

Evaluation of *Azospirillum's spp* Performance as Nitrogen-Fixing Bacteria in Potato Rhizosphere at Various Nitrogen Levels

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Abstract

One of the elements that restricts plant growth is nitrogen (N), which is primarily provided exogenously through fertilizer application. By fixing atmospheric N in the soil, diazotrophic rhizobacteria have been shown to enhance plant development. A field experiment was conducted in a field in Babylon province, 40 km north of Hilla city, during the spring season of 2024-2025, to cultivate the Dutch-origin Boran potato variety in silty loam soil. The study aimed to investigate the response of potato (*Solanum tuberosum* L.) to the nitrogen-fixing bacteria *Azospirillum spp.* under different nitrogen levels. The study included two factors: first, the addition of *Azospirillum* bacteria at three levels (0, 10, and 20 ml) to the tubers and the soil (designated A0, A1, and A2); and second, the addition of nitrogen at four levels (0, 100, 200, 300) kg ha⁻¹, coded N0, N1, N2, N3. The experiment was conducted according to a randomized complete block design (RCBD) with three replicates of 36 experimental units. Soil samples were collected for biological measurements (total bacterial counts after germination and total bacterial counts before harvest). The treatment A2N3 achieved the highest values of bacteria after germination at 2.436×10^{-9} CFU g⁻¹ soil, compared to the control treatment A0N0, which achieved 1.498×10^{-9} CFU g⁻¹ soil. As such the number of the same bacteria were maximized before flowering compared to the control. The results showed that the treatment A2N3 had the highest indicators for (plant height, chlorophyll index, and vegetative dry weight), the values were 55.713 cm, 58.113 spad, and 5.283 $\mu\text{g ha}^{-1}$, respectively, compared to the A0N0 control treatment, which had values of 44.317 cm, 35.260 spad, and 2.233 $\mu\text{g ha}^{-1}$, respectively. This study demonstrates that adding diazotrophic *Azospirillum spp.* to potato systems can enhance plant growth and N use efficiency, creating opportunities to increase potato crop growth with less N fertilizer input, but such events were completely dependent on the higher doses of bacteria inoculated in both of soils and potato. .

Keywords: potato tubers, nitrogen fertilizer, *Azospirillum*, nitrogen fixing, and plant growth regulator

Introduction

Biofertilizers play a vital role in sustainable agriculture and are part of an effective soil microbiome (Al-Balkhi, 2005; Al-Maliki et al., 2020). Biofertilizers contain nitrogen-fixing bacteria that are symbiotic or associative, such as *Azospirillum ssp.*, and their growth-

promoting secretions lead to increased plant growth and yield (Al-Samarrai and Faris, 2018). The use of biofertilizers is considered a sustainable technology that reduces pollution by utilizing natural sources (Al-Karboli et al., 2017; Al-Maliki et al., 2020; Al-Maliki, and Al-Shamary, (2022). *Azospirillum* bacteria are among the most efficient free-living

nitrogen-fixing bacteria. They fix atmospheric nitrogen in the soil and increase its uptake by plants. They also secrete growth-promoting substances such as gibberellins, cytokinins, and IAA hormone. Furthermore, they increase the uptake of water and nutrients such as potassium and phosphorus by plants (Mehnaz, 2015). Azospirillum is considered the most efficient type of bacteria in nitrogen fixation (Foriani et al., 1995). Nitrogen is the most important nutrient limiting factor for agricultural crop production. Although it constitutes up to 78% of atmospheric gases, higher plants cannot utilize it unless it is fixed by prokaryotic microorganisms (prokaryotes) that possess the nitrogenase enzyme, which reduces atmospheric nitrogen and converts it into ammonia (Tisdal et al., 1993). The trend is towards increasing the amount of nitrogen added to the soil using biofertilization. Biofertilizers will solve many food, pollution, and cost problems, given that they currently contribute 65% of the nitrogen used in agriculture and their importance in crop production will increase in the future (Vance, 1997). Potatoes are an annual herbaceous plant belonging to the nightshade family. They are a good source of vitamins B1, B2, and B3, and minerals such as potassium, phosphorus, and magnesium. They also contain folic acid, pantothenic acid, riboflavin, dietary

antioxidants, and dietary fiber beneficial to health (FAW, 2008). (Biofertilizers will solve many food, pollution, and cost problems, given that they currently contribute 65% of the nitrogen used in agriculture and their importance will increase in the future). In order to assess the efficacy of Azospirillum spp. doses with varying nitrogen levels on potato plants in field settings, the current study looked at their capacity for nitrogen fixation.

