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Functional Characterization of the Bacterial Inoculant *Azospirillum lipoferum* and Its Effect on the Growth and Yield of Maize Crop

Sulaf Hamid Taimooze[1*]
[1]University of Al-Qadisiyah/College of Science, Email: sulaf.taimooze@qu.edu.iq

Abstract: Within a series of field experiments, an experiment was conducted in one of the fields of Al-Qadisiyah Governorate, Al-Shamiyah District, for the purpose of observing the effect of the *Azospirillum lipoferum* bacteria vaccine on the growth indicators in the yield of two varieties of yellow corn (Levantine corn and Gelatinous corn). The results of the statistical analysis showed that the bacterial inoculum treatment was significantly superior for all the studied traits that were used within the scope of the study, such as plant height, number of grains in the ear, leaf area index, number of leaves, and yield of one plant over the control treatment to which no bacterial inoculum was added. The plant height was recorded in Table (2). The plant height was recorded in Table (2) as (168.30), the number of leaves (7.01), the leaf index (1.92), the number of grains in the head (1236.22), the weight of 1000/g (25.03), and the yield of one plant/g (29.27), respectively. As for adding the bacterial inoculum, the results were plant height (185.11), the number of leaves (8.00), and the leaf area index (2.25) and the number of grains in the head (1344.15) and the weight of 1000/g (27.71) and the yield of one plant/g (35.89). When studying the effect of the interaction between the bacterial inoculum and the varieties in Table (4), the interaction between the bacterial inoculum and the gelatinous variety was significantly superior for all the studied traits except for the trait of the weight of 1000 grains, which did not constitute any significant difference between the two varieties, as it constituted (28.11) g for the Gelatinous corn, while Levantine corn corn (26.96) g.

Keywords: *Azospirillum lipoferum*, Yellow Corn, Bacterial Inoculation, Plant Growth, Maize Varieties, Leaf Area Index, Yield Components.

Research Paper

***Corresponding Author:**
Sulaf Hamid Taimooze
University of Al-Qadisiyah/College of Science

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INTRODUCTION

Yellow corn is one of the oldest cultivated plants in the world. It is characterized by its slightly sweet taste and its white-brown color. Studies have targeted this crop to exploit all available capabilities to increase productivity and obtain all its resources of oils and other things in exchange for the steady increase in population numbers and to exploit it as an oil alternative to oil crops such as sunflowers and yellow corn is why many researchers in various research centers have adopted many modern scientific technologies that can make an effective contribution to driving the wheel of economic production [1, 2].

Many biopesticides, including *Trichoderma harzianum*, have been used to improve plant growth, increase productivity, and combat fungi and other pathogens that can affect this crop. Also, pesticides made from the bacterium *Azospirillum* sp., whether used alone or in combination with other types of biobacteria, have had a significant and positive impact on plant growth in the soil. In general and specifically in sandy soil through the provision of essential nutrients of nitrogen and phosphorus on the one hand, and on the other hand, these bacterial vaccines have an important role in binding sandy soil particles and increasing their aggregates due to the absorption of these cells on the surfaces of sand particles [3-5].

Therefore, this study aimed to demonstrate the effect of *Azospirillum lipoferum* bacteria on the quality characteristics and growth yield of yellow corn crop by addressing the functional description of this bacteria.

MATERIALS AND METHODS

The experiment was conducted in Al-Qadisiyah Governorate / on one of the farms affiliated with Al-Shamiyah District for the autumn season 2022-2023 in soil whose characteristics are shown in Table (1) with the aim of determining the effect of bacterial inoculum treatments on growth indicators in yellow corn and its productivity as follows:

1. **Collecting Soil and Root Samples for the Purpose of Bacterial Isolation:** Soil samples were collected from the rhizosphere region. The roots of millet plants and rhizosphere soil were carefully removed from a field located in Al-Shamiya/Al-Qadisiyah District. The samples were placed in clean plastic bags sterilized with alcohol and kept in the refrigerator until used for isolation purposes. Also, samples were taken from the surface layer (0-30 cm) of soil far from the root to conduct chemical, physical and biological tests shown in (Table 1).
2. **Isolation and Purification of *Azospirillum* Spp.:** Millet roots were taken after removing the rhizosphere soil. Some roots were cut into small pieces 5-8 mm long and transferred using sterile forceps into test tubes containing sterile nitrogen-free malate (NFB) semi-solid medium to obtain *Azospirillum* isolates according to [6, 7]. When the ring growth appeared in the nitrogen-free medium, three successive transfers were made to the same medium and incubated at 30°C for 48 hours. Then, the apparent growth was transferred in Nfb medium using the carrier to the dishes containing Rogo Congo (R.C) medium to which Convo red dye was added. It was planted by the striping method and the dishes were incubated at 37°C for 72 hours. According to method [19], after the appearance of small crimson colonies on the medium, each colony was purified again by re-drawing it on the same medium to ensure its purity and observing the shapes of the colonies belonging to the isolate. The shapes of their cells were examined microscopically, and then the isolates were preserved on the slant nutrient agar medium for the purpose of conducting diagnostic tests on them.
3. **Bacterial Diagnostic Methods:** The biochemical, morphological, and microscopic characteristics of nitrogen-fixing *Azospirillum* bacteria isolates were studied, taking into account the characteristics associated with the identification of the genus *Azospirillum* and its related species [8, 9].

Table No. 1: Some chemical, physical and biological properties of the soils used in the study

Al-Shamiyah District / Al-Qadisiyah Governorate	Characteristics	
185	g.kg^{-1}	Clay
519		Silt
302		Sand
Mixture	Soil texture	
7.2	pH	
3.11	ds.m^{-1} E.C	
7.2	Organic matter g.kg^{-1}	
37.1	mg.kg^{-1}	Nitrogen availability
7.92		Phosphorus availability
$10^6 \times 2.9$	Dry soil cfu. gm^{-1}	Total bacteria
$10^4 \times 84$		*Azospirillum* bacteria

*Results represent an average of three replicates.

1. Seed treatment with *Azospirillum* sp. L. isolates. The bioinoculum bacteria were obtained from the soil planted with millet after being isolated on Nutrient agar. The isolate was evaluated by conducting biochemical tests and counting the number of colonies in a bacterial counting device. The isolate's efficiency was confirmed [10].

RESULTS AND DISCUSSION

1. Isolation and identification of *Azospirillum sp*. bacteria: [4]. *Azospirillum sp*. bacteria are isolated from an area planted with millet in the city of Diwaniyah. *Azospirillum sp*. bacteria were isolated from within the roots of the millet plant.

By studying the cultural, microscopic and biochemical characteristics of the studied isolates shown in Table (3), and depending on the special criteria for differentiating between species according to researchers [11-14], it appeared that all isolates belong to the species (*Azospirillum* spp)) as shown in (Table 2).

Table 2: Some microscopic and biochemical characteristics for diagnosing isolates of the genus (*Azospirillum* spp)

Biochemical properties					Microscopic features			Morphological characteristics of colonies			Isolation number
pH 7.5)(	PH (6)	NaCl%3	Catalase	Oxidase	Pigment جرام	motility	shape	Color	shape	density	
++	++	+	+	++	G^{-ve}	Spiral	Bacilli	red	Dry	+++	A1
+	+	+	++	++	G^{-ve}	Spiral	Bacilli	red	Dry	+	A2
++	+	+	++	++	G^{-ve}	spiral	Bacilli	red	Dry	+	A3
+	+	+	+	+	G^{-ve}	spiral	Bacilli	Pink	shiny	++	A4

(-) No growth (+) Poor growth (++) Average growth (+++) Dense growth

1. Effect of Bacterial Inoculum on Growth Indicators of Yellow Corn

The results of Table (3), when analyzed statistically, indicated that the bacterial inoculum treatment significantly outperformed the control treatment, showing a clear effect on plant traits such as height, number of leaves, and leaf area (185.11 and 168.30 cm). Meanwhile, the average number of leaves was 8.00 and 7.01 leaves per plant, while the average leaf area was 2.25 and 1.92 cm for the bacterial inoculum and control treatments, respectively.

The reason for the emergence of these results may be due to the presence of growth regulators secreted by these bacteria around the seeds, which have a positive effect on the seeds and help protect the seedlings, whether before or after emergence, from fungal infection, thus increasing plant growth. This study is consistent with what was found by [15, 16]. Because these bacteria produce a growth regulator known as gibberellins, they play a major role in increasing growth indicators through stimulating processes within plant cells.

