RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (2) 851-860, 2025
http://doi.org/10.54910/sabrao2025.57.2.41
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

EFFECT OF SULFUR, THIOBACILLUS BACTERIA, AND NANO-ZINC ON BIOCHEMICAL CHARACTERISTICS AND GRAIN YIELD OF MAIZE IN CALCAREOUS SOIL

E.A.O. AL-TAEE* and A.A. ALAMERY

Department of Field Crops, College of Agriculture, University of Kerbala, Kerbala, Iraq
*****Corresponding author's emails: emadadelll1990@gmail.com
Email address of co-author: abas.hussian@uokerbala.edu.iq

SUMMARY

The presented study sought to determine the effect of sulfur, thiobacillus bacteria, and nano-zinc on biochemical and grain yield traits of maize (*Zea mays* L.) grown in calcareous soils. The experiment, laid out in a randomized complete block design with three factors and three replications, commenced in the spring of 2022 at the Ibn-Al-Bitar Vocational Preparatory School, Holy Kerbala Governorate, Iraq. The first factor comprised three doses of agricultural sulfur (0, 1500, and 3000 kg ha^{-1}), the second was two thiobacillus bacteria levels (control - no addition, and bacterium), and the third factor was the nano-zinc concentrations (0, 50, and 100 mg L^{-1}). The sulfur addition (3000 kg ha^{-1}) showed the highest grain yield (8.06 mg ha^{-1}) with percent of nitrogen (1.72%), phosphorus (0.53%), protein (10.79%), and sulfur (0.270%) in maize grains. The thiobacillus bacteria also exhibited the maximum 500-grain weight and grain yield (154.64 g and 7.52 mg ha^{-1}, respectively). For nano-zinc, the optimum level (100 mg L^{-1}) excelled in nitrogen (1.62%), phosphorus (0.41%), protein (10.79%), and sulfur (0.239%) in maize grains and 500-grain weight (155.53 g); however, nano-zinc (50 mg L^{-1}) outshone in grain yield (8.01 mg L^{-1}). The combine application of three factors also improved most biochemical traits of maize.

Keywords: Maize (*Zea mays* L.), agricultural sulfur, thiobacillus bacteria, nano-zinc, biochemical traits, grain yield

Key findings: Results showed sulfur's highest level (3000 kg ha^{-1}) was superior in enhancing maize (*Zea mays* L.) grain yield and biochemical traits. The treatments with bacteria (thiobacillus) and nano-zinc (100 mg L^{-1}) also excelled in all maize traits.

Communicating Editor: Dr. A.N. Farhood

Manuscript received: January 02, 2024; Accepted: April 21, 2024.

Citation: Al-Tee EAO, Alamery AA (2025). Effect of sulfur, thiobacillus bacteria, and nano-zinc on biochemical characteristics and grain yield of maize in calcareous soil. *SABRAO J. Breed. Genet.* 57(2): 851-860. http://doi.org/10.54910/sabrao2025.57.2.41.

INTRODUCTION

Maize (*Zea mays* L.) is one of the economically important grain crops, as it ranks third after wheat and rice, with more than one-third of the world's population depends on, as its basic food. The crop, grown for its high nutritional value for humans and animals alike, is a major source of carbohydrates, desirable oil content, protein, vitamins, cellulose, and several mineral elements, making it an indispensable commodity (Abd-El-Fattah *et al.,* 2023).

As a four-carbon crop, maize is one of the most soil-stressful crops. A comprehensive fertilization program is essential, along with fundamental ingredients. Chemical fertilizers are vital for determining production per unit area, and their value grows with soil conditions, lacking critical components and organic compounds (Lin *et al*., 2022). Numerous studies have demonstrated dry and semi-arid soils, particularly Iraqi soils, with high calcium carbonate and pH, are alkaline, limiting nutritional availability, especially micronutrients (Jabbar and AL-Ziyadi, 2021).

As the plant cannot complete its life cycle without these essential components, following procedures is necessary to boost their readiness to enhance physiological processes, plant growth, and productivity. One way is adding agricultural sulfur to the soil, also involved in various soil processes (Hoshan and Yassin, 2023). This is because several chemical, organic, and inorganic forms of sulfur exist, and many microbes convert organic sulfur into plant-ready minerals. Studies showed microorganisms may oxidize sulfur organically in soil and for autotrophic bacteria under specific circumstances (Al-Taee and Alamery, 2024).