Materials and Methods

The spring season field experiment was conducted in a private field in the project area of Babylon Governorate, located approximately 40 km north of the city of Hilla, between longitudes 44.5°E and latitude 32.7°W. The soil is a silty loam. Several soil samples were randomly taken from the field before plowing to a depth of 0-30 cm. The soil samples were thoroughly mixed, and a composite sample was obtained, air-dried, ground, and sieved through a 2 mm sieve. This composite sample was used to estimate some of the chemical, physical, and biological properties of the study soil. Table 1 shows some of the results of the chemical, physical, and biological analyses of the field soil before planting, including:

Table 1: Chemical, Physical, and Biological Properties of the Soil Before Planting

traits	units	values
Electrical conductivity ((Ece	Ds.m ⁻¹	7.8
(Soil acidity (pH	---	7.7
Organic matter in the soil	%	0.50
Dissolved ions, positive and negative		
Calcium	mmol L ⁻¹	17.88
Magnesium	mmol L ⁻¹	10.02

Sodium	mmol L ⁻¹	22.86
Potassium	mmol L ⁻¹	0.83
Sulfate	mmol L ⁻¹	4.32
Chloride	mmol L ⁻¹	50.16
Bicarbonate	mmol L ⁻¹	Nil
Carbonate	mmol L ⁻¹	4.41
available nutrients		
Nitrogen	mg.kg ⁻¹ soil	22.4
Phosphorus	mg.kg ⁻¹ soil	7.22
Potassium	mg.kg ⁻¹ soil	85.7
traits	units	values
Soil separator		
Sand		300
silt	g.kg ⁻¹ soil	608
Clay		92
texture		Silty loam
Total bacterial counts	CFUg ⁻¹ Soil dry	⁹ 10×2.6
Total fungal counts	CFUg ⁻¹ Soil dry	⁹ 10×1.3
Nitrogen-fixing bacteria counts	CFUg ⁻¹ Soil dry	⁹ 10×0.6

The field experiment was conducted on an area of 861 m² (41 m × 21 m). The experimental site was plowed twice perpendicularly to a depth of 25 cm using a moldboard plow, followed by leveling and grading. The field was divided into three main sections, each containing 12 experimental units. Each experimental unit measured 6.25 m² and consisted of three rows, each 3 m long and 75 cm apart, according to Muharram and Abdul (1987). A 1 m spacing was maintained between treatments to control fertilization and

prevent cross-fertilization. The experiment was conducted using a randomized complete block design (RCBD), which included two factors: first, the application of Azosoprillum bacteria at three levels (0, 10, and 20 ml) per plant immediately after emergence, with a known inoculum density of 910, designated A2, A1, and A0 respectively; and second, the application of nitrogen at four levels. (300, 200, 100, 0) kg ha⁻¹, denoted by N3, N2, N1, N0 respectively, thus making the number of

experimental units 3) main sections (\times) 12 experimental units (36 = experimental units).

Soil tests:

Soil samples were collected and stored in polyethylene bags sterilized with ethyl alcohol and transported to the laboratory and then to the refrigerator until their use in isolating the nitrogen-fixing bacterium *Azospirillum* spp. The bacterial isolate was obtained, its efficiency tested, and it was isolated at the Agricultural Research Center in Zaafaraniya, Baghdad. The dilution and plate counting method was used to calculate:

1- The total bacterial count in the soil at the post-germination stage using Soil Extract Agar medium, as described in Baruah (2000).

