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Effect of Azotobacter viellandii biofilm and levels of organic fertilizer on the growth and yield of barley *Hordeum vulgare* L.

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Abstract

A field experiment was conducted in Al-Muthanna Governorate within latitude(31.368475N) and longitude (45.259249E) during the winter season 2023_2024 to know the effect of the biofilm of Azotobacter viellandii bacteria and levels of organic fertilizer made from the Nile flower plant on the growth and yield of barley. The experiment included two factors: the first factor was two levels of biofilm (B0) without inoculum and (B1)) the biofilm of Azotobacter viellandii bacteria. The second factor included 3 levels of Nile flower compost: the first level (C0) without addition, the second level (C1) 1.5% by volume of organic fertilizer, and the third level 2% by volume of organic fertilizer. Laboratory experiments showed that ten isolates of Azotobacter bacteria were obtained and two isolates were identified as Azotobacter viellandii. The results of adding biofilm showed that treatment B1 was superior in plant height, but did not excel in the biological yield, 1000-grain weight, and dry weight. While the results of adding organic fertilizer to the Nile flower plant showed that treatment C2 was superior in the trait (plant height, 1000-grain weight), and treatment C1 was superior in the biological yield. As for the interaction between the two factors, there were no significant differences.

Keywords: Biofilm, Azotobacter viellandii, barley

Introduction

Iraq is located in dry and semi-dry areas, and as a result of drought conditions and lack of organic matter, there is a deficiency in the supply of important nutrients for plant growth. Another problem is the excessive use of chemical fertilizers, which has negative effects, including increasing the cost to the farmer, in addition to the fact that the use of nitrogen fertilizers causes nitrates to leak into groundwater. Therefore, there is a need to use modern strategies and techniques aimed at increasing crop production and improving their quality through the use of organic fertilizers and biofertilizers, which are clean agricultural technologies to reduce sources of pollution and are a natural resource. They are also a suitable alternative or complement to mineral fertilizers (2,1).

Biofertilizers are modern technologies in the agricultural field and contain one or more types of microscopic organisms that are added to seeds, soil, plants, or both. They are used to improve the physical, chemical, and biological properties of the soil and maintain the balance of nutrients in agricultural lands and convert them into ready-to-plant forms and provide them to the plant during its life cycle. Thus, part of the mineral fertilizers can be dispensed with, thus increasing production and reducing agricultural production costs. (3)

Among the biofertilizers used are Azotobacter bacteria vaccines that fix nitrogen freely in addition to their secretion of some hormones, enzymes, and growth regulators (4). Currently, there is a tendency to use organisms that are resistant to soil conditions such as drought and lack of organic matter, as they are characterized by their resistance to difficult conditions and their survival in the soil for a longer period, as organisms that produce biofilms have been used and have the ability to provide protection from external stress and reduce microbial competition, in addition to the positive effects on plant growth, yield and crop quality (5)

Organic fertilizers have an important and positive role and are a source of major and minor elements necessary for plant growth. The nutrient content of organic fertilizers varies depending on their source, and the value of these fertilizers is not only estimated by their nutrient content, but also by their readiness, in addition to their improvement of the various soil properties (6).

One of the plants that has spread at the present time is the Nile flower plant, Echhornia crassipes, which is a perennial aquatic plant that floats on the surface of the water. The Crassipes species belonging to the genus Echhornia is the widespread species in Iraq, and it causes great economic damage when it spreads in dam water and water reservoirs, and it has become a problem that threatens the aquatic environment, so specialists have resorted to stopping the spread of this plant. The Nile flower plant has a positive and beneficial side as it can be a source of many nutrients and can be used as an organic fertilizer (7). Barley (Hordeum spp) is an ancient field crop as it was known before wheat. Barley is grown all over the world because it can withstand harsh environmental conditions as well as because of its low nutritional

requirements. It still represents an alternative to wheat in food. The irrigated area planted with barley has increased in the world, while the areas planted with rain have decreased due to fluctuating rainfall (8).

Materials and method

Laboratory experiments

Ten soil samples were collected from the rhizosphere region from different locations in the governorates (Muthanna, Diwaniyah, Najaf, Hillah) for the purpose of isolating and diagnosing Azotobacter bacteria. The samples were stored in sterile polyethylene bags until use.

