Combination Effects of Rhizobium sp. and Nano-Fe on Growth, Nodulation, and Nutrient Uptake of Chickpea (*Cicer arietinum* L.)

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Abstract

The objective of this study is to investigate the effects of Fe Nano fertilizer and Rhizobium inoculation on nodulation, nitrogen fixation, and the plant growth of chickpea (*Cicer arietinum* L.). The soil samples were collected from the fields that were previously planted with chickpea. The study included testing the interaction influence of five levels of Nano Fe and three soil inoculation treatments using three replicates. The results indicated that Nano Fe application and soil inoculation rhizobium had a significant effect on plant growth number of seeds per plant and the seed protein content of chickpea. The plant height is greatly increased in the sterilizing process. The shoot height and root lengths of seedlings both increased significantly. The rhizobium bacteria have a positive impact on plant proteins and significantly increase the amount of protein in the plant. In conclusion, the results provided compelling evidence that the presence of Fe Nano fertilizer in nodules enhances nodulation and nitrogen fixation, improving the symbiotic performance between Rhizobium (chickpea) and the common bean plant.

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Introduction

Nano (Fe) is one of the most important micronutrients and the third most limiting nutrient needed for plant growth and development it is very essential and widely used in biochemical processes from photosynthesis to respiration. Fe is intricate in the systems of electron transport and is crucial for maintaining the chloroplast organization (Broadley *et al*., 2012; Briat *et al*., 2015).

The first observable symptom of a Fe deficit is a change in the chlorophyll (Chl) content of young leaves. These changes are not only related to the loss of Chl but also to the gathering and emergence of other mechanisms of the photosynthetic apparatus and Cropping systems use Fe fertilizers in the soil to alleviate the symptoms of deficiency around the world (Márquez-Quiroz et al., 2015). This system is also utilized to fulfill commitments for bio - fortification. The majority of Fe's transformation to the oxidized ferric process occurs in aerobic soil, which is immovable and flexible in soil. By increasing the soil's pH, Fe-bearing minerals with solubilities in the range of 7.4 to 8.5 slowly dissolve, gram commonly known as chickpea or Bengal gram is the most important pulse crop of India Chickpeas require phosphorous, sulphur, nitrogen and zinc for a successful production. When planning applications it is best to know the paddocks soil pH and fertilizer history. Consult with your

agronomist or have a soil test undertaken for these nutrients (Granja and Covarrubias, 2018).

Rhizobium's classification is based on the "cross incolation principle," which states that a significant portion of the original taxonomy of rhizobia was based on host specificity (Fred, Baldwin, and McCoy). The symbiotic nodulation (nod) genes of rhizobia, which are the causes of the host range and had a significant impact in rhizobia taxonomy, are therefore crucial. Recently, tools have been developed to create more accurate classifications using phylogenetic studies in conjunction with molecular techniques including DNA hybridization, DNA sequencing, and typing procedures (Vandamme *et al.,* 1996).

Chickpeas are poor competitors with weeds during their slow early growth stage. It is highly recommended to implement good broad leaf weed control in the previous year's crop. Once established they are an excellent break crop from diseases, weeds and pests. (Chen, *et al*, 2011).

Calcareous soils can induce Fe-deficiency chlorosis in several forages including many forage legumes (Baker *et al*., 1991). Chlorotic plants have reduced rates of photosynthesis caused by reduced chlorophyll content in the leaves. Frankeland Wei (1994) described several characteristics of resistance to Fe-deficiency chlorosis in subterranean clover (*Trifolium subterraneum* L.) including a greater root: shoot: ratio, lower concentrations of tissue P that may interact with tissue Fe, mechanisms for immobilizing Fe in the soil, reduced Fe requirements for plant metabolism, and greater efficiency of Fe use.

Usually suffer from a lack of micronutrients, especially zinc and iron. Zinc deficiency is most pronounced in maize, especially under high yield intensive cultivation systems. Zinc sulphate is an effective zinc source and is the most popular form in use.

