

The effect of bio fertilizer type on the chemical properties of two okra variety *Abelmoschus esculentus* L. grown in greenhouses

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I. Abstract:

The field experiment was conducted in plastic houses for growing okra (Hasnawi and Batra) in the research station of the College of Agriculture and Marshlands at Thi Qar University with four levels of biofertilizer : Treatment O (control treatment without addition bacteria), Treatment A (with addition of *Azotobacter chroococcum* inoculum) at a density of 1.1×10^{-9} cells/ml, Treatment B (with addition of *Pseudomonas fluorescens*) at a density of 3.6×10^{-9} cells/ml, and Treatment C (a mixture of *Azotobacter chro.* + *Pseudomonas fluor.*) at a density of 4.7×10^{-9} cells/ml. The following results were obtained:

1- The Hasnawi okra variety (A) significantly outperformed the Bitter okra variety (B) in nitrogen, phosphorus, and potassium percentages, and in carbohydrate and chlorophyll concentrations, while no significant difference was found in vitamin C concentration between the two okra varieties.

2- The bio fertilizer treatment C (*Azotobacter chro.* + *Pseudomonas fluor.*) significantly outperformed all treatments (A, B, and C) in nitrogen, potassium, chlorophyll, and carbohydrate percentages, with a p-value of 0.05, while no significant difference was found between treatment (C) and treatment (B). However, a significant difference was found between treatment (C) and treatment (A, O) in vitamin C content.

3- The best interaction was obtained for the mixed bio fertilizer C treatment (*Azot. chroo.* + *Pseu. fluor.*) with the Hasnawi variety (A) for all the studied treatments and for the percentage of

nitrogen, phosphorus, potassium, the amount of carbohydrates and chlorophyll at a probability level of p-value of 0.05 while no significant difference was obtained with vitamin C.

Keywords: Bacteria, bio fertilizer, okra, plastic house

II. Introduction:

Okra plant (*Abelmoschus esculents* L). is one of the most important summer vegetable crops in Iraq and world. It grows in most agricultural lands and belongs to the Malvaceae family (Boras *et al.*, 2011). The plant has medicinal properties, and interest in cultivating the crop and obtaining its fresh pods has increased. The plant contains proteins, carbohydrates, minerals, and vitamins (A, B, C, E, K) (Abdul Qader *et al.*, 2010). This plant is considered a good source of important nutrients such as calcium, magnesium, potassium, iron, and zinc. It also contains riboflavin and thiamine, in addition to other medicinal uses (Olivera *et al.*, 2014). It is widely used in daily life, such as in the manufacture of fishing lines and adhesives used in paper industries (Al-Moussawi, 2012). Due to the world's population growth, this has led to increased demand for the production of various food crops in greenhouses and increased productivity, particularly okra, which is grown in winter to meet community needs. Bio fertilizers have been used in this field to reduce soil, water, and air pollution, increase crop productivity, maintain public health, and create a clean environment. Therefore, it has become possible to reduce chemical fertilizers and even eliminate some during cultivation to ensure increased plant growth and productivity.

The research aims to:

1. Investigate the response of two okra cultivars to these types of biofertilizers when grown in greenhouses, increasing their growth and productivity.
2. Investigate the effect of the type of biofertilizer on improving the chemical properties of okra.
3. Investigate the interaction between the type of biofertilizer and the plant cultivar, and their relationship to the chemical properties of the plant.

III. Materials and Methods:

A- Soil Sample Preparation

Soil samples were taken from the work site after being cleaned, ground, sifted, and passed through a 2-millimeter sieve for the purpose of preparing, preserving, and measuring their chemical and physical properties, as shown in Table (1).



Table (1): Physical and Chemical Properties of Field Soil

Units	Value	Kind analysis
dsm^{-1}	5.0	Ece
---	7.10	Ph
Ppm	35	Available- n
Ppm	275	Available -k
Ppm	21.0	Available phosphorus
%	0.90	Organic matter
%	26	Sand
%	14	Clay
%	60	Silt
---	Silty loam	Soil texture

1- Soil pH:

The soil pH was measured in the soil paste extract using a pH meter as described by Page *et al.*, 1982.

