Al-Muthanna J. For Agric Sci   Print ISSN: 2226-4086   Online ISSN:2572-5149
Vol. 12 , Issue 2. 2025

https://muthjas.mu.edu.iq/   http://doi.org/10.52113/mjas04/12.2/36

Effect of adding *Thiobacillus thioparus* and *Azospirillum brasilense* bacteria and agricultural sulfur levels on soil NPK and sulfate availability

Zainab Hassan Sahip [1] **Ghanem Bahlol Noni** [2] **Anmar Hamoudi Kadhim** [3]

[1,2,3]College of Agriculture, University of Al-Muthanna/ iraq

E- mail : agr.soil.zainab2025@mu.edu.iq E- mail : ghanem-bahlol@mu.edu.iq
E- mail : anmarjhayl@mu.edu.iq
Received on 01/07/2024 Accepted on 10/9/2024 Published on 15/6/ 2025

Abstract

A field experiment was conducted in Al-Muthanna Governorate, between latitudes 31.368–475°N and longitudes 45.259–249°E, during the 2024-2025 winter season, to study the effect of adding bioinotropic inoculants of *T. thioparus* and *A. brasilense* and agricultural sulfur levels on NPK and sulfate concentrations in the soil. The experiment included two factors: The first factor was the addition of the bioinoculation, with four treatments (B0: no addition, B1: *T. thioparus*, B2: *A. brasilense*, B3: two-bacterial interference). The second factor was sulfur levels (C0: no addition, C1: 1000 kg ha^{-1}, C2: 2000 kg ha^{-1}, C3: 3000 kg ha^{-1}). The results showed successful isolation of the target bacteria. Treatment B3 recorded the highest increase in soil NPK and sulfate concentrations compared to the other treatments. The addition of sulfur contributed to improving soil nutrient content, with treatment C3 showing superior NPK and sulfate availability in the soil. The interaction between the bioinopolymer and sulfur levels resulted in significant differences in soil NPK and sulfate concentrations.

Keywords: Bio-inoculation, *Thiobacillus thioparus*, *Azospirillum brasilense*, agricultural sulfur,NPK,sulfates..

Introduction

One of the most prominent problems facing agricultural lands in Iraq is the scarcity of essential nutrients.This deficiency leads to a significant decline in soil fertility, to compensate for this lack of nutrients, excessive and unbalanced use of chemical fertilizers is sometimes resorted, these fertilizers have a negative impact on water and soil. Therefore, many researchers around the world have sought solutions to mitigate these challenges, by the development of long-term strategic plans and visions aimed at achieving sustainable agriculture and increasing and improving agricultural production (Ali and AlMousawe, 2023).

Microorganisms play a vital role in soil health when suitable environmental conditions are present. They contribute to the bio-oxidation of sulfur. *Thiobacillus thioparus* is one of the most important beneficial bacteria in this process. It colonizes the root zone and oxidizes inorganic sulfur into sulfate ions that can be absorbed by plants (Mathilde *et al*., 2019; Al Salman and Al-Gharawi, 2019). Autotrophic bacteria of the genus *Thiobacillus spp*. are also among the most prominent organisms responsible for this process, converting sulfur into sulfuric acid. This leads to a decrease in soil pH, thus increasing nutrient availability and improving alkaline soil properties. Sulfur acts as a chemical improver for the soil (Valdebentio-Rolack *et al*., 2011; AL-Barakat *et al*., 2018).

Excessive use of chemical fertilizers causes nitrogen loss through leaching or volatilization as gases, leading to environmental pollution. Therefore, using biofertilizers containing nitrogen-fixing bacteria such as Azospirillum is an effective solution. These bacteria work to fix atmospheric nitrogen in the soil in conjunction with plant roots (Patra *et al*., 2017; Asghari *et al*., 2020).

Iraqi soils are of the calcareous type, containing high percentages of calcium carbonate ranging from 10% to 45%. Their pH tends towards alkalinity, negatively affecting the availability of essential nutrients for plant growth. Therefore, recent studies have focused on using soil amendments with an acidic effect, such as agricultural sulfur, to mitigate the problems of calcareous soils and increase the soil's ability to provide essential nutrients to plants, leading to improved growth and increased productivity (Noni and Abd, 2021; Sawadi, and Noni,2023).