2- The total bacterial count in the soil at the pre-harvest stage using Soil Extract Agar medium. (in Baruah, 2000).

Vegetative traits indicators for the studied traits

3- Plant height (cm)

On April 16, 2025, as signs of maturity began to appear on the potato plants, 5 plants were randomly measured from the middle of each experimental unit, from the soil surface to the growing tips, using a measuring tape and according to the average.

4- Dry weight of the vegetative parts (Mg ha^{-1})

This was measured by cutting five plants randomly selected from each experimental unit at their soil contact point. The cuttings were then thoroughly washed with water and dried in an electric oven at 25°C/h until the weight stabilized (Al-Sahaf, 1989). The average weight of the five plants was then calculated, and the dry weight of the experimental unit was derived from this average. This weight was then expressed as a percentage per hectare, calculated as follows:

Percentage of dry weight of the vegetative parts = (dry weight / fresh weight) \times 100

5- Chlorophyll content in leaves (spad unit)

This was measured using a Chlorophyllmeter (502-Spad) and estimated as the average of ten readings from two intermediate sources for each experimental unit during the flowering stage.

Results and Discussion

Total Bacterial Counts in the Post-Growth Stage (Bacterial Cells/g Soil)

Table 2 shows the results of the soil bacterial counts during the growth stage. The single treatment for *Azospirillum* bacteria reached its highest value at level A2, achieving 2.067×10^9 cells/g dry soil, compared to the control treatment A0, which gave 1.340×10^9 cells/g dry soil. Treatment A1, with its low biodensity, gave 1.783×10^9 cells/g dry soil. When different nitrogen levels were added to the single treatments, as shown in the same table, single treatment N3 achieved the highest value at 1.999×10^9 cells/g dry soil, which was not significantly different from the next single treatment, N2, which gave 1.837. The two low-level bacterial treatments showed the following results. N1 and N0 had values of (1.559, 1.537) $10^9 \times$ cells g dry soil⁻¹ respectively. It was also shown that the bi-interaction between levels of bio-inoculum of *Azospirillum* bacteria and the addition of different levels of nitrogen had a significant effect on increasing the number of bacteria in the post-germination stage. The A2N3 treatment achieved the highest value, reaching $2.436 \times 10^9 \times$ cells g dry soil⁻¹, which did not differ significantly from the bi- interaction treatment (A2N2), which reached a value of $2.260 \times 10^9 \times$ cells g dry soil⁻¹. Meanwhile, the bi- interaction treatment, the control treatment, and A0N0 had the lowest value, which was $1.469 \times 10^9 \times$ cells g dry soil⁻¹ of the number of *Azospirillum* bacteria in the post-germination stage.

Table 2 Effect of adding Azospirillum bacteria under different levels of nitrogen and the interaction between them on the number of bacteria in the soil (after germination) Bacterial 10^9 cell g soil⁻¹.

Nitrogen levels (N)	Azospirillum bacteria(A)			average N
	A0	A1	A2	
N0	1.469	1.498	1.645	1.537
N1	1.203	1.548	1.928	1.559
N2	1.305	1.947	2.260	1.837
N3	1.386	2.142	2.436	1.999
average A	1.340	1.783	2.067	
L.S.D	A	N*A		N
)0.05(0.0971	0.1943		0.1122

Effect of adding nitrogen-fixing Azospirillum bacteria under different nitrogen levels and the interaction between them on bacterial populations during the pre-flowering period (cell g) in dry soil -1.

Table 3 shows that the effect of the Azospirillum bioinoculation under different nitrogen levels significantly increased the number of bacteria in the soil at the pre-flowering stage. The highest single-treatment bacterial count was achieved at level A2, reaching 4.449×10^9 cells/g dry soil⁻¹, compared to the control treatment A0, which reached 2.533×10^9 cells/g dry soil⁻¹. Treatment A1 reached 4.232×10^9 cells/g dry soil⁻¹. When different nitrogen levels were added to the single-treatments, as shown in the same table, the single treatment N3 gave the highest count at 4.226×10^9 cells/g dry

