Evaluation of manufactured bio-fertilizers in growth and yield of oats

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Abstract

A field experiment was conducted during the 2025-2026 winter season at the Field Crops Department, College of Agriculture, Al-Qasim Green University, in Babylon Governorate. The aim was to evaluate the effects of manufactured bacterial, fungal, and algal bio-fertilizers on some growth and yield traits of oats, and comparison with imported bio-fertilizers To compare fertilizers manufactured at low cost with expensive imported fertilizers. A randomized complete block design (RCBD) was used. The plots included the Shifa variety and the following fertilizer treatments: A0: control (no bio-fertilizer), A1: imported bacterial fertilizer, A2: imported fungal fertilizer, A3: imported algal fertilizer, A4: manufactured bacterial fertilizer, A5: manufactured fungal fertilizer, and A6: manufactured algal fertilizer. The fertilizers were manufactured in the graduate studies laboratory of the Field Crops Department, College of Agriculture, Al-Qasim Green University, and injected into the soil after two weeks from the planting. The results of the experiment showed that treatment A6 excelled in a number of tillers. m^{-2}, chlorophyll content, number of panicles. m^{-2}, number of grains per panicle, total grain yield, and grain protein content. This treatment was not differ significantly from A5 treatment. Manufactured bio-fertilizers did not differ in their effect on plants; rather, fungal and algal fertilizers were superior despite their lower cost.

Keywords: Oats, fungal bio-fertilizer, algal bio-fertilizer, bacterial bio-fertilizer

1- Introduction

Oats rank seventh among cereal crops in terms of importance and productivity. Global oat production exceeds 22 million tons annually, cultivated on an estimated 9 million hectares. Russia leads the world in oat

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cultivation [1]. Recently, sustainable development concepts have emerged that it rely on the use of natural resources for increasing of a production with the protecting of environment from pollution through the use of the fertilizers that enhance agricultural output[2]. Bio-fertilizers are modern agricultural inputs that utilize on beneficial microorganisms such as bacteria, fungi, and algae to improve soil fertility and promote of the plant growth. These fertilizers characterize from their ability to fix of the atmospheric nitrogen, decompose of the insoluble phosphates, and secrete of the natural growth stimulants, So this is making them an adjunct to the chemical fertilizers. Bio-fertilizers are fertilizers that include microbial inoculants of bacteria, fungal and algal, which have the ability to increase the availability of some essential nutrients for plant growth, such as nitrogen, phosphorus, potassium, sulfur, and iron [3]

Trichoderma asperellum is a widespread fungus found in various agricultural environments, it forms an part of the agricultural ecosystem. So it plays a vital role in supplying plants with micro- and macro-mineral nutrients for increasing of growth and productivity [4]. Furthermore, its effective in enhancing of the production of plant growth hormones and volatile compounds. Trichoderma has been found to produce plant growth hormones and volatile compounds that positively influence in the plant growth. These include hormones such as indole-3-acetic acid (IAA) and gibberellic acid, which are essential for the elongation of plant roots and shoots.

Adding algae is an important method for improving of the soil properties and plant growth. In some countries, algae are used as food and an energy source due to their nutrients, carbohydrates, proteins, and fats, which improve of the soil's natural properties. They also promote of the tillering. Plant hormones present in algae (such as cytokinins) help in increase of the number of lateral shoots (tillers), which directly leads to an increase in the number of panicles and an increase in the total yield. [5].

Finally, developing and manufacturing fertilizers locally supports efforts to achieve self-sufficiency in agricultural inputs, reducing dependence on foreign markets and global price fluctuations, and enhancing the quality of crops produced, making them safer and more competitive in the market. So this study was aimed to compare laboratory-manufactured fertilizers with traditional fertilizers, with the aim of developing sustainable and effective agricultural alternatives that support food security and reduce the negative environmental impact in local agricultural environments such as Babylon Governorate, in addition to the possibility of manufacturing these fertilizers in Iraq.

