



Response of maize grown in calcareous soils to levels of agricultural sulfur, thiobacillus bacteria and nano-zinc

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Abstract

A field experiment was conducted in spring season of 2022 according to a randomized complete block design with three replications (RCBD). The experiment included three factors. The first included three levels of agricultural sulfur (0, 1500, and 3000 kg ha⁻¹). The second factor included two levels of thiobacillus bacteria, which are the control treatment (no addition) and the treatment with bacteria. The third factor included three concentrations of nano-zinc (0, 50, and 100 mg L⁻¹). The results showed an excellent level of sulfur (3000 kg ha⁻¹) in the grain yield (8.06 Mg ha⁻¹), the percentage of nitrogen in the grains (1.72%), the percentage of phosphorus in the grains (0.53%) and the percentage of protein in grains is (10.79%), and the percentage of sulfur in grains is (0.270%). As the results showed that the second factor was superior when adding thiobacillus bacteria in the weight of 500 grains and the total grain yield (154.64 g and 7.52 Mg ha⁻¹, respectively). As for nano-zinc, the third level (100 mg L⁻¹) excelled in percentage of nitrogen (1.62%), phosphorus (0.41%), protein (10.79%) and sulfur (0.239%) in Grains, and the weight of 500 grains (155.53 g), with the exception of the total yield characteristic in which the level excelled (50 mg L⁻¹) with an average of (8.01 mg L⁻¹). The interaction between the three factors also gave a positive effect on most of the traits under study.

Keywords: Maize, Calcareous soils, Agricultural sulfur, Thiobacillus bacteria, Nano-zinc.

Introduction

Soils vary widely in their content of calcium carbonate salts, and when their content in the soil exceeds 15%, they are called calcareous basic soils. This is the result of the presence of abundant dissolved calcium deposits. Calcareous soils are formed by the passage of acidic water (rainwater) through the limestone rocks to the bottom of the soil, attracting it with its calcium carbonate. These carbonates exist in the form of small grains, forming clear layers spread throughout the soil sector. They are often hard, but they collapse when watered, harden, and dry [1]. Therefore, a high percentage of calcium carbonate in the soil is considered to have a negative impact on plant nutrition by monitoring symptoms of element deficiency due to the low percentage of organic



matter and lack of clay It is often low in fertility because it contains a low percentage of organic materials and basic nutrients, and water retention can affect this Negatively affects the soil's ability to support plants with necessary nutrients [2].

That reclaiming such soils is necessary to increase their plant cover,, several mechanisms can be used, one of which is adding agricultural sulfur and mixing it well with the soil, along with adequate irrigation a period before planting, in order to ensure its good decomposition and the formation of sulfuric acid, which contributes to reducing the basicity of the limestone soil and improving its suitability for growth. Plants develop through the interaction of sulfate with calcium and magnesium salts to form more acidic compounds and release hydrogen ions (H), which are important in improving soil quality and providing nutrients with moderate and low pH [3]. This is done with the help of several microorganisms that work to decompose sulfur and transform it into useful forms for the soil and plants, including thiobacillus bacteria. The use of sulfur-oxidizing bacteria in cultivating calcareous and alkaline soil is considered an effective strategy for improving soil quality and increasing plant productivity through their effective role in several vital processes that produce It converts organic sulfur into mineral ready for absorption, so it is preferable to inoculate the soil with thiobacillus bacteria, in order to reduce problems related to high calcium concentration and pH in the soil by improving the acidity level in the soil. This works to improve the availability of nutrients to plants and thus fill their need for nutrients necessary [4]. Sulfur has a clear role on the growth and yield of maize. In a study that [5] included adding agricultural sulfur at four levels (500, 1000, 1500 and 2000 kg ha⁻¹), where the level (2000 kg ha⁻¹) showed its superiority in several characteristics, including plant height (200.17), compared to the control treatment (188.27), leaf area (6270.48), compared to the control treatment (5513.6), and chlorophyll content of leaves (38.9), compared to the control treatment (28.47).