The availability of NPK and sulfates in the soil is an important indicator of its fertility. Nitrogen is one of the most mobile and variable elements, influenced by microbial activity and organic decomposition processes. Phosphorus is affected by soil pH and its ability to bind to calcium or iron. Potassium is bound to the structure of clay minerals and becomes available through biological activity and root growth. Sulfates represent the available form of sulfur for plants. They are primarily formed by the oxidation of sulfur by oxidizing bacteria (Havlin *et al*., 2014).

Based on the above, the current study aims to demonstrate the effect of adding bioinotropics of *Thiobacillus thioparus* and *Azospirillum brasilense* bacteria and levels of agricultural sulfur on the availability of NPK and sulfates in the soil.

Material and methods

Laboratory Experiments:

Seven soil samples were collected from the rhizosphere region of various locations in the Muthanna Governorate for the purpose of isolating and identifying *Thiobacillus thioparus* and *Azospirillum brasilense* bacteria. The samples were stored in special bags until use.

Isolation and Purification of Azospermum Bacteria:

Azoospermum bacteria were isolated from the soil adjacent to plant roots after being immersed with the roots in a container containing 90 ml of sterile water. A series of dilutions was then made according to

(Baldani and Dobereiner 1980). 1 ml of the dilutions (10-5, 10-7) was transferred to tubes containing 9 ml of nitrogen-free culture medium (N.F.B.), autoclaved at 121°C for 20 minutes and a pressure of 15 psi. Then, they were incubated for 48 hours at 30°C according to Krieg and Dobereiner (1984). The color of the medium changed from green to blue, with the appearance of a white pellicle, indicating Azospermum growth. Three successive transfers were then made to the same media. The bacteria were purified by transferring the visible growth using a transfer loop onto media, Congo red staining was added to the (RC). The scarlet-colored colonies were transferred to the same medium to ensure purity and observe the colony shapes. The isolates were then stored on agar for diagnostic testing (Baldani and Dobereiner, 1980).

Isolation and Identification of Thiobacillus Bacteria:

Soil samples collected from the above-mentioned agricultural areas were placed in 1 kg anvils. 50 gm of agricultural sulfur was added to activate *Thiobacillus* bacteria. The samples were left for 15 days, with irrigation to maintain soil moisture. A series of tenfold dilutions of the soil samples was prepared by adding 10 g of each soil sample to 90 ml of sterile water in a 250 ml beaker. The samples were mixed well, and then serial dilutions (10-1-10-7) were performed by transferring 1 ml of the soil suspension to a test tube containing 9 ml of sterile water. The process was repeated until the 10-7 dilution was reached. 1 ml of each dilution was taken and inoculated into test tubes containing 9 ml of liquid *Thiobacillus* media, with three replicates for each dilution. The tubes were incubated aerobically at 28°C for 10 days. 25 days. The appearance of sediments on the surface of the medium, ring-shaped growth, complete or incomplete film-like growth, sediments on the walls of the tubes, turbidity, the precipitation of sulfur particles, and a decrease in the pH of the medium, positive indicators of the growth of sulfur-oxidizing bacteria (Vidyalakshmi and Sridar, 2007). 1.0 ml of the tubes that showed growth were taken, and spread on the surface of a Petri dish containing solid media. The plates were incubated at 28°C for 2-3 days. The plates were re-stained using the staining method to obtain pure colonies of bacteria. The colonies were then obtained with a yellow or yellowish-white color. These colonies were isolated in pure form on solid media until the rest of the diagnostic tests were completed. They were coded and numbered according to the region from which they were isolated.

Field Experiment:

The experiment was conducted according to a randomized complete block design (RCBD) after perpendicular tillage. Three blocks were placed 1.5 m apart. Each block contained 16 experimental units, each measuring 4 m (2 m x 2 m). The distance between experimental units was 1 m, and the number of experimental units in the experiment was 48. Agricultural sulfur fertilizer was added to the experimental units as a percentage of volume. Seeds inoculated with the bio-inoculum of the identified bacteria were planted according to the treatments in rows, 10 cm apart. The number of rows was 8, with a seed rate of 100 kg ha^{-1} (Guidance Bulletin, 2012). The land was planted on November 30, 2024.

