



(RESEARCH ARTICLE)



## The biological impact of *Azospirillum spp.* isolated from the Samawa desert on several growth indices of wheat crops

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### Abstract

Biostimulants are one of the important components of the sustainability of agriculture production. Additionally, they replace chemical fertilizers and mitigate environmental pollution and soil contamination. *Azospirillum* is a free-living bacterial, which can be used as a fertilizer and promote growth. This study is designed to evaluate the biological impact of isolated *Azospirillum spp.* from the Samawa desert on several growth indices of wheat crops. Four strains of nitrogen-fixing bacterium were isolated from the Samawa desert. The bacterial isolates were identified according to colonies' morphology, microscopical features, and biochemical characteristics as *Azospirillum spp.* Three isolates were classified as *A. lipoferum* according to their superior nitrogen fixing efficiency. Then, three isolates were designated A1, A2, and A3 and utilized to inoculate wheat groups. The inoculants served to fulfill a portion of the nitrogen requirements of the wheat crop via the execution of a field experiment. Cultivation occurred in pots, with three replications as follows: A0 without inoculant, A1, A2, and A3 with three different isolates. Meanwhile, nitrogen fertilization was N1, N2, and N3 for 0, 0.25, and 0.5 % of the fertilizer guideline. Significant effects were seen on the plant height for the *Azospirillum spp* group compared to the control group. The plant height, dry weight of shoots, leave area, and Chlorophyll content (SPAD) revealed variations between the treated and control groups. The results also showed that isolate A3 revealed better efficacy in enhancing plant height compared to other isolates. The increase was 92.53 cm in plant dry weight with a 9.60 grams total increase. Further, isolate A3 demonstrated superiority over the other strains in augmenting leaf area with a 47.47 cm<sup>2</sup> total increase and 38.34 spad chlorophyll content increment. In conclusion, this study approved the growth improvement of wheat plants with bacterium inoculum. The authors recommend future studies to investigate more features of *Azospirillum spp.*, such as biostimulants.

**Keywords:** *Azospirillum Spp*; *A. Lipoferum*; Biostimulants; Nitrogen; Samawa; Wheat Crops

### 1. Introduction

In recent years, there has been a global inclination towards employing bio fertilization technology to mitigate pollution caused by chemical fertilizers. The biofertilization approach employs live microorganisms as fertilizers and inoculants, including *Azospirillum* bacteria that associate with plant roots [1, 2].

Grassy plants serve as a bacterial inoculant, supplying and activating bacterial cells while fulfilling a portion of the nitrogen requirements for plants. Additionally, they secrete hormones such as indole acetic acid, gibberellins, and cytokinin, which facilitate the growth and development of the root system. This enhancement results in improved absorption of water and nutrients by the roots, ultimately reducing production costs and pollution [3]. *Azospirillum* is a bacterium sourced from several global regions and is regarded as the most effective non-symbiotic nitrogen-fixing

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organism. It is linked to the roots of wheat plants, exhibiting a negative result for Gram staining, possessing a bent rod morphology, and demonstrating a positive oxidase reaction [4]. Nitrogen fixed by microorganisms enhances soil nutrient levels and improves the absorption of water and minerals. These organisms enhance soil fertility and mitigate agricultural environmental issues. The wheat crop, *Triticum aestivum* L., is a crucial grain crop globally, supplying over 25% of protein and calories, serving as the primary food source for more than 40 countries and over 35% of the global population. The worldwide harvested area totals 215.49 million hectares, with a production of 670.87 million tonnes. In Iraq, it ranks as a significant winter crop, with a harvested area of 1.20 million hectares and a yield of 2.40 million tonnes in 2012 [5]. Review of literature revealed scarce publication regarding the impact of *Azospirillum* spp. on several growth indices of wheat Crops including the plant height, dry weight of shoots (plant-1 g) and Leaves area. Consequently, this study designed to isolate and investigate the microorganisms from the Samawa desert and study their impact as growth enhancers without the necessity artificial fertilizers, which have detrimental environmental effects.

