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THE ROLE OF NITROGEN AND PHOSPHORUS BIOFERTILIZERS IN THE UPTAKE OF SOME NUTRIENTS AND GROWTH OF CHICKPEAS (CICER ARIETINUM L)

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Abstract

This study examined the effect of co-inoculating phosphorus- solubilizing and nitrogen-fixing bacteria on chickpea (*Cicer arietinum* L) growth and nutrient intake at various phosphorus levels. Rock phosphate was used as a source of phosphorus. The experiment was conducted from the November 2021 to March 2022 growing season at the Bakrajo Horticulture Department of the College of Agricultural Engineering Science, University of Sulaimani. With three replications for each treatment, the experiment was carried out in silty clay soil using a completed randomized design (CRD). After five months the plants were measured for their height and chlorophyll intensity. The chickpea plants exhibited significantly greater plant height, chlorophyll intensity, nodule numbers, root dry weight, and shoot dry weight from the treatments. The highest values were 78.00 cm, 76 SPAD, 77.00 g, 6.00 g, and 14.34 g, respectively compared to the control group. The highest values for chickpea shoot N, P, K, Fe, Zn, and Mn content were 26.00 g kg^{-1}, 11.36 g kg^{-1}, 26.00 g kg^{-1}, 445.33 $\mu g g^{-1}$, 46.25 $\mu g g^{-1}$, and 41 $\mu g g^{-1}$, respectively, observed at p levels of 160 kg P ha^{-1}. Additionally, *Rhizobium leguminosarum* inoculation alone increased plant height, chlorophyll intensity, number of nodules, root and shoot dry weights of the chickpea plants but less than with co-inoculation. Compared to the control, inoculation with *Pseudomonas putida* had the least significant increase in plant height, chlorophyll intensity, nodule number, root dry weight, and shoot dry weight. The corresponding values were 62.33 cm, 43.30 SPADS, 51.00, 3.98 g, and 10.40 g at the highest P levels. On the other hand, they decreased at the greatest p-values. The nutrients N, P, K, Fe, Zn, and Mn for chickpea shoots had the highest values at 18.60 g kg^{-1}, 10.00 g kg^{-1}, 17.63 g kg^{-1}, 298.00 $\mu g g^{-1}$, 34, 76 $\mu g g^{-1}$, and 18 $\mu g g^{-1}$, respectively.

Keywords: Rhizobium, Pseudomonas, Biofertilizer, Phosphorus, Chickpea.

دور التسميد الحيوي النتروجيني والفوسفاتي في امتصاص بعض العناصر الغذائية ونمو الحمص (Cicer arietinum L)

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الخلاصة

تأثير التلقيح الثنائي بالقاح البكتيريا المثبتة للنيتروجين والبكتيريا المذيبة للفوسفور في نمو وامتصاص العناصر الغذائية لنبات الحمص (*Cicer arietinum* L) عند مستويات مختلفة من الفسفور (0، 40، 80، 120 ،160) كغم فسفور لكل هكتار، والصخر الفوسفاتي كمصدر للفوسفات. أجريت تجربة السنادين البولي أثلين في البيت البلاستيكي في كلية علوم الهندسة الزراعية، جامعة السليمانية، بكرجو خلال موسم النمو من نوفمبر 2021 إلى مارس 2022. أجريت التجربة في تجربة عاملية بالتصميم العشوائي الكامل (CRD) في تربة غرينية طينية، بثلاثة مكررات لكل معاملة، وبعد خمسة أشهر من النمو تم قياس ارتفاع النباتات وشدة الكلوروفيل وبعد ذلك تم حصاد النباتات، تم فصل الجذور عن الجزء الخضري وبعد ذلك تم عزل العقد الجذرية وبعد الغسل بالماء تم عدها. أظهرت النتائج زيادة معنوية في ارتفاع النباتات وشدة الكلوروفيل وعدد العقد الجذرية والوزن الجاف للجذور والوزن الجاف للنباتات الحمص مقارنة مع المقارنة. وأعلى قيمة كانت (87 سم، 76 spad، 77، 6.00 غم لكل سندانة و14 غم لكل سندانة) تم تسجيلها على التوالي عند أعلى مستويات الفسفور (160 كغم فسفور هكتار$^{-1}$)، في حين أن أعلى قيمة للعناصر الغذائي N، P، K، Fe، Zn وMn كانت 26.00 غم كغم$^{-1}$، 11.36 غم كغم$^{-1}$، 26.0غم كغم$^{-1}$، و445.33 ميكروغرام$^{-1}$ و46.25 ميكروغرام$^{-1}$ و41 ميكروغرام غم$^{-1}$ مسجلة على التوالي عند أعلى مستوى من الفسفور. كما أدى التلقيح بـ*Rhizobium Legeuminosarum* إلى زيادة معنوية في ارتفاع النبات وشدة الكلوروفيل وعدد العقد الجذرية والوزن الجاف للجذور والوزن الجاف للمجموع الخضري

وN، P، K، Fe، Zn، Mn لنباتات الحمص ولكنه كان أقل من التلقيح المشترك. عندما تم تلقيح التربة ببكتيريا *Pseudomonas putida* أظهرت النتائج أقل زيادة معنوية في ارتفاع النبات وشدة الكلوروفيل وعدد العقد الجذرية والوزن الجاف للجذور والوزن الجاف للمجموع الخضري في نباتات الحمص مقارنة مع المقارنة، وكانت أعلى قيمة 62.33 سم، 43.30 spad، 51.00، 3.98غم و10.40 غم على التوالي عند أعلى مستوى P.بينما كانت أعلى قيمة للعناصر الغذائية N وP وK وFe وZn وMn في نباتات الحمص هي 18.60غم كغم$^{-1}$ و10.00غم كغم$^{-1}$ و17.63غم كغم$^{-1}$ و298.0 ميكروغرام$^{-1}$ و34.76 ميكروغرام$^{-1}$ و18 ميكروغرام غم$^{-1}$على التوالي، عند أعلى مستوى p.

كلمات مفتاحية: Rhizobium، Pseudomonas، Biofertilizer، الفسفور ، الحمص.

Introduction

The chickpea (*Cicer arietinum* L) is the third most important pulse crop worldwide with a production level of 15.08 million tons and covering 14.84 million hectares of cultivated area (13). A good source of carbohydrate, protein, fibers, Ca, Mg, N, P, K, Fe, Zn, and vitamins (23) it is a key factor in human nutrition. It also contributes to sustainable agriculture practices due to its ability to fix atmospheric N through a process called nitrogen fixation, which increases the amount of fixed nitrogen in the environment, and sets them apart from cereal crops. Chickpea production in Iraq has decreased over the decades due to insufficient biological nitrogen fixation or a lack of native soil rhizobia inhabitants (12). Increased applications of costly chemical fertilizers to raise crop yields may degrade soil health and cause environmental pollution (26). Microbial inoculants, composed of beneficial microorganisms that increase nutrient availability, encourage plant growth, and enhance overall plant health, have received much attention for use in sustainable agriculture practices in recent years (26).