Excess nitrogen can damage delicate plant species, unbalancing the ecosystem. Most plants cannot tolerate synthetic fertilizers or high levels of nitrogen. Nitrogen pollution causes nitrogen-tolerant species to thrive and outcompete more sensitive wild plants and fungi. The aim of this study is to investigate the effects of Fe Nano fertilizer and Rhizobium inoculation on nodulation, nitrogen fixation, and the plant growth of chickpea and give information about Fe Nano fertilizer, Rhizobium and the procedures of the soil samplings.

Materials and methods

Soil sampling

The soil samples were collected from the fields that were previously planted with chickpea in Suleimani, Kurdistan region, Iraq, during 2021–2022. On October 13th, 2021, in Bakrajo, in the grid coordinates N 3532 42.9" and E 4521 12.9," soil samples were removed at 1 cm and taken soil in 30 cm depth. Soil was put in 4 ml of CIVI. The samples were disinfected using an autoclave device for three days under well-protected conditions and then prepared for planting

Pot preparation

The complete Randomized Design with three replicates was used to study the influence of 5 levels of Nano fertilizer (0, 22, 44, 66, and 88 mg/pot) and sterilized soil Plus levels on Nano fertilizers, non- sterilized soil with levels of Nano and non-sterilized soil plus levels of Nano and Rhizobium, it means the number of combination treatment was 15 with 3; replicates and a total number of experimental units or pots equal to 45. On 24th November 2021, the sowing was done (8 seeds/pot) after the plant growth, we pulled out four of them from the pot. The irrigation

was done whenever needed. The harvesting was done on June 6, 2022.

-Number of interaction treatments =Levels of Nano Fe * soil treatments
-Number of interaction treatments =5 * 3 =15 interaction treatments
-Number of replicates = 3

Total number of experimental units =15*3 = 45 experimental units as shown in Table 1 which represents the number of interaction treatments per replicate or plot.
Nano Fe0=0 mg/pot
Nano Fe1=22mg/pot
Nano Fe2=44 mg/pot
Nano Fe3=66 mg/pot
Nano Fe4=88 mg/pot

Table 1. Layout of the experiment

Nano Fe	Soil treatments		
	Inoculated soil	non-inoculated soil	Sterilized soil
Fe0	Fe0 inoculated soil R1	Fe0 non-inoculated soil R1	Fe0 Sterilized soil R1
Fe0	Fe0 inoculated soil R2	Fe0 non-inoculated soil R2	Fe0 Sterilized soil R2
Fe0	Fe0 inoculated soil R3	Fe0 non-inoculated soil R3	Fe0 Sterilized soil R3
Fe1	Fe1 inoculated soil R1	Fe1 non-inoculated soil R1	Fe1 Sterilized soil R1
Fe1	Fe1 inoculated soil R2	Fe1 non-inoculated soil R2	Fe1 sterilized soil R2
Fe1	Fe1 inoculated soil R3	Fe1 non-inoculated soil R3	Fe1 sterilized soil R3
Fe2	Fe2 inoculated soil R1	Fe2 non-inoculated soil R1	Fe2 sterilized soil R1
Fe2	Fe2 inoculated soil R2	Fe2 non-inoculated soil R2	Fe2 sterilized soil R2
Fe2	Fe2 inoculated soil R3	Fe2 non-inoculated soil R3	Fe2 sterilized soil R3
Fe3	Fe3 inoculated soil R1	Fe3 non-inoculated soil R1	Fe3 sterilized soil R1
Fe3	Fe3 inoculated soil R2	Fe3 non-inoculated soil R2	Fe3 sterilized soil R2
Fe3	Fe3 inoculated soil R3	Fe3 non-inoculated soil R3	Fe3 sterilized soil R3
Fe4	Fe4 inoculated soil R1	Fe4 non-inoculated soil R1	Fe4 sterilized soil R1
Fe4	Fe4 inoculated soil R2	Fe4 non-inoculated soil R2	Fe4 sterilized soil R2
Fe4	Fe4 inoculated soil R3	Fe4 non-inoculated soil R3	Fe4 sterilized soil R3

Planting

The seeds were planted on November 24th, 2021. Nano fertilizer was added to 45 pots. The pots were initially irrigated. After translocation of the pots to the field, supplementary irrigation was dependent. We took the height of the plant at the end of the study before harvesting by centimeter for each replication which contains three plants in the same pot. The plants were harvested on June 6, 2022.