2- Electrical conductivity of salts (ECe):

The electrical conductivity of the soil was measured in the saturated soil paste extract using a conductivity-brige device as described by (Page *et al.*, 1982).

3- Organic matter and organic carbon:

Organic carbon and organic matter in the soil were determined using the Walkely-Blak method described by Page *et al.*, 1982. This was done by oxidizing the organic matter with potassium chromate ($\text{K}_2\text{Cr}_2\text{O}_7$) in the presence of concentrated H_2SO_4 acid containing silver sulfate, then titrating with 0.5 N aqueous ferrous sulfate using O-phenothroline as an indicator. Organic matter was calculated from the product of organic carbon (1.724).

$$\text{Toc}\% = \frac{N1V1 - N2V2 \times 0.003 \times 100 \times 1.9}{\text{Weight of soil}}$$

$$\text{o.n}\% = \text{Toc} \times 1.724$$

4- Soil texture:

Soil texture was estimated using a burette method, as described in (Black, 1965).

5- Available nitrogen ($\text{NH}_4 = \text{NO}_3^-$):

Nitrate and ammonium were extracted from the soil using MKCl solution according to the method of (Bremner and Keendy, 1966) and were estimated using steam distillation according to the microkjeldahl method.

6- Bacterial counts were estimated using agar plate dilutions and plate counts using a bacterial nutrient medium, as described in (Black, 1965).

B- Experimental site.

The field experiment was conducted during the 2020-2021 agricultural season in an unheated greenhouse measuring 9 x 51 m and covering an area of 459 m² in the College of Agriculture, University of Thi Qar, specifically in the Mustafawiyya Agricultural Area. This is to study the effect of different types of biofertilizers on the physical and chemical properties of two okra varieties grown in greenhouses.

C- Soil description. Agricultural operations were carried out in the soil of the plastic house after it was plowed, smoothed, divided and animal manure (cows) was placed in an amount of 5 kg per experimental unit of 2.8 m² for all treatments according to the 2×4×3 design, i.e. the two varieties of okra Hasnawi and Batrah, and four types of bio-fertilizer, which is without addition and with the addition of bacteria (*Azotobacter chroo.*) at a concentration of 1.1×10^{-9} and the other treatment with the addition of bacteria (*Pseudomonas fluor.*) at a concentration of 3.6×10^{-9} and the fourth treatment is a mixture of bacteria (*Azot.chroo. + Pseu.fluor.*) at a concentration of 4.7×10^{-9} - and the addition of NPK fertilizers in an amount of (50-80-15) gm/fertilizer unit respectively, and the soil was irrigated by drip irrigation with Ec 1.3 mmol/cm and pH 7.1, while the characteristics of the organic fertilizer after Analysis: C\N 13.56, pH 6.4, organic carbon 343 g/kg⁻¹, and total nitrogen 25.3 g/kg⁻¹.

D- Digestion of plant samples:

leaf samples with 0.2 g were dried, ground and digested according to the method of Greser and Parson (1979). Concentrated 4 ml of 98% sulfuric acid was added to the sample and left for 24 hours until it turned black. 1 ml of sulfuric acid (H_2SO_4) was added, followed by 4% perchloric acid (HClO_4). The sample was placed on a hot plate and then heated at 350°C until a clear solution was obtained. The sample was transferred to a 50 ml beaker and the volume was made up with distilled water, ready for measuring the percentage of nutrients (NPK).

1- Percentage of nitrogen: The percentage was calculated using the Kjeldahl apparatus for digested samples, as mentioned in (Page *et al.*, 1982).

2- Phosphorus percentage: The percentage was calculated using the ammonium molybdate and ammonium vanadate method using a spectrophotometer, and the color intensity of the solution was measured at a wavelength of 882 nm (Sommer and Olson, 1982).

