Role of agricultural sulfur, *Thiobacillus* bacteria and nano-zinc in the availability of some microelements in the soil and some qualitative traits of maize

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Abstract

A field experiment was conducted in one of the fields of Ibn Al-Bitar Vocational Preparatory school in Al-Husseiniyah district in the Holy governorate of Kerbala for the spring season of 2022 according to a randomized complete block design with three replications (R.C.B.D). The experiment included three factors. The first included three levels of agricultural sulfur (0, 1500 and 3000 kg ha^{-1}), symbolized by (S0, S1 and S2, respectively). The second factor included two levels of *thiobacillus* bacteria: the control treatment (no addition) and the treatment with bacteria, symbolized by (T0 and T1). The third factor included three concentrations of nano-zinc (0, 50 and 100 mg L^{-1}), symbolized by (Zn0, Zn1 and Zn2, respectively). The results of the experiment showed that the addition of sulfur had a significant effect, as the level of 3000 kg ha^{-1} was superior in the concentration of iron and zinc in the soil, protein, carbohydrates and sulfur in the grains, with averages of (4.32 and 2.88 mg kg^{-1}, 10.79 and 76.38 and 0.272% respectively). Also, the treatment with Thiobacillus bacteria showed superiority in the concentration of zinc in the soil and the concentration of protein and sulfur in the grains with averages of (2.55 mg kg^{-1}, 10.25 and 0.248%). As for nano zinc, the third concentration (100 mg L^{-1}) was superior in the concentration of protein, carbohydrates and sulfur in the grains with averages of (10.15, 74.00 and 0.239%). It was also observed that some interactions were significant in most of the traits studied.

Keywords: Maize, Agricultural sulfur, *Thiobacillus* bacteria, nano-zinc

Introduction

Maize (*Zea mays* L.) is one of the most important economic grain crops globally, belonging to the Poaceae family. It ranks third after wheat and rice, serving as a primary food source for more than one-third of the world's population. The crop is cultivated for its high nutritional value for both humans and animals. It is a major source of carbohydrates and also contains moderate amounts of oil, protein, essential amino acids, healthy fats, various vitamins, and cellulose, additionally, it includes some essential minerals such as phosphorus, magnesium, and potassium, making it an indispensable commodity [1].

Maize is considered one of the most soil-exhausting crops, requiring a comprehensive fertilization program and the essential nutrients necessary to ensure its growth and productivity [2]. Many experts have explained that soils in arid and semi-arid regions, including Iraqi soils, often contain high levels of calcium carbonate, which may negatively impact the availability of nutrients to plants [3]. Therefore, precise scientific methods must be followed to increase the availability of nutrients in the soil, which are important in increasing the physiological processes of plants and thus increasing the improvement of their growth and development, since plants cannot complete their life cycle naturally without them. Among these methods is adding agricultural sulfur to the soil, as it is one of the common methods for improving soil properties [4]. This is due to the diversity of organic and inorganic sulfur forms, and the multiplicity of microorganisms in the soil, which primarily encourage the transformation of organic sulfur forms into minerals ready for plant absorption. When the appropriate conditions of nutrients, ventilation and temperature are available, microorganisms play a decisive role in the biological oxidation of sulfur in the soil, including autotrophic chemolithic bacteria of the genus *Thiobacillus*. Sulfur is transformed by these organisms, with the availability of moisture and sufficient time, into sulfuric acid (H_2SO_4). The latter, in turn, works to reduce the pH in basic soil, which contributes to improving soil properties. This, in turn, contributes to increasing the readiness and efficiency of essential nutrients, especially the minor ones, in the soil [5]. In a study of three levels of sulfur fertilizers (0, 20 and 40 kg S ha^{-1}), the 40 kg S ha^{-1} level recorded the highest average protein content in grains, reaching 10.97%, compared to the control treatment, which recorded 9.46% [6].

There has been increasing interest in foliar nutrition of plants in recent years, as it is one of the means that increase the productivity of the unit area, as the search for new sources of nutrition in spraying Micronutrients are among the essential elements for plant growth that it needs in small quantities compared to the basic elements it needs [7]. Including nano zinc, which affects the vital and physiological processes within the plant, as it is the basis for its growth and development and enters into the composition of many vital enzymes that affect the metabolic and growth processes within the plant [8]. Therefore, this study aimed to determine the best level of agricultural sulfur addition and its effect on the availability of some micronutrients in the soil, as well as to know the extent of the effect of inoculation with *T.thioparus* bacteria on sulfur oxidation processes and its effect on the qualitative characteristics of the maize crop, in addition to knowing the extent of the response to spraying with nano zinc and its effect on the qualitative characteristics of the maize crop.