Soil Chemical analysis

The soil analyses included pH, EC, Nitrogen, Phosphorus, potassium, Calcium, Magnesium, Sodium, carbonate, Bicarbonate, Chloride, and Organic matter determined according to (Granja *et al*., 1996) method.

Statistical analysis

The statistical analysis was performed using IBM SPSS (version 27.0). Statistical significance was considered at P <0.05. Test to comparisons among the mean values, and differences between the mean treatments and interaction treatments.

Result and Discussions

The results are shown in Figure 1 we took the height of the plant at the end of the study before harvesting by centimeter for each replication which contains three plants in the same pot. A considerable increase in plant height at various pots when sterilized as compared to pots not sterilized soil. This indicated that was the ideal amount for plant height. In a study conducted by Lili Mao (2022), soil was soaked and prepared prior to autoclaving, and air-dried soil was utilized for irradiation. The changes as a result of sterilization were compared to the corresponding unsterilized soil. The autoclaving process caused a minor reduction in soil pH. Both sterilization methods significantly altered several of the soil's chemical properties, although the effects of sterilization were greater than those of non-sterilization. The soil height is greatly increased in the sterilizing process. Scientists contend that unsterilized soil is preferable because it produces outcomes that are more likely to reflect farmers' intended practices (Zhang, 2020). Another proposal is to compare the results of both sterilized and unsterilized treatments, especially for farmers. Sterilizing garden soil before planting is always a good practice to ensure the best growth and health of your plants because dirt can harbor pests, illnesses, and weed seeds (Zhang *et al.*, 2016).

However, for chickpea, the shoot height and root lengths of seedlings both increased dramatically (Figure 1). Chickpea and was shown to significantly reduce both shoot height and root length when exposed to bacterial treatments. Another bacterial treatment resulted in either longer roots or taller shoots.

According to research conducted by Pide *et al.* (2022), nanomaterials can boost crop productivity by enhancing the availability of fertilizer nutrients in soil and nutrient uptake by plants. Table 2 shows some important physical and chemical properties of the soil used in the experiment

Table 2. Some important physical and chemical properties of the soil used in the experiment

Soil physical properties	Units	Values
Depth	cm	30
sand	%	%5.01
clay		%44.07
silt		%50.92
texture		Silty clay

Soil chemical properties		
EC	dS m^{-1}	0.4
PH		8.5
N	%	0.25
Available p	ppm	18.128
K +	mmolc $.L^{-1}$	0.138
Na^{+}		0.160
Ca^{+2}		1.5
Mg^{+2}		0.55
Cl^{-}		0.1
HCO_3^{-}		3.5
$CO3^{-2}$		0.1
O.M	g kg^{-1}	16.6
$CaCO_3$		220