2- Materials and Methods

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A field experiment was conducted during the 2025-2026 winter season at the Field Crops Department, College of Agriculture, Al-Qasim Green University, in Babylon Governorate. The aim was to study and evaluate the effects of manufactured bacterial, fungal, and algal bio-fertilizers on growth and yield of oats. A randomized complete block design (RCBD) was used. The aim of the study was to propagate bacteria, fungi, and algae Manufactured (used as bio-fertilizers added to the soil for plant growth) in culture media prepared from low-cost and readily available materials. The source of the fungi, bacteria, and algae is imported bio-fertilizers. The harizanum Trichoderma harzianum fungal, Bacillus subtilis bacteria and Chlorella vulgaris algae were used.

Culture media used in the study:

Potato culture medium for fungal growth

The culture medium was prepared by mashing potatoes and drying them in a convection oven.

The drying process was complete by exposure to dry air in the shade.

The dried potatoes were ground into potato powder.

39 g of potato powder was dissolved in 1 liter of water, according to the recommendations for industrial media.

The liquid medium was distributed into 250 ml glass flasks, and their openings were sealed with cotton plugs.

The culture media were sterilized in an autoclave at 121°C and 1 bar pressure for 20 minutes.

After sterilization, the antibiotic chloramphenicol was added to the culture medium at a rate of 100 mg/L.

The media were cooled and stored in a refrigerator at 4°C until use.

Bran infusion medium (natural homemade medium) for bacteria

Ingredients

100 g of the ground wheat bran (carbohydrate source).

1 liter water (preferably unchlorinated or boiled and cooled).

10 g of the sugar (quick energy source).

1 teaspoon baker's yeast (optional, as a nitrogen source).

Steps

Soak the ground wheat bran in water for 24 hours.

Drain the liquid and add sugar and yeast.

Cover the container with a breathable cloth and leave it in a warm place (25-30°C) for 2-3 days until a white foam appears on the surface.

This "soaked" liquid is used as a liquid medium for bacterial growth.

Creating of the Conditions for Algae Growth

1. Prepare a 5-liter glass tank.
2. Sterilize the tank with 70% ethanol.
3. Add sterile, filtered water free of impurities.
4. Add nitrogen-containing nutrients to stimulate the algae life cycle.
5. Connect the internal lighting and oxygenation system to the tank.

Steps

Algae samples were obtained by scraping the growing layer from the glass walls of the aquarium.

Samples were transferred to a clean

glass container filled with water from the same aquarium.

A preliminary filtering of the suspended solids was performed using a fine porous gauze pad to remove larger impurities.

The algae were purified by allowing the suspended solids to settle, skimming off the supernatant, and adding distilled water.

This washing process was repeated several times to ensure the removal of dissolved salts and impurities.

The purified sample was stored in sterile glass bottles for later use in subsequent research experiments.

Activation and Proliferation of the Bacteria

1. Bacteria were inoculated into nutrient agar plates using a sterile loop and incubated at 37°C for 24 hours.

2. A young colony was transferred to a glass flask containing the pre-prepared liquid nutrient broth.

3. The flask was placed on a heat magnetic stirrer to ensure homogeneity and good aeration for 24–48 hours at 37°C until a clear turbidity, it was represented the bacterial suspension.

4. The suspension was stored at 4°C until use.

Activation and Proliferation of the Fungi

1. The fungus was inoculated onto pre-prepared potato dextrose agar (PDA) agar plates.

2. The plates were incubated at 25 ± 2 °C for 5–7 days until growth was complete and characteristic green spores appeared.

3. To prepare the fungal suspension for use in treatments, the spores were skimmed from the surface of the solid medium and transferred to the liquid medium.

4. The suspension was filtered through several layers of sterile gauze to obtain a clear spore suspension free of mycelial remains.

5. The suspension was stored at 4 °C until use.

Bacterial counting

Use a bacterial counter to count the number of bacteria in the colonies. The bacterial count was at the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad.

Device Components

1.Illuminated base: Provides side lighting (often LED) to improve colony contrast.

2.Probe: Used to touch the colony, automatically counting.

3.Digital display: Shows the cumulative count (usually from 0 to 999).

4.Magnifying glass: To facilitate viewing small colonies.