Thiobacillus chemicals convert sulfur into sulfuric acid, which also lowers the soil reaction (pH) and conditions the soil's basic and calcareous qualities. Similarly, it enhances the crucial nutrients' availability by replacing some chemical and organic fertilizers (Nguyen *et al.,* 2022; Alaarage and Alamery, 2023). As part of the production of three essential amino acids, i.e., cysteine, cystine, and methionine, sulfur is crucial to plant growth and development. Vitamin B thiamine's synthesis also occurs with it, with this enzyme needed for respiration, along with sulfur in biotin and coenzyme A synthesis (De-Rose *et al*., 2023).

Foliar application of nutrients needs attention, which can increase the productivity per unit area. Moreover, the search for new nutrition sources involves adding micronutrients, which the plant requires in small quantities compared with macro-nutrients (N, P, and K) to compensate for some basic elements' deficiency (Singh *et al*., 2021). These nutrients include nano-zinc, vital for plant growth and development, enhancing resistance, and synthesizing various enzymes. It also prevents plant cells from oxidizing and boosts plant growth and production (Seyed-Sharifi *et al*., 2020). The presented study determined how different levels of agricultural sulfur affect the biochemical properties and grain yield of maize, as well as, how thiobacillus bacteria influence sulfur oxidation and convert it into plant-absorbable forms. Additionally, the study examined how nano-zinc modifies maize growth and yield.

MATERIALS AND METHODS

This study sought to determine the effect of sulfur, thiobacillus bacteria, and nano-zinc on biochemical and grain yield traits of maize (*Zea mays* L.) grown in calcareous soils. The experiment layout had a randomized complete block design with three factors and three replications. It commenced in the spring of 2022 at the Ibn-Al-Bitar Vocational Preparatory School, Holy Kerbala Governorate, Iraq (latitude: 32' 36' 57.71 North, longitude: 44' 29.57 East). The experiment had three variables. The first factor comprised three doses of agricultural sulfur (0, 1500, and 3000 kg ha^{-1} designated as S0, S1, and S2). The second factor has two thiobacillus bacteria levels (control - no addition, and bacterium labeled as T0 and T1), while the third was the nano-zinc concentrations (0, 50, and 100 mg L^{-1} presented as Zn0, Zn1, and Zn2, respectively).

The maize seeds' infection with thiobacillus bacteria occurred before sowing them in the field, at 25 cm and 75 cm apart within and between the rows, with an experimental unit area of 2 m × 3 m. As per maize crop recommendations, the application of N, P, and K fertilizes was as 100, 87.2, and 66.4 kg ha^{-1}, respectively. The phosphate and potassium fertilizers treatment occurred during soil preparation and sowing. The urea fertilizer application was in two batches, according to the treatments. All field practices of the maize crop proceeded, as per recommendation of the Ministry of Agriculture, Iraq.

Data recorded

For grain nitrogen proportion (%), the digested dried and powdered samples of 0.2 g from each experimental unit continued with the nitrogen (%) calculation using a Micro Kjeldahl device at the University of Kerbala, Kerbala, Iraq (George *et al*., 2013). Grain phosphorus (%)measurement utilized the spectrophotometer using ammonium molybdate and ammonium phendate at 410 nanometers to quantify grain phosphorus (Al-Sahhaf, 1989). Grain protein (%)calculation employed the following equation:

Protein % = nitrogen centenary × 6.25

According to Bhargava and Raghupathi (1993), sulfur (%) calculation in maize grains added gum acacia (0.5%) and acetic acid with distilled water in a 1:1 ratio, and measured with a spectrophotometer at 420 nm. The computation of 500-grain weight (g) and the total grain yield (mg ha^{-1}) also ensued.

Statistical analysis

Analysis of all recorded data for various parameters underwent the analysis of variance (ANOVA), as per the RCBD design (Al-Mohammadi and Al-Mohammadi, 2012). The least significant difference ($LSD_{0.05}$) test used compared and separated further the treatment means. All the analyses ran under the statistics software GenStat12.

RESULTS AND DISCUSSION

Nitrogen

Sulfur addition indicated significantly varied values of the maize grain nitrogen percentages (Table 1). The sulfur level (3000 kg ha^{-1}) achieved the highest nitrogen percentage

Table 1. Effect of agricultural sulfur, thiobacillus bacteria, and nano-zinc and their interaction on the nitrogen percentage in maize grains.