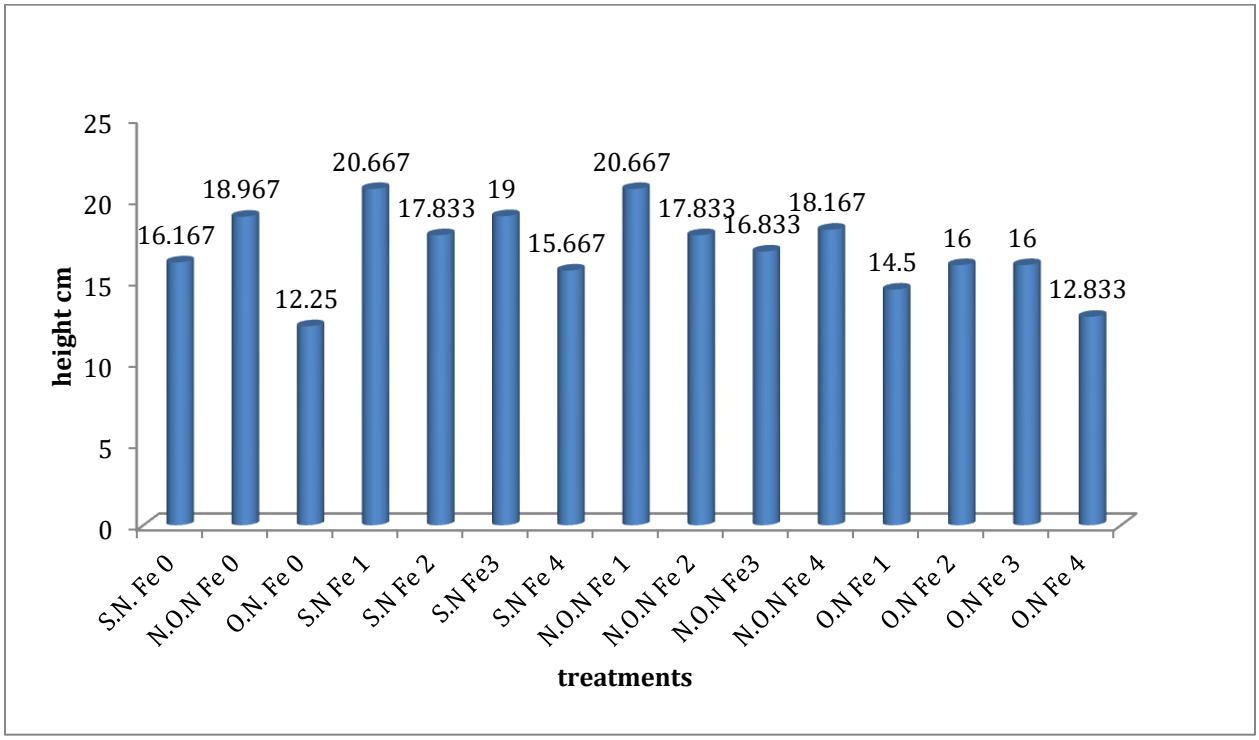

Figure 1. Effect of Rhizobium bacteria inoculated and non-inoculated and their interaction on chickpea height (cm) at different Fe Nano fertilizer levels

S.N.Fe= Non-inoculated by Rhizobium sterilize soil.
N.O.N.Fe =Non-inoculated by Rhizobium.
O.N.Fe = Inoculated by Rhizobium.

The results of the number of seeds generated across the treatments are shown in Figure 2. This data shows that the quantity of seeds is at its maximum level; the result is in agreement with that reported by Yun *et al.* (2020). Additionally, it has been discovered that through chemical or thermal treatments, soil disinfection eliminates pathogens, weed seeds, and dangerous organisms from mineral soils and potting mixes (Wood *et al.*, 2021).

This is helpful when soils are frequently used for seed germination, cutting propagation, or the growth of young plants. Additionally, because soil sanitation is so beneficial to plants, it can significantly increase the amount of seeds present. This illustrates how many seeds are present in sterilized soil compared to unsterilized soil in a plant. Additionally, the chickpea, which is accumulated by rhizobium bacteria, has fewer seeds.

This is true more for symbiotic microbes. Non-specific rhizobium applied as fertilizer does not result in root grafting or greater crop yields. Overall, it is evident that the Nano Fe is highly beneficial for the plants in that it enhances the number of seeds produced by the plants.