Fertilizer was added according to the fertilizer recommendation of 120 kg N ha^{-1} (Kharbit and Hashem, 2017). Phosphate and potassium fertilizer were added according to the full recommendation before planting. As for nitrogen fertilizer, it was added at half the recommendation and part in two batches, the first (25%) was added two weeks after germination (Morsi and Abdel-Gawad, 1967) after germination and the second half (25%) at the branching stage.

Experimental Factors:

The factorial experiment included two factors:

Factor 1: Fertilization with a bio-inoculum of *T. thioparus* and *A. brasilense*, comprising four levels. **B0:** No inoculum, **B1:** *Thiobacillus thioparus*, **B2:** *Azospirillum brasilense,* **B4:** *T. thioparus* and A. *brasilense*.

Factor 2: Contains four levels of 4 levels of agricultural sulfur concentrations. **C0:** No inoculum, **C1:** First concentration of agricultural sulfur 1000 kg ha^{-1}. **C2:** Second concentration of agricultural sulfur 2000 kg ha^{-1}. **C3:** Third concentration of agricultural sulfur 3000 kg ha^{-1}.

The studied characteristics

Soil nitrogen after harvest: The amount of available nitrogen was estimated using the Micro Kieldahl apparatus and the method used by Bremner (Page *et al*., 1982).

Soil phosphorus after harvest: The amount of phosphorus in the soil was estimated using the Olsen method (Page *et al*., 1982).

Soil potassium after harvest: Potassium was extracted by means of ammonium acetate (1N NH_4 OAC) pH 7 and estimated using a photometer flame (Black, 1965).

Post-harvest soil sulfate: It was determined by extracting SO4-S with a 0.15% $CaC_{l2}.2H_2O$ solution (Williams and Steinbergs, 1959). The sulfate concentration in the extracts was then measured by turbidity using barium chloride ($BaC_{l2}.2H_2O$). A Libra S5 biochrome spectrophotometer was used at a wavelength of 470 nm.

Table 1: Soil analysis before planting.

Items	Value	Unit
ECe	5.40	dsm^{-1}
pH	7.60	---
organic matter	0.60	%
Available nitrogen	18.40	mg kg^{-1}
Available phosphorus	14.30	
Available potassium	159.83	
Soluble calcium	12.00	Mmol L^{-1}
Soluble magnesium	5.40	
Soluble sodium	21.30	
Carbonates	Nill	
Bicarbonates	12.50	
Sulfates	35.60	
Chloride	17.00	
Bulk Density	1.80	gm cm^{3}

True Density	2.50	
Porosity	32.00	
Sand	193.40	gm kg^{-1} soil
Clay	241.40	
Silt	559.80	
Soil texture	Silty loam	
Total bacterial counts	4.90×10^6	CFU gm^{-1} dry soil
Azoospermum counts	3.40×10^4	
Thiobacillus counts	0.642×10^3	

Results and Discussion:

Identification of Isolates of Species belonging to the genus Azospirillum brasilense:

After performing microscopic, morphological, and biochemical culture tests on the studied isolates, ten bacterial isolates were obtained. These isolates were characterized by the formation of white ring-shaped growths 1-1.5 cm below the surface of nitrogen-free semi-solid Nfb culture media. In addition, the medium changed color from green to blue. This change was due to ammonia formation during nitrogen fixation. The bacteria were characterized as curved rods, Gram-negative, with convex, shiny colonies with smooth or toothed edges. They were non-sticky and red when grown on RC medium containing Congo stain. Based on the aforementioned characteristics and what has been mentioned in scientific studies and sources, these characteristics belong exclusively to the genus Azospirillum.

Azospirillum isolates were divided into their respective species based on the special keys for differentiating between species provided in Dobereiner and Day (1976); Tarrand *et al*. (1978); Lamm and Neyra (1981); Krieg and Dobereiner (1984); Reinhold *et al.* (1987); Khammas *et al*. (1989); and Holt *et al*. (1994) from a study of some morphological characteristics, the growth capacity of these isolates in some differentiating culture media, their biochemical characteristics, and their ability to consume certain carbohydrates as carbon sources (Table 2), these characteristics were compared with those of the species belonging to the genus *Azospirillum*. It can be concluded that the isolates belong to the species *A. brasilense*.

Table 2: Results of some differential biochemical tests for *Azospirillum brasilense* bacteria.

