growth plants were harvested and the roots were washed very carefully to remove the sand. Then the number of root nodules plant-1 was calculated and the dimensions of nodules plant* were calculated, and repeated it for three times.

Evolution of Nitrogenase enzyme

The nitrogenase enzyme was evaluated using the method described by Hardy et al. (2021), employing a closed-system approach. After the roots were carefully separated from each plant sample subjected to the different irrigation water treatments, they were washed with distilled water. The nodules were then carefully removed from the roots and placed in glass bottles containing 250 mL of mannitol solution. The bottles were sealed with rubber stoppers, and 15 mL of acetylene was injected into each bottle. Subsequently, the samples were incubated for 2 h. The analysis was performed using gas chromatography (GC) to determine the concentration of ethylene produced through acetylene reduction.

Nitrogen assay

To determine the nitrogen content in roots, 5 mg of dried root tissue was digested using concentrated sulfuric acid. The final volume was then adjusted to 10 mL with distilled water. Subsequently, the samples were

analyzed using the Kjeldahl method, as described by Bennett (1993).

Statically analysis

Three replicates were used in this experiment, randomized complete block design RCBD was employed, and ANOVA was used to analyze the data at a significance level of probability $p \leq 0.05$, the data analysis were conducted in SPSS version 2025.

Results and Materials

Salinity effect

Based on the results were obtained, as shown in **Table 4**. the characteristics of the root nodules, with 5 types of water treatments, we observe significant reduction 80% in salinity treatment, at level 7dsm⁻¹, compared with the control treatment appeared clear ,when treating the plant with water stored in pottory pots, a high level of improvement by 67%, was also observed improvement with 78% in the magnetic treatment and with interference of (pottory +magnetic water) by 85% compared with salinity treatments. The improvement appeared in all characteristics of nodules number, diameter, fresh weight and dry weight. was related with improvement in absorption of nitrogen, So the appearance of nodules was swollen and pink in color, compared to the salinity treatment which showed reduced in a size and pale to brown in color **Figures (1,2,3,4)**.

Table 4 - Characteristics of nodules with different of water treatments of *Vicia faba* plant.

Treatments	N.no	N.dim	N.Fw	N.Dw	Mean
	/plant	mm/plant	mg/plant	mg/plant	
C	75.82 ^a	3.81 ^a	30.25 ^a	14.81 ^a	31.17 ^a
S	40.12 ^b	1.30 ^d	10.21 ^s	3.41 ^s	13.76 ^s
P	86.33 ^c	5.46 ^f	50.94 ^d	22.09 ^x	41.20 ^c
M	89.33 ⁿ	5.69 ^g	80.44 ^y	41.11 ^g	58.11 ^f
MP	103.33 ^t	5.89 ^g	89.33 ^t	48.12 ^r	61.66 ^y
St.	23.78	1.47	33.25 ^h	18.49	-
P≤0.05	0.056	0.051	0.020	0.031	-

-The mean values of different water types followed by different letters are expressively different at $P \leq 0.05$. Data represent the mean of 3 replicates $\pm$ SE. N.no=number of nodules; N. dim = diameter of nodules; N. Fw =nodules fresh weight; N. Dw = nodules dry weight; N. Dim = nodule diameter.

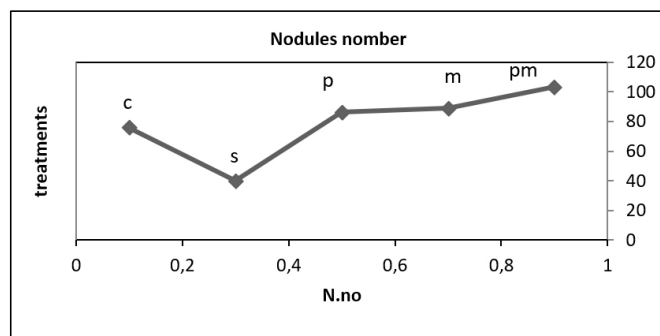


Figure 1 - Showed differences in nodules number(N.no) of *Vicia faba* L. plant under water treatments, *significant differences at level $p \leq 0.05$ **c**: control, **s**: salinity water, **p**: pottery, **m**: magnetic water, **pm**: magnetic water and pottery water.

