The Effect of inoculation with local isolates of Azotobacter chroococcum bacteria and levels of chelated iron in the growth and yield of (Zea mays L(.

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Abstract:

The study included crop cultivation Zea mays L in Al-Muthanna Governorate / Al-Majd District during the 2023-2024 agricultural season The study aimed effect of inoculation with local isolates of Azotobacter chroococcum bacteria and levels of chelated iron on the growth and yield (Zea maysL(. .The study included conducting a laboratory experiment which included the isolation and identification of Azotobacter chroococcum bacteria from the rhizosphere soil. The experiment was applied in (R.C.B.D.) The first factor represented the biovaccine with three isolates of A. chroococcum bacteria and was given the Symbols S0, S1, S2, S3, and the second factor was adding levels of chelated iron fertilizer 0, 8,16 kg h-1, and was given Symbols F0, F1, F2. The results of the statistical analysis showed significant differences (P≤0.05) between the studied traits, Where isolation S3 excelled, namely the chlorophyll content, weight of 500 seeds, grain yield, dry weight, concentration Nitrogen, phosphorus, potassium and iron in the plant, and the concentration of nitrogen, phosphorus, potassium and iron ready in the soil and the number of Azotobacter bacteria .The treatment of adding chelated iron F2 fertilizer outperformed and recorded the highest values in each of the weight of 500 grains, grain yield, dry weight, concentration, phosphorus and potassium. And iron in the plant, and the concentration of nitrogen, phosphorus, potassium, and ready iron in the soil. The S3F2 The overlap coefficient excelled in each of the weight of 500 grains, grain yield, the concentration of phosphorus and iron in the plant, and the ready concentration of nitrogen in the soil. Keywords: Azotobacter chroococcum, Chelated iron, Zea mays L

Introduction:

Nitrogen is one of the major nutrients necessary for the production of agricultural crops, and although it represents 79% of the air, it is in the form of N2, which is an inert gas (that is, chemically inactive) and most forms of life cannot benefit from it .

Prokaryotic microorganisms (Procaryotes) which possess the enzyme nitrogenase, carry out the process of biological fixation of atmospheric nitrogen, which is reduced to ammonia [1]. The process of biological stabilization is carried out by symbiotic organisms such as Rhizobium spp, which coexist with leguminous plants, as well as Frankia actinomycetes, which infect some perennial trees, and non-symbiotic or freeliving organisms such as Azotobacter spp, Cyanobacteria, and commensal organisms. Or associative, such as Azospirillum spp, in which interest has increased recently due to its association with the roots of many cereal crops and its positive effects on the plant and yield.

The Azotobacter chroococcum bacteria is one of the genera of free-living bacteria with the ability to fix atmospheric nitrogen. Its use has expanded as a biofertilizer with a large number of crops, in addition to its ability to secrete some hormones, enzymes, vitamins, and growth regulators [2]. The amount of nitrogen fixed by Azotobacter bacteria is estimated at 20 km N h-1 year-1 [3.]

Iron is considered the fourth most abundant element on the face of the Earth, and iron also forms an important part of the nitrogenase enzyme secreted by the bacterium Azotobacter chroococcum, which has an effect in fixing nitrogen in nitrogen-fixing soils. It was shown [4] that Iraqi soils in general are characterized as tending to alkaline and with a high content. Of carbonate, it is also characterized by a low content of organic matter. The sum of these characteristics makes Iraqi soils low in readiness for microelements, including iron, as chelating compounds increase the addition of micronutrients to plant roots by increasing the total concentration of dissolved elements, which consequently leads to an increase in the supply of the element. Nutrients on the root surface [5] as chelated iron Fe-EDDHA is one of the most important chelated fertilizers used in modern agricultural technology.

It Zea mays L is considered one of the most important grain and industrial crops that are grown on large areas in the world. It is considered the third crop in the world after wheat and rice in terms of economic importance, area and production. Corn is used in various industries and in the fields of energy and biofuel production and in the feed industry. It has a high nutritional value for humans as it is extracted Including starch and oil and containing protein, vitamin A and vitamin B1 B2 [6] It is widely cultivated in Iraq, as the cultivated area in 2016 reached (76.00) thousand hectares and a production of 3.415 mega grams h-1 [7] Despite this, the production rate is still low compared to global production. It does not meet the country's need. Therefore, means must be provided that lead to increasing production in quantity and quality in the country.