soil⁻¹, while the two low-level bacterial treatments, N2 and N1, had values of 4.106 and 3.746×10^9 cells/g dry soil⁻¹, respectively. In comparison to the control treatment, which gave 2.872×10^9 cells per g dry soil, the statistical table also shows that the bi- interaction between different levels of Azospirillum bioinopolymer and the addition of varying nitrogen levels significantly increased bacterial counts in the pre-flowering stage. Treatment A2N3 achieved the highest value at 4.993×10^9 cells per g dry soil, which was not significantly different from the bi- interaction treatments A2N2 and A1N3 (4.863 and 4.817 cells per g dry soil, respectively). The bi- interaction treatment A0N0 gave the lowest value, at 1.720 cells per g dry soil, in the pre-flowering stage.

Table 3 Effect of adding the nitrogen-fixing Azospirillum bioinoculant under different nitrogen levels and the interaction between them on the number of bacteria (before flowering) 10^9 cell g dry soil⁻¹.

Nitrogen levels (N)	Azospirillum bacteria(A)			average N
	A0	A1	A2	
N0	1.720	3.277	3.620	2.872
N1	2.763	4.157	4.320	3.746
N2	2.780	4.677	4.863	4.106
N3	2.870	4.817	4.993	4.226
average A	2.533	4.232	4.449	
L.S.D (0.05)	Azospirillum	interaction		Nitrogen
	A	N*A		N
	0.1505	0.3010		0.1738

The results of the statistical analysis in Table 4 indicated that all the study factors, including the addition of Azospirillum bacteria, the addition of different levels of nitrogen, and the interaction between them, showed a significant effect on the plant height trait (cm). The highest value was in the single treatment A2, which achieved a value of 54.709 cm, compared to the two single treatments A1 and A0, whose values reached (51.981) and 48.593) cm respectively. The addition of nitrogen levels also led to a significant increase in the plant height trait of the potato crop, and the highest value was in the single treatment N3, which achieved a value of 54.902 cm, which did not differ

significantly from the single treatment N2, whose value reached 54.245 cm, compared to the treatments N1 and N0, whose values reached 52.025 and 45.872 cm respectively. As can be seen from the same table, the interaction between the addition of the Azospirillum bacterial inoculum and different nitrogen levels significantly increased the plant height of potatoes. The highest value was observed in the A2N3 interaction treatment, which achieved 58.127 cm. This is significantly different from the A1N3 and A2N2 interactions, which gave values of 57.713 and 57.420 cm, respectively. The lowest plant height value in the A0N0 control treatment was 44.317 cm.

Table 4: Effect of adding nitrogen-fixing Azospirillum bacteria under different nitrogen levels and their interaction on plant height (cm).

Nitrogen levels (N)	Azospirillum bacteria(A)			average N
	A0	A1	A2	
N0	44.317	45.590	47.710	45.872
N1	48.357	52.140	55.580	52.025
N2	50.833	54.483	57.420	54.245
N3	50.867	55.713	58.127	54.902
average A	48.593	51.981	54.709	
L.S.D 0.05	Azospirillum	interaction		Nitrogen
	A	N*A		N
	0.4068	0.8136		0,4697

Dry Weight of vegetative growth (Mg ha⁻¹)

The results of the statistical analysis in Table 5 show that the addition of Azospirillum bacteria and different levels of nitrogen significantly affected the dry weight of vegetative growth. The highest value was observed in the single treatment (A2) with the addition of 20 ml of Azospirillum bacterial inoculant, achieving a value of 4.548 Mg ha⁻¹. This was compared to the two treatments (A1 and A0) with the addition of 10 ml of Azospirillum biofertilizer, which gave values of 3.891 and 3.651 Mg ha⁻¹, respectively. Furthermore, the application of nitrogen at the full

recommended level significantly increased the dry weight of the potato shoots. The highest value was observed in the single treatment (N3), achieving a value of 5.111 Mg ha⁻¹, compared to the single treatment (N0) without fertilizer. The nitrogen treatment resulted in the lowest vegetative dry weight, at 2.622 Mg ha⁻¹. As can be seen from the same table, the bi- interaction of adding Azospirillum bacteria at three levels and applying nitrogen at one and a half times the recommended fertilizer amount significantly increased the vegetative dry weight of potato plants. The highest value was achieved with the bi-interaction treatment A2N3, reaching 5.283 Mg

ha⁻¹, which was not significantly different from the bi- interaction treatment A2N2, which reached 5.220 µg ha⁻¹. The lowest

vegetative dry weight was observed with the control treatment A0N0, at 2.233 µg ha⁻¹.