Tests	Symbol of isolation						
	AB1	AB2	AB3	AB4	AB5	AB6	AB7
Gram stain test	_	_	_	_	_	_	_
Oxidase enzyme test	+	+	+	+	+	+	+
Movement test	+	+	+	+	+	+	+
Catalase enzyme test	+	+	+	+	+	+	+
Methyl Red test	-	+	-	-	-	-	+
Nitrate reduction test	+	-	+	+	+	-	+
Starch hydrolysis test	+	+	+	+	+	+	+
Gelatin hydrolysis test	+	+	+	+	+	+	+

Voges proskauer test		+	-	+	+	+	+	-
Citrate Vtilization test		+	-	+	+	-	-	-
Urease test		-	-	-	-	-	+	-
pectin hydrolysis		+	-	+	+	-	+	-
Biotin need		-	+	-	-	-	-	+
Use of carbon sources	**Glucose**	-	+	-	-	-	-	-
	Lactos	-	+	-	-	-	-	+
	Rhamnos	-	+	+	-	-	-	-
	Maltose	-	-	+	-	-	-	+
Nacl	**%3**	+	-	+	+	+	+	+
Indole Production Test		+	-	+	+	+	-	-

(-) No growth (+) There is growth

Isolation and Identification of Thiobacillus thioparus:

Table (3) shows some of the cultural and microscopic characteristics of the bacterial isolates isolated from the soil and rhizosphere of some field crops. Most of the colonies of these bacterial isolates were smooth, medium to large in size, with a convex surface, circular, and yellowish-white in color. The yellowish-white pigment was clearly visible over time. As for the results of examining the Gram-stained slides, the cells were Gram-negative, rod-shaped, or spherical, and motile. They formed deposits on the surface of the liquid Starkey (sulfur) and thiosulfate culture medium, and showed ring growth, complete or incomplete film-like growth, deposits on the walls of the test tubes, or turbidity in the medium. The bacterial isolates were identified using some of the biochemical tests shown (Table 3). All isolates yielded positive results for the catalase test and negative results for the thiosulfate test. For the oxidase test, the isolates also showed a negative result for the Fox-Proskauer test. The isolates produced a positive result for the methyl red test, except for isolate AB2, which produced a negative result. The isolates demonstrated an inability to consume citrate, produce gelatinase, produce indole, or reduce nitrate. In addition, they were unable to hydrolyze starch, and demonstrated motility. The results also showed the bacteria's inability to produce the urease enzyme, except for isolate AB2, which produced a positive result. All isolates grew in sulfur and thiosulfate media at 32°C.

Table 3: Results of biochemical tests for all *Thiobacillus thioparus* isolates.

Tests	**Symbol of isolation**						
	AB1	**AB2**	**AB3**	**AB4**	**AB5**	**AB6**	**AB7**
Gram stain test	-	-	-	-	-	-	-
Oxidase enzyme test	-	-	-	-	-	-	-
Movement test	+	+	+	+	+	+	+
Catalase enzyme test	+	+	+	+	+	+	+
Methyl Red test	+	-	+	+	+	+	+
Nitrate reduction test	-	-	-	-	-	+	-
Starch hydrolysis test	-	-	-	-	-	-	-
Gelatin hydrolysis test	-	-	-	-	-	-	-
Voges proskauer test	-	+	-	-	-	-	-
Citrate Vtilization test	-	-	-	-	-	-	-

Urease test	-	-	-	-	-	-	+
Indole Production Test	-	-	-	-	-	-	-
Growth at 32°C	+	+	+	+	+	+	+

(-) No growth (+) There is growth

Field Experiment:

Soil Available Nitrogen Concentration (mg kg soil^{-1}):

Table (4) shows significant differences in soil available nitrogen concentration after harvest when inoculated with *T. thioparus*. and *A. brasilense*. The highest value of available nitrogen was recorded in treatment B3, with a maximum average of 34.80 mg/kg soil, representing a 125.53% increase. This is compared to the control treatment, which yielded the lowest average of 15.43 mg kg soil^{-1}. This may be attributed to the role of *A. brasilense* bacteria, which improves the chemical and physical properties of the soil, as well as its ability to perform atmospheric nitrogen fixation, and its secretion of organic compounds, which lowers soil pH, significantly contributing to an increase in soil available nitrogen concentration (Martin, 2022).