Materials and methods:

A field experiment was carried out during the spring season (2023-2024) in Al-Muthanna Governorate, within a circle of latitude (N 39.469188) and longitude (E 55.281100). The field soil was of a mixed, alluvial texture, to study the effect of biofertilization of Azotobacter chroococcum bacteria. Levels of chelated iron and growth and productivity of yellow maize.

Preparing and preparing the soil for planting.

After the land was selected for the experiment, the land was prepared after performing perpendicular plowing operations on it, smoothing and leveling it, and then the land was divided into three large sectors (Blocks). Each sector was divided into experimental units in the form of panels (Plots), and the area of the experimental units was equal to (2 * 3)m2 for one experimental unit. The units were divided into four corridors, and the distance between one corridor and another was 75 cm and between one hole and another was 20 cm. An area of 1 m was left between each experimental unit and another within one sector, and a distance of 1.5 m between each sector in order to prevent interference of experimental factors and Then the yellow corn seeds were planted, which were divided into four sections, and each section of these seeds was placed in sterile plastic pots, then a previously banned 10% gum arabic solution was added to them, then the bacterial bioinoculum for the bacteria of the first isolate (A.chroococcum) was added to the seeds of the first section, and the bioinoculum was added. The bacterial inoculum was added to the bacteria of the second isolate (A.chroococcum) to the seeds of the second section, and the inoculum was added to the bacteria of the third isolate (A.chroococcum) to the seeds of the third section, while the

seeds of the fourth section were left without inoculation, and the seeds were mixed well with the vital vaccines. On the date of 15/3/2024, 3 seeds were placed in each hole, and the waterways were opened for the purpose of irrigation, and service operations were carried out according to the need of the crop. Weeding operations were also carried out to get rid of the harmful bushes manually, and then the process of thinning the plant took place a week after germination, with one plant for each hole.

Fertilization

Mineral fertilizers were also added according to the recommended quantities, mixed with the the soil. 50% of nitrogen fertilizer recommendation was 320 kg h-1 in the form of urea fertilizer 46% N, and phosphate fertilizer was added in an amount of 100 kg h-1 in the form of triple superphosphate P2O5 44%. (TSP) and potassium fertilizer was added at a rate of 150 kg h-1 in the form of potassium sulphate fertilizer (41.5% K) [8] Nitrogen fertilizer was added in two batches, one batch at planting and one batch 30 days after planting, while only phosphate and potassium fertilizer were added. All at once before planting, and after (21) days of germination, ground chelated iron was added at three levels (0, 8, 16) kg h-1 Fe-EDDHA (6% Fe.(

Field experiment factors

First: Biofertilizer was added at four levels

The isolates were selected based on testing their efficiency and highest percentage of atmospheric nitrogen fixation.

.1S0: without the addition of the biovaccine (control(

.2S1: The first Azotobacter chroococcum isolate

.3S2: The second isolate, Azotobacter chroococcum

.4S3: Azotobacter chroococcum

Second: Fertilizing with chelated iron F at three levels

.1F0: Iron fertilization level 0 kg h-1

.2F1: Iron fertilization level 8.0 kg h-1

.3F2: Iron fertilization level is 16.0 kg h-1

Plant measurements

Chlorophyll content (Spad (

The amount of chlorophyll in leaves was measured in the field using a SPAD 502 device during the flowering stage [9.]

Grain yield (tons h-1(

The grain yield was calculated according to the following equation, and after drying the sample until the weight was stable based on a moisture content of 15.5% [10.[

Grain yield = (grain rate per plant x plant density h-1(

Determination of nitrogen by plant

The quantity was estimated according to the method [11] using a Micro Keldhal device after the harvesting stage.

Determination of phosphorus by plant

The concentration of phosphorus in the plant was estimated using an Olsen sommers spectro photometer as described [12] after the harvest stage.