3- Potassium percentage: The percentage was estimated using a flame photometer as mentioned (Page *et al.*, 1982).

E - Experimental Measurements:

1- Leaf Chlorophyll Content (mg/gm 100g⁻¹): The chlorophyll content of the leaves was determined after taking 1 gram of plant tissue, adding 10 ml of 80% acetone, and crushing the tissue with a ceramic mortar (Goodwin, 1976). The tissue was then placed in a test tube in a centrifuge for 10 minutes, then filtered and placed in a spectrophotometer to read at wavelengths of 663 and 645 nm. This was calculated according to the following equation:

$$\text{Total Chlorophyll} = 100(V \times W \times 1000) \times (D_{663} \times 8.02 + D_{645} \times 20.2)$$

Where D represents the reading of the instrument, W represents the weight of the sample, and V represents the final volume of the extract.

2- Carbohydrate content of leaves (mg/100g): The carbohydrate content was calculated after taking 0.5g of the dried and ground sample, adding 75ml of distilled water for 1 hour at 90°C, then removing it to laboratory temperature, filtering it, taking 5ml of the filtrate, adding 25ml of distilled water, then taking 1ml of the solution, adding 1ml of 5% phenol and 5ml of sulfuric acid. A spectrophotometer was used at a wavelength of 490nm, and carbohydrates were calculated using the modified sulfuric acid colorimetric method described by (Dubois *et al.*, 1956) using the following equation:

Soluble carbohydrates = X value × dilution × volume in liters (0.075) / sample weight (0.5) × 100. The X value is extracted from the standard curve equation.

3- Vitamin C ascorbic acid (100 mg/gm): A 50 g sample was taken from the fresh sample, and 50 ml of 6% oxalic acid was added to it. The sample was mixed for a quarter of an hour with an electric mixer. The sample was filtered, and 50 ml of 3% oxalic acid was added to it. After that, 5 ml of the solution was taken and ground to 2,6 Dichlorophenol indophenol according to the method (A.O.A.C 1970).



IV. Results and Discussion:

1- Leaf Nitrogen Content (%):

Table (2) shows the effect of biofertilizers on the percentage of nitrogen in the leaves of okra cultivars. The Hasnawi (A) cultivar outperformed the Batrah (B) cultivar, with the nitrogen content of the A cultivar reaching 1.95%, while the B cultivar yielded 1.81%. The significant effect on the variation in traits is due to the variation in genetic response between cultivars (Suder *et al.*, 2010). The same table showed that the types of biofertilizer had a significant effect on increasing the nitrogen content of leaves, and treatment C (*Azot. chroo.* + *Pseu. fluor.*) was superior and obtained the highest level, reaching 2.16% at a probability level of $\sim p 0.05$ over all treatments A,B,O. Treatment B differed significantly from treatment A (*Azot. chroo.*) This difference may be due to the response to the role of added bacteria in the growth of the root system of the okra plant, as well as to the production of phytohormones and increased decomposition of organic matter and strengthening the plant with the main elements NPK and their transfer into the plant. This is consistent with what was obtained by (Cleyet-Marel *et al.*, 2001). As for the two-way interaction between the varieties and types of biofertilizers, this was significantly affected at a significance level of $\sim p 0.05$, as treatment C (*Azot. Chroo.* + *Pseu. Fluor.*) gave the highest values for interaction with the Hasnawi variety. It reached 2.23% and was significantly superior to all binary overlapping treatments, while the lowest overlapping rate was in the BO treatment, i.e. the category of the piece, with the comparison treatment, which reached 1.52%.