The addition of agricultural sulfur at different levels resulted in a significant difference in soil nitrogen content. Treatment C3 was superior, yielding the highest average of 34.80 mg kg soil^{-1}, representing a 28.77% increase. This is in comparison to the control treatment, which yielded the lowest average of 22.17 mg kg soil^{-1}. This may be attributed to the role of agricultural sulfur, which increases soil nitrogen availability. This is due to sulfur oxidation and pH reduction, which improves the solubility of nitrogen compounds. Furthermore, it activates microorganisms and increases nitrogen mineralization rates, converting it into readily absorbable mineral forms (Zhao *et al*., 2021).

The results indicated significant differences in the bilateral interaction between the bio-inoculant treatments and the levels of agricultural sulfur added to the soil. The B3C3 combination yielded the highest average of 46.62 mg kg soil^{-1}, compared to the control treatment, which yielded the lowest average of 14.73 mg kg soil^{-1}. This may be attributed to the interaction between the addition of agricultural sulfur and the bio-inoculant to the soil. This interaction increases the concentration of available nitrogen in the soil. Sulfur helps to lower pH and improve nutrient solubility. Meanwhile, nitrogen-fixing and degrading bacteria facilitate the mineralization process and the conversion of nitrogen into readily absorbable mineral forms. This results in an increase in the soil's available nitrogen content (Bashan and de-Bashan, 2010; Al-Omran *et al*., 2018; Jabbar, 2020).

Table 4: Effect of adding bio-inoculum of *T. thioparus* and *A. brasilense* bacteria and levels of agricultural sulfur on Soil Available Nitrogen Concentration (mg kg soil^{-1}).

Agricultural sulfur	Bio-inoculum				Mean
	B0	B1	B2	B3	
C0	15.28	14.73	16.33	15.38	22.17
C1	22.53	23.42	28.30	25.05	24.17

C2	26.98	27.13	26.52	27.13	27.12
C3	23.87	31.42	27.32	46.62	28.55
Mean	15.43	24.82	26.94	34.80	
L.S.D$_{0.05}$	**C**		**B**		**BC**
	2.398		2.398		4.795

Soil phosphorus concentration (mg kg soil^{-1}):

Table (5) shows a significant effect of adding the bioinotropic inoculant of *T. thioparus* and *A. brasilense* bacteria, along with agricultural sulfur levels, on increasing soil phosphorus concentration (mg kg soil^{-1}). Treatment B3 outperformed treatment B0, with an average phosphorus concentration of 25.19 mg kg soil^{-1}, representing a 61.26% increase compared to the control treatment of 15.62 mg kg soil^{-1}. This may be attributed to the role of *A. brasilense* bacteria in increasing the availability and solubility of phosphorus in the soil. This is achieved through the secretion of organic acids, which lower soil pH. These results are consistent with Wiggins *et al*. (2022).

Significant differences were found in the levels of agricultural sulfur application, with treatment C3 showing the highest results. It yielded the highest average of 25.85 mg kg soil^{-1}, representing a 55.62% increase compared to the control treatment, which averaged 16.61 mg kg soil^{-1}. The increase in available phosphorus in the soil due to higher sulfur levels may be attributed to the bio-oxidation of sulfur by certain microorganisms and the formation of sulfuric acid. This acid contributes to the dissolution of some phosphorus-containing compounds. Furthermore, the released hydrogen ions play a role in lowering the soil pH, thus increasing phosphorus availability (Saloum and Ali, 2011; Hassan and Al-Barkat, 2023).

The results showed no significant differences in the average concentration of available phosphorus in the soil when using a dual inoculant of *T. thioparus* and *A. brasilense* bacteria and levels of agricultural sulfur in the percentage of available phosphorus in the soil.

Table 5: Effect of adding bio-inoculum of *T. thioparus* and *A. brasilense* bacteria and levels of agricultural sulfur on Soil phosphorus concentration (mg kg soil^{-1}).