Co-inoculating chickpea seeds with *Rhizobium leguminosarum* and *Pseudomonas putida* based on competitive and efficient rhizobia fixing atmospheric N combined with P solubilizing bacteria may provide plants with N and P in an environmentally sustainable manner. It offers an eco-friendly agricultural process, stimulates plant growth and nutrients uptake, as well as mitigates environmental pollution from the use of chemical fertilizers (5). Most soils in the Iraqi-Kurdistan reign are calcareous with a high amount of CaCO3, which may fix available phosphorus. Also, soluble nitrogen sources may be leached or lost by denitrification and volatilization. This research evaluated the effect of nitrogen fixing and phosphorus solubilizing biofertilizer with phosphorus on chickpea growth and nutrient uptake.

Materials and Methods

This experiment was conducted during the November 2021 to March 2022 growing season in the greenhouses of the University of Sulaimani’s Bakrajo Horticulture Department, College of Agricultural Engineering Sciences (GPS readings 35 53 7.9 N, 45 36 4.5 E). It assessed the co-inoculation effect of Rhizobium leguminosarum and

Pseudomonas putida on the nutrient uptake and growth response of chickpea (Cicer arietinum L.) at P levels of 0, 40, 80, 120, and 160 kg P ha^{-1} using rock phosphate as the source of phosphorus. The experiment site was excavated to a depth of 0–20 cm depth and the soil completely crushed and air-dried to pass through a 4 mm sieve. The soil was classified as vertisols with a silty clay texture (68.80 sand, 553.20 silt, and 378.00 clay g kg^{-1}) based on the USDA Soil Taxonomy. It was determined to have 3.40 P mg kg^{-1} of variable P, 31.20 g kg^{-1} of solubility K, 15.30 g kg of organic matter, 256 g kg^{-1} of CaCO3, total nitrogen 0.11 g kg^{-1}, pH of 7.42 (1:2.5 soil: water suspension), and an EC of 0.17 dSm^{-1}. The CFU of total bacteria 2.48 x 10-8 and fungi were 1.35 x 10-6.

Five kilograms of unsterilized soil were transferred into 25- and 35-cm diameter sterilized plastic containers. All soil, potted or otherwise, received five milligrams of P at 0, 40, 80, 120, and 160 kg P ha^{-1} in the form of rock phosphate. After immersing the chickpea seeds in a 95% solution of ethanol for five minutes, they were surface sterilized, and then carefully cleaned with sterile, distilled water. Each of the containers was then filled with eight uniformly-sized, pre-selected, healthy seedlings. The Rhizobium leguminosarum-inoculated pots were obtained by adding 2 ml of inoculum, 2 ml of Pseudomonas putida, 4 ml for the interactions with the planting holes in each pot, and 2 ml of bacterial inoculum to some of the pots. The seeds that emerged were subsequently covered with thick soil. With three replicates for each treatment, the pots were set up in a completely randomized design (CRD) on plastic house benches. The seedlings were reduced to four per container after germination. The soil was regularly weighted in pots throughout the study period to maintain it at 70% field capacity. The crops were irrigated twice daily with tap water if required during later growth stages. The height of the plants and chlorophyll intensity (SPAD) were recorded after five months of growth. The shoots were removed from the roots washed and counted, and their root dry weight measured after drying for 72 hours at 65º C to a constant weight. The macronutrients (N, P, and K) and micronutrients (Fe, Zn, and Mn) were identified by processing the shoot dry weight after it passed through a 0.5 mm screen. The data were statistically analyzed using the XLSTAT version 12 software, specifically the 2019-2.2 edition. A Duncan multiple tests at P 0.05 was used to compare the averages.

Results and Discussion

Plant height (cm): Table 1 shows a significant effect was achieved when the soil was co-inoculated with the *Rhizobium leguminosarum* and *Pseudomonas putida* bacteria in terms of increased chickpea plant height at higher P levels compared to the non-inoculated plants (control). In addition, the heights of both inoculated and non-inoculated plants increased with higher P levels. These findings are consistent with those reported by (1 and 2).

When both bacteria were co-inoculants of the soil, the highest plant height value 78.0 cm was obtained at the highest P level (160 kg P h^{-1}), while the lowest value at 40.00 cm was in the control treatment. On the other hand, for non-inoculated plants, the greatest height was at the highest P level (160 kg P h^{-1}), and the lowest at 0 kg P h^{-1}. When the two bacteria were co-inoculated into the soil, chickpea plant heights increased. This may be due to the ability of Rhizobium leguminosarum to fix nitrogen from the atmosphere and provide the plant with accessible nitrogen, which increases plant height and biomass. On the other hand, plants treated with Pseudomonas putida produce greater amounts of hormones that are known to impact plant height, including auxin, gibberellin, acetic acid, and IAA. In addition, it could be because the bacteria provides various organic acids that dissolve available P and make it available to plants, increasing their height and growth. The highest value for the plants inoculated with Rhizobium leguminosarum was 76.10 cm while the lowest was 54.50 cm. For Pseudomonas putida they were 62.33 cm and 48.00 cm, respectively at with the lowest P level.

Root dry weight (g): Table 1 shows that soil co-inoculated with Rhizobium leguminosarum and Pseudomonas putida significantly increased chickpea root dry weight at different P levels compared with the control. The highest value at 6.00 g pot^{-1} was recorded at highest P levels (160 kg P h^{-1}), whereas the control value (1.95 g pot^{-1}) had the lowest value. However, in the inoculated plants, the maximum chickpea root dry weight was recorded at the highest P levels (160 kg P h^{-1}), while the lowest was for the control (1.95 g pot^{-1}). The presence of the Rhizobium leguminosarum bacteria in co-inoculated chickpea plants may have contributed to their increased root dry weights. This is because the bacteria form nodules and fix nitrogen, which increases the amount of N supplied to the plant and is essential for photosynthetic rate, plant growth, and root dry weight. Alternatively, it might be due to the bacteria's capacity to secrete various organic acids, primarily glycemic and keto gluconic acid. They also have the effect of raising the release of phosphates from both organic and inorganic substances and lowering the pH of the soil rhizosphere. This increases the amount of available P, which increases plant uptake of P and increases root dry weight (8). However, for the Rhizobium leguminosarum-inoculated plants, the highest root dry weight was 4.95 g while the lowest was 1.95 g followed by those inoculated with Pseudomonas putida where the values were 3.98 g and 1.95 g, respectively at the lowest P level.