2. Yield and Components of (Yellow Corn)

The results of Table (3) showed that the bacterial inoculum treatment was superior to the control treatment (which was free of bacteria treatment). The results showed a significant effect on the characteristics of the yield and its components (number of grains per head, weight of 1000 grains, and grain yield). The bacterial inoculum treatment gave an average number of grains per head of (1344.12) grains, while the average weight of 1000 grains was 29.27 grams, and the yield per plant was 89.35 grams.

When analyzing the results, it was found that the significant increase in growth characteristics, especially leaf area, blocked a large amount of light and thus increased the process of photosynthesis, which led to an increase in the plant's stock of materials. This process led to the creation of a better opportunity to produce a large amount of flowers, as these stored materials had an impact on the increase in the weight of the grain.

As for the grain yield of a single plant, it increased significantly in the bacterial vaccine treatment as a result of the increase in the components of the yield (number of grains per head and weight of 1000 grains). These obtained results were consistent with many studies in which the effect of the bacterial vaccine was mentioned, as adding the bacterial vaccine to the plant has the ability to increase the yield as a result of the materials secreted by the soil [17-20].

Table 3: Effectiveness of bacterial vaccine on some growth traits, yield and its components

Yield per plant/g	Weight 1000/g	Number of grains per head	Leaf area index	Number of leaves /Leaf	Plant height (cm)	treatment
29.27 B	25.03 B	1236.22 b	1.92 b	7.01 b	168.30 b	without treatment (control)
35.89 q	27.71 q	1344.15 q	2.25 q	8.00 q	185.11 q	Treating seeds with bacterial inoculum

*Duncan's test of differences, which indicates significant differences at a 5% probability level (different letters).

3. The Effectiveness of the Interaction between the Bacterial Vaccine and the Varieties

From table (3), the results indicated that there is a significant increase in the growth characteristics when the seeds of the Levantine corn and Gelatinouscorn varieties are treated with the bacterial inoculation over the control treatment and it was observed that there is an overlap between the Levantine corn variety and the treatment with the bacterial inoculation on the averages for the height of the plants (172.24) and the number of leaves (8.77 leaves/plant) as well as the index of leaf area , The reason for this may be due to the fact that these bacteria produce different types of growth regulators such as ethylene, which play a major role in accelerating growth and the ability to stimulate ready-made resistance in I.S.R. plants against pathogens. And this study agreed with [21-23], who pointed out that the increase in the growth characteristics of field crops increases when they are treated with bacterial vaccines as well as with the addition of some substances such as calcium 3CaCo.

4. The Yield and Its Components When Treated with the Bacterial Inoculum

Analyzed the results shown in table (4) which statistically led to the existence of significant differences between the bacterial inoculation treatment when interfering with the yellow corn varieties and its effect on the number of grains in the ear and the yield per plant compared to the control treatment and it is clear that the interference process was reflected on the 1000 grain weight of the grain. And in general, the bacterial inoculant treatment and the Levantine corn variety had dominance over all other interferences, as the average number of grains per head was (1335-14) a grain/head and the average yield per plant was [36-59], grams, and this may be due to the superiority of the interference process and its effect on growth characteristics. It may also be explained that this bacteria possesses a set of effective and competitive mechanisms where it produces Sidrophorces compounds which form a compound with iron and therefore leads to an increase in yield and its components. This was in agreement with [24-27].

But the absence of significant differences in the characteristics of (1000) grains may be due to the process of competition for the results of photosynthesis between the seeds on the corn cob, which reflected negatively on the weight of the (1000) grains.

Table 4: The interaction between bacterial inoculum and two varieties of corn and its effect on growth traits, yield and its components

Yield per plant/g	Weight 1000/g	Number of grains per head	Leaf area index	Number of leaves /Leaf	Plant height {cm)	Corn varieties	treatment
29.31	26.28	1195.13 c	1.95 c	7.10 c	151.12 cd	Levantine corn	المقارنة
34.23	27.12	1299.11 b	2.45 b	7.81 b	161.11 b	Gelatinous corn	
31.99	26.96	1222.11 c	2.24 b	7.91 b	159.20 bc	Levantine corn	اللقاح البكتيري
36.59	28.11	1335.14 a	3.32 a	8.77 a	172.24 a	Gelatinous corn	

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