Materials and Methods

A field experiment was conducted during the spring season of 2023 in one of the fields of Ibn Al-Bitar Vocational School in Al-Husseiniyah District, Kerbala, Iraq (Latitude: 32°N, Longitude: 44°E). The experiment included three factors: the first factor involved three levels of sulfur fertilizer, which were control, 1500 and 3000 kg ha^{-1}, denoted as (S0, S1 and S2) respectively. The second factor was the application or non-application of *Thiobacillus* bacteria, denoted as (T0 and T1) respectively. The third factor was nano zinc at three concentrations: distilled water (control, 50 mg L^{-1} and 100 mg L^{-1}, denoted as (Zn0, Zn1 and Zn2). The experiment was implemented according to a randomized complete block design (RCBD) with factorial experiments and three replications. The seeds were planted in holes on the lines, with a distance between each line (75 cm) and a distance between each hole (25 cm). Nitrogen fertilizer was added in the form of urea fertilizer at a rate of 100 kg ha^{-1} in two batches. Phosphate fertilizer was also added at a rate of 87.2 kg ha^{-1}, while potassium fertilizer was added in two batches at a rate of 66.4 kg ha^{-1}. All agricultural operations were carried out according to the recommendations approved by the Ministry of Agriculture.

Sample preparation

Plant samples were taken from the dry matter and seeds, dried and ground, so that they would be representative samples of the community, to carry out the digestion process according to the method of [9], where the sample is taken with a weight of 0.2 g, concentrated sulfuric acid is added to the sample in an amount of 3.5 cm^3 and then left for 24 hours, after which 1 cm^3 of perchloric acid is added, then the mixture is heated over low heat until the solution becomes clear and transparent, after which the sample is left for some time until it cools and is transferred quantitatively to a glass flask. The volume is completed to 50 cm^3.

Data recorded

The concentration of available iron and zinc in soil:

Soil samples were taken from the experimental units at 0-30 cm depth. The soil was air-dried, ground, and passed through a 2 mm sieve, and then stored in containers for the necessary analyses. The ions of these elements in the soil were determined using the method described by [10], This involved adding 20 mL of a DTPA extraction solution (0.005 M) with a pH of 7.3 to 10 g of soil, shaking for two hours, and then filtering. The elements were then quantified using an atomic absorption spectrophotometer Type (PG 990).

Protein concentration (%):

The protein concentration was calculated according to the method of [11], using the following equation:

Protein percentage % = Nitrogen percentage % x 6.25

Carbohydrate concentration (%):

The carbohydrate concentration was determined according to the method of [12], a 1 g sample of ground dry grains was taken, and 50 mL of boiling distilled water was added. The sample was then placed in a water bath for half an hour at a temperature of 80°C. Afterward, the sample was filtered, and the filtrate was diluted to 50 mL with distilled water. Then, 1 mL of 5% phenol reagent and 1 mL of the filtrate were mixed well. Subsequently, 5 mL of concentrated sulfuric acid was added, followed by the addition of 10 mL of distilled water for dilution. The intensity of the color was then measured using a spectrophotometer at a wavelength of 488 nm.

Sulfur concentration (%):

The sulfur concentration in the grains was estimated by adding 0.5% gum acacia, glacial acetic acid, and distilled water in a 1:1 ratio, followed by the addition of barium chloride ($BaCl_2.2H_2O$). The concentration was then measured using a spectrophotometer at a wavelength of 420 nm, according to the method described by [13].

Statistical analysis

The data were statistically analyzed using analysis of variance in the order of factorial experiments within the randomized complete block design (RCBD). The arithmetic means of the treatments were compared using the least significant difference at the probability level (0.05) and using the statistical program Genstate to determine the nature of the differences between the treatments [14].