Shoot dry weight (g): As shown in Table 1, the shoot dry weight of chickpea increased significantly with co-inoculation of the soil with the two bacteria (Rhizobium leguminosarum and Pseudomonas putida) compared with the control, and increased with higher dosages of P in both co- inoculated and non-inoculated plants. In the former, the maximum value for shoot dry weight was 160 kg P h^{-1} while the lowest at 4.18 g pot^{-1} was for the control treatment. Conversely, soil inoculation with only Rhizobium leguminosarum produced the highest shoot dry weight at 11.25 g pot^{-1} compared to the control’s 4.18 g pot^{-1} while inoculation with Pseudomonas putida generated the highest value at 10.40 g pot^{-1} compared with the 4.18 g pot^{-1} for the control. These results agree with (9). Therefore, the ability of Rhizobium leguminosarum to fix atmospheric nitrogen, which supplies available nitrogen leads to increased uptake and increased plant growth. This increases shoot dry weight, or the production of phytohormones, which produces greater chickpea plant growth at different P levels (19). Also, it may be due to the beneficial effect of the Pseudomonas putida which could be attributed to the potential effect of increasing the availability of P through the excretion of different organic acids on dissolving rock phosphates which supply P to the soil making it available for plant growth and higher shoot dry weight (8).

Table 1: Effects of phosphorus on the height and dry weight of the roots and shoots of chickpea plants with Rhizobium leguminosarum and Pseudomonas putida inoculations.

Handling	Plant height (cm)	Root dry weight (g pot^{-1})	Shoot dry weight (g pot^{-1})
P0 control	40c	1.9n	4.34p
P40	46i	2.37m	4.82o
P80	51.34i	2.72i	5.33n
P120	55.66hi	2.87k	5.84m
P160	58.66fg	3.18j	6.77k
P0+R	54i	3.38i	6.96k
P40+R	59.66fg	3.67h	8.60i
P80+R	64.33e	4.46f	9.35g
P120+R	73.66c	4.75e	10.93d
P160+R	76.33c	4.95d	11.34c
P0+Ps	45.66i	2.49m	6.50l
P40+Ps	51.33i	2.96k	7.61j
P80+Ps	57.33gh	3.34i	8.84h
P120+Ps	61.33ef	3.37h	10.47c
P160+Ps	64.33e	3.94g	9.87f
P0+R+Ps	60.33fg	3.76g	7.50j
P40+R+Ps	68.66fg	4.52f	8.90h
P80+R+Ps	83b	5.17c	11.50c
P120+R+Ps	84b	5.76b	12.45b
P160+R+Ps	88.33a	6.03a	14.34a

P0: control; P: phosphorus (kg ha^{-1}); R: *Rhizobium leguminosarum*; Ps: *Pseudomonas putida.*

Number of nodules: Data on nodule number in the chickpea plants are presented in Table 2. It shows that co-inoculating the soil with both bacteria at different phosphorus levels significantly increased the number of plant nodules compared with the control, as well as with higher applications of phosphorus. This is consistent with the findings of (5 and 25). The maximum value of 77.00 nodule pot^{-1} was recorded at the highest phosphorus level (160 kg P ha^{-1}), while the lowest at 14.00-nodule pot^{-1} was at 0 kg P ha^{-1}. The largest increase in nodule numbers was observed in co-inoculation. In non–inoculated plants nodule numbers increased with higher phosphorus levels with the maximum at 44.33 and the minimum at 4.00 for the control. The increased nodule number resulting from co-inoculation could be attributed to the bacteria in dissolving rock phosphate and providing the soil with accessible phosphorus, both of which increase nodulation (5 and 14).

The results show that chickpea plants inoculated with only Rhizobium leguminosarum had significantly more nodules at different levels of phosphorus. The largest value at 59.00 was at the highest P level, while the lowest at 14.00 was for the control. In comparison to the control, Pseudomonas putida considerably increased the number of nodules at varied phosphorus levels. The 51.00-nodule pot^{-1} had the highest recorded value at the maximum phosphorus level while the 14.00 nodules had the lowest recorded phosphorus level. The elevated nodule number from the higher P levels in the plants may be due its ability to improve and increase root growth and root biomass, and help bacteria rhizobium activity to form more nodule which increase nitrogen fixation (16).

Chlorophyll intensity (SPAD): Table 2 shows that co-inoculation of chickpea with both bacteria significantly increased leaf chlorophyll intensity especially with greater phosphorus application. The maximum chlorophyll intensity produced was 76.00 (SPAD) for the 160 kg P ha^{-1} compared to the control's 14.33 (SPAD) at 0 kg P ha^{-1}. Additionally, the maximum chlorophyll intensity value (SPAD) in non-inoculated soil was 45.33 at 160 kg P ha^{-1}, while the lowest was 14.33 (SPAD). The increase in chlorophyll intensity due to dual inoculation may be attributed to Rhizobium leguminosarum's ability to enhance soil nitrogen through N2-fixation (16). Additionally, Pseudomonas putida may contribute by solubilizing unavailable phosphorus into an available form, thereby increasing phosphorus availability in the soil. The heightened nitrogen and phosphorus levels in the soil enhances the plant's ability to uptake nitrogen, magnesium (Mg), iron (Fe), and phosphorus (P) thereby fostering increased chlorophyll content in the chickpea plants. This results in greener plants as they contain higher levels of nitrogen, phosphorus, potassium (K), and sulfur (S), which promote greater chlorophyll synthesis rates, ultimately leading to a significant enhancement in chlorophyll intensity in the plants (3).

The results demonstrate that plants inoculated with Rhizobium leguminosarum significantly increased leaf chlorophyll intensity of chickpea plants whereby the P levels is increased in contrast to the control. In contrast to the control value of 14.33 (SPAD), the maximum value for shoot chlorophyll intensity was 66.33 (SPAD). The results indicate a significant increase in chlorophyll intensity in chickpea plants when the soil is inoculated with Pseudomonas putida. In addition, chlorophyll intensity showed a positive correlation with increasing phosphorus (P) levels at 43.30 (SPAD) for the highest and 14.33 (SPAD) for the control.

Table 2: Effect of Rhizobium leguminosarum, Pseudomonas putida and their interactions with phosphorus on chickpea nodules number and chlorophyll intensity.

Treatment	Nodule numbers	Chlorophyll intensity (SPAD)
P0 control	14 m	13o
P40	18.73i	23m
P80	29j	30jk
P120	39.73h	38gh
P160	44.3g	45ef
P0+R	22.7k	26l
P40+R	33j	36hi
P80+R	48h	47c
P120+R	55fg	53c
P160+R	58.34e	65b
P0+Ps	21k	18n
P40+Ps	20.7i	28kl
P80+Ps	29ef	32j
P120+Ps	45.77cd	43f
P160+Ps	49c	39g
P0+R+Ps	29j	34i
P40+R+Ps	38h	44ef
P80+R+Ps	55c	53d
P120+R+Ps	66b	66b
P160+R+Ps	77a	78a

P0: control; P: phosphorus (kg ha^{-1}); R: *Rhizobium leguminosarum*; Ps: *Pseudomonas putida*; Rh+Ps: *Rhizobium leguminosarum+ Pseudomonas putida* mean the same letters are not significant at ($P \le 0.05$).