Table (2): The effect of biofertilizers on the percentage of nitrogen (%) in the leaves of the two okra varieties

mean	<i>Azot. +Pseu.</i> (C)	<i>Pseu.fluor.</i> (B)	<i>Azot.chroo.</i> (A)	With out (O)	Class
1.95	2.23	2.03	1.87	1.66	A (Al-Hasnawi)
1.81	2.07	1.94	1.69	1.52	B (Al-Batra)
L.S.D _{0.05} C*B	2.16	1.98	1.78	1.59	mean
0.039	0.073				L.S.D _{0.05} B
L.S.D _{0.05} Class	0.051				

2- Leaf Phosphorus Content (%): Table (3) shows a significant difference between okra cultivars grown in greenhouses in leaf phosphorus content. The Hasnawi (A) cultivar significantly outperformed the Batra (B) cultivar in phosphorus content, reaching 0.27% for the Hasnawi cultivar and 0.20% for the Batra cultivar. This difference is due to the genetic characteristics of

the cultivar, which allowed it to absorb more elements than the other cultivars, thus increasing the percentage of the element (Abu Dahi and Younis, 1988). The same table showed the significant effect of biofertilizers for all addition treatments A.B.C compared to the comparison treatment, as well as the presence of a significant difference between the treatments themselves. Treatment C (*Azot.chroo.+Pseu.fluor.*) outperformed all treatments, as it reached 0.40%. This is due to the increased absorption of phosphorus by the roots due to phosphorus-dissolving microorganisms, the increased density of vegetative and root plant growth, and the increased absorption of elements by the plant (Al-Mandlawi, 2002), in addition to the contribution of *Pseu.fluor* bacteria. To dissolve the deposited phosphorus and increase its absorption inside the plant (Raddad, 1997) in addition to the secretion of hormones and growth regulators (Alkarak *et al.*, 1997) and phenols that encourage vegetative and root growth, which are secreted by the bacteria *Azot.chroo* (Bashir *et al.*, 2004). It was noted that the joint addition of nitrogen-fixing and phosphorus-dissolving bacteria increased the phosphorus concentration in the inoculated plant. The dual interaction between the varieties and biofertilizers significantly affected the amount of phosphorus in the leaves, and the treatment of Al-Hasnawi variety A with the inoculated treatment C (*Azot.chroo.+Pseu.fluor*) significantly outperformed all the treatments interacting with it, and the highest percentage of phosphorus reached 0.45%, while the lowest value was obtained by the interacting treatment B and the comparison treatment O, which reached 0.11%.

Table (3): The effect of biofertilizers on the percentage of phosphorus (%) in the leaves of the two okra varieties.

mean	<i>Azot. +Pseu.</i> (C)	<i>Pseu.fluor.</i> (B)	<i>Azot.chroo.</i> (A)	With out (O)	Class
0.27	0.45	0.25	0.23	0.15	A (Al-Hasnawi)
0.20	0.34	0.55	0.21	0.11	B (Al-Batra)
L.S.D _{0.05} C*B	0.40	0.24	0.18	0.13	mean
0.039	0.027				L.S.D _{0.05} B
L.S.D _{0.05} Class	0.019				

3- Potassium content of leaves (%): Table (4) showed a significant increase in potassium values in the leaves of the A-Hasnawi okra variety compared to the B-Tara okra variety, reaching (1.44 and 1.65%). The significant superiority was due to the genetic variation of the variety and its impact on the response to this trait (Aktas *et al.*, 2009). The same table showed a significant difference in potassium percentages in the okra leaves after adding the bacterial inoculum to all treatments. Treatments A,B,C significantly outperformed the control treatment O, then treatment C (*Azot.chrom.+Pseu.fluor.*) significantly outperformed treatment A.B. Then the treatments differed from each other. This is due to the ability of organisms to fix nitrogen, increase phosphorus and potassium, and improve plant quality characteristics (Al-Balkhi, 2005), as well as the increase in growth regulators such as auxins, gibberellins, and cytokines, which encourage the plant to absorb nutrients (Al-Shaibani ,2005). The table shows the bilateral interaction between the varieties and biofertilizers and their significant effect on increasing the production of potassium percentage in plant leaves, where treatment A of the Al-Hasnawi variety interacted with treatment C (*Azot. chroo. + Pseu. Fluor.*) gave the highest values, reaching 2.18%, significantly superior to all the interactions of the varieties and biofertilizers. As for the lowest interaction values, it is treatment B interacted with the comparison treatment, reaching 1.14%.