Results and discussion

Available iron concentration in soil (mg kg^{-1} soil)

The results of Table 1 show that there is a significant effect when adding levels of agricultural sulfur (S) on the amount of available iron in the soil, as treatment S2 gave the highest value of 4.32 mg kg^{-1} soil, while the comparison treatment S0 (without fertilization) gave the lowest value of 2.85 mg kg^{-1} soil. The reason for the increase in the availability of iron in the soil is attributed to the presence of an acidic medium as a result of adding agricultural sulfur, forming sulfuric acid and releasing hydrogen ions [14].

It is also noted from the same table that there were no significant differences when inoculated with *T.thioparus* bacteria as well as when sprayed with nano zinc. The same table also shows that there were no significant differences in the binary interaction between sulfur and *T.thioparus* bacteria (S*T). While there were significant differences in the binary interaction between sulfur and nano zinc (S*Zn)), where the treatment S2Zn2 recorded the highest value, which amounted to 4.78 mg kg^{-1} soil, compared to the treatment S0Zn0, which gave the lowest value, which amounted to 2.76 mg kg^{-1} soil, and did not differ significantly from the treatments S0Zn1, S1Zn1 and S0Zn2. The results also showed that there was a non-significant binary interaction between *T.thioparus* bacteria and nano zinc (T*Zn). The same table also shows that there were no significant differences in the triple interaction coefficients for agricultural sulfur, *T.thioparus* bacteria and nano zinc (S*T*Zn).

Table (1): Effect of agricultural sulfur, inoculation with *T. thioparus* bacteria, nano zinc and their interaction on iron availability in soil (mg kg^{-1} soil)

Sulfur (S)	Nano-zinc (Zn)	*Thiobacillus* (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	2.64	2.84	2.76
	Zn 1	2.93	2.89	2.84
	Zn 2	2.84	2.95	2.94
S1	Zn 0	3.88	2.97	3.89
	Zn 1	2.84	3.91	2.89
	Zn 2	2.80	3.58	3.21
S2	Zn 0	3.92	3.94	3.92
	Zn 1	4.95	3.92	4.28
	Zn 2	4.62	4.62	4.78
				Means (S)
Interaction (S*T)	S0	2.80	2.89	2.85
	S1	3.23	3.43	3.33
	S2	4.27	4.38	4.32
				Means (Zn)
Interaction (Zn*T)	Zn0	3.48	3.57	3.52
	Zn1	3.25	3.42	3.33
	Zn2	3.57	3.71	3.64
Means (T)		3.43	3.57	

L.S.D$_{0.05}$						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.19	N.S	N.S	0.48	N.S	N.S	N.S

Available zinc concentration in soil (mg kg^{-1} soil)

The results of Table 2 indicated a significant effect when adding levels of agricultural sulfur (S), as treatment S2 gave the highest value, which amounted to 2.88 mg kg^{-1} soil, which did not differ significantly from treatment S1, while the comparison treatment S0 (without fertilization) gave the lowest value, which amounted to 1.54 mg kg^{-1} soil. This may be attributed to the fact that increasing sulfur levels reduced the degree of soil reaction and increased its acidity, which in turn increased the availability of trace elements, including zinc [16].

It is also noted from the same table that the inoculation treatment with *T.thioparus* bacteria gave the highest concentration of zinc in the soil, which amounted to 2.55 mg kg^{-1} soil, compared to the non-inoculation treatment T0, which gave the lowest concentration of zinc, which amounted to 2.27 mg kg^{-1} soil. This may be attributed to microbial activity through the use of *T.thioparus* in the soil, which increases the reaction of sulfur and its oxidation in the soil, leading to a decrease in the pH value of the soil and its reflection on the increased availability of trace elements, including zinc, and its increased absorption by the plant [15].

The results of the same table showed no significant effect at the levels of nano zinc (Zn) spraying on the concentration of available zinc in the soil. The same table also shows no significant differences in the binary interaction between sulfur and *T.thioparus* bacteria (S*T). The results also showed significant differences in the binary interaction between sulfur and nano zinc (S*Zn), as the treatment S2Zn2 recorded the highest value of available zinc, which amounted to 2.97 mg kg^{-1} soil, which did not differ significantly from the treatments S1Zn1, S1Zn0, S2Zn0, S2Zn1 and S1Zn2, compared to the treatment S0Zn0, which gave the lowest value of available zinc, which amounted to 1.14 mg kg^{-1} soil, which did not differ significantly from S0Zn1. There is a non-significant binary interaction between *T.thioparus* bacteria and nano zinc (T*Zn). The same table also shows that there were no significant differences in the triple interaction coefficients for agricultural sulfur, *T.thioparus* bacteria, and nano zinc (S*T*Zn).