Shoot nitrogen content (g kg^{-1}): Inoculating chickpea plants with Rhizobium leguminosarum and Pseudomonas putida produces significantly higher shoot nitrogen content as compared to the control (Figure 1). Also, increasing the rate of P, raised N content. Significant changes were found in the co-inoculated and non-inoculated plant (control) treatments (8). Following inoculation with Rhizobium leguminosarum, the plant's nitrogen content increased significantly in comparison to the control treatment. Treatment with the greatest phosphorus level had the highest result for nitrogen content at 21.50 g kg, while for the lowest P it was only 12.15 g kg^{-1}. Compared to non-inoculated plants, inoculating chickpea with Pseudomonas putida enhances N content, while in the control treatment at the lowest nitrogen content value 14.0 g kg^{-1} and 160 kg P ha^{-1} produced the greatest value 18.60 g kg^{-1}.

The importance of co-inoculation with Rhizobium leguminosarum, which provides the plant with accessible nitrogen through N2 fixation, can be seen in the co-inoculation plants' growing N concentration. This could also be attributed to the synthesis of hormones like auxin, which encourages plant development and increased nitrogen uptake. Additionally, Pseudomonas putida has the ability to create organic acids and enzymes that dissolve rock phosphate, increasing the quantity of P available in the soil solution and promoting plant growth. It increases the plant's capacity to take in additional nitrogen as well (10).

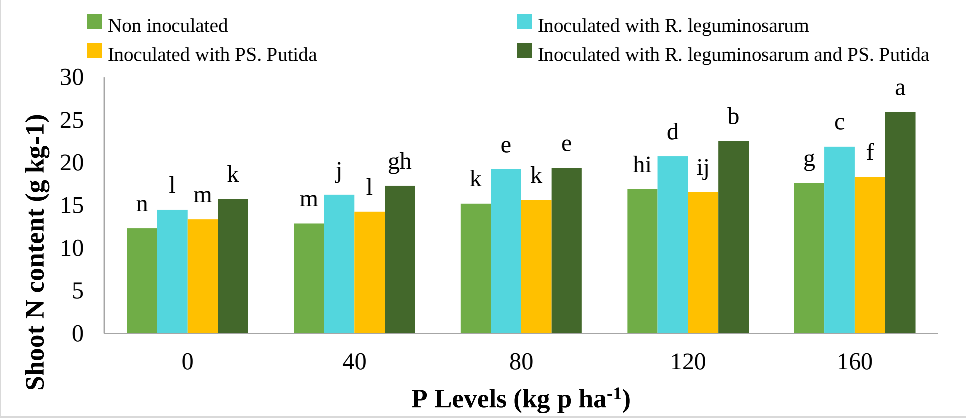

Figure 1: Effect of both inoculated and non-inoculated Rhizobium leguminosarum and Pseudomonas putida on chickpea nitrogen at different phosphorus levels.

The results for shoot nitrogen content at various P levels are shown in Figure 2 as the coefficient of determination (R2). Treatment with both bacteria produced the highest value 0.9988 followed by only Rhizobium leguminosarum at 0.9978, while the lowest number with only Pseudomonas putida treatment was 0.9941.

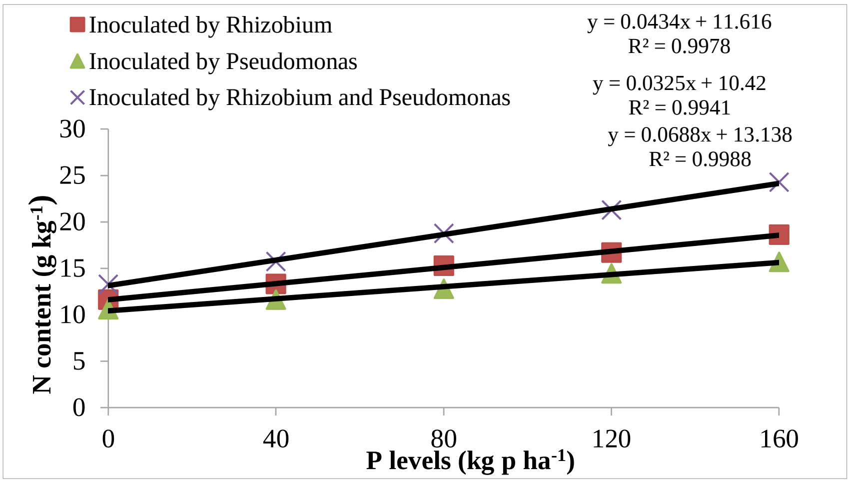

Figure 2: Relationship between chickpea N content (g kg^{-1}) and phosphorous levels for chickpea plants inoculated with Rhizobium leguminosarum and Pseudomonas putida and their interactions.

Shoot phosphorus content (g kg^{-1}): Injecting both bacteria into the chickpea plant led to considerably higher amounts of phosphorus in the shoots compared to the control. The results demonstrate notable variations in the shoot phosphorus content between treatments. Phosphorus content in plants rose with higher P levels. At 160 kg P ha^{-1}, the co-inoculation treatment exhibited greater phosphorus content (11.36 g kg^{-1}) while the control group had the lowest value at 3.53 g kg^{-1}. Conversely, at 0 kg P ha^{-1} and 160 kg P h^{-1}, the P content in the inoculated plants was 3.53 g kg^{-1} and 7.40 g kg^{-1}, respectively. These results correspond with (1). Figure 3 shows that inoculating chickpea plants with Rhizobium leguminosarum significantly changed their phosphorus concentrations at different rates compared to the control.

Figure 3 shows that chickpea plants treated with Pseudomonas putida showed considerable increases in phosphorus concentrations at higher rates of phosphorus applications compared to the control. This is consistent with the findings of (15). The height value for P concentration was 10.00 g kg^{-1} at the highest P level (160 kg P ha^{-1}), and 3.53 g kg^{-1} at the lowest. A possible explanation for the rising shoot P content in co-inoculated chickpea plants is that Rhizobium inoculum enhances N supply by N2-fixation (1) while the Pseudomonas putida lowers soil pH and releases various organic acids including lactic, citric, and oxalic acids, among others, to dissolve rock P. This process raises the quantity of accessible P in the soil solution and increases plant P uptake (15).