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction S x Zn
		T0	T1	
S0 (0 ha^{-1})	Zn 0	1.42	1.52	1.47
	Zn 1	1.43	1.54	1.48
	Zn 2	1.46	1.53	1.50
S1 (1500 kg ha^{-1})	Zn 0	1.55	1.58	1.56
	Zn 1	1.54	1.60	1.57
	Zn 2	1.54	1.64	1.59
S2 (3000 kg ha^{-1})	Zn 0	1.63	1.72	1.67
	Zn 1	1.68	1.76	1.72
	Zn 2	1.70	1.85	1.78
				Means S (%)
Interaction (S x T)	S0	1.44	1.53	1.48
	S1	1.54	1.61	1.57
	S2	1.67	1.78	1.72
				Means Zn (%)
Interaction (Zn x T)	Zn0	1.53	1.60	1.57
	Zn1	1.55	1.63	1.59
	Zn2	1.57	1.67	1.62
Means T (%)		1.55	1.64	
$LSD_{0.05}$ S: 0.015, Zn: 0.015, T: 0.012, S x Zn:0.026 , S x T: 0.021, Zn x T: N.S, S x Zn x T: 0.037				

(1.72%), while the control treatment showed the lowest percentage (1.48%), with a 16.21% rise. Adding agricultural sulfur decreases the soil pH and forms the sulfuric acid, which dissolves complex compounds and salts, carrying nutrients and converting them into forms ready for absorption in the soil solution, including the macroelement nitrogen (Al-Fahdawi *et al.,* 2020). By introducing bacteria, the utmost nitrogen percentage (1.64%) emerged versus the lowest (1.55%) in the control treatment, with a 5.80% rise. The reason is the thiobacillus bacteria converts sulfur into sulfate, which may be due to its active involvement. It releases hydrogen ions from sulfuric acid to lower soil alkalinity and boosts the nutrients' availability in all forms (Gomah *et al*., 2014).

The nano-zinc (100 mg L^{-1}) foliar application provided the highest percentage of nitrogen in maize grains (1.62%) compared with the control treatment, giving the lowest percentage (1.57%), with a percent increase of 3.18%. When plants indicate insufficient zinc in the soil, this ensures difficult absorption of various nutrients with a low pH, and neutral nitrogen becomes ineffective even when it is available in sufficient quantities in the soil. Therefore, it is preferable to spray the zinc and improve its availability because it enhances the plants' ability to make optimal use of nitrogen. Zinc is part of a group of enzymes that contribute to regulating and activating the absorption of available nitrogen in the soil by crop plants (El-Ghareib *et al.,* 2014). As for the triple interaction, it was also significant, and the nitrogen reached the maximum percentage (1.85%) in the sulfur level (3000 kg ha^{-1}) with nano-zinc (100 mg L^{-1}) and treated with thiobacillus bacteria. However, the lowest nitrogen percentage (1.42%) resulted in the interaction of the control treatments of the three factors.

Phosphorus

The sulfur (3000 kg ha^{-1}) application showed the highest percentage of phosphorus in maize grains (0.53%), whereas the control treatment (without fertilization) delivered the lowest (0.26%), with a rise of 103.84% (Table 2). The role of agricultural sulfur in forming sulfuric acid (H_2SO_4) through biochemical oxidation increases the concentration of H ions in the soil, which dissolves some phosphate compounds and releases the phosphorus

Table 2. Effect of agricultural sulfur, thiobacillus bacteria, and nano-zinc and their interaction on the phosphorus percentage in maize grains.

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction S x Zn
		T0	T1	
S0 (0 ha^{-1})	Zn 0	0.22	0.27	0.24
	Zn 1	0.27	0.28	0.27
	Zn 2	0.26	0.30	0.28
S1 (1500 kg ha^{-1})	Zn 0	0.33	0.40	0.36
	Zn 1	0.40	0.43	0.41
	Zn 2	0.35	0.44	0.39
S2 (3000 kg ha^{-1})	Zn 0	0.46	0.53	0.49
	Zn 1	0.45	0.61	0.53
	Zn 2	0.47	0.66	0.56
				Means S (%)
Interaction (S x T)	S0	0.25	0.28	0.26
	S1	0.36	0.42	0.39
	S2	0.46	0.60	0.53
				Means Zn (%)
Interaction (Zn x T)	Zn0	0.33	0.40	0.37
	Zn1	0.37	0.44	0.40
	Zn2	0.36	0.46	0.41
Means T (%)		0.35	0.43	
$LSD_{0.05}$ S: 0.009, Zn: 0.009, T: 0.008, S x Zn:0.016 , S x T: 0.013, Zn x T: 0.013, S x Zn x T: 0.024				