Table (2): Effect of agricultural sulfur, *T. thioparus* inoculation, nano-zinc and their interaction on soil zinc availability (mg kg^{-1} soil).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	0.84	1.20	1.14
	Zn 1	2.03	1.44	1.41
	Zn 2	1.62	2.09	2.06
S1	Zn 0	2.91	2.83	2.89
	Zn 1	2.37	2.87	2.96
	Zn 2	3.10	2.84	2.60
S2	Zn 0	2.31	2.72	2.87
	Zn 1	3.25	3.43	2.80
	Zn 2	2.88	2.69	2.97
				Means (S)
Interaction (S*T)	S0	1.36	1.72	1.54
	S1	1.70	2.93	2.82
	S2	2.76	3.00	2.88
				Means (Zn)
Interaction (Zn*T)	Zn0	2.02	2.58	2.30
	Zn1	2.25	2.53	2.39
	Zn2	2.55	2.54	2.54

Means (T)			2.27	2.55		
L.S.D0.05						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.32	N.S	0.26	0.56	N.S	N.S	N.S

Protein concentration in grains (%)

The results presented in Table 3 show significant differences in the protein content of the grains. The treatment S2 resulted in the highest protein content of 10.79%, while the control treatment S0 (without fertilization) yielded the lowest protein content at 9.29%, with an increase of 16.14%. The increase in protein content in the grains with sulfur treatment S2 might be attributed to sulfur's role in balancing soil pH, which in turn enhances the availability of nutrients that are more accessible at a neutral pH, including nitrogen. This increased nitrogen availability in the soil leads to greater absorption by the plant and storage in the grains, alternatively the increase could be due to sulfur's positive effect on the formation of essential amino acids (cysteine, cystine and methionine) that are crucial for protein synthesis, thereby boosting its content in the grains [17].

As shown in the same table, the bacterial inoculation treatment T1 resulted in the highest percentage of protein content, of 10.25%, compared to the non-inoculated treatment T0, which had the lowest percentage of 9.71%, with an increase of 5.56%. This increase may be attributed to the role of the sulfur-oxidizing bacterium *T. thioparus*, which converts sulfur into its readily available form as sulfates. These sulfates are essential in the synthesis of protein precursors. Additionally, the bacteria play a role in releasing hydrogen ions, which enhances the availability of nutrients, including nitrogen, leading to its increased translocation to the grains, where it serves as the primary component of protein [18].

The results from the same table indicate that foliar application of nano zinc has a significant effect on protein content. The treatment Zn2 yielded the highest protein percentage, of 10.15%, compared to the non-sprayed treatment Zn0, which recorded the lowest percentage at 9.82%, with an increase of 3.36%. This increase might be due to zinc's role in enhancing nitrogen availability, which is a key indicator of increased activity of protein-synthesizing enzymes in the vegetative parts. These compounds are then translocated to the grains for storage [19]. Additionally, zinc is involved in the formation of proteins within the plant, contributing to the development of new tissues and cells [6].

The same table reveals significant differences in the interaction between sulfur and the bacteria (*S**T). The treatment S2T1 recorded the highest protein percentage of 11.12%, compared to the treatment S0T0, which had the lowest percentage of 9.00%. The results also show significant differences in the interaction between sulfur and nano zinc (*S**Zn), with the treatment S2Zn2 achieving the highest percentage of 11.12%, compared to the treatment S0Zn0, which had the lowest percentage of 9.19%. However, the interaction between *T. thioparus* bacteria and nano zinc (*T*Zn* interaction) was not significant. The table also highlights significant differences in the triple interaction among agricultural sulfur, *T. thioparus* bacterial inoculation, and nano zinc (*S**T*Zn), with the treatment S2T1Zn2 yielding the highest protein percentage of 11.60%, compared to the treatment S0T0Zn0, which recorded the lowest percentage at 8.87%.