Operation

1 ml from liquid bacterial media was used in petri dish. A Petri dish is placed on the illuminated base, and the technician touches each colony with the probe. The device records the count, saving time and effort compared to traditional manual counting.

Measuring of the Colony Diameter (Solid Medium) of Fungi

1. fungus was cultured in the center of a Petri dish containing a solid nutrient

medium.

2. The fungus was inoculated at a suitable temperature (25°C).

3. The diameter of the fungal colony was measured by using a smart distance application at regular intervals (24 hours).

4. This measurement was used to assess the linear growth rate, and a growth curve is plotted based on the colony diameter.

Dry Weight Measurement of Algae

1. Algae suspension treatments were numbered and oven-dried (105°C) for 1 hour, then cooled in a desiccator and taken of the initial weight (W1).

2. 10 ml of suspension was filtered through the filter by using a vacuum pump and a membrane filter (0.2 - 1.2 micrometers).

3. The filtered part of the algae was washed with distilled water to remove dissolved salts and residual nutrients.

4. Then the filtered part of the algae was placed in a drying oven at 75°C for 1–24 hours (until weight stabilizes).

5. The filtered part of the algae is cooled again in the desiccator and weighed of final weight (W2).

6. The dry weight of algae ($g.L^{-1}$) is calculated using the equation:

W_d = dry weight, W2= final weight, W1= Initial weight and V= suspension size.

The plots included the Shifa variety and the following fertilizer treatments: A0: control treatment without bio-fertilizer, A1: imported bacterial fertilizer, A2: imported fungal fertilizer, A3: imported algal fertilizer, A4: manufactured bacterial fertilizer, A5: manufactured fungal fertilizer, and A6: manufactured algal fertilizer. The fertilizers were manufactured in the graduate studies laboratory of the Field Crops Department, College of Agriculture, Al-Qasim Green University, and the bio-fertilizers were injected into the soil after two weeks from the planting. The field soil was plowed, smoothed, and leveled, then divided into three replicates, each containing three experimental units of 4 m² (2m x 2m). Phosphate fertilizer (P_2O_5 45%) was added at a rate of 80 kg ha^{-1} [6] mixed with the soil. Sowing was carried out in rows spaced 20 cm apart at a seeding rate of 100 kg ha^{-1} [7]. Nitrogen fertilizer (urea 46% N) was added at a rate of 120 kg ha^{-1} [8] in three equal applications: the first after emergence, the second at the beginning of the branching stage, and the third when the plants had flowered. The soil was injected with bio-fertilizers two weeks after sowing. The data were collected and analyzed by using the ready-made statistical software GenStat V.20 according to the method adopted by [9]. The means were compared by using the least significant difference test at a probability level of 5%. Some growth and yield characteristics were measured: **1. Number of Tillers (tillers m^{-2})**

The number of tillers was determined by randomly selecting a 1 m² area from the central rows of each experimental unit. All tillers within the selected area were counted, and the total number of

tillers per square meter was recorded.

2. Leaf Chlorophyll Content (SPAD)

The relative chlorophyll content was estimated in the flag leaf, as it represents the primary source of carbohydrates for subsequent grain filling. To minimize human error, advanced digital image analysis techniques were employed. Clear digital images of the flag leaf were captured and analyzed using the **Color Picker** application on a smart device, following the methodology described by**[11]**

3. Number of Panicles per Square Meter (panicles m^{-2})

The number of panicles was determined by direct counting. All panicles within the previously designated and harvested 1 m^2 area of each experimental unit were counted, and the total number of panicles per square meter was recorded.

4. Number of Grains per Panicle (grains $panicle^{-1}$)

The number of grains per panicle was determined from a random sample of ten panicles collected from the designated area of each experimental unit. The grains were manually separated from each panicle, counted individually, and the average number of grains per panicle was calculated for each treatment.

5. Grain Yield (t ha^{-1})

The grains were completely separated from the plants harvested from the previously designated 1 m^2 area of each experimental unit using manual threshing and cleaning procedures to ensure that no grain was lost. After removing impurities and chaff, the clean grain weight was accurately determined. Grain yield was then converted from the harvested area (1 m^2) to tons per hectare (t ha^{-1}).