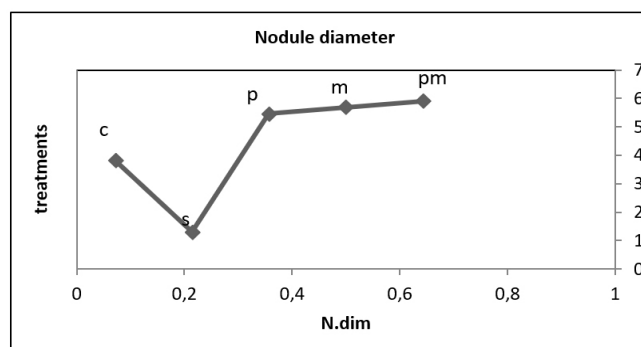


Figure 2 - Showed differences in nodules diameter (N.no) of *Vicia faba* L. plant under water treatments, *significant differences at level $p \leq 0.05$ **c**: control, **s**: salinity water, **p**: pottery, **m**: magnetic water, **pm**: magnetic water and pottery water.

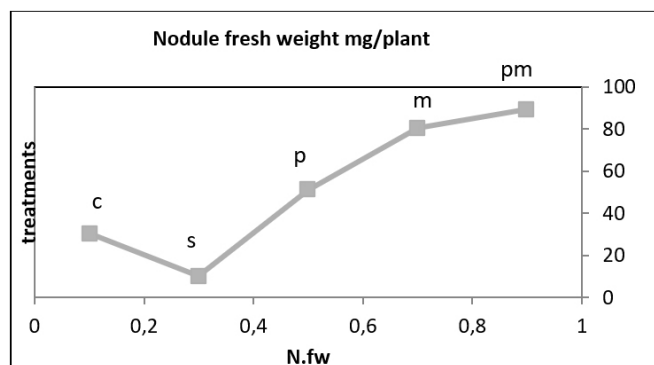


Figure 3 - Showed differences in nodules fresh weight (N.fw) of *Vicia faba* L. plant under water treatments. *significant differences at level $p \leq 0.05$ **c**: control, **s**: salinity water, **p**: pottery, **m**: magnetic water, **pm**: magnetic water and pottery water.

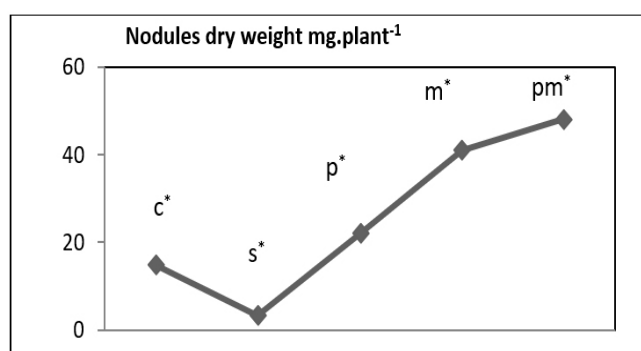


Figure 4 Showed differences in nodules fresh weight mg.plant⁻¹ (N.dr.w) of *Vicia faba* L. plant under water treatments. *significant differences at level $p \leq 0.05$ **c**: control, **s**: salinity water, **p**: pottery, **m**: magnetic water, **pm**: magnetic water and pottery water.

The soil surrounding the roots is very sensitive to the growth of root nodules, high salinity levels in the soil cause an imbalance in nutrient absorption by the roots (Hozayn, et al., 2022; El-Beltagi, et al., 2025), therefore, signs of wilting and reduction in the number, size and weight of root nodules appear accordingly. The interaction of root hairs with the surrounding soil is sensitive and very necessary for the growth of the rhizobium and essential for fixing and absorbing the nitrogen needed for the growth of *Vicia faba* plants.

Effect of Magnetic and pottery water

As shown in Table 4 and Figures 1, 2, 3, and 4, a significant improvement in root nodule growth was observed following treatment with magnetized water, pottery water, and their combined application. The most significant improvement was observed in the interaction treatment compared with the salinity treatment, as evidenced by increased nitrogen absorption, which was reflected in the overall improvement in plant growth indicators, root nodule characteristics, and nitrogen

content in the roots. These results suggest that plant resistance to salinity was improved. These findings were consistent with those reported by Hozayn et al. (2022) in *Cicer arietinum* and Hozayn et al. (2024) in faba bean plants, respectively, who concluded that plant growth improved under saline conditions following the application of magnetized water. This enhancement is associated with increased plant resistance, which is related to greater nutrient solubility and absorption by the roots. Therefore, improvements were observed in all root nodule growth characteristics, which were associated with the overall growth of the plants.