element, making it easier for plants to absorb (Alamery *et al.,* 2019). The added bacteria gave the ultimate percentage of phosphorus (0.43%) compared with the control treatment, which gave the lowest percentage (0.35%), with an increase of 22.85%. This may be due to the positive role of biofertilizers, such as, sulfur-oxidizing bacteria thiobacillus, in the readiness of nutrients through the secretion of special enzymes facilitating the release of precipitated elements. It also enhanced the presence of oxygen in the soil with a small percentage of moisture, by modifying the degree of soil interaction to allow the plants' absorption of basic elements (Lateef *et al.,* 2019), including phosphorus, and its ease of availability and absorption (Al-Bayati *et al.*, 2016; Alafeea *et al.*, 2019).

The maize with foliar application of nano-zinc (100 mg L^{-1}) exhibited the highest phosphorus content (0.41%) compared with the control treatment, providing the lowest percentage (0.37%), with an increase of 10.81% (Table 2). Perhaps, the increase in the phosphorus percentage in grains was due to the positive effect of enriching the readiness of nutrients, including the available nitrogen and potassium in the soil (Table 1). They are the elements working to enhance the expansion of the root and shoot systems, and thus, positively affecting the release and absorption of the phosphorus element in the soil (Ali *et al.,* 2014). For the triple interaction, the supreme interaction effects and phosphorus (0.66%) appeared with the combine application of sulfur (3000 kg ha^{-1}), nano-zinc (100 mg L^{-1}), and treating with thiobacillus bacteria. Meanwhile, the lowest phosphorus (0.22%) was evident in the interaction of the control treatments.

Protein

The results revealed significant differences among the sulfur levels for the maize grains' protein content (Table 3). The application of sulfur (3000 kg ha^{-1}) achieved the highest protein percentage (10.79%), while the control treatment had the lowest percentage (9.29%), with an increase of 16.14%. The increase in the grains' protein with sulfur treatment may be due to its role in balancing the degree of soil reaction (PH). This, in turn, boosts the readiness of elements, as facilitated by a neutral pH, including nitrogen in the soil (Table 1). It may also refer to the positive effect of sulfur in creating essential amino acids (cysteine, cystine, and methionine), crucial in protein synthesis, and, thus, increasing the grains' protein content (Thirupathi *et al.,*

Table 3. Effect of agricultural sulfur, thiobacillus bacteria, and nano-zinc and their interaction on the protein percentage in maize grains.

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction S x Zn
		T0	T1	
S0 (0 ha^{-1})	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 (1500 kg ha^{-1})	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 (3000 kg ha^{-1})	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 x 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 x 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	

$LSD_{0.05}$ S: 0.096, Zn: 0.096, T: 0.078, S x Zn: 0.166 , S x T: 0.136, Zn x T: N.S, S x Zn x T: 0.236

Table 4. Effect of agricultural sulfur, thiobacillus bacteria, and nano-zinc and their interaction on the sulfur percentage in maize grains.

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction S x Zn
		T0	T1	
S0 (0 ha^{-1})	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 (1500 kg ha^{-1})	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 (3000 kg ha^{-1})	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 x 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 x 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	
$LSD_{0.05}$ S: 0.013, Zn: 0.013, T: 0.010, S x Zn: 0.022 , S x T: N.S, Zn x T: N.S, S x Zn x T: 0.032				

2016). The bacteria addition also showed the optimum protein (10.25%) compared with the control treatment (9.71%), with a percent increase of 5.56%. This may be because the thiobacillus bacteria supplemented the work of sulfur in improving the physical and chemical properties of the soil, including raising soil readiness and encouraging the nutrients' absorption, most notably nitrogen (Table 1). The increased nitrogen from the synthesis of enzymes, amino acids, and nitrogenous bases, enhanced protein formation in crop plants and storing in the sink (Ansori and Gholami, 2015).

The maize spraying with nano-zinc significantly affected the protein percentage (Table 3). The application of nano-zinc (100 mg L^{-1}) gave the maximum percentage (10.15%) compared with the control treatment (9.82%), with an increase of 3.36%. The rise in grains' protein may be due to the zinc entering the metabolism process, and, thus, it helped boost the assimilation of nutrients, including proteins within the plants (El-Ghareib *et al.,* 2014). The triple interaction was also remarkable, with the highest protein content (11.60%) obtained with the combined application of sulfur (3000 kg ha^{-1}), nano-zinc (100 mg L^{-1}), and treating with thiobacillus bacteria. Meanwhile, the lowest interaction (8.87%) emerged in the control treatments of the three factors.