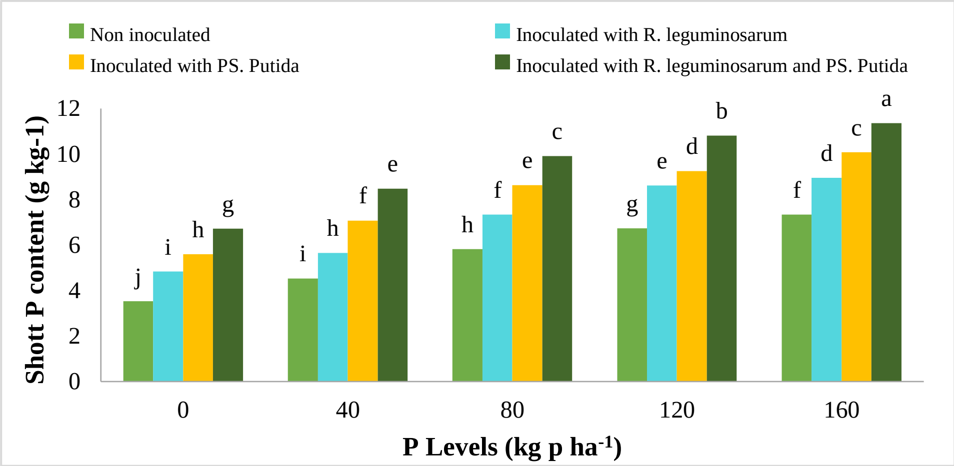

Figure 3: Effect of both inoculated and non-inoculated Rhizobium leguminosarum and Pseudomonas putida on chickpea phosphorus at varying levels of phosphorus.

The R^2 coefficient for shoot phosphorus at varying P levels is displayed in Figure 4. The highest value was achieved through inoculation with both bacteria 0.9557 followed by *Pseudomonas putida* treatment 0.967, and the lowest value was recorded for the *Rhizobium leguminosarum* treatment 0.9632.

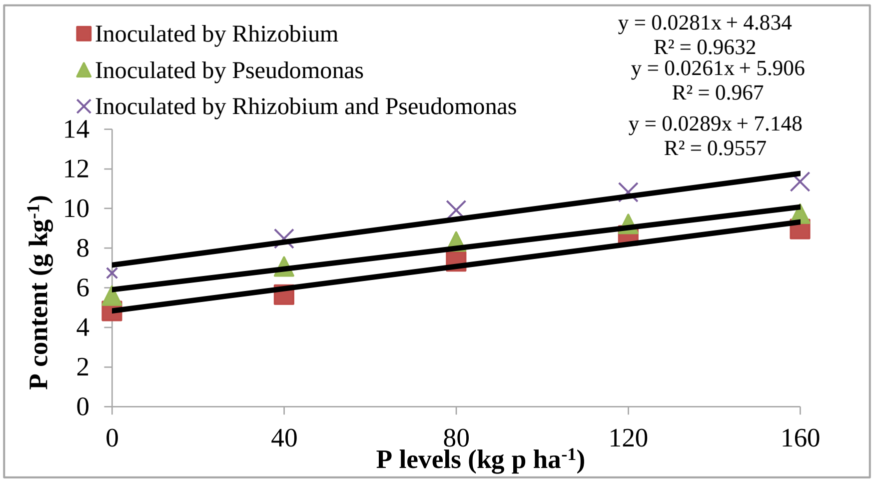

Figure 4: Relationship between chickpea P content (g kg^{-1}) and phosphorous levels for chickpea plants inoculated with Rhizobium leguminosarum and Pseudomonas putida and their interactions.

Shoot potassium content (g kg^{-1}): The findings in Figure 5 demonstrate notable variations in K content at different P levels with plants co-inoculated with both bacteria having significantly higher K content compared to the control. This is consistent with the findings of (1 and 18). The co-inoculation statistics are shown in Figure 5. The highest K content value was 26.00 g kg^{-1} at the highest P level (160 kg P ha^{-1}), while the lowest was in the control group at 11.87 g kg^{-1}. After inoculation with only Rhizobium leguminosarum, the highest value for K content in the chickpea plant was 13.23 g kg^{-1} at 160 kg P ha^{-1} when the soil was not inoculated, as opposed to 11.87 g kg^{-1} in the control. Despite being injected with Pseudomonas putida, K content was 21.90 g kg^{-1} as opposed to the control (12.30 g kg^{-1}). K content ranged from 17.63 g kg^{-1} to the lowest at 12.30 g kg^{-1} in the control group.

The increased K content from co-inoculation may be due to the role of the bacteria Rhizobium leguminosarum in providing available N through N2-fixation and increasing uptake by plants, which enhances high plant biomass and the ability of the plant to absorb mineral nutrition (19). However, k-solubilizing bacteria, which dissolves it from insoluble forms and converts it to accessible forms by creating various organic acids, may be the cause of the rising K content in chickpea plants (6 and 19). Alternatively, it could be because the Pseudomonas putida dissolves rock phosphate, increasing the P in the soil and facilitating plant development and biomass as well as the ability of the plants to take up and store more K from the soil (24).

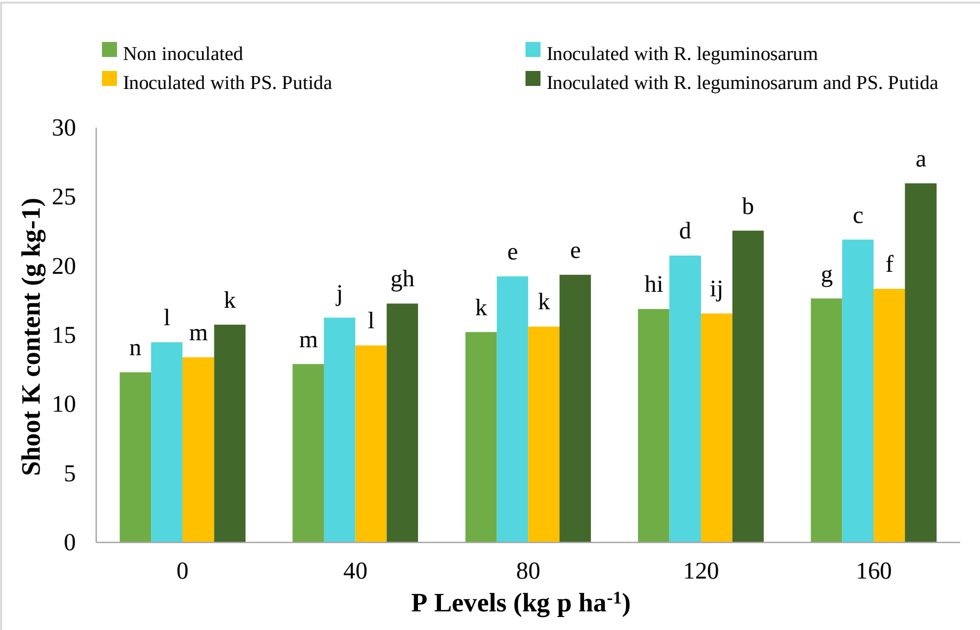

Figure 5: Effect of both inoculated and non-inoculated Rhizobium leguminosarum and Pseudomonas putida on chickpea potassium at varying phosphorus levels.