Table 5: Effect of adding nitrogen-fixing Azospirillum bacteria at different nitrogen levels and their interaction on vegetative dry weight (µg ha⁻¹).

Nitrogen levels (N)	Azospirillum bacteria(A)			average N
	A0	A1	A2	
N0	2.233	2.463	3.170	2.622
N1	3.590	3.853	4.520	3.988
N2	3.850	4.127	5.220	4.399
N3	4.930	5.120	5.283	5.111
average A	3.651	3.891	4.548	
L.S.D 0.05	Azospirillum	interaction		Nitrogen
	A	N*A		N
	0.0978	0.1956		0.1129

Chlorophyll content in leaves (Spad units)

The results of the statistical analysis in Table 6 indicate that the addition of Azospirillum

bacteria and different levels of nitrogen significantly affected the chlorophyll content of potato leaves. The addition of

Azospirillum bacteria increased the chlorophyll index, with the highest value being in the single treatment A2, which achieved a value of 51.835 spad units, compared to the single treatments A1 and A0, which had values of 48.849 and 43.951 spad units, respectively. Similarly, the addition of different levels of nitrogen significantly increased the chlorophyll content of potato leaves, with the highest value being in the single treatment N3, which achieved a value of 54.454 spad units, not significantly different from the single treatment N2, which had a value of 54.049 spad units. Compared to the two indivi-

treatments N1 and 0N, which gave values of 46.733 and 37.610 spad units, respectively, as can be seen from the same table, the interaction between the addition of the Azospirillum bacterial bioinotrope and different nitrogen levels significantly increased the chlorophyll content of potato leaves. The highest value was observed with the A2N3 interaction treatment, which achieved 58.113 spad units, compared to the A2N2 and A1N3 treatments, which gave values of 57.803 and 56.177 spad units, respectively. The control treatment, A0N0, gave the lowest chlorophyll content in potato leaves, at 35.260 spad units.

Table 6: Effect of adding nitrogen-fixing Azospirillum bacteria under different nitrogen levels and the interaction between them on chlorophyll content in leaves (spad units).

Nitrogen levels (N)	Azospirillum bacteria(A)			average N
	A0	A1	A2	
N0	35.260	37.333	40.237	37.610
N1	42.807	46.207	51.187	46.733
N2	48.663	55.680	57.803	54.049
N3	49.073	56.177	58.113	54.454
average A	43.951	48.849	51.835	

L.S.D	Azospirillum	interaction	Nitrogen
0.05	A	N*A	N
	0.1482	0.2964	0.1711

Statistical Tables 2 and 3 show that inoculation with different levels of nitrogen-fixing Azospirillum bacteria and the addition of different levels of nitrogen significantly increased Azospirillum bacterial populations in the soil at all growth stages of potatoes, both post-emergence and pre-flowering. This increase, particularly in the pre-flowering stage, may be attributed to the fact that bacterial numbers increased with plant maturity, reaching their peak at this stage due to increased plant size and root penetration, allowing them to access larger quantities of nutrients. Additionally, the number of bacterial cells increases during the pre-flowering and flowering stages compared to the vegetative growth stage, due to the secretion of sugars, organic and amino acids, vitamins, and other compounds by the roots. These results and explanations are consistent with those indicated by Al-Maamouri (2020). Furthermore, the statistical analysis tables show that adding different levels of nitrogen significantly increased bacterial populations during the various growth stages of potatoes. Especially during the pre-flowering period, indicating the role of nitrogen in activating and increasing bacterial numbers. It was found that the higher the rate of addition of Azospirilla bacteria, the higher the average activity and density of bacterial numbers in the soil. Studies indicated a direct relationship between nitrogen addition rates and bacterial numbers, especially in the pre-flowering stage, where the higher the concentration of nitrogen addition, the higher the activity and density of bacteria in the soil (Yang et al., 2009). The bacteria work to oxidize nitrogen in the soil