1- Isolation and purification of Azotobacter bacteria

Soil dilutions were prepared by adding 10 grams of soil to 90 ml of sterile water, then a series of dilutions were carried out until 10-7 according to (9), then 1 ml of the dilutions (10-5 and 10-7) was taken to inoculate tubes containing 9 ml of the liquid medium free of nitrogen (Sucrose mineral-salt broth) sterilized in an autoclave at a temperature of 121 °C for 20 minutes and a pressure of 15 pounds / inch, then the test tubes were incubated in the incubator for a week at a temperature of 30 °C and the appearance of ring growth or the presence of turbidity is a positive sign of Azotobacter growth, then 1 ml was taken from the tubes that gave an indicator of Azotobacter growth and spread in a plate containing the solid medium free of nitrogen Sucrose mineral-salt agar with 2% agar added to it and incubated for a week at a temperature of 30 them, the planning was repeated three times to obtain pure isolates of Azotobacter bacteria, after which the isolates were saved for the purpose of conducting diagnostic tests on them.

2- Experiment of the ability of bacteria to produce and assemble biofilm

Azotobacter bacteria were grown in tubes containing Tryptic soya broth medium and the same isolates were also grown in tubes containing nutrient broth, and after incubation the thickness of the biofilm was measured after pouring the contents of the tubes and adding safranin dye with a concentration of 1% and leaving it for a minute and then pouring the dye, as the appearance of the red ring is an indication of the formation of the biofilm (10). After confirming the ability of bacteria to form biofilm, the membrane of the identified bacteria was collected by growing it on Tryptic soya broth medium and collecting the formed membrane in Scrow tubes containing Phosphate Buffer Saline (PBS) solution prepared by adding 8 g of Nacl, 0.2 g of Kcl, 0.2 g of KH2PO4, and 1.5 g of NaH2PO4 and pH 7.2. Then the biofilm was placed in the oven at 50°C for three days and was kept in the refrigerator until use (11). Field experiment

The experiment was carried out using a complete randomized block design (RCBD) after perpendicular tillage and leveling of the field soil, with three replicates, each replicate having 6 experimental units. The area of the experimental unit is 4 m2, the distance between one sector and another is

1.5 m, while the distance between the experimental units is 1 m, and the number of experimental units is 18 experimental units. Compost was added to the experimental units as a volume ratio, then the seeds inoculated with the biofilm of the identified bacteria were planted in the form of lines, the distance between one line and another is 10 cm and the seed ratio is 100 kg ha-1 12)), and the land was planted on 11/20/2023.

Nitrogen was added at half the fertilizer recommendation and part in two batches, the first (25%) was added two weeks after germination and the second half (25%) at the branching stage according to (13), and the recommendation of 120 kg N ha-1 was used (14).

Experimental factors: The experiment included two factors as shown
The first factor: fertilization with the biofilm of Azotobacter viellandii bacteria and included two levels
B0: without inoculation.
B1: Biofilm of Azotobacter viellandii bacteria.
The second factor: included three levels of organic Nile flower fertilizer.
C0: without addition.
C2: 1.5% concentration by volume of organic fertilizer for Nile flower.
C2: 2% concentration by volume of organic fertilizer for Nile flower.
The studied characteristics

1- Plant height (cm)
The plant height was measured at the harvest stage using a measuring tape from the soil surface to the end of the spike without the ear.

2- Dry weight of the plant (g)

Ten plants were taken from each experimental unit and dried for 72 hours at a temperature of 65°C until the weight was fixed and their dry weight was calculated.

3- Bio-yield (megagram.ha-1)

The two middle lines were harvested and the plants were weighed as a whole (grains with straw), and the weight was converted from grams.m2 to megagram.ha^{-1}

4- Weight of 1000 grains (g)

A thousand grains were randomly collected from the grain yield of each experimental unit and weighed with a sensitive balance after being counted manually.

Soil analyses before planting

Soil samples were taken from the field and represented all treatments and replicates at a depth of (0-30) cm and randomly, then air-dried and ground, then mixed well to obtain a composite sample and passed through a sieve with a diameter of 2 mm holes, then the necessary analyses were conducted before planting.