A significant improvement ($p \leq 0.05$) in nodule characteristics was observed following treatment with pottery water and its combination with magnetized water (135 mT) in *Vicia faba* plants subjected to irrigation with saline water (7 dS m^{-1}). In addition, storing water in pottery vessels can increase the concentrations of calcium, magnesium, and iron in the water. These elements originate from the materials composing the pottery vessels and dissolve in the water during storage (Ben Hassen et al., 2020; Sutiyaniti & Rachmawati, 2021). Furthermore, water stored in pottery vessels undergoes cooling through evaporative heat loss, particularly during periods of high temperatures. Consequently, the cooled water provides suitable conditions for the activity of several metabolic enzymes, which also play an important role in plant growth. Hussein and Abo Al-Saoud (2024) also reported improved growth in *Vicia faba* and garlic plants following treatment with magnetized water. The results are presented in Table 3. A positive correlation was observed between the application of magnetized water, pottery water, and their combination and root nodule characteristics. Therefore, this approach can be used to reduce the effects of salinity on *Vicia faba* plants. Similarly, Rahayu et al. (2022), in *Capsicum annuum*, and Hossain (2023), in cherry tomatoes, reported improvements in

plant growth following treatment with pottery water, whereas Hamza and Saeed (2025) reported improved vegetative growth in *Vicia faba* following the combined application of magnetized water and pottery water.

Evolution of Nitrogen and Nitrogenase enzyme

Figure 5 illustrates the changes in nitrogen concentration and nitrogenase enzyme activity under different irrigation water treatments. A significant improvement ($p \leq 0.05$) was observed in the nitrogen concentration in the roots of *Vicia faba*, with an increase of 74%, and in nitrogenase activity, with an increase of 75%, following irrigation with magnetic water, pottery water, and their combination, compared with the salinity treatment. In contrast, salinity significantly reduced nitrogen concentration in the roots by 80% and nitrogenase activity by 87% (**Figure 6**). Similar negative effects of salinity on nitrogen concentration and nitrogenase activity have been reported by Severin et al. (2012), Hessini et al. (2012), and Figueroa-Galvis et al. (2024) in studies involving mangrove and maize plants.

Exposure of water to a magnetic field has been reported to influence certain physicochemical properties, including surface tension and viscosity, depending on the treatment conditions. These changes may affect water-solute interactions and nutrient availability, potentially contributing to improved nutrient uptake and plant physiological performance under stress conditions. In leguminous plants, biological nitrogen fixation is carried out by symbiotic rhizobia within root nodules, where the nitrogenase enzyme catalyzes the conversion of atmospheric nitrogen (N_2) into ammonia (NH_3), which can subsequently be assimilated by the plant. Furthermore, iron and molybdenum are essential elements involved in nitrogenase function and symbiotic nitrogen fixation. Therefore, improved nutrient availability and favorable conditions for rhizobial activity may

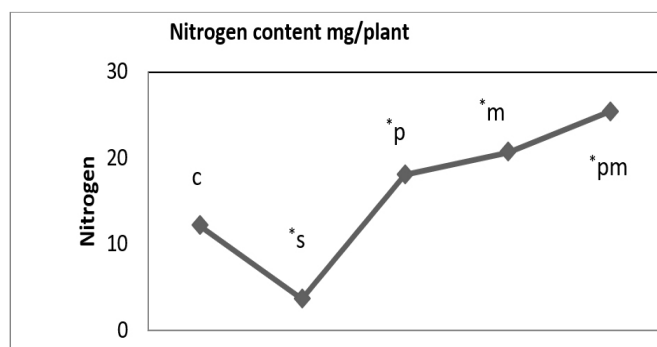


Figure 5 - Explain effect water treatments on Nitrogen mg plant⁻¹ content on *Vicia faba* L. *significant differences at level $p \leq 0.05$ plant, c: control, s: salinity water, p: pottery,