Agricultural sulfur	Bio-inoculum				Mean
	B0	**B1**	**B2**	**B3**	
C0	11.43	13.03	11.43	26.57	16.61
C1	13.63	18.30	17.82	22.08	19.75
C2	21.58	25.91	22.90	22.69	19.83
C3	19.78	21.77	27.16	32.06	25.85
Mean	15.62	17.96	23.27	25.19	
L.S.D$_{0.05}$	**C**		**B**		**BC**
	5.108		5.108		N.S

Soil Potassium Concentration (mg kg soil^{-1}):

Table (6) shows the effect of adding bioinotropes of *T. thioparus* and *A. brasilense*, along with agricultural sulfur levels, on soil potassium concentration (mg kg soil^{-1}). Significant differences were found between the bioinotrope treatments. Treatment B3 outperformed treatment B0, with an average potassium concentration of 205.2 mg kg soil^{-1}, representing a 37.62% increase. This is compared to the control treatment, which recorded 149.1 mg kg soil^{-1}. This may be attributed to the fact that adding bioinotropes such as *A. brasilense* or *T. thioparus*. These microorganisms improve soil fertility through various mechanisms, including the secretion of organic acids and plant growth regulators that increase the solubility of minerals, including potassium. In addition, they increase root growth and absorption efficiency (Bashan and de-Bashan, 2010). In addition, the use of bio-inoculation in calcareous soils contributed to a relative reduction in pH and an increase in the concentration of available nutrients, including potassium, as a result of the metabolic activity of the bacteria (Abdullah *et al*., 2020).

The results showed no significant effect of fertilization with agricultural sulfur levels and a binary interaction between the bioinoculation of *T. thioparus* and *A. brasilense* bacteria and agricultural sulfur levels on soil potassium concentration.

Table 6: Effect of adding bio-inoculum of *T. thioparus* and *A. brasilense* bacteria and levels of agricultural sulfur on Soil Potassium Concentration (mg kg soil^{-1}).

Agricultural sulfur	Bio-inoculum				Mean
	B0	B1	B2	B3	
C0	146.4	157.9	145.9	146.4	173.0
C1	168.8	165.9	168.0	164.6	172.0
C2	167.5	172.2	169.8	183.6	170.1
C3	209.4	191.8	196.6	223.0	179.4
Mean	149.1	166.8	173.3	205.2	
$L.S.D_{0.05}$	C		B		BC
	N.S		12.59		N.S

Soil sulfate concentration (mg kg soil^{-1}):

Table (7) shows significant differences in the effect of bioinoculation with *T. thioparus* and A. brasilense on soil sulfate concentration (mg kg soil^{-1}). Treatment B3 was superior, yielding the highest average concentration of 13.73 mg kg soil^{-1}, representing a 70.34% increase. This is in contrast to the control treatment, which yielded the lowest average concentration of 8.06 mg kg soil^{-1}. This difference may be attributed to the fact that the bacterial bioinoculation containing Thiobacillus bacteria, reduces soil pH, thereby increasing oxidation and sulfuric acid production (Besharati *et al*., 2007).

The addition of agricultural sulfur fertilizer to the soil resulted in the superiority of the C3 treatment. The application of 4000 kg ha^{-1} yielded the highest average of 13.34 mg kg soil^{-1}. This may be attributed to the fact that the addition of sulfur, combined with favorable environmental conditions, increased the population of sulfur-oxidizing bacteria of the species *T. thioparus*. These bacteria released a greater quantity of sulfates.

Significant differences between the dual interaction of bioinoculation with *T. thioparus* and *A. brasilense* bacteria and agricultural sulfur fertilization of the soil. The interaction level was higher between treatment B3 and agricultural sulfur treatment C3, which yielded the highest average of 14.39 mg kg soil^{-1}. This was in comparison with treatment B1C0, which yielded the lowest average of 4.12 mg kg soil^{-1}. This may be attributed to the fact that the interaction between the addition of agricultural sulfur and the bioinoculation, led to an increase in available sulfates in the soil, due to enhanced activity of sulfur-oxidizing bacteria and increased decomposition of organic matter, as well as a reduction in pH, which facilitated the solubility of sulfates and their conversion into a form readily available for absorption (Subbarao, 2014; Al-Omran *et al*., 2018).

Table 7: Effect of adding bio-inoculum of *T. thioparus* and *A. brasilense* bacteria and levels of agricultural sulfur on Soil sulfate concentration (mg kg soil^{-1}).