Table (1) Soil analysis before planting

Unit	Value	Attribute		S
dsm^{-1}	5.5		Ec_e	1
--------	7.9		PH	2
%	0.5		Organic matter	3
mg kg-1	19.3		Nitrogen	4
	13		Phosphorus	5
	168.97		Potassium	6
mmol/L-1	Nill		Carbonates	10
	12.8		Bicarbonates	11
kg of soil 1	195.7	Sand	Soil Separators	17
	239.1	Silt		
	565.2	Clay		
	Clay		texture	18
	$5.6*10^6$		Total Bacterial Counts	19
	$1.1*10^2$		Azotobacter numbers	20

Results and Discussion

Isolation and Identification of Azotobacter

After conducting microscopic, morphological and biochemical culture tests for the studied isolates, ten bacterial isolates belonging to the genus Azotobacter were obtained. The cells of this genus were characterized by having multiple shapes, including rod-shaped and spherical, single or double or in the form of quadruple clusters, motile and positive for Gram stain, and were characterized by forming a white membrane

on the surface of the liquid culture medium, while the colonies growing on the solid medium (Sucrose Mineral Salts) were characterized by being opaque, elevated, convex, sticky, shiny, and of medium to large sizes. Through these characteristics and based on what was mentioned in the studies, these characteristics are consistent with the cultural and microscopic characteristics of the genus Azotobacter (15, 16, 9, 17).

Isolates of the species belonging to the genus Azotobacter were diagnosed through some biochemical tests, especially the ability of bacteria to grow in Burke's medium, which is a distinctive characteristic of the species (A.vinelandii) and the ability of the isolates to consume carbon sources, which are shown in Table (3). Through studying some phenotypic characteristics, especially the characteristic of pigment formation in solid cultures, the species (A.chroococcum) was distinguished by its secretion of brown pigment, and the species (A.vinelandii) was distinguished by its secretion of green pigment, while the species (A.beijerinkii) was distinguished by its secretion of yellow pigment.

Table (2) Results of some biochemical and differential tests to distinguish the species belonging to the genus Azotobacter spp

AF10	AF9	AF8	AF7	AF6	AF5	AF4	AF3	AF2	AF1	Isolation number / Biochemical tests		.No
+	+	+	+	+	+	+	+	+	+	Gram stain		1
+	+	+	+	+	+	+	+	+	+	Oxidase		2
+	+	+	+	+	+	-	+	+	+	Catalase		3
+	+	+	+	+	+	+	+	+	+	Methyl Red		4
+	+	+	-	+	+	+	+	+	+	Starch hydrolysis test		5
+	+	+	+	+	+	+	+	+	+	Gelatin hydrolysis test		6
+	-	-	-	-	+	-	-	-	+	Voges proskauer test		7
+	+	+	+	+	-	-	+	+	-	Urease test		8
+	+	-	+	-	-	-	-	-	-	Beark test		9
+	+	+	+	+	+	+	+	-	+	Lactose	Use of carbon sources	10
+	-	+	+	+	+	+	+	+	+	Rhaminose		

++	+	+	++	++	++	++	+++	+	-	Maltose		
+	+	+	+	+	+	+	+	+	+	3%	Nacl	11
+	+	-	-	-	-	-	-	-	-	4%		

Negative - Positive +

Field experiment

1- Plant height (cm. plant)

The results of Table (3) indicated the effect of the biofilm of Azotobacter viellandii bacteria and the levels of organic fertilizer on the plant height trait, indicating significant differences between the biofilm treatments, as treatment B1 outperformed treatment B0, as the average plant height reached 92.83 cm, with an increase rate of 7% compared to the comparison treatment, which recorded 86.70 cm. This may be attributed to the role of the Azotobacter viellandii bacteria, which produce biofilms, in increasing the fixation of atmospheric nitrogen, in addition to its role in providing major and minor nutrients, and then it works to produce growth regulators, auxins, and IAA, which are necessary for cell elongation, and the role of biofilms in enhancing the absorption of nutrients, and then reflecting them positively on plant traits and plant height, which is consistent with (19,18).

The results of Table (3) showed significant differences between the organic fertilizer treatments, as treatment C2 outperformed the two treatments (C0), and the average plant height reached 97.50 cm, with an increase of 21.49% compared to the comparison treatment, which recorded 80.25 cm. This may be due to the organic fertilizer containing the necessary elements for plant growth, including nitrogen in sufficient quantities to stimulate vital processes and increase vegetative growth, in addition to the role of phosphorus in increasing the growth and development of cell division, which is then positively reflected in increasing plant height, which is consistent with (20,21).

The results of Table (3) also showed that there were no significant differences in the interaction between the biofilm treatments and organic fertilizer levels in the plant height trait.