Table (3): Effect of agricultural sulfur, inoculation with *T.thioparus* bacteria, nano zinc and their interaction on protein concentration in grains (%).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	8.87	9.52	9.19
	Zn 1	8.97	9.62	9.30
	Zn 2	9.16	9.60	9.38
S1	Zn 0	9.70	9.89	9.80
	Zn 1	9.66	10.02	9.84
	Zn 2	9.62	10.27	9.94
S2	Zn 0	10.18	10.75	10.46
	Zn 1	10.54	11.02	10.78
	Zn 2	10.64	11.60	11.12
				Means (S)
Interaction (S*T)	S0	9.00	9.58	9.29
	S1	9.66	10.06	9.86
	S2	10.45	11.12	10.79
				Means (Zn)
Interaction (Zn*T)	Zn0	9.59	10.05	9.82
	Zn1	9.72	10.22	9.97
	Zn2	9.81	10.49	10.15
Means (T)		9.71	10.25	

L.S.D0.05						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.096	0.096	0.078	0.166	0.136	N.S	0.236

Carbohydrate concentration in grains (%)

The results presented in Table 4 indicate a significant effect on the carbohydrate content in the grains. The S2 treatment produced the highest percentage of 76.38%, while the S0 treatment (without fertilization) resulted in the lowest percentage of 70.23%, representing an increase of 8.75%. This increase could be attributed to sulfur's role in enhancing the availability of nutrients in the soil solution and stimulating their absorption. Sulfur indirectly increases carbohydrate content in the grains by boosting the absorption and translocation of nutrients to the vegetative parts, which are then accumulated in the grains during their formation stages, additionally, sulfur plays a crucial role in improving plant vitality, growth, and development, enhancing their ability to perform photosynthesis effectively, which in turn increases the production of complex compounds such as glucose and sucrose [20], both of which are key components of carbohydrates.

As observed in the same table, there were no significant differences between the means of *T. thioparus* bacteria in terms of carbohydrate content in the grains. However, the results indicate a significant effect of nano zinc foliar application on carbohydrate content, with the treatment Zn2 achieving the highest percentage of 74.00%, compared to the non-sprayed treatment Zn0, which recorded the lowest percentage at 72.24%, representing an increase of 2.43%. Zinc is a crucial element that acts as a regulatory or structural component of a wide range of enzymes and proteins involved in various biological and chemical pathways responsible for carbohydrate metabolism, including photosynthesis and the conversion of complex sugars into starch, protein metabolism, and auxin regulation. Zinc serves as a cofactor for enzymes responsible for regulating photosynthesis in plants by activating enzymes related to the conversion of carbon dioxide (CO_2) and water (H_2O) into carbohydrates. These carbohydrates are subsequently translocated during grain formation stages and stored within the grains, where they constitute the largest proportion [19].

There was also a significant interaction between *T. thioparus* bacteria and nano zinc (*T*Zn* interaction), where the treatment T1Zn2 recorded the highest percentage of 74.38%, compared to the treatment T1Zn0, which had the lowest percentage at 72.00%. This value did not significantly differ from the treatment T0Zn0. Furthermore, the table reveals significant differences in the three-way interaction among agricultural sulfur, *T. thioparus* bacteria, and nano zinc (*S*T*Zn* interaction). The treatment S2T1Zn2 resulted in the highest carbohydrate percentage of 79.62%, compared to the treatment S0T1Zn1, which had the lowest percentage of 69.84%.

Table (4): Effect of agricultural sulfur, inoculation with *T.thioparus* bacteria, nano zinc and their interaction on carbohydrate concentration in grains (%).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	70.21	70.28	70.03
	Zn 1	70.55	69.84	70.08
	Zn 2	69.88	70.66	70.60
S1	Zn 0	72.31	72.95	72.15
	Zn 1	73.66	72.00	73.19
	Zn 2	73.44	72.88	73.27
S2	Zn 0	74.93	76.00	74.54
	Zn 1	76.66	74.16	76.46

	Zn 2	76.92	79.62	78.14
				Means (S)
Interaction (S*T)	S0	70.35	70.12	70.23
	S1	72.97	72.77	72.87
	S2	75.86	76.90	76.38
				Means (Zn)
Interaction (Zn*T)	Zn0	72.48	72.00	72.24
	Zn1	73.08	73.41	73.24
	Zn2	73.62	74.38	74.00
Means (T)		73.06	73.26	