Table (4): The effect of biofertilizers on the percentage of potassium (%) in the leaves of the two okra varieties.

mean	<i>Azot. +Pseu.</i> (C)	<i>Pseu.fluor.</i> (B)	<i>Azot.chroo.</i> (A)	With out (O)	Class
1.65	2.18	1.70	1.43	1.30	A (Al-Hasnawi)
1.44	1.88	1.45	1.28	1.14	B (Al-Batra)
L.S.D _{0.05} C*B	2.03	1.58	1.35	1.22	mean
0.126	0.089				L.S.D _{0.05} B
L.S.D _{0.05} Class	0.063				

4- Leaf chlorophyll content (mg/100g⁻¹): Table (5) shows that the Hasnawi variety A significantly outperformed the B Batrah variety, at the probability level, as the leaf chlorophyll content for the Hasnawi variety reached 74.90 and 63.63 mg/100g⁻¹ for the Batrah variety, respectively. The reason for this difference is due to the role of different genetic traits between the two varieties and the role of their response to this trait (Aktas *et al.*, 2009). This is consistent with what was obtained by (Al-Hajjaji *et al.*, 2021), who noted the superiority of the Hasnawi variety over the Batrah variety grown in a greenhouse. The same table showed that adding

different types of biofertilizer with a constant level of organic fertilizer had a significant effect on the chlorophyll content of leaves, as the amount of chlorophyll for treatment A (*Azot.chrom.*), treatment B (*Pseu.fluor.*), and treatment C (*Azot.chroo.+Pseu.fluor.*) was 68.02, 76.91, and 81.32 mg 100 g⁻¹, respectively, compared to the treatment without addition of 60.63 mg 100 g⁻¹. The reason for the superiority of treatment C (*Azot.chroo.+Pseu.fluor.*) over treatment A and treatment B is due to the ability of different microorganisms to fix atmospheric nitrogen, which helps in increasing its absorption and thus increasing the chlorophyll content. In addition, the difference in bacteria in increasing the decomposition of organic matter and the speed of release of different nutrients such as nitrogen, phosphorus, and potassium, which led to an increase in chlorophyll in the treatments, and this is consistent with (Mustafa, 2002 and Al-Hijami *et al.*, 2021). As for the interaction between the varieties and types of biofertilizers, the same table showed that the best significant superiority was achieved by the combined treatment A Al-Hasnawi and the bacterial mixture C (*Azot.chroo.+Pseu.fluor.*), which reached 84.80 mg 100 g⁻¹ and outperformed all the combined treatments in chlorophyll content, with the exception of the combined treatment of Al-Hasnawi variety A and treatment B (*Pseu.fluor.*), which **reached 81.03 mg 100 g⁻¹**.

Table (5): The effect of biofertilizers on the amount of chlorophyll (mg/100 g⁻¹) in the leaves of the two okra varieties

mean	<i>Azot. +Pseu.</i> (C)	<i>Pseu.fluor.</i> (B)	<i>Azot.chroo.</i> (A)	With out (O)	Class
74.90	84.80	81.03	71.41	62.38	A (Al-Hasnawi)
68.53	77.83	72.78	64.63	58.88	B (Al-Batra)
L.S.D _{0.05} C*B	81.32	76.91	68.02	60.63	mean
4.006	2.833				L.S.D _{0.05} B
L.S.D _{0.05} Class	2.00				