6. Seed Protein Content (%)

Seed protein content was determined using the **Semi-micro Kjeldahl** method to estimate total nitrogen. Protein percentage was calculated according to the following equation:

Protein content (% dry weight basis) = Seed nitrogen (%) × 6.25

following the procedures described by **[10]**

3- Results and Discussion

3-1 Number of Tillers (tillers.m^{-2})

Table (1) showed a significant superiority in the number of tillers in the treatments of the synthetic fungal and algal bio-fertilizers. A6 treatment recorded the highest rate 402.9 tillers.m^{-2} and did not differ significantly from A5 treatment, which differed significantly from the other bio-fertilizer treatments. A0, A1, A2, and A3 did not differ significantly, while the A0 treatment recorded the lowest rate 362.5 tillers.m^{-2}. Manufactured bio-fertilizers showed similar data to imported bio-fertilizers in the number of tillers, with the superiority of manufactured fungal and algal fertilizers. So the possibility of manufacturing these fertilizers by using simple means and giving high number of tillers [12]; [13]. Active fungi play a role in secreting enzyme inducers in the root zone, which

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stimulate an increase in the formation of basal buds that grow with the availability of nutrients to form new tillers [14]. Active algae also play a major role in increasing the availability of nutrients in the root zone, and nitrogen makes most providing and available for absorption by the plant [15].

Table (1). Effect of biofertilizer treatments on the number of tillers (tillers m^{-2}).

Biofertilizer treatments	A_0	A_1	A_2	A_3	A_4	A_5	A_6
Treatment Mean	362.5	370.3	379.6	386.6	374.7	393.6	402.9
L S D 0.05	15.17						

3-2 Leaf Chlorophyll Content (SPAD)

The results in Table (2) showed a significant superiority of the manufactured bio-fertilizer treatments in leaf chlorophyll content. A6 treatment recorded high average was 42.79 SPAD, and did not differ significantly from A5 treatment, which differed significantly from the other bio-fertilizer treatments. A0, A1, A2, and A3 treatments did not differ significantly, while the A0 recorded the lowest rate 31.60 SPAD. Manufactured bio-fertilizers differed in this characteristic from their imported counterparts, and this in turn greatly encourages the possibility of manufacturing these fertilizers in the laboratory. Manufactured bacterial, fungal, or algal bio-fertilizers played a role in increasing of the formation of chloroplasts due to the direct or indirect effect of algae in feeding the plant and supplying it with the biosynthesis compounds for chloroplasts [16] . Similarly, fungal or bacterial bio-fertilizers played a supporting role through enzymatic stimulation and increasing the plant's efficiency in absorbing compounds and affecting the activity of plant hormones [17].

Table 2. Effect of biofertilizer treatments on leaf chlorophyll content (SPAD).

Biofertilizer treatments	A_0	A_1	A_2	A_3	A_4	A_5	A_6
Treatment Mean	31.60	33.43	38.28	39.77	35.93	41.20	42.79
L S D 0.05	3.837						

3-3 Number of panicles (panicles.m^{-2})

The Table (3) showed a significant superior in the manufactured bio-fertilizer treatments in the number of panicles. A6 treatment recorded the highest average 374 panicles.m^{-2}, and did not differ significantly from treatment A5, which differed significantly from the other bio-fertilizer treatments. A0 and A1 treatments did not differ significantly, while A0 recorded the lowest average 313 panicles.m^{-2}. Manufactured bio-fertilizers showed compatibility with imported fertilizers with the manufactured fertilizers having due to their availability in the soil. Manufactured fungal or algal fertilizers resulted in a higher number of panicles because these fertilizers increased the number of tillers and chlorophyll content, which in turn increased the percentage of active tillers and significantly affected the number of panicles per unit area. Algal fertilizers provided greater accumulation of elements in the root zone due to their ability to synthesize compounds and the availability of elements [18]. While, the enzymatic activity of the fungi contributed for providing a suitable environment in the root zone for the absorption of compounds and increased biological stimulation of the plant towards of branching and the formation of flower buds[19].