Agricultural sulfur	Bio-inoculum				Mean
	B0	B1	B2	B3	
C0	5.26	4.12	10.73	12.12	8.08
C1	6.92	11.71	13.15	8.65	9.79
C2	8.58	12.23	13.14	14.39	12.77
C3	11.55	11.10	14.07	18.20	13.34
Mean	8.06	10.11	12.09	13.73	
$L.S.D_{0.05}$	C		B		BC
	1.675		1.675		3.349

References:

1. Abdullah, S.H., M.A. Ahmed and A.R. Mustafa. (2020). Effect of bacterial inoculation and the addition of agricultural sulfur on soil fertility and wheat productivity in calcareous soils. Basrah Journal of Agricultural Sciences, 18(2), 45-56.
2. Al Salman, N.T.Sh., and Al-Gharawi. J.K.M., 2019. Effect of Eucalyptus leaves water extract on some productive traits of broilers. Plant Archives Vol. 19, Supplement 1, pp. 920-923.
3. AL-Barakat, H.N.K., ALshujairy, Q.A.T. and AL-Hedny S. 2018. Effect of Bio-fertilization and the addition of humic and fulvic acid in availability of phosphorus, some minor elements in soil and growth of white maize plant, sorghum bicolor L. Plant Archives Vol. 18 No. 2, 2018 pp. 2777-2785.
4. Ali, A.F.A. and I.I.H. AlMousawe (2023). Problems of agricultural land uses for plant production in AlQasim district. Ibn Khaldoun Journal for Studies and Researches, 3(8): 171-197.
5. Al-Omran, A.M., Al-Wabel, M.I. and El-Maghraby, S.E. (2018). Influence of elemental sulfur on soil properties and nutrient availability in calcareous soils. Arab Gulf Journal of Scientific Research, 36(2), 45-55.
6. Asghari, B., Khademian R., and Sedaghati B., 2020. Plant growth promoting rhizobacteria (PGPR) confer drought resistance and

stimulate biosynthesis of secondary metabolites in pennyroyal (*Mentha pulegium* L.) under water shortage condition. journal Elsevier. Scientia Horticulturae.263: 109-132.
7. Baldani, V.L.D., and Dobereiner, J., 1980. Host- Plant specificity in the infection of cerealy with Azospirillum spp. Soil Biol. Biochem. 12 : 433-439.
8. Bashan, Y. and de-Bashan, L.E. (2010). How the plant growth-promoting bacterium Azospirillum promotes plant growth—a critical assessment. Advances in Agronomy, 108, 77-136.
9. Besharati, R., M. Hussein and A. Karim. (2007). Study on the effect of microbial and chemical factors on soil fertility. Iranian Journal of Agricultural Sciences, 15(3), 120-130.
10. Black, C.A. (1965). Methods of soil analysis. Part 2. Chemical and Microbiological properties. Am. Soc. Agron., Inc. Madison, Wisconson, USA.
11. Dobereiner, J. and Day, J. (1976). Associative Symbiosis in tropical grasses. characterization of Microorganisms and dinitrogen. fixing sites. In: Newton, W.E., and Nymans, C.J. cedsj. Proceeding of the Ist international symposium on nitrogen fixation. Vol.2.pp.518-538.
12. Guidance Bulletin. 2012. Agricultural Extension Department. Pages 36. Baghdad, Iraq.
13. Hassan, T. and Al-Barkat H.N.K. 2023. Effect of Agricultural Polymer and Levels of NPK on some Chemical Traits and Availability of some Nutrients, Growth and Yield of Wheat (Triticum aestivum L.). IOP Conf. Series: Earth and Environmental Science 1158 (2023) 022016.
14. Havlin J.L., Beaton J.D., Tisdale S.L., and Nelson W.L., 2005. Soil Fertility and Fertilizers An introduction to Nutrient Management 7th Edition. Prentice Hall. New Jersey.
15. Holt, J., Krieg, N.R., Sneath, P.H.A., Staley, J.T. and Williams, S.T., 1994. Bergey's manual determinative bacteriology. 9th Ed . Williams and Wilkins, USA.
16. Jabbar, M. (2020). Effect of agricultural sulfur levels on wheat growth and yield under field conditions. Iraqi Journal of Agricultural Sciences, 51(3), 215–226.
17. Khammas, K.M., Ageron E., Grimont P.A.D., and Kaiser P., 1989. Azospirillum irakense sp. Nov., A nitrogen-fixing bacterium associated with rice roots and rhizosphere. soil. Res. Microbiol., 140: 679-693.
18. Kharbit, H.K., and Hashim, K.I., 2017. Forage Crops Book. Ministry of Higher Education and Scientific Research. University of Baghdad. College of Agriculture. Number of pages: 298.
19. Krieg, N.R. and Dobereiner J., 1984. Genus Azospirillum in: Krieg, N.R. and Holt, J.G. (eds.). Beery's Manual of systematic bacteriology, vol. I. pp, 94-104. William and Wilkins, Baltimore London.
20. Lamm, R., and Neyra, C.A., 1984. Differentiation of Azospirillum species by polyacrylamide gel