Determination of potassium in plants

Potassium in the extracted plant was estimated using a Flamephotometer device according to [12] after the harvesting stage.

Determination of iron in plants

It was estimated using an atomic absorption device during the flowering stage, as mentioned in [13]

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value	Unit of measurement	Adjective
5.5	¹ dsm ⁻	Electrical conductivity (ECe)
7.6	_	Degree of soil reaction (pH)
0.8	%	Organic matter
Concentration of positive a	and negative dissolved ion	ns in a soil:water extract
1:1		
6.08		⁺² Ca
2.273		Mg^{+2}
3.49	$M \text{ mol.}L^{-1}$	Na ⁺
1.02		K ⁺
2.8		HCO ₃
12.9		$SO_4^{=}$
28.5		Cl
Nil		CO_3^{-2}
3.555	am ka ⁻¹ soil	Ready iron
25.9	giii.kg son	Ready nitrogen
12	-	Ready phosphorus
168	-	Ready potassium
Soil separators		
37.5	om leo ⁻¹ soil	Sand
50		Alluvial
12.5		Clay
Silty loam	-	Histology
5.54×10 ⁶	Cfu g ⁻¹ dry soil	Total bacteria
2.21×10 ⁴		bacteria Azotobacter

Table	(1):	Some	chemical,	physical	and	biological	characteristics	of	the	study	soil	before
plantii	ng											

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Results and discussion

Chlorophyll

Table No. (2) shows that adding the bioinoculum to isolates of the bacteria A. chroococcum led to a significant increase in the chlorophyll content in the yellow corn plant, as the highest rate reached 51.80 spad when inoculated with isolate S3 compared to the control treatment S0, in which the chlorophyll rate reached 44.56. spad. The reason for this may be attributed to the role of the bacteria A. chroococcum in fixing nitrogen and increasing its concentration in the leaves, which is considered the basis for the formation of chlorophyll, as well as encouraging root

growth, which in turn increased the rate of absorption of water and nutrients, which corresponds to an increase in the overall vegetative total in the plant through the number of leaves. and paper area. These results are consistent with [14]. The results in Table (2) indicated that there was no significant effect of adding levels of chelated iron fertilizer Fe-EDDHA and the binary interaction between the A.chroococcum biovaccine and levels of chelated iron on chlorophyll content.

Table (2)	The	effect	of	bacterial	bioinoculum	and	levels	of	chelated	iron	fertilizer	and	their
interaction	n on c	chloro	phy	yll (spad)	content								

Mean S	F ₂	F ₁	F ₀	F
44.56	44.63	46.03	43.02	S ₀
45.78	47.51	46.18	43.65	S ₁
48.50	50.82	45.97	48.69	S ₂
51.80	54.19	52.52	48.70	S ₃
	49.29	47.68	46.02	Mean F
F*S=N.S	F=N.S	S=3.943		L.S.D(0.05)

Grain yield ton/h1-

The results of Table No. (3) indicated that there was a significant difference in the characteristics of the total grain yield of yellow maize plants when using the bioinoculum for isolates of the bacteria A. chroococcum, as treatment S3 excelled, as it gave the highest average amounting to 6.71 tons h-1, and the percentage increase was 139.64% compared to Measurement treatment S0, which gave the lowest average of 2.80 tons h-1. The increase may be attributed to the ability of the isolates from the bacteria A. chroococcum to produce many growth regulators, including indole acetic acid and its positive role in root system activity, which enhances the ability to absorb nutrients in Table (3) also shows that adding chelated iron fertilizer to the soil resulted in superiority to the F2 treatment, as the addition treatment of 16 kg h-1

excelled and gave the highest average of 6.41 tons h-1 with a percentage increase of 68.24% compared to the no-addition F0 treatment, which gave the lowest average. It reached 3.81 tons h-1. The reason for the increase in yield may be attributed to the role of iron in the manufacture of chlorophyll, in addition to addition to its ability to fix nitrogen. Which plays an important role in increasing the yield. The results of the table agree with [15 .[being involved in the synthesis of ferredoxin, which is the first reductive compound in the electron transport chain for the process of photosynthesis, which was reflected in an increase in the products of photosynthesis and their distribution to the reproductive parts, and these results are consistent. With [16.[On the other hand, Table No. (3) shows that there are significant differences between the dual interaction of bio-inoculation and fertilization with chelated iron added to the soil