Sulfur

The findings showed considerable differences in sulfur application for maize grains' sulfur (Table 4). The sulfur level (3000 kg ha^{-1}) achieved the highest percentage of sulfur (0.272%), while the control treatment provided the lowest percentage (0.194%), with a percent increase of 40.20%. The increased grain sulfur percentage was due to the elevated sulfur application and its conversion after its addition into dissolved forms easily absorbed as sulfate in the soil. Afterward, its absorption is quicker through the roots, easily transferring to plant tissues and grains (Jabir and Habeeb, 2017). The added bacteria gave the premier sulfur content (0.248%) versus the treatment with no addition (control), displaying the lowest percentage (0.207%), with a percent increase of 19.80%. This may also refer to the positive role of the bacteria thiobacillus involved in the enzymes. It accelerated the decomposition of sulfur compounds, stimulated oxidation reactions, and contributed to converting reduced sulfur into sulfate for ready absorption through the roots, and then, it transferred to the grains (Gomah *et al.,* 2014).

The maize spraying with nano-zinc had a substantial effect on the percentage of sulfur in grains (Table 4). The sulfur (100 mg L^{-1}) gave the highest percentage of sulfur (0.239%). However, it did not differ significantly from the sulfur concentration at 50 mg L^{-1}, which gave a percentage of 0.227%, compared with the control treatment (0.217%), with a percent increase of 10.13%. The grains' elevated sulfur may be due to the higher concentrations of nano-zinc, which contributes to enhancing the absorption of nutrients, including sulfur. Nanotechnology may lead to raising the efficiency of plants' absorption of zinc and other elements by improving their availability and utilization (Aref, 2011). As for the triple interaction, it was significant, and the highest sulfur percentage (0.308%) appeared with the combined application of sulfur level (3000 kg ha^{-1}), nano-zinc (100 mg L^{-1}), and treating thiobacillus bacteria. Inversely, the lowest percentage (0.165%) was evident with the nano-zinc concentration at 50 mg L^{-1} and the control treatments of sulfur and thiobacillus bacteria.

The 500-grain weight

The outcomes implied sulfur levels significantly differed for the 500-grain weight in maize (Table 5). The sulfur level (1500 kg ha^{-1}) achieved the highest average of 500-grain weight (154.26 g). However, it did not vary extensively from the control treatment (152.32 g), while the sulfur (3000 kg ha^{-1}) achieved the lowest average (149.92 g), with a percent increase of 2.81%. It could be due to agricultural sulfur and its role in the photosynthesis process. Thus, it increased the activity of vegetative growth characteristics (leaf area and chlorophyll content), which led to an escalation in the period required for grain filling, as reflected in the grain weight. Through these metabolic processes occurring during the plant growth and development helped assembly in grains (Jassim and Kateb, 2016).

The bacterial treatment caused notable differences in the 500-grain weight in maize, giving the highest average (154.64 g) compared with the control treatment (149.70 g), with a percent increase of 3.29%. It may refer to the contribution of biofertilizers to raising grain productivity by improving soil properties and providing essential nutrients to

Table 5. Effect of agricultural sulfur, thiobacillus bacteria, and nano-zinc and their interaction on the weight of 500 grains in maize.

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T) T0	T1	Interaction S x Zn
S0 (0 ha^{-1})	Zn 0	136.24	158.53	147.38
	Zn 1	136.57	162.48	149.53
	Zn 2	150.40	181.36	165.88
S1 (1500 kg ha^{-1})	Zn 0	148.53	142.31	145.42
	Zn 1	170.39	139.99	155.19
	Zn 2	170.53	142.20	156.37
S2 (3000 kg ha^{-1})	Zn 0	163.67	147.23	155.45
	Zn 1	139.41	160.54	149.98
	Zn 2	131.58	157.08	144.33
				Means S (g)
Interaction (S x T)	S0	163.15	141.50	152.32
	S1	141.07	167.46	154.26
	S2	144.89	154.95	149.92
				Means Zn (g)
Interaction (Zn x T)	Zn0	149.48	149.36	149.42
	Zn1	148.79	154.34	151.56
	Zn2	150.84	160.22	155.53
Means T (g)		149.70	154.64	
$LSD_{0.05}$ S: 3.35, Zn: 3.35, T: 2.73, S x Zn: 5.80 , S x T: 4.74, Zn x T: 4.74, S x Zn x T: 8.21				

crop plants. By using correctly, it can lead to boosting the efficiency of plants' absorption of nutrients (Tables 1, 2, and 3) and enhancing their growth and development, and thus, reflecting positively on yield components, including grain weight (Ganzour *et al.,* 2020).