(Yang et al., 2009). Statistical analysis also showed an important role in increasing bacterial numbers, as the use of the biofertilizer Azospirilla resulted in a significant increase in bacterial numbers. This is due to its important role in preparing nutrients that contribute to increasing the number of bacterial cells, which increases the availability of nutrients around the root system. The results of this experiment agreed with what Kamal et al. reported. (2016). The combined effect of inoculation with nitrogen-fixing bacteria and the addition of nitrogen significantly increased bacterial colonies. This may be attributed to the synergistic role of chemical fertilizer (nitrogen) and nitrogen-fixing bacteria in providing the necessary nutrients and organic matter for microorganisms. As can be seen from Tables 4, 5, and 6, the addition of Ossosphere bacteria and several levels of nitrogen, along with their interactions, led to an increase in all vegetative indicators of potato crops, namely (plant height, chlorophyll index, and dry weight of the shoot). The added levels of bacterial inoculum improved vegetative growth characteristics as the levels of addition increased. This is attributed to the bacteria's positive role in photosynthesis. Furthermore, nitrogen is essential for many cellular metabolic products that stimulate cell division and elongation, and increase the transport of carbon metabolism products from their sites of synthesis to sites of need within the plant. This positively impacted vegetative growth indicators, such as plant height and dry weight of the shoot, resulting from the division and elongation of leaf cells. The increase in

vegetative growth indicators, including the chlorophyll index, is due to the importance of this nutrient in chlorophyll formation, despite its non-participation in chlorophyll synthesis (Saadi-AL, 2006; Ali et al., 2014). The increase in the dry weight of the potato plant's vegetative mass is attributed to the improvement of all vegetative growth characteristics, leading to an increase in dry matter and subsequently, an increase in the dry weight of the vegetative mass (Al-Jadri,2017).

This may also be attributed to the fact that these bacteria improve soil properties, including fertility, thereby enhancing the plant's growth medium, especially the roots. This leads to increased root growth and nutrient absorption from the soil solution, which positively impacts plant growth. Furthermore, the increased germination rate, occurring in a shorter time compared to control treatments, may be due to the higher temperature around the tubers caused by the soil and its retention of organic matter. The increased content of these nutrients in the leaves plays a crucial role in regulating the activity of hormones that control growth and cell division processes, stimulating vital activities, and building a healthy vegetative mass. This positively impacts the volume of manufactured nutrients necessary for building and forming plant tissues, thus increasing plant height, leaf area, and chlorophyll index. This, in turn, leads to an increase in the dry weight of the vegetative mass, which aligns with the findings of Al-Muhammadi. (2009) and (Al-Fadhli, 2011). The interaction of the study factors also plays a prominent role in increasing nutrient availability, photosynthesis, and root growth, in addition to its indirect role in increasing the efficiency of available fertilizers through the acidic environment, which reduces soil pH. Furthermore, it has a direct role in improving all vital plant activities (Nardi et al., 2004). The significant role of the twofold interaction of the study factors in regulating optimal plant growth development leads to improved soil

properties and increased fertility, which positively impacts all indicators of vegetative growth in crops, consistent with (Bhat et al., 2018) and (Al-Khafaji, 2023).

Conclusion:

This study demonstrated that Azospirillum inoculation enhanced potato plants' N uptake, N utilization, and N usage efficiency in field conditions, ultimately boosting plant growth. Therefore, using Azospirillum sp. can significantly increase potato output; however, the amount of nitrogen fertilizer and the concentrations of bacteria introduced into the soil determine this. Therefore, while higher concentrations of bacteria must be applied, the strains presented here can be used as biofertilizers to improve plant growth.

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