The search for new sources of nutrition and developing techniques for applying micronutrients to leaves in order to provide them to plants easily and quickly for absorption is important. Many nutritious products have been developed for foliar nutrition that contain balanced combinations of micronutrients, which play a crucial role in various diseases. Physiological and biochemical processes within plants [6]. One of the most important of these nutrients is nano-zinc, as it is involved in the formation of many vital enzymes and proteins, which makes it necessary for many vital physiological processes in the plant that help it regulate its growth and development. It is also involved in the synthesis of indole acetic acid (IAA) in the growing apex of plants and in the regulation of hormones. Plants contribute to stimulating the formation of chlorophyll, activating the process of photosynthesis, and participating in the processes of respiration and metabolism. In addition, zinc plays a role in the process of transporting and storing carbohydrates in the plant [7]. Nanotechnology is one of the most important means of increasing the rate of plant absorption of nutrients and water by entering plant tissues easily, reducing fertilizer loss, and improving their effective use. Therefore, nanofertilization is one of the modern strategies that can contribute to improving growth and production of plants and environmental sustainability [8]. In a study he



carried out [9] for several levels of spraying with zinc (0, 25, 50, 75 and 100 ml L⁻¹), it was found that the spraying treatment (50 ml L⁻¹) was superior in several characteristics, including plant height (184 cm), leaf area (730 cm²), and total grain yield (208 g plant⁻¹). The current study aims to reduce the basicity of calcareous soils using agricultural sulfur and the influence of thiobacillus bacteria, which oxidize it, and to know the effect of adding nano-zinc on growth indicators and yield of maize.

Materials and Methods

A field experiment was carried out during the spring season of 2023 in one of the fields of Ibn Al-Bitar vocational preparatory school in Al-Husseiniyah district, Kerbala, Iraq (latitude: 32 36' 57.71" north, longitude: 44 01' 29.57" east) according to a randomized complete block design (RCBD). In the order of the factorial experiments and with three replications, the experiment included three factors. The first factor included three levels of agricultural sulfur, which are 0, 1500, and 3000 kg ha⁻¹, and are designated (S0, S1, and S2, respectively). The second factor included two levels of thiobacillus bacteria, which are the comparison treatment (no addition) and treatment with bacteria and their symbols (T0 and T1, respectively). The third factor included three levels of nano-zinc spraying (0, 50, and 100 mg L⁻¹, symbolized as 0Zn, Zn1, and Zn2, respectively). The seeds were planted in a hollow on lines, the distance between one hole and another was 25 cm and the distance between one line and another was 75 cm. All agricultural operations were carried out according to the recommendations approved by the Ministry of Agriculture.

Data recorded

Plant height (cm) was measured for five randomly selected plants from each experimental unit, and cob height (cm) was measured from the soil surface to the main cob for five randomly selected plants from each experimental unit. The leaf area of the plant (cm²) was also calculated upon completion of flowering for five plants from each experimental unit.

The chlorophyll content in the leaves (mg g⁻¹) was also measured according to a method [10] the 200 mg of green leaves were used, cut by sterilized scissors, and then ground in a ceramic mortar in the presence of 6 ml of acetone at a concentration of 80%. When the color of the precipitate became free from the green dye, and then the filtrate was separated from the precipitate by a centrifuge for 10 minutes at a speed of 1600 rpm. The extract was placed in dark tubes so that the dye would not oxidize in the light, and the volume was completed by adding acetone. A sample (Blank) was also prepared, as this sample contains all the materials used in the experiment except for the plant sample, and then the optical density of the filter was measured through a spectrophotometer at wavelengths 645 and 663 nm, where chlorophyll was estimated in plant leaves based on mg g⁻¹ fresh plant tissue by using the following equation:

$$\text{Total Chlorophyll} = \frac{(22.2 \times D_{645} + 8.02 \times D_{663}) \times V}{W \times 1000}$$



In addition, some other characteristics were measured, such as the number of rows in the ear (row ear^{-1}), the number of grains in the ear (grain ear^{-1}), the weight of 500 grain (g), and the total grain yield (Mg ha^{-1}).

Statistical analysis

The data were collected from the field experiment and the results were statistically analyzed according to the analysis of variance (ANOVA) as per the RCBD design [11]. The least significant difference ($\text{L.S.D}_{0.05}$) test was used to compare and separate the mean. The statistics software GenStat12 was employed.

Results and Discussion

Plant height (cm)

The results of table (1) showed a significant effect when adding levels of agricultural sulfur (S) on plant height (cm), as treatment S2 recorded the highest average of 159.92 cm, while the comparison treatment S0 (without fertilization) recorded the lowest average of 146.76 cm, with percentage increase of 8.96%. This discrepancy in plant height may be due to an increase in the amount of agricultural sulfur added to the soil due to the role of sulfur in improving the vegetative growth of plants by improving soil fertility as a result of the formation of an acidic environment in the soil by adjusting the soil (pH), which increased the readiness and absorption of nutrients. [12], including nitrogen and its importance in the formation of nitrogenous bases and protein, which work to increase the size of the cells and the speed of their division, which leads to an increase in the vegetative system, and thus this increase is observed in the height of the plant. The resulting increase in the height of the plant when sulfur was added, it increased the readiness of N and other elements, in addition to the effect of sulfur as food in the form of sulfate [13]. In addition to the role of nutritional sulfur as a result of its contribution to the process of photosynthesis and the formation of nutrients such as protein and amino acids, which contribute to the increase and expansion of cells, which leads to an increase in vegetative growth as a result of the divisions that occur within the apical meristem cells, and thus results in an increase in the height of the plant, and this is consistent with Findings of [14].