Furthermore, the results showed maize spraying with nano-zinc had a significant effect on the 500-grain weight in maize. The sulfur 100 mg L^{-1} indicated the maximum average of the 500-grain weight (155.53 g) versus the control treatment (149.42 g), with a percent increase of 4.08%. With the positive role of zinc in increasing chlorophyll synthesis and leaf area, it boosts the photosynthesis and respiration processes, enriching plant activities to absorb more water and nutrients (Al-Mohammadi *et al.,* 2015). The triple interaction also proved significant, with the highest 500-grain weight (181.36 g) provided by the interaction of the control treatment of sulfur with nano-zinc (100 mg L^{-1}) and the addition of thiobacillus bacteria. However, the lowest 500-grain weight (131.58 g) was noteworthy at the sulfur level 3000 kg ha^{-1} with nano-zinc 100 mg L^{-1} and the control treatment of thiobacillus bacteria.

Grain yield

The sulfur levels showed a significant difference for grain yield in maize (Table 6). The sulfur level 3000 kg ha^{-1} displayed the highest average grain yield (8.06 mg ha^{-1}), while the control treatment recorded with the lowest average (5.95 mg ha^{-1}), with a percent increase of 41.51% in grain yield. The increase in average yield may be because of the sulfur's positive role in improving the soil quality and its chemical composition. Therefore, it raises the availability of nutrients in the soil solution and their absorption by the plants, and thus, providing optimal conditions for the growth and development of plants. It transferred the products of photosynthesis from the sources of their synthesis to the components of the crop, thus, boosting the grain yield (Ahmed, 2016).

The maize seeds treated with bacteria revealed the maximum average grain yield (7.52 mg ha^{-1}) compared with the control treatment having the lowest average (6.54 mg ha^{-1}), with a percent increase of 14.98% (Table 6). This can be due to the use of sulfur-oxidizing bacteria thiobacillus, which improved the soil by raising the available essential

Table 6. Effect of agricultural sulfur, thiobacillus bacteria, and nano-zinc and their interaction on the grain yield in maize.

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction S x Zn
		T0	T1	
S0 (0 ha^{-1})	Zn 0	5.25	6.13	5.69
	Zn 1	5.64	6.89	6.26
	Zn 2	5.32	5.89	5.60
S1 (1500 kg ha^{-1})	Zn 0	4.85	6.94	5.89
	Zn 1	6.77	7.87	7.32
	Zn 2	6.90	7.94	7.42
S2 (3000 kg ha^{-1})	Zn 0	6.97	7.81	7.39
	Zn 1	7.04	8.50	7.77
	Zn 2	7.41	8.63	8.02
				Means S (Mg ha^{-1})
Interaction (S x T)	S0	5.18	6.72	5.95
	S1	7.00	7.20	7.10
	S2	7.53	8.59	8.06
				Means Zn (Mg ha^{-1})
Interaction (Zn x T)	Zn0	6.69	7.55	7.12
	Zn1	7.91	8.12	8.01
	Zn2	7.76	8.11	7.93
Means T (Mg ha^{-1})		6.54	7.52	
$LSD_{0.05}$ S: 0.640, Zn: 0.640, T: 0.523, S x Zn: 1.109 , S x T: 0.906, Zn x T: 0.906, S x Zn x T: 1.569				

macro- and microelements with a neutral pH (Tables 1 and 2). Such conditions provided organic material and enhanced their readiness for the plants. Past studies also indicated a significant increase in yield by inoculating the soil with bacteria and using biofertilizers (Malekzadeh *et al.*, 2016).

The maize spraying with nano-zinc remarkably influenced its grain yield. The nano-zinc concentration 50 mg L^{-1} achieved the highest average grain yield (8.01 mg ha^{-1}). However, it did not differ significantly from the nano-zinc 100 mg L^{-1}, which gave an average yield of 7.93 mg ha^{-1} compared with the control treatment providing the lowest average (7.12 mg ha^{-1}), with a percent increase of 12.50%. Zinc has several roles that could contribute to raising grain productivity through its positive effects on the synthesis of chlorophyll, which boosts the photosynthesis in crop plants. The interaction effects were noteworthy for grain yield in maize. The triple interaction coefficients for the sulfur fertilizer (3000 kg ha^{-1}), thiobacillus bacteria, and nano-zinc (100 mg L^{-1}) showed the utmost interaction with maximum grain yield (8.63 mg ha^{-1}). However, the least grain yield (4.85 mg ha^{-1}) was evident at the sulfur level 1500 kg ha^{-1} with the control treatments of nano-zinc and thiobacillus bacteria. In plants, the manufactured foodstuffs' fast transmittal to the rest of the plant parts improved growth rate and reflected positively on the grain yield in maize (*Zea mays* L.) (Al-Mohammadi *et al.*, 2015; Hoshan, 2024).