Table (3)The effect of bacterial bio-inoculum and levels of chelated iron fertilizer and their interaction on grain yield ton/h-1

Mean S	F ₂	F ₁	F ₀	F
2.80	4.55	2.06	1.79	S ₀
4.06	6.59	3.43	2.15	S ₁
5.94	6.72	5.86	5.22	S ₂
6.71	7.79	6.27	6.08	S ₃
	6.41	4.40	3.81	Mean F
F*S=1.131	F=0.565	S=0.653		L.S.D(0.05)

Nitrogen concentration in grains%

The results of the statistical analysis of Table significant (4) showed that there were differences when inoculated with A. chroococcum isolates in the concentration of nitrogen in the grains of yellow maize plants, as treatment S3 was significantly superior to the comparison treatment, as it recorded the highest average of 3.120% compared to the measurement treatment, which recorded less. The average was 2.166% and the reason for this may be attributed to the isolation of bacteria A. chroococcum by the process of fixing atmospheric nitrogen, thus increasing the soil's ready-made nitrogen content and stimulating root growth with enzymes that stimulate the growth and formation of a dense root system. Therefore, the ability to absorb nutrients, including nitrogen, increases, and the results are consistent with a study conducted by [17.] The results in Table (4) also indicated that there was no significant difference in chelated iron fertilization, the binary interaction in bacterial bioinoculum, and levels of chelated iron fertilization in the nitrogen concentration in grains

Mean S	F ₂	F ₁	F ₀	F
				S
2.166	2.300	2.527	1.670	S ₀
2 5 2 6	2.640	2 422	2 502	S
2.320	2.040	2.435	2.303	\mathfrak{s}_1
2.544	2.783	2.307	2.543	S ₂
3.120	3.177	3.210	2.973	S ₃
	2.725	2.619	2.422	Mean F
F*S=N.S	F= N.S	S= 0.4481	1	L.S.D(0.05)

Table (4) The effect of bacterial bi	ioinoculum a	nd levels	of chelated	iron	fertilizer	and	their
interaction on nitrogen concentration	on in grains						

Chelated iron concentration (mg kg-1

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The results of Table (5) indicated that there was a significant difference in the effect of bioinoculation with bacteria. A. chroococcum on iron concentration in leaves, as treatment S3 excelled, giving the highest average amounting to 87.68 mg kg-1, with an increase rate of 19.45% compared to the measurement treatment, which gave the lowest average amounting to 73.40 mg kg-1. The increase attributed to the effect may be of biofertilization with bacteria A. chroococcum in increasing the readiness of iron in the soil comes from the ability of the bacteria Azotobacter chroococcum to secrete high amounts of microbial siderophores, which improve the readiness of iron in the soil and its average amounting to 93.45 mg kg-1, with an increase rate of 48.14% compared to the noaddition treatment (measurement absorption by the plant. Hence, it is clear that organisms in the soil play an important role in the amount of iron absorbed by the plant in Under conditions of deficiency, significant changes occur in the degree of soil interaction and then in

various growth parameters, such as stimulating the growth and development of the root group, as well as the production of growth regulators that increase the readiness of nutrients, especially iron, for the plant. These results are consistent with [18.]

Table (5) also shows that adding chelated iron fertilizer to the soil resulted in superiority to the F2 treatment, as the addition treatment of 16 kg h-1 excelled and gave the highest treatment). Which gave the lowest average of 63.08 mg kg-1. This may be due to increasing the amount of iron added directly to the soil and then increasing its content in plant leaves, which has a role in activating vital activities in the leaves, which was reflected in the

absorbed amount of iron. These results are consistent with [19.[

Table (5) indicated that there were significant differences between the bilateral interaction of bioinoculation with bacteria A. chroococcum and chelated iron fertilization