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## 2. Methods and Materials

### 2.1. Isolation and identification of *Azospirillum* spp.

To isolate bacteria, four soil samples were collected from the desert and stored in polyethylene bags for preservation until needed. Ten grams of soil were combined with ninety milliliters of sterile distilled water to provide a  $10^{-1}$  dilution in test tubes containing Semisolid Nitrogen (Nfb) medium. Sterile malate medium for *Azospirillum* isolates as per [6]

Following the emergence of annular growth in Nfb medium, three consecutive transfers were conducted on the same medium and incubated at 30 °C for 48 hours. Subsequently, purification was achieved by transferring the visible growth in Nfb medium onto plates containing Congo Red medium, supplemented with Congo Red dye, and cultured utilising the planning method, with the plates incubated at 37 °C. For a duration of 72 hours, following previously described method [7]. Each colony was re-purified by re-culturing on the same medium to confirm its purity, while observing the morphological characteristics of the colonies and microscopically examining the cellular shapes. Subsequently, the isolates were preserved on agar medium, commonly referred to as Slant, for the purpose of conducting diagnostic tests.

### 2.2. Preparing the inoculant and applying it to the Peatmoss

An inoculant was formulated from the nitrogen-fixing *Azospirillum* bacteria by culturing isolate A1 in a 500 ml flask containing Nfb medium, which was sterilised using an autoclave at 121 °C and 15 psi for 20 minutes. The culture was then incubated at 28 °C for 48 hours. Subsequently, 50 ml of the inoculant was incorporated into the carrier (Peatmoss), and the mixture was thoroughly agitated to ensure uniform distribution and homogeneity of the inoculant within the Peatmoss [8, 9, 10].

### 2.3. Preparation of anvils and seeds

The anvils, each weighing 5 kg, were manufactured in a quantity of 9 anvils, and dirt was added after its softening. Phosphorus and potassium were applied in accordance with the fertilizer guidelines for wheat prior to planting, while nitrogen was administered in two increments: the first one-month post-germination and the second at 50% flowering. Milliliters of gum acacia solution at a 20% concentration were combined with water to facilitate pollen adherence to the seeds. Subsequently, the mixture of pollen and peat moss was incorporated with the wheat seeds, swirled thoroughly, and allowed to rest for one and a half hours prior to planting.

### 2.4. Field experience

The field experiment was conducted at home use nine anvils, comprising three without additives, three supplemented with pollen, and three receiving the complete fertilizer suggestion. The experiment was monitored, and irrigation and weeding activities were conducted as necessary.

**Table 1** The physical and chemical properties of the soil

Adjective	Value	unit
pH		7.6
Ec <sub>e</sub>	dSm <sup>-1</sup>	3.8
O.M	gkg <sup>-1</sup>	1.3
N available	mg kg <sup>-1</sup> soil	32.5
P available		13.5
K available		111.8
Clay	g kg <sup>-1</sup>	20.8
Salt		60.7
Sand soil texture		10.5 Sility Lom

### 3. Results and Discussion

#### 3.1. Identification of *Azospirillum* bacterial isolates

Four isolates were extracted from the desert soil in Al-Muthanna Governorate. It is classified within the genus *Azospirillum*. Through the examination of the morphological, biochemistry, and microscopic attributes of the analysed isolates, as presented in Table 3. They were distinguished by vibrioid or short spiral shapes. The isolated bacteria exhibited a vibratory motion akin to that of a snake and is notable for its capacity to fix nitrogen in a semi-solid nitrogen-free culture medium (Nfb). It forms a thin, white pellicle growth beneath the surface of the medium at a depth of 1-1.5 cm, which begins to ascend, ultimately reaching 2-3 mm below the surface after 48 hours of incubation at 30°C. This indicates its ability to fix nitrogen under conditions of limited aeration, a distinctive characteristic of this genus. The colour of the (Nfb) medium, including bromothymol blue dye, transitioned from light green at pH (6.8) to basic blue as a result of ammonia production during nitrogen fixation [11,12]. The colonies of these isolates, when grown on solid RC medium containing Congo red dye, absorb the dye and display a scarlet red colouration, differentiating them from other nitrogen-fixing bacterial taxa in terms of colour [13].