5- Leaf content of total soluble carbohydrates (mg/100g⁻¹): Table (6) showed that the leaf content of total soluble carbohydrates was significantly higher for the A-Hasnawi variety than the B-Batrah variety, reaching 14.92 and 13.58 mg/100g⁻¹, respectively. This is due to the genetic differences and variations of the cultivated varieties responsible for the chemical components of the okra plant (Mahdi *et al.*, 2016 and Al-Hijami *et al.*, 2021). The addition of biofertilizers significantly affected the amount of carbohydrates in the leaves, as treatment C (*Azot. Chroo. + Pseu. Fluor*) significantly outperformed all added treatments, giving the highest rate of 19.72

mg/100g⁻¹. As for treatments A (*Azot. Chroo.*) and B (*Pseu. Fluor.*), treatment B outperformed A. This is due to the role of... Bacteria improve chemical properties and carbohydrate levels, as well as balance major nutrients NPK, in addition to the role of added bacteria in increasing vegetative growth, photosynthesis, and transporting manufactured materials from their feeding sites to their storage sites (Yazdani *et al.*, 2009; Al-Hijami *et al.*, 2021). The same table showed the two-way interaction between plant varieties and types of biofertilizers, where treatment C (*Azot. chroo.* + *Pseu. fluor.*) was significantly superior to all other interacting treatments, reaching 20.76 mg 100 g⁻¹, while treatment O had the lowest values, reaching 8.54 mg 100 g⁻¹.

Table (6): The effect of biofertilizers on total carbohydrates (mg/100 g⁻¹) of the leaves of the two okra cultivars

mean	<i>Azot. +Pseu.</i> (C)	<i>Pseu.fluor.</i> (B)	<i>Azot.chroo.</i> (A)	With out (O)	Class
14.92	20.76	15.47	14.11	9.36	A (Al-Hasnawi)
13.58	18.67	14.60	12.54	8.54	B (Al-Batra)
L.S.D _{0.05} C*B	19.72	15.03	13.32	8.95	mean
1.607	1.136				L.S.D _{0.05} B
L.S.D _{0.05} Class	0.80				

6- Ascorbic acid vitamin C (mg/100gm⁻¹): The results of Table (7) showed no significant differences between varieties A and B in the amount of ascorbic acid, as the amount of acid reached 28 and 27.41 mg/100gm⁻¹, respectively. Treatment A (*Azot.chroo.*) and treatment B (*Pseu.fluor.*) significantly outperformed each other and outperformed treatment O, the control treatment, in the values of ascorbic acid. Treatment C (*Azot.chroo.+Pseu.fluor.*) also outperformed the control treatment only. The same table showed that treatments A, B, and C outperformed treatment O in the amount of ascorbic acid, due to the role of microorganisms in improving soil and plant characteristics, increasing soil fertility, plant nutrition, increasing vegetative growth, increasing element availability, and increasing metabolic products, thus increasing ascorbic acid in okra fruits (Al-Ashry *et al.*, 2001). Al-Hajjaji *et al.*, 2021), as for the bilateral interaction between the varieties and biofertilizers in increasing ascorbic acid, the table showed that treatments B and C and okra varieties A and B had a significant superiority over all

the remaining interacting treatments, and treatments A and O did not significantly outperform each other in the amount of ascorbic acid.

Table (7): The effect of biofertilizers on the amount of ascorbic acid (100 mg/g-1) in the leaves of the two okra varieties

mean	<i>Azot. +Pseu.</i> (C)	<i>Pseu.fluor.</i> (B)	<i>Azot.chroo.</i> (A)	With out (O)	Class
28.00	35.67	33.33	24.67	18.33	A (Al-Hasnawi)
27.42	34.00	33.33	25.33	17.00	B (Al-Batra)
L.S.D _{0.05} C*B	34.83	33.33	25.33	17.67	Average
4.236	2.995				L.S.D _{0.05} B
L.S.D _{0.05} Class	2.118				

Conclusions and Recommendations:

1. We conclude that all biofertilizers added to the soil increased okra productivity, and we recommend using them at high concentrations and inoculum levels.
2. Adding these biofertilizers (bacteria) to various low-yielding plant species to increase their productivity.
3. We recommend preserving such inoculums and bacterial species that promote plant growth in laboratories (known), i.e., in banks established to preserve these species and transfer them to other locations, given their known effectiveness in increasing productivity.

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