Figure 6 shows the R2 values for shoot potassium content at different P values. Inoculation with both the bacteria produced the highest value 0.8954, followed by Rhizobium leguminosarum 0.977 and Pseudomonas putida treatments at 0.986.

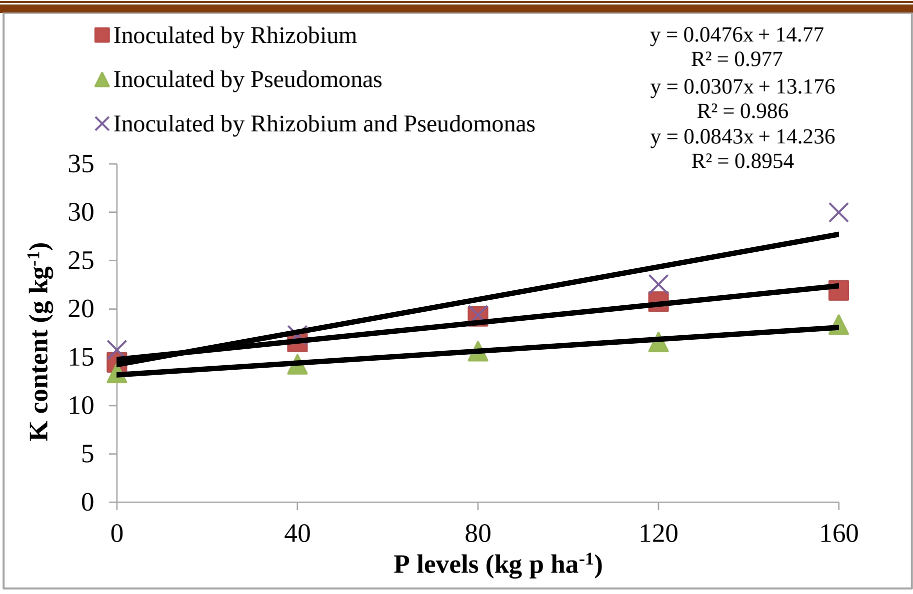

Figure 6: Relationship between phosphorus levels and K content (g kg^{-1}) of chickpea plants inoculated with Pseudomonas putida and Rhizobium leguminosarum and their interactions.

Shoot Fe content (µg g^{-1}): Figure 7 indicates that co-inoculating with both bacteria had a substantial impact on the Fe content of the chickpea plants. The highest Fe concentration was in the co-inoculation plants compared to the control treatment, with Fe increasing with higher P levels. The highest shoot Fe content was 445.33 µg g^{-1} observed in co-inoculation at 160 kg P ha^{-1} compared to control (149.66 µg g^{-1}). The results show that different phosphorus levels produced significant differences between the treatments, as noted by (17). The ability of Rhizobium leguminosarum to enhance plant nitrogen intake through nitrogen fixation, and the importance of iron for legume-rhizobium symbioses and as a co-factor for the nitrogenized enzyme, may explain the higher Fe content in the co-inoculated treatments (22). Furthermore, Pseudomonas putida increases the amount of P in the soil by generating various organic acids, which are important for the mineralization of phosphate rock through the action of acid phosphatase. Hence, N and P are the two vital macronutrients that support plant growth and improve the chickpea plant's capacity to absorb more micronutrients, particularly Fe, which are then stored in shoot plants (12 and 21).

As compared to the control, chickpea plants inoculated with Rhizobium leguminosarum showed considerable increases in Fe concentrations at different P levels, findings that are consistent with (9). In comparison to the control value of 149.66 µg g^{-1}, the highest value for Fe concentration was 393.33 µg g^{-1} at 160 kg P ha^{-1} (11). Pseudomonas putida-inoculated soil came next, with the highest Fe content at 282.00 µg g^{-1} compared to 149.66 µg g^{-1} for the control.

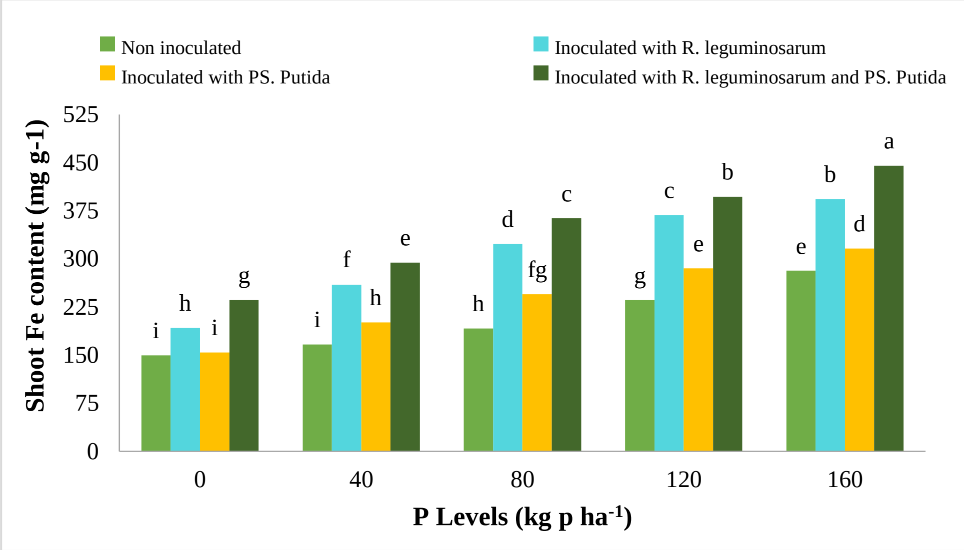

Figure 7: Relationship between inoculated and non-inoculated Rhizobium leguminosarum and Pseudomonas putida in chickpea iron content at different phosphorus levels.

Figure 8 displays the coefficient of determination (R2) for shoot Iron content at various P levels. Inoculating with both bacteria produced the greatest value 0.9883, while Rhizobium leguminosarum came in second at 0.8109 and Pseudomonas putida treatment had the lowest value 0.9944.