**Table 2** General and differential tests that were carried out for isolates of *Azoospirillum* bacteria

The test	isolates	
	<i>A.lipoferum</i>	<i>A.brasilense</i>
	A2 ‘ A3‘ A4	A1
Gram stain	-	-
Catalase test	+	+
Oxidase test	+	+
Reduction of nitrate (NO <sub>3</sub> ) to nitrite (NO <sub>2</sub> )	+	+
growth at PH6	+	+
Grow at pH 7.5	+	+
Denitrification	+	+
growth with 3% Nacl	+	+

Growth on RC medium in the presence of methyl red dye	-	-
Colonies formed on RC medium in the presence of Congo red dye	With smooth edges, the dye is concentrated in the center of the colony	With wavy or smooth edges, the dye spreads throughout most of the colony

**Table 3** Some microscopic and phenotypic characteristics of isolates of the genus *Azospirillum*

Adjective	Notes
growth medium	NFB
Cell pool	single
cell movement	Cork piercing movement and some undulation
shape of the cells	Slightly curved rod
Interaction with chromium dye	-

3.1.1. Plant height (cm)

Table (4) demonstrated that bacterial inoculation with *Azospirillum* isolates significantly influenced plant height in comparison to the control treatment lacking inoculation. The isolate *Azospirillum* A3 exhibited a growth rate of 97.43cm, surpassing the control treatment and outperforming all other isolates utilised in the investigation, The rationale for this may stem from the utilisation of *Azospirillum* bacteria, which fix atmospheric nitrogen and augment the nitrogen availability in the soil, thereby promoting the production and secretion of natural growth regulators in plants, which significantly contributes to their growth and height. This aligns with the findings of [14, 15] . The table indicates substantial variations in plant height when inoculated with *Azospirillum* isolates and subjected to varying nitrogen levels, with increases of 86.46, 84.71, and 82.58 cm observed at nitrogen levels N3, N2, and N1, respectively.

**Table 4** Effect of inoculation with local isolates of *Azospirillum* bacteria on plant height (cm)

A \ N	N1	N2	N3	mean
A0	66.80	72.07	74.99	71.28
A1	72.46	79.54	79.76	77.25
A2	85.17	88.66	90.53	88.12
A3	92.74	87.42	97.43	92.53
Mean	79.29	81.92	85.67	
L.S.D 0.05	A= 2.23 , N=1.93 , A*N=3.86			

3.1.2. Dry weight of shoots (plant<sup>-1</sup> g)

The results presented in Table (5) indicate significant differences in the dry weight of the shoot when inoculated with *Azospirillum*. The inoculation treatments demonstrated superior performance with *Azospirillum* isolates, recording an increase of 9.86 when inoculated with isolate A3, in contrast to the control treatment, which yielded the lowest mean dry weight of the shoot at 5.5%. This increase in dry weight can be attributed to the nitrogen-fixing capabilities of *Azospirillum* , which enhance the nitrogen nutritional status of the plant. Additionally, the role of these bacteria in promoting plant growth and secreting growth regulators contributes to the plant's enhanced absorption of macronutrients {NPK}, thereby increasing the dry weight of the shoot [16]. The interaction between inoculation with *Azospirillum* isolates and nitrogen levels The table indicates that nitrogen significantly influenced the dry weight of the shoot, with nitrogen levels N3 and N2 resulting in an increase in dry weight, showing percentage increases of 8.34 and 8.84, respectively. In a sequential comparison with the control treatment, which recorded the lowest mean of 7.95, the increase in the dry weight of the shoot can be attributed to the plant's response to the absorption of macronutrients.

This response had a significant effect, as the A3N3 level achieved the highest mean of 10.70, in contrast to the comparison treatment A0N1, which yielded the lowest mean of 5.50. Elevated potassium transfer rates to the aerial components contribute significantly to dry matter accumulation, in contrast to the control treatment plants.