electrophoresis of cellular proteins. Canadian Journal of Microbiology, 30, 958–967.
21. Martin, K. 2022. Effect of sulfur application on growth and productivity traits of wheat. Agronomy, 15(9), 1012.
22. Mathilde, M., 2019. Edith Joseph, in Advances in Applied Microbiology.
23. Morsi, M.A. and Abdel-Gawad A.A., 1967. Field Crops. Part Two: Field Crops Cultivation, Anglo-Egyptian Library, Cairo, Egypt, pp. 468-498.
24. Noni, G.B. and Abd A.H. 2021. The Effect of Efficacy Local Isolates of Paenibacillus Bacteria on Available Soil Phosphorus and Growth of Cultivars Wheat (*Triticum Aestivum* L.). IOP Conf. Series: Earth and Environmental Science 923 (2021) 012073.
25.
26. Page, A.L.; R.H. Miller and D.R. Keeney. (1982). Methods of soil analysis. Part (2) 2nd .ed. Agrnomy series 9.
27. Patra, A.K., Mishra, K.N., Garnayak L.M., and Mohanty A.K., 2017. Influence of long-term organic nutrient management on soil quality and crop productivity in rice (Oryza sativa)-potato (Solanum tuberosum L.)-okra (Abelmoschus esculentus) cropping system under irrigated condition. Indian Journal of Agronomy, 62: 268-274.
28. Reinhold, B., Hurek T., Fendrick I., Pot B., Gillis M., Kersters K., Sthielemans R., and Deley J., 1987. Azospirillum halopraeferens sp.nov.of Kallar grass (leptochloa fusca L.Kunth). Int. J. Sys. Bactriol., 37: 43-51.
29. Saloum, M.O. and S.Z. Ali. 2011. Effect of agricultural sulfur levels on phosphorus availability and growth of cauliflower (*Brassica oleracea* L.) under surface and drip irrigation conditions. Diyala Journal of Agricultural Sciences - 3(2): 457-467.
30. Sawadi, S.Z. and Noni G.B. 2023. Effect of Azospirillum brasilense Addition and Organic Matter on Irrigation Efficiency, NPK Availability and Wheat Yield (Triticum aestivum L.). IOP Conf. Series: Earth and Environmental Science 1158 (2023) 022029.
31. Subbarao, G.V. (2014). Role of sulfur in crop production and soil microorganisms. Advances in Agronomy, 123, 153–169.
32. Tarrand, J.J., Krieg N.R., and Dobereiner J., 1978. Ataxonomic study of the Azospirillum lipoferum group with description of anew genus, gen. nov. and two species, *Azospirillum Lipoferum* (Beijerink) comb. Nov. and *Azospirillum brasilense* sp. Nov, can.J. microbiol. (24): 967-980.
33. Valdebentio-Rolack E.H., T.C. Aaya, L.E. Abarzua, N.M. Ruiz-Tagle, K.E. Sossa, G.E. Aroca and H.E. Urrutia. 2011. Thiosulphate oxidation by Thiobacillus thioparus and Halothiobacillus neapolitanus strains isolated from the Petrochemical industry. Electro. J. Biotechno. Chile Univ. 14(1):Fulltext-10.
34. Vidyalakshmi, R., and Sridar R., 20007. Isolation and characterization of sulphur

oxidizing bacteria. Microbiological Research, 162 (2): 139-153.

35. Wiggins, A., Smith, B., and Johnson, C. (2022). Title of the study or article. Journal Name, Volume(Issue), page range.
36. Williams, C.H. and A. Steinbergs. 1959. Soil sulphur fractions as chemical indices of available sulphur in some Australian soils. Aust. J. Agric. Res. 10: 340-352.
37. Zhao, F.J., McGrath, S.P., and Blake-Kalff, M.M.A., 2021. Crop responses to sulphur fertilization in Europe. Soil Use and Management, 37(1), 1–12.