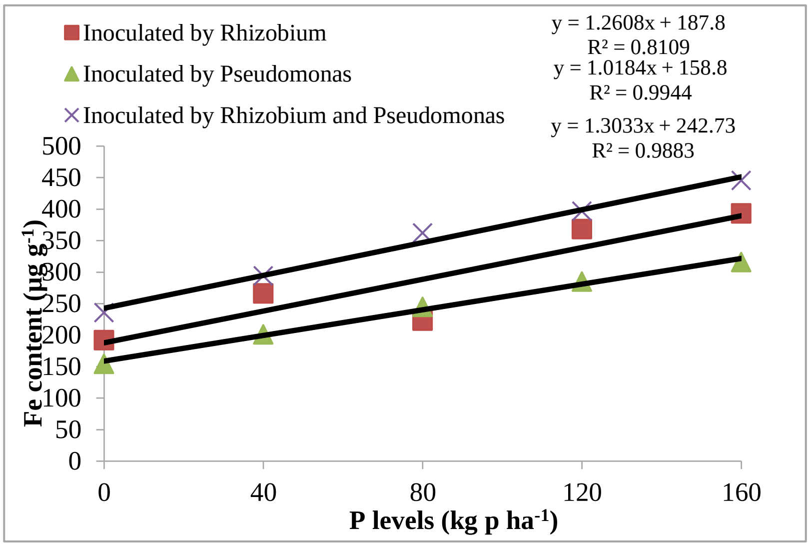

Figure 8: Relationship between shoot Fe content (µg g^{-1}) and phosphorous levels for chickpea plant inoculated with Rhizobium leguminosarum and Pseudomonas putida and their interactions.

Shoot Zn content (µg g^{-1}): As clearly seen in Figure 9, co-inoculating chickpea plants with Pseudomonas putida and Rhizobium leguminosarum enhanced zinc content in the shoots across all treatments at varying P levels but increased with higher P application rates. At 160 kg P ha^{-1}, the co-inoculation treatment recorded the maximum value of shoot zinc at 46.25 µg g^{-1}, whereas the control treatment at 0 kg P ha^{-1} had the lowest of 15.35 µg g^{-1}. Conversely, at 160 kg P ha^{-1} in the non-inoculated treatments, peak Zn concentration was observed at 33.10 µg g^{-1} as opposed to 15.35 µg g^{-1} for the 0 kg P ha^{-1}. This may be attributed to the Rhizobium leguminosarum bacterium fixing and supplying accessible zinc to increase its content in the co-inoculated plants (1) or because Pseudomonas putida lowers soil pH and promotes biochemical phosphate mineralization by dissolving available P in the soil and supplying different organic acids (28). The two main macronutrients for enhancing plant growth are nitrogen and phosphorus. They stimulate the uptake of nitrogen and phosphorus which improve the plants' capacity to absorb more nutrients, particularly zinc that accumulates in the shoots.

The resultant zinc content on shoot chickpea plants treated with Rhizobium leguminosarum is depicted in Figure 9 which shows that it increases significantly at higher phosphorus rates. The chickpea plant's Zn content reached its maximum value of 36.27 µg g^{-1} when derived from 160 kg P ha^{-1}, whereas it was lowest with 15.35 µg g^{-1} for the control. However, the maximum Zn content (34.76 µg g^{-1}) was obtained at the highest P level in soil inoculated with Pseudomonas putida as against 15.35 µg g^{-1} for the control. The high Zn in chickpea may be due to the ability of this bacterium in making more p available to the plant allowing the root to absorb nutrients including Zn (20).

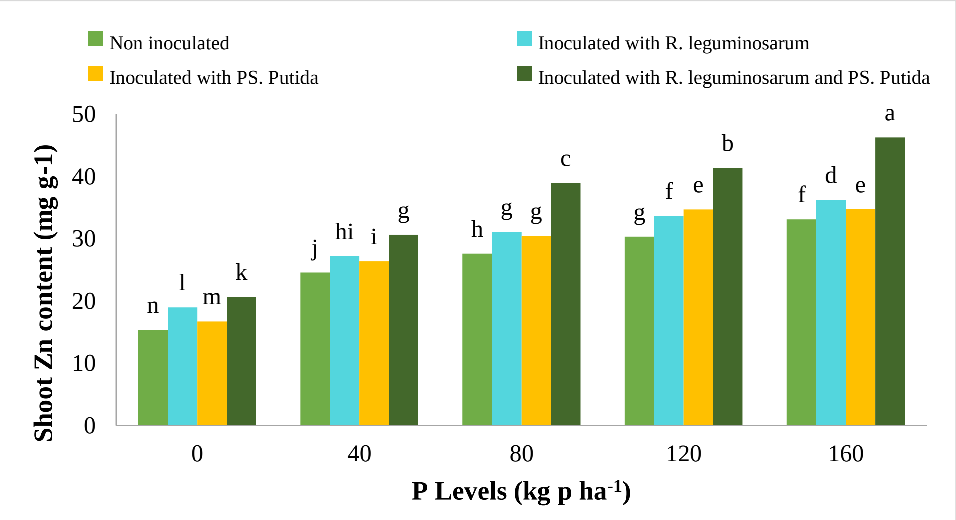

Figure 9: Effect of inoculation and non-inoculation of *Rhizobium leguminosarum* and *Pseudomonas putida* on the zinc content of chickpeas at varying phosphorus levels.

Figure 10 show the coefficient of determination (R2) for shoot Zinc content at various P levels. Treatment with both bacteria produced the maximum value of 0.9457, followed by 0.8783 with only Rhizobium leguminosarum and finally Pseudomonas putida treatment produced the lowest value 0.9146.

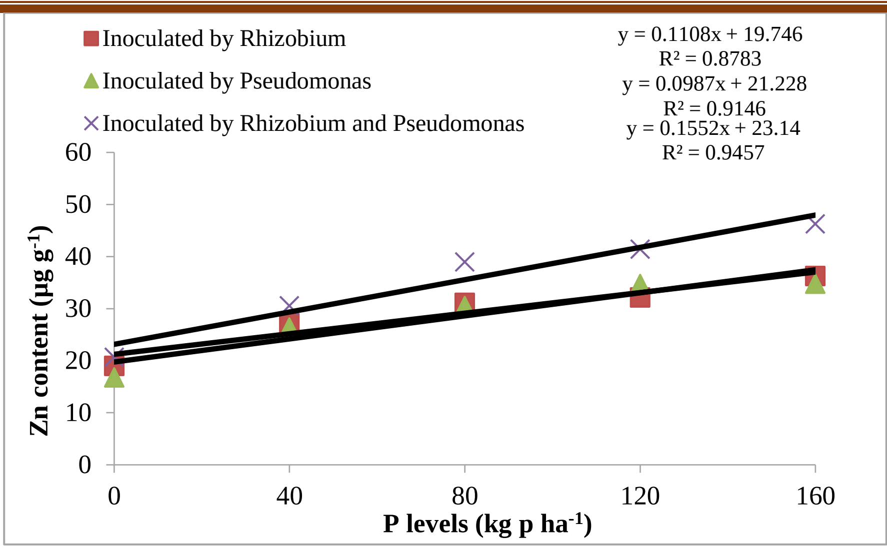

Figure 10: Relationship between shoot Zn content (µg g^{-1}) and phosphorous levels for chickpea plants inoculated with Rhizobium leguminosarum and Pseudomonas putida and their interactions.