**Table 5** Effect of inoculation with local isolates of *Azospirillum* and nitrogen fertilizer on the dry weight of shoots of wheat plants at flowering (plant gm<sup>-1</sup>)

<b>A \ N</b>	<b>N1</b>	<b>N2</b>	<b>N3</b>	<b>mean</b>
A0	5.5	6.06	6.53	6.03
A1	7.13	7.26	7.63	7.34
A2	8.66	8.83	9.03	8.84
A3	9.36	9.60	9.86	9.60
Mean	7.66	7.93	8.26	
L.S.D 0.05	A=0.24 , N= 0.21 , A*N= 0.42			

3.1.3. Leaves area (cm<sup>2</sup>)

The findings presented in Table (6) indicate notable differences in leaf area when subjected to inoculation with the *Azospirillum* isolate. The treatments demonstrated superior performance with the *Azospirillum* isolate, achieving an increase rate of 48.59 when inoculated with isolate A3. In contrast, the measurement treatment yielded the lowest mean for leaf area, recorded at 38.33. The third isolate, A3, surpassed the other isolates. The rise is attributed to nitrogen's crucial role in plant physiological functions, since it participates in the creation of nucleic acids and cytochrome enzymes essential for respiration and photosynthesis [17]. Nitrogen significantly influenced leaf area, with levels N3 and N2 resulting in increases of 44.52 and 45.53, respectively, compared to the control treatment.

**Table 6** Effect of inoculation with local isolates of *Azospirillum* bacteria on the leaf area of wheat plant (cm<sup>2</sup>)

<b>A \ N</b>	<b>N1</b>	<b>N2</b>	<b>N3</b>	<b>mean</b>
A0	38.33	38.58	39.39	38.76
A1	40.04	42.26	43.58	41.96
A2	45.21	45.58	46.23	45.67
A3	46.48	47.35	48.59	47.47
Mean	42.51	43.44	44.44	
L.S.D 0.05	A =0.08 N = 0.07 A*N = 0.15			

3.1.4. Chlorophyll content (SPAD)

The results presented in Table 7 indicated significant differences in chlorophyll content following inoculation with *Azospirillum* isolates. The *Azospirillum* isolate A3 demonstrated superior performance, yielding the highest mean of 39.73 spad, in contrast to the control treatment A0, which recorded the lowest mean of 24.28. Furthermore, isolate A3 outperformed all other isolates employed in the study. This increase is attributed to the application of biofertilizers, particularly *Azospirillum* bacteria in conjunction with varying levels of nitrogen fertiliser, which enhances nutrient provision, hormone synthesis, and the uptake of nutrients and water throughout the growth stages. These findings align with those reported by [17]. The results presented in Table (7) indicated significant differences in chlorophyll content when inoculating with *Azospirillum* isolates and applying varying levels of nitrogen fertilization. Specifically, nitrogen fertilisation levels N3 and N2 yielded the highest means of 33.54 and 35.39 spad, respectively, in contrast to the control treatment, which recorded the lowest mean of 32.60 spad.

**Table 7** Effect of inoculation with local isolates of *Azospirillum* bacteria on the chlorophyll content of wheat plant (SPAD)

A \ N	N1	N2	N3	mean
A0	24.28	25.45	27.65	25.79
A1	28.74	29.81	30.38	29.64
A2	32.67	33.83	35.78	34.09
A3	36.74	38.56	39.73	38.34
Mean	30.60	31.91	33.38	
L.S.D 0.05	A = 0.04 , N = 0.03 , A*N = 0.06			

#### 4. Conclusions

In conclusion, the results of the current study approved the isolation of bacteria. The Bacterial inoculate revealed the best effects on wheat plant in the studied traits. It revealed an increase in the dry weight of the vegetation, the leaf area, and that the increase in nitrogen levels affected the chlorophyll content and the plant height.

#### Compliance with ethical standards

##### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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