L.S.D0.05						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.40	0.40	N.S	0.70	0.57	0.57	0.99

Sulfur concentration in grains (%)

The results presented in Table 20 show significant differences in the sulfur content of the grains. Treatment S2 achieved the highest sulfur percentage of 0.272%, while the treatment S0 (without fertilization) recorded the lowest percentage of 0.194%, representing an increase of 40.2%. The increase in sulfur content in the grains is attributed to the enhanced reduction of available sulfur due to the application of agricultural sulfur. Over time, this sulfur is converted into soluble forms, making it readily available for absorption as sulfates in the soil. Once absorbed through the roots, it is translocated to the plant tissues, thereby increasing the concentration of this element in the grains [21].

The same table confirms that the bacterial inoculation treatment T1 resulted in the highest sulfur content of 0.248%, compared to the non-inoculated treatment T0, which had the lowest content of 0.207%, representing an increase of 19.8%. This increase may be attributed to the role of *T. thioparus* bacteria, which work in conjunction with specific enzymes that accelerate the breakdown of sulfur compounds, stimulate oxidation reactions, and contribute to the conversion of reduced sulfur into sulfates that are readily absorbed by the roots, these sulfates then participate in the plant's growth and development stages before being translocated to the grains [22].

The same table indicates that nano zinc foliar application has a significant effect on sulfur content in the grains. The treatment Zn2 resulted in the highest sulfur percentage of 0.239%, which was not significantly different from the treatment Zn1 of 0.227%. In contrast, the non-sprayed treatment Zn0 recorded the lowest sulfur percentage of 0.217%, representing an increase of 10.13% with the treatment Zn2. This increase in sulfur concentration in the grains may be attributed to the higher levels of nano zinc, which enhances the absorption of nutrients from the soil, including sulfur. This effect is due to the activation of various enzymes, particularly Carbonic Anhydrase [23], leading to the formation of a robust root system capable of absorbing nutrients more effectively.

The table indicates that there were no significant differences in the interaction between sulfur and *T. thioparus* bacteria (S*T). However, significant differences were observed in the interaction between sulfur and nano zinc (S*Zn), with the treatment S2Zn1 achieving the highest sulfur percentage of 0.292%, which was not significantly different from the treatment S2Zn2. This was in contrast to the treatment S0Zn1, which recorded the lowest percentage of 0.168%. There was also no significant interaction between *T. thioparus* bacteria and nano zinc (T*Zn). Additionally, the table reveals significant differences in the three-way interaction among agricultural sulfur, *T. thioparus* bacterial inoculation, and nano zinc (S*T*Zn). The treatment S2T1Zn2 yielded the highest sulfur percentage of 0.308%, which was not significantly different from the treatment S2T1Zn1. This was compared to the treatment S0T0Zn1, which had the lowest sulfur percentage of 0.165%.

Table (5): Effect of agricultural sulfur, inoculation with *T.thioparus* bacteria, nano zinc and their interaction on sulfur concentration in grains (%).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	0.171	0.223	0.197
	Zn 1	0.165	0.171	0.168
	Zn 2	0.182	0.255	0.219
S1	Zn 0	0.189	0.212	0.200
	Zn 1	0.191	0.251	0.221
	Zn 2	0.218	0.239	0.228
S2	Zn 0	0.238	0.271	0.254
	Zn 1	0.283	0.301	0.292
	Zn 2	0.231	0.308	0.270
				Means (S)
Interaction (S*T)	S0	0.172	0.216	0.194
	S1	0.199	0.234	0.217
	S2	0.250	0.293	0.272
				Means (Zn)
Interaction (Zn*T)	Zn0	0.199	0.235	0.217
	Zn1	0.213	0.241	0.227
	Zn2	0.210	0.267	0.239
Means (T)		0.207	0.248	
$L.S.D_{0.05}$				

S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.013	0.013	0.010	0.022	N.S	N.S	0.032

The study findings emphasize the importance of utilizing agricultural sulfur as a soil conditioner for limestone soils in Iraq. They also highlight the necessity of incorporating *Thiobacillus* bacteria to improve and expedite the oxidation process. Furthermore, the application of nano-zinc is advised, given that calcareous soils generally lack essential nutrients, including zinc.

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