Table (3) Effect of the biofilm of Azotobacter viellandii and organic fertilizer levels on plant height (cm. plant)

Mean	C2	C1	C0	Organic fertilizer / Biofilm

86.70	97.20	88.30	74.60	B0
92.83	97.80	94.80	85.90	B1
	97.50	91.55	80.25	Mean
BC		**C**	**B**	**L.S.D.(0.05)**
N.S		**7.35**	**7.35**	

2-5-3 Dry weight (g. plant)

The results of Table (4) Effect of the biofilm of Azotobacter viellandii and organic fertilizer on the dry weight (g. plant) indicated that there were no significant differences between the biofilm treatments and organic fertilizer levels and the interaction between them in the dry weight of the plant

Table (4) Effect of the biofilm of Azotobacter viellandii and organic fertilizer levels on the dry weight (g. plant)

Mean	C2	C1	C0	Organic fertilizer / Biofilm
7.42	7.73	8.92	5.63	B0
7.43	7.95	7.28	7.08	B1
	7.84	8.10	6.35	Mean
BC		**C**	**B**	**L.S.D.(0.05)**
N.S		**N.S**	**N.S**	

3- Bio-yield (megagram. ha-1)

The results of Table (5) indicated the effect of the biofilm of Azotobacter viellandii and the levels of organic fertilizer on the bio-yield characteristic, indicating that there were no significant differences between the bio-film treatments.

The results of Table (5) showed that there were significant differences between the organic fertilizer treatments, as treatment C1 outperformed treatment (C0), and the average bio-yield of the plant reached 12.09 megagram. ha-1, with an increase rate of 26.86% compared to the comparison

treatment, which recorded 9.53 megagram. Hectare-1 This may be attributed to the role of organic fertilizer and its high content of nutrients that increase microbial activity, which secretes organic acids and growth-stimulating hormones such as auxins and gibberellins, which stimulate plant growth and increase the availability of nutrients and their role in the physiological processes that take place inside the plant and its reflection on the plant's absorption of nutrients in the quantities it needs for its growth and thus improve the plant's characteristics, including the bio-product, or the reason may be due to the high cation exchange capacity of Nile flower compost and thus holding the nutrients and preserving them from loss throughout the plant's growth period as a result of the organic matter containing carboxyl, phenol and hydroxyl groups, which have the ability to absorb positive ions, and this is consistent with (22).

The results of Table (5) also showed that there were no significant differences in the interaction between the biofilm treatments and organic fertilizer levels in the bio-product characteristic.

Table (5) Effect of biofilm of Azotobacter viellandii and and organic fertilizer levels on the bio-yield (megagram/ha)

Mean	C2	C1	C0	Organic fertilizer / Biofilm
10.98	12.10	12.33	8.52	B0
11.32	11.58	11.85	10.54	B1
	11.84	12.09	9.53	Mean
BC		**C**	**B**	**L.S.D.(0.05)**
N.S		**1.46**	**N.S**	

4- Weight of 1000 grains (g)

The results of Table (6) of the effect of the biofilm of the bacteria Azotobacter viellandii and the levels of organic fertilizer on the characteristic of 1000 grains (g) indicated that there were no significant differences between the biofilm treatments.

The results of Table (6) showed significant differences between the organic fertilizer

treatments, as treatment C2 outperformed (C1, C0), as the average weight of 1000 grains reached 51.04 g, with an increase of 20.15% compared to the comparison treatment, which recorded 42.48 g. This may be attributed to the role of organic fertilizer in providing the necessary nutrients for plant growth and their role in the physiological processes within the plant, increasing the efficiency of photosynthesis processes and transferring its products from manufacturing sites to storage sites in grains, increasing energy production and forming energy compounds ATP, building starch, sugars, lipids, proteins and other compounds that are stored in grains, which then leads to an increase in their weight. This is consistent with (20,21). The results of Table (6) also showed that there were no significant differences in the interaction between the biofilm treatments and organic fertilizer levels in the weight of 1000 grains (g)

Table (6) Effect of biofilm of Azotobacter viellandii and organic fertilizer levels on the weight of 1000 grains (g)

Mean	C2	C1	C0	Organic fertilizer / Biofilm
45.25	48.81	46.33	40.63	B0
47.74	53.28	49.40	44.33	B1
	51.04	47.86	42.48	Mean
BC		**C**	**B**	**L.S.D.(0.05)**
N.S		**2.54**	**N.S**	

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