Table 3. Effect of biofertilizer treatments on total grain yield (tons ha^{-1}).

Biofertilizer treatment	A_0	A_1	A_2	A_3	A_4	A_5	A_6
Treatment Mean	313.0	324.0	341.0	346.0	330.7	369.0	374.0
L S D 0.05	18.10						

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3-4- Number of grains per panicle (grain.panicle^{-1})

In Table (4), the results indicated a significant effect of the fungal and algal bio-fertilizer treatments on the number of grains per panicle. A6 treatment gave high value 49.27 grain. panicle^{-1}, which did not differ significantly from A5. A0 and A1 treatments did not differ, as well as A2 and A3 did not also differ significantly. The control treatment A0 gave low value 43.08 grain. panicle^{-1}. Manufactured algal or fungal fertilizers showed superiority in increasing of the number of grains per panicle compared to imported fertilizers. This is attributed to the role of these organisms in increasing the number of florets and the fertility rate of flowers [20], in addition to the role of these superior treatments in providing essential compounds and increasing photosynthetic rates due to the high chlorophyll content in the leaves [21].

Table 4. Effect of biofertilizer treatments on grains per panicle (grains panicle^{-1}).

Biofertilizetreatments	A_0	A_1	A_2	A_3	A_4	A_5	A_6
Treatment Mean	43.08	43.67	43.60	45.27	43.73	48.40	49.27
L S D 0.05	2.341						

3-5- Total Grain Yield (ton ha^{-1})

The results in Table (5) appeared a significant superior of the manufactured fungal and bacterial bio-fertilizer treatments on the total grain yield, with treatment A6 which recorded the highest value 4.813 tons ha^{-1}, which did not differ significantly from A5. The treatments A0, A1, and A4 did not differ significantly, while the control treatment A0 recorded the lowest value 4.063 tons ha^{-1}. The manufactured bio-fertilizer treatments did not differ from the imported bio-fertilizer treatments in terms of yield components, with superior of the fungal and algal treatments . This superior was evident in the grain yield due to the number of panicles Table (3) and the number of grains per panicle Table(4)

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Table 5. Effect of biofertilizer treatments on total grain yield (t ha^{-1}).

Biofertilizer treatments	A_0	A_1	A_2	A_3	A_4	A_5	A_6
Treatment Mean	4.063	4.187	4.733	4.887	4.137	4.740	4.813
L S D 0.05	0.079						

3-6- Grains Content of protein(%)

The results showed in Table (6) a significant effect of the fungal and algal bio-fertilizer treatments on protein content. A6 treatment appeared high value 10.6%, which did not differ significantly from A5 treatment. A1 and A4 treatments did not differ significantly, and A2, A3, and A4 also did not differ significantly, while the control A0 recorded low value 8.51%. The manufactured bio-fertilizers did not differ from their imported counterparts in their effect on increasing grain content of protein. In fact, the superior treatments of both manufactured and imported bio-fertilizers showed a high increase in the protein. This is attributed to the role of the bio-fertilizer in providing readily available nitrogen in the soil [22], which is essential for amino acid formation. Additionally, the role of the nutrient-rich fungus in accumulating compounds, which increases protein content [21].This trait is linked to the plant's photosynthetic efficiency (as evidenced by the superior chlorophyll content in the leaves of the treatments).

Table 6. Effect of biofertilizer treatments on grain protein content (%).

Biofertilizer treatments	A_0	A_1	A_2	A_3	A_4	A_5	A_6
Treatment Mean	8.510	9.273	9.560	9.807	9.497	10.223	10.590
L S D 0.05	0.7278						

4- **Recommendations**

The effect of the laboratory-manufactured fungal and algal bio-fertilizers did not differ from the effect of imported fertilizers, but rather they were significantly superior

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in all studied characteristics due to be active fertilizers. Therefore, we recommend the possibility of using the laboratory-manufactured fungal and algal bio-fertilizers, which are manufactured by simple
means and at a lower cost than imported fertilizers.

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