CONCLUSIONS

The study revealed the importance of sulfur as a conditioner for Iraqi limestone soils. Likewise, it detailed the need to add thiobacillus bacteria to enhance and accelerate the oxidation process, in addition to foliar application of nano-zinc, because the calcareous soil lacked nutrients, including zinc.

REFERENCES

Abd-El-Fattah DA, Maze M, Ali BA, Awed NM (2023). Role of mycorrhizae in enhancing the economic revenue of water and phosphorus use efficiency in sweet corn (*Zea mays* L. var. *saccharata*) plants. *J. Saudi Soc. Agric. Sci.* 22(3): 174-186.

Ahmed FWA (2016). Effect of the time and level of adding agricultural sulfur on the absorption of phosphorus, iron, and zinc and the growth and yield of two varieties of maize (*Zea mays* L.). *Al-Qadisiyah J. Agric. Sci.* 2(6): 136-150.

Alaarage S, Alamery AA (2023). Role of bio-azotobacter and nitrogen fertilizers on growth and yield traits of sorghum (*Sorghum bicolor* L.). *SABRAO J. Breed. Genet*. 55(4): 1311-1320.

Alafeea RAA, Alamery AA, Kalaf IT (2019). Effect of bio fertilizers on increasing the efficiency of using chemical fertilizers on the yield component of maize (*Zea mays* L.). *Plant Arch*. 19(2): 303-306.

Alamery AA, Lateef SM, Almosawy AN, Alhassaany MH, Almosawy MM (2019). Effect of phosphate bio fertilizers on increasing the efficiency of the use of phosphate mineral fertilizers and its effect on some growth properties of broccoli (*Brassica oleracea* var. *italica*). *Biochem. Cell. Arch*. 19. 2740-2751.

Al-Bayati AHI, Al-Rubaie IMR, Al-Maamiri AAK (2016). Effect of incubation time on the biological oxidation of agricultural sulfur on some chemical properties of alluvial clay soil. *Diyala J. Agric Sci*. 8 (2): 117-124.

Al-Fahdawi WA, Ahmed FW, Cheyed SH (2020). Effect of agricultural sulfur on availability of NPK in the soil growth and yield of corn (*Zea mays* L.). *Indian J. Ecol*. 47(12): 275-280.

Ali NES, Rahi HS, Shaker AA (2014). Soil Fertility. Ministry of Higher Education and Scientific Research - University of Baghdad, Iraq. pp. 307.

Al-Mohammadi SAF, Al-Dulaimi RMH, Al-Dulaimi TMB (2015). Effect of foliar feeding with zinc and irrigation periods on some growth and productivity traits of maize (*Zea mays* L.) plants. *Anbar J. Agric. Sci.* 1(13): 265-279.

Al-Mohammadi SM, Al-Mohammadi FM (2012). Statistics and Experimental Design. Dar Osama for Publishing and Distribution, Oman, Jordan. pp. 355.

Al-Sahhaf FH (1989). Applied Plant Nutrition. Ministry of Higher Education and Scientific Research. Dar al-Hikma, University of Baghdad, Iraq. pp. 380.

Al-Taee EAO, Alamery AAH (2024). Response of maize grown in calcareous soils to levels of agricultural sulfur, thiobacillus bacteria and nano-zinc. *J. Kerbala Agric. Sci.* 11(1): 65-86.

Ansori A, Gholami A (2015). Improved nutrient uptake and growth of maize in response to inoculation with thiobacillus and mycorrhiza on an alkaline soil. *Commun. Soil Sci. Plant Anal.* 46(17): 2111-2126.

Aref F (2011). Zinc and boron content by maize leaves from soil and foliar application of zinc sulfate and boric acid in zinc and boron deficient soils. *Middle-East J. Sci. Res*. 7(4): 610-618.

Bhargava BS, Raghupathi HB (1993). Analysis of Plant Materials for Macro and Micronutrients. Binng Printers L- 14, Lajpat Nagor, New Delhi. pp. 49-82.