Shoot Mn content (µg g^{-1}): Figure 11 shows that the co-inoculation of chickpea plants with Rhizobium leguminosarum and Pseudomonas putida resulted in the highest chickpea Mn content (41 µg g^{-1}) at 120 kg P ha^{-1} when compared to the non-inoculated control (18 µg g^{-1}) of 0 kg P ha^{-1}. Additionally, significant increases in Mn content were recorded at higher P application levels. With Rhizobium leguminosarum, the greatest P level (160 kg P ha^{-1}) produced a higher Mn content (36. µg g^{-1}) in shoot chickpea plants compared to the control (18 µg g^{-1}). Inoculation with only Pseudomonas putida led to a Mn concentration of 30 µg g^{-1} at higher P levels. However, Mn concentrations in the inoculated plants at zero kg P ha^{-1} registered 18 µg g^{-1}.

The higher Mn content in chickpea plants under treatment may be due to the co-inoculation. Nitrogen fix from the atmosphere in Rhizobium leguminosarum provides the plant with more nitrogen and encourages vegetative growth. This aids the uptake of micronutrients, including Mn, which are accumulated in the shoots and soil. Alternatively, this may be because the bacteria forms a symbiotic relationship with leguminous plants, thereby improving Mn uptake (7). Also, Pseudomonas putida may improve nitrogen fixation ability by solubilizing unavailable P into available forms and mobilizing trace elements like Mn in the plants (4).

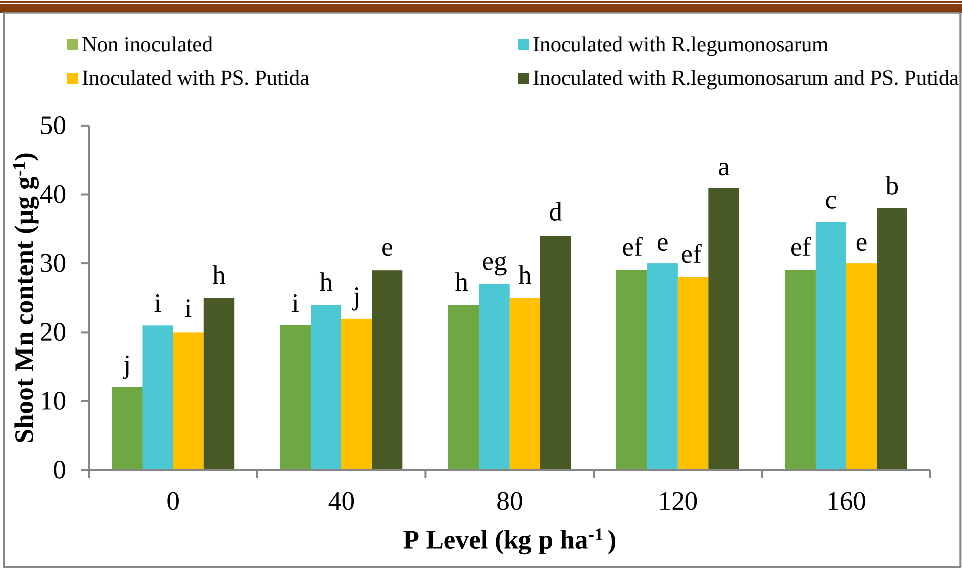

Figure 11: Effect of inoculation and non-inoculation of Rhizobium leguminosarum and Pseudomonas putida on manganese content of chickpeas at varying phosphorus levels.

Figure 12 show the coefficient of determination (R^2) for shoot manganese content at various P levels. Treatment with *Pseudomonas putida* bacteria produced the maximum value 0.9961, followed by *Rhizobium leguminosarum* at 0.9941, and the lowest value 0.846 in both bacteria.

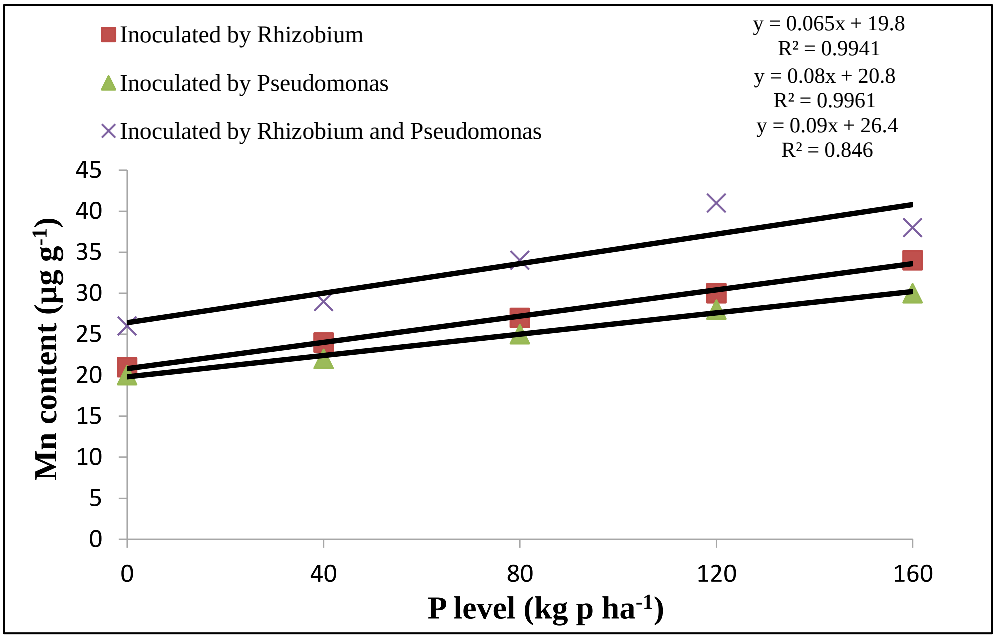

Figure 12: Correlation between phosphorus levels and chickpea Mn content (µg g^{-1}) for plants inoculated with Pseudomonas putida and Rhizobium leguminosarum and their interactions.

Conclusions

Co-inoculating with *Rhizobium leguminosarum* and *Pseudomonas putida* enhances plant growth in chickpeas resulting in notable increases in their height, chlorophyll intensity, nodule numbers, root and shoot dry weights, and N, P, K, Fe, Zn, and Mn content. Adding 160 kg P ha^{-1} rock phosphate and co-inoculating with the two bacteria enhances chickpea plant growth and raises nutrient content. Additionally, inoculating only *Rhizobium leguminosarum* significantly raised those indicators in the chickpea plants but not as much as co-inoculation with both bacteria. Compared to control, soil inoculated with *Pseudomonas putida* produced the lowest increases for the same parameters.

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Omar Ali Fattah: methodology, writing, original draft preparation, reviewing and editing. Author has read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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