Table (5) Effect of bacterial bioinoculum and levels of chelated iron fertilizer and their interaction on chelated iron concentration (mg kg-1(

Mean S	F ₂	F ₁	F ₀	F S
73.40	91.93	79.61	48.66	S ₀
80.68	92.02	83.63	66.38	S ₁
81.65	90.46	89.63	64.86	S ₂
87.68	99.38	91.24	72.41`	S ₃
	93.45	86.03	63.08	Mean F
F*S= 5.121	F= 2.561	S= 2.957		L.S.D(0.05)

Phosphorus concentration in grains %

The results of Table (6) indicated that the bioinoculation increased the concentration of phosphorus in the grains, as the S3 inoculation treatment recorded the highest average of 0.442% compared to the measurement treatment, which reached the lowest average of 0.262%. The reason for this may be attributed to the effectiveness of A. chroococcum in stimulating the root to absorb phosphorus, thus Increased concentrations absorbed by the plant and the results of the study are consistent with [20.]

Table (6) also shows that adding levels of chelated iron fertilizer to the soil led to the superiority of the F2 treatment and gave the highest average of 0.322% compared to the

no-addition treatment (measurement treatment), which gave the lowest average of 0.270%. The reason for the increase in phosphorus concentration in the grains may be due to The important role of iron in activating the processes of respiration and carbon metabolism is a result of its contribution to the synthesis of basic compounds and components in the plant cell, including cytochromes, and thus was reflected in an increase in the plant's absorption of phosphorus, and these results were consistent with [21.[

Table (6) shows that there are also significant differences in the treatment of interaction between the vaccine and isolates of bacteria A. chroococcum and chelated iron fertilizer levels exceed the level of interaction between the S3 treatment and the F2 chelated iron treatment, which gave the highest average of 0.506% compared to the measurement treatment S0F0, which recorded the lowest average of 0.236%,

and the reason is due to the increase between the bacterial biofertilizers and the chelated fertilisers

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Mean S	F ₂	F ₁	F ₀	F
0.262	0.246	0.303	0.236	S ₀
0.254	0.266	0.270	0.226	S ₁
0.328	0.270	0.436	0.280	S ₂
0.442	0.506	0.480	0.340	S ₃
	0.322	0.372	0.270	Mean F
F*S=0.088	F= 0.044	S= 0.050		L.S.D(0.05)

Table (6) The effect of bacterial bioinoculum and levels of chelated iron fertilizer and their interaction on phosphorus concentration in grains

Potassium concentration in grains %

Table (7) shows the effect of A. chroococcum bio-inoculation on the potassium content in grains. Treatment S3 achieved the highest average of 2.828%, with an increase rate of 11.46% compared to the measurement treatment, which gave the lowest average of 2.539%. Treatment S3 outperformed the rest of the treatments. Without a significant difference, the reason for this may be attributed to the ability of the A. chroococcum bacteria to freely fix atmospheric nitrogen, in addition to producing many hormones and growth regulators, which in turn stimulate the plant to absorb various nutrients, including potassium. These results may be consistent with what was indicated by [22.]

The results in Table (7) also indicated that there were significant differences in the concentration of potassium in the grains when chelated iron was added to the soil. This led to the superiority of the F2 treatment, which gave the highest average of 2.762%, with an increase rate of 6.15% compared to the no-add treatment, which gave less. The average was 2.607%. The reason for this may be attributed to the vital role of iron in activating many enzymes and thus activating vital processes in including the processes the plant. of respiration and carbon metabolism, which were affected in the leaves and thus reflected in the grains. These results are consistent with [23, 24.]

Mean S	F ₂	F ₁	F ₀	F
2.539	2.713	2.520	2.383	S ₀
2.729	2.737	2.713	2.737	S ₁
2.669	2.677	2.763	2.567	S ₂
2.828	2.920	2.823	2.740	S ₃
	2.762	2.705	2.607	Mean F
F*S=N*S	F=0.0957	S=0.1	106	L.S.D(0.05)

Table (7) The effect of bacterial	bioinoculum ar	d levels o	f chelated	iron	fertilizer	and	their
interaction on potassium concentr	ation in grains						

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