De-Rose S, Kuga Y, Sillo F, Fochi V, Sakamoto N, Calevo J, Balestrini R (2023). Plant and fungal gene expression coupled with stable isotope labeling provide novel information on sulfur uptake and metabolism in orchid mycorrhizal protocorms. *Plant J*. 116(2): 416-431.

El-Ghareib EA, El-Sayed MA, Mesbah EE, Azzam KA (2014). Effect of foliar spraying with dolfan and zinc on yield and yield components of maize (*Zea mays* L.) under different nitrogen fertilizer rates. *Middle East J.* 3(3): 465-471.

Ganzour S, Ghabour T, Hemeid NM, Khatab KA (2020). Effect of biofertilizers on maize (*Zea mays* L.) growth and yield under calcareous soil conditions. *Egyptian J. Soil Sci.* 60(4): 469-483.

George E, Rolf S, John R (2013). Methods of soil, plant, and water analysis: A manual for the West Asia and North Africa region. *Int. Center for Agric. Res. in the Dry Areas* (ICARDA). pp. 244.

Gomah HH, Mahmoud SM, El-Rewainy HM, Abdrabou MR (2014). Soil solarization and inoculation with sulphur oxidizing bacteria and their effects on some soil properties. *J. Microbiol. Biochem. Technol*. 3(2): 11-12.

Hoshan MN (2024). Interaction effects of leaching, salinity, sulfur, and organic matter on physical properties of soil planted with maize crop. *SABRAO J. Breed. Genet.* 56(1): 310-322. http://doi.org/10.54910/sabrao2024.56.1.28.

Hoshan MN, Yassin MM (2023). The interaction effect of leaching requirements, salinity of irrigation water, levels of added sulfur and type of organic matter on soil salinity cultivated with maize plants (*Zea mays* L.). *Univ. Thi-Qar J. Agric. Res*. 12(1): 1-21.

Jabbar AK, Al-Ziyadi DQ (2021). Effect of sulfur-oxidizing bacteria thiobacillus thioparus and different levels of agricultural sulfur on wheat yield (*Triticum aestivium* L.). In: *IOP Conf. Ser: Earth and Environ. Sci.* 923(1):12-74.

Jabir A, Habeeb K (2017). Effect of sulfur sources, levels and time of addition on the growth and yield of corn (*Zea mays* L.). *Al-Qadisiyah J. Agric. Sci.* 7(1): 1-12.

Jassim AH, Kateb IM (2016). Effect of supplemental nitrogen fertilizer treatments on the yield and its components of four genotypes of maize. *Al-Furat J. Agric. Sci.* 8(3): 126-135.

Lateef SM, Alamery AA, Alhassaany MH, Almosawy AN, Almosawy MM (2019). Role of bio-fertilizers and phosphate levels on some growth and yield properties of broccoli (*Brassica oleracea* var. *italica*). *Plant Arch*. 19(2): 1564-1568.

Lin S, Pi Y, Long D, Duan J, Zhu X, Wang X, He J, Zhu Y (2022). Effect of organic and chemical nitrogen fertilizers on the crop yield and fertilizer use efficiency of soybean–maize intercropping systems. *Agriculture* 12(9): 14-28.

Malekzadeh T, Besharati H, Savaghebi G (2016). Effects of sulfur and thiobacillus bacteria application on some nutrients availability in soils with different buffering capacities. *J. Sol Biol.* 3(2): 187-202.

Nguyen PM, Do PT, Pham YB, Doan TO, Nguyen XC, Lee WK, Ngo HH (2022). Roles, mechanism of action, and potential applications of sulfur-oxidizing bacteria for environmental bioremediation. *Sci. Total Environ.* 852(7): 158-203.

Seyed-Sharifi R, Khalilzadeh R, Pirzad A, Anwar S (2020). Effects of biofertilizers and nano zinc-iron oxide on yield and physicochemical properties of wheat under water deficit conditions. *Commun. Soil Sci. Plant Anal.* 51(19): 2511-2524.

Singh J, Partap R, Singh A (2021). Effect of nitrogen and zinc on growth and yield of maize (*Zea mays* L.). *Int. J. Bio-Resour. Stress Manag*. 12(3): 179-185.

Thirupathi I, Sagar GEV, Devi KS, Sharma SHK (2016). Effect of nitrogen and sulphur levels on growth, yield, quality and economics of single cross hybrid maize (*Zea mays* L.). *Int. J. Sci. Environ. Technol.* 5(5): 2989-2998.