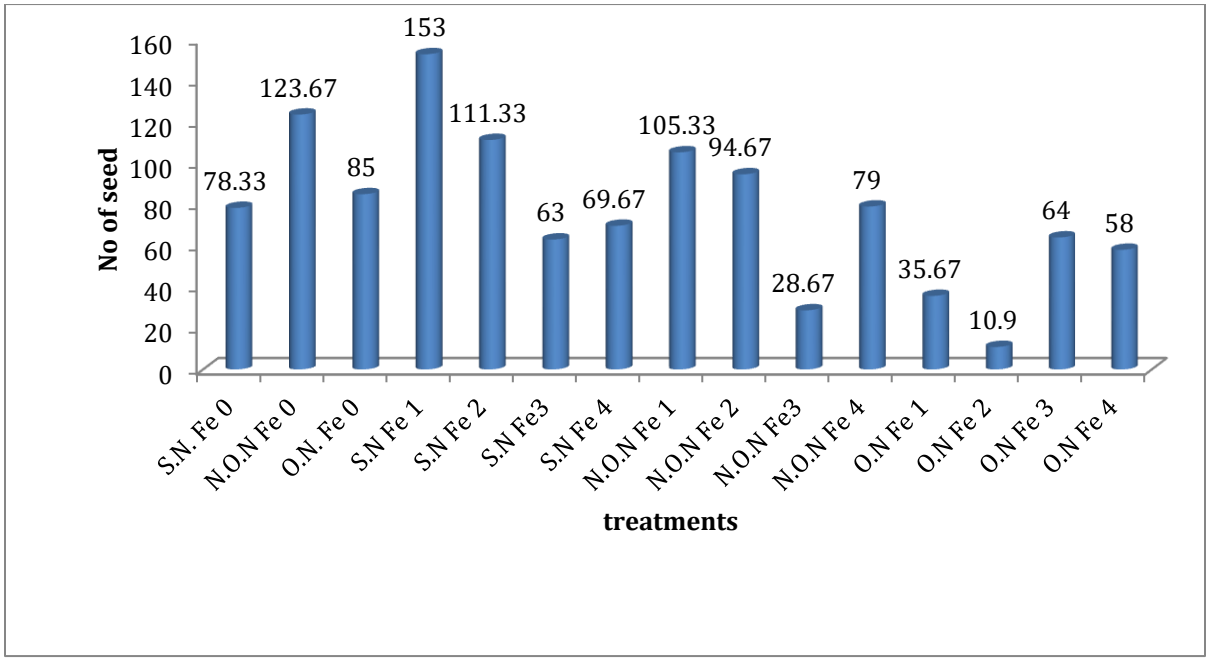

Figure 2. Effect of Rhizobium bacteria inoculated and non-inoculated and their interaction on chickpea number of seed at different Fe nanofertilizer levels

In Figure 3, our results are somehow different from the others because the soil was sterilized in the pots. Nitrogen is high while the soil sterilized pot is also in the lowest range. Nitrogen regularly boosts root growth and phosphorus foraging capacity. Some of the nitrogen's effects are connected to how it affects plant top growth and how it concurrently increases phosphorus absorption (Lindemann, 2020). Nitrogen and Nano-

fertilizers both greatly enhance soil quality, plant growth, and crop production of high-quality fruits and grains.

Successful Rhizobium-legume symbioses boost the uptake of BNF into soil ecosystems. The main source of fixed nitrogen in land-based systems is rhizobium-legume symbioses, which can contribute to more than half of the biological source of fixed nitrogen (Hamdi and Zahran, 2015).