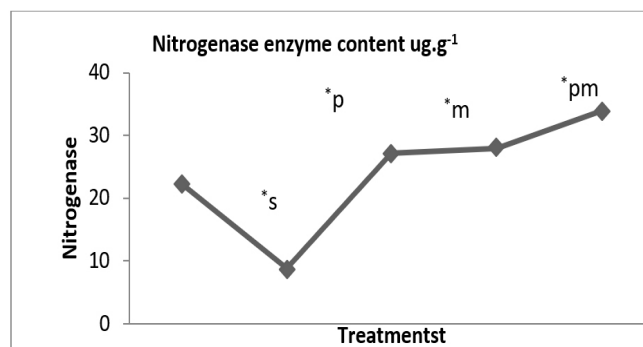


Figure 6 - Explain effect water treatments on Nitrogenase ug.g⁻¹ activity on *Vicia faba* L. plant, *significant differences at level $p \leq 0.05$ c: control, s: salinity water, p: pottery, m: magnetic.

contribute to increased nitrogen accumulation and nitrogenase activity.

In the present study, the magnetic water treatments were associated with significantly higher nitrogen concentrations and nitrogenase activity compared with the salinity treatment, which negatively affected both parameters. Similar beneficial effects of magnetically treated water on nitrogen-related physiological processes have been reported by Xiumei et al. (2019) in *Populus*, Abu-Saied et al. (2023) in cucumber, and Yao et al. (2024) in lettuce. However, the mechanisms underlying these responses require further investigation.

Water stored in pottery containers may also undergo changes in its physicochemical characteristics, including temperature and mineral composition, depending on the properties of the ceramic material and the surrounding environmental conditions. The potential cooling effect associated with evaporative water loss from porous pottery could influence the root-zone environment, although its direct contribution to nitrogenase activity has not been established in the present study. Wang et al. (2024) reported that moisture conditions in the root environment influence nitrogen fixation in alfalfa plants, suggesting that adequate soil moisture may contribute to maintaining favorable conditions for symbiotic nitrogen fixation.

In contrast, Gu et al. (2023) reported that salinity negatively affects nitrogen fixation, nitrogenase activity, and nutrient availability in soil, potentially compromising plant growth and the establishment of effective rhizobial symbiosis. Favorable conditions for rhizobial activity may support root nodule development and nitrogen fixation. Furthermore, the reddish coloration of active nodules is associated with the presence of leghemoglobin, a protein involved in regulating oxygen availability and protecting nitrogenase activity. Therefore, the larger and redder nodules observed under the water treatments, compared with those under salinity stress, may indicate more favorable conditions for symbiotic nitrogen fixation.

Overall, the results suggest that magnetic water and pottery-treated water may contribute to improving nitrogen accumulation and nitrogenase activity in *Vicia faba* under saline conditions, whereas salinity stress negatively affects these parameters. Nevertheless, additional physicochemical and physiological analyses are required to establish the mechanisms responsible for the observed responses.

Productivity

Through the experiment conducted in testing magnetic water and pottery water in reducing salinity

irrigation water on root nodules growth and nitrogenase enzyme activity were investigated, Found that plants that have been irrigation with pottery water and magnetic water have positive effect and promoted the nodules growth and increase in nitrogenase activity, but when pairing the two treatments of pottery an magnetic water have more productivity of nodules and more activity of nitrogenase, So the content of nitrogen in roots is increased accordingly.

Conclusion

The current study was conducted on *Vicia faba* L. plants and examined the negative effects of saline irrigation water on the morphological characteristics of root nodules. The study demonstrated improvements in all nodule characteristics, including nodule number, diameter, fresh weight, and dry weight, as well as increased nitrogen content and nitrogenase activity. All these nodule parameters showed greater improvements when pottery water was combined with magnetized water. These findings suggest that the combined treatment represents a new approach to promoting sustainability and enhancing plant growth.

Authors' Contributions

The experimental procedures were conducted by Dr. Shatha Mohammed, while the results were interpreted by Dr. Zainab, who also contributed to the phenotypic measurements of root nodules and the statistical analysis.

All authors read and approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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