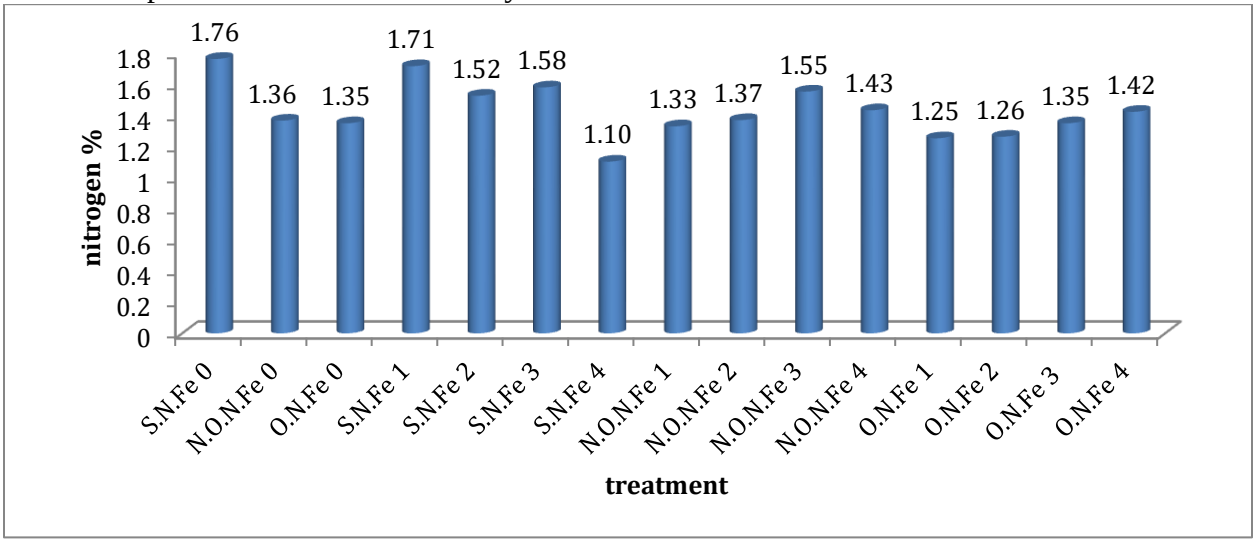

Figure 3. Effect of Rhizobium bacteria inoculated and non-inoculated and their interaction on chickpea Nitrogen at different Fe Nano fertilizer levels

Figure 4 shows that the rhizobium bacteria have a positive impact on the plant protein and significantly increase the amount of protein in the plant (Christopher *et al.*, 2017) research shows that bacteria can use their gene expression machinery to create foreign proteins from inserted genes. Protein synthesis in bacteria has considerably facilitated our understanding of how proteins function. Additionally, it enabled the bacteria to manufacture a significant amount of medically significant proteins.

The main nitrogen source in soil organic matter is the only nitrogen source available to bacteria. Very little research has been done to clarify how complex organic nitrogen functions as a source of nitrogen for plants. Moreover, it has been stated that the key nutrients plants require to build protein are glucose and nitrates, which are absorbed by roots from the soil, not sterilized dirt. Amino acids are created when glucose and nitrate are mixed together. Numerous amino acids are joined to create proteins during protein synthesis.

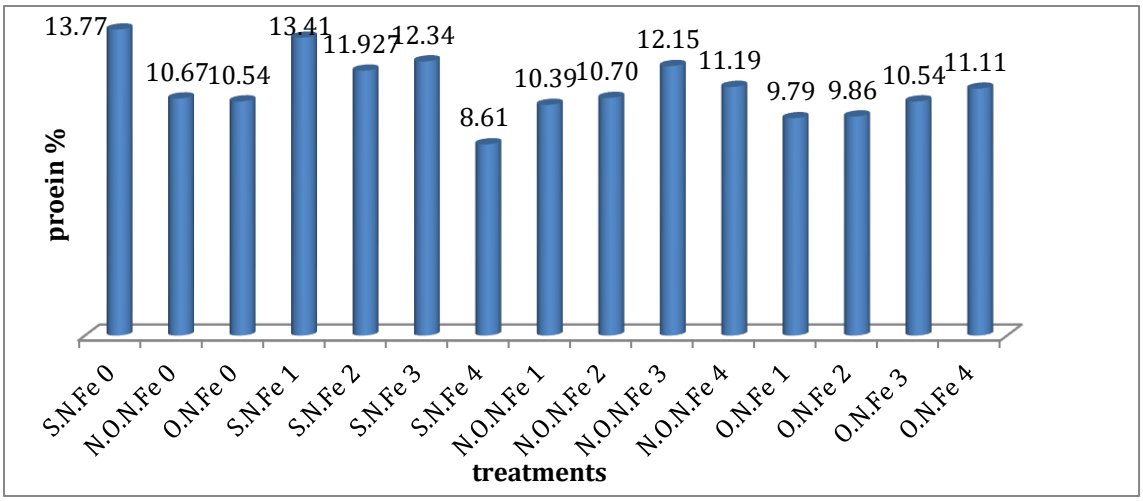

Figure 4. Effect of Rhizobium bacteria inoculated and non-inoculated and their interaction on chickpea protein at different Fe Nano fertilizer levels

Conclusion

The result of Nano Fe was highly beneficial for the plants (chickpea) as it enhances the number of seeds produced by the plants. The results showed a significant increase in plant height at various pot sizes when sterilized compared to pots without check pea seed inoculation. The soil height is greatly increased in the sterilizing process. The shoot height and root lengths of seedlings both increased significantly. Also, the result revealed the chickpea significantly reduced both shoot height and root length when exposed to bacterial treatments. The nitrogen was found to increase in the pot with unsterilized soil and lower in sterilized ones. The rhizobium bacteria positively impact the plant protein and significantly increase the amount of protein in the pots.

Conflict of interest

The authors declare there are no conflicts of interest among all authors.

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