The same table also shows that the treatment of adding bacteria T1 gave highest average with plant height reach 160.48 cm, compared to the treatment without adding T0, which gave the lowest average of 147.20 cm, with percentage increase of 9.02%. Sulfur-oxidizing bacteria are considered a biological mediator between sulfur in the soil and plants. They enhance plants' absorption of sulfur and contribute to their growth and development. When sulfur is present in sufficient quantities in the soil, these bacteria Subscribe with plants in sulfur oxidation. They oxidize the organic sulfur present to thiosulfate. and then into sulfate molecules (Sulfate). These difficult compounds are transformed into easily absorbed sulfate ions (SO_4^{-2}), which is an available form for absorption by plants [15]. When bacteria oxidize sulfur, they release other associated nutrients. With sulfur, such as nitrogen, phosphorus, and microelements, which contribute to increasing the activity of vital processes within the plant and thus increasing



the efficiency of the photosynthesis process and then increasing the accumulation of dry matter, which is reflected in increasing the height of the plant [16].

The results table (1) showed that spraying with nano-zinc had a significant effect on plant height. The Zn₂ treatment gave the highest average of 167.17 cm, compared to the treatment without adding Zn₀, which gave the lowest average of 137.52 cm, with percentage increase of 21.56%. It is likely to affect plant height in several areas in the early stages, including zinc, which activates many enzymes in plants. These enzymes have an effective role in the growth processes and development of the vegetative system of plants, including the formation of hormones, proteins, and chlorophyll, which is necessary for the formation of dry matter through biological processes. Within the plant, it works to increase growth and is reflected positively in the length of the plant [17]. This may be attributed to the role of zinc in the synthesis of the amino acid tryptophan, which is considered the basic substance for making the plant hormone auxin, which is located in the growing apex of plants, which is responsible for Plant height through its necessary role in increasing cell division, elongation, and the formation of the largest number of cells, which in turn leads to increased plant growth and thus reflects positively on plant height. This result is consistent with the findings of [18].

Table (1): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on plant height of maize crop (cm).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	127.90	148.19	138.04
	Zn 1	140.05	157.86	148.96
	Zn 2	152.58	168.66	160.62
S1	Zn 0	126.29	140.17	133.23
	Zn 1	162.54	156.32	159.43
	Zn 2	160.99	169.76	165.37
S2	Zn 0	143.96	152.92	148.44
	Zn 1	152.80	168.19	160.49
	Zn 2	161.09	171.00	166.04
				Means (S)
Interaction (S*T)	S0	132.09	161.43	146.76
	S1	151.22	147.45	149.33
	S2	150.60	169.24	159.92
				Means (Zn)
	Zn0	130.96	144.09	137.52
	Zn1	154.48	160.16	157.32



Interaction (Zn*T)	Zn2	160.47	173.86	167.17		
Means (T)		147.20	160.48			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
3.54	3.54	2.89	6.13	5.00	5.00	8.67

The same table shows that there are significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T). as the S2T1 treatment gave the highest average (169.24 cm) compared to the S0T0 treatment, which gave the lowest average (132.09 cm). The results also showed that there are significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), where the S2Zn2 treatment recorded the highest average (166.04 cm) compared to the S1Zn0 treatment, which gave the lowest average (133.23 cm). There is also a significant bilateral interaction between thiobacillus bacteria and nano-zinc (T*Zn), as the T1Zn2 treatment was significantly superior by giving it the highest average of 173.86 cm, while the T0Zn0 treatment gave the lowest average of 130.96 cm. The table also shows that there are significant differences in the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where treatment S2T1Zn2 gave the highest average of 171.00 cm compared to treatment S1T0Zn0, which gave the lowest average of 126.29 cm.

Ear height (cm)

The results of table (2) showed that there were significant differences when adding levels of agricultural sulfur (S) in stem height (cm), where treatment S2 gave the highest average of 106.17 cm, while the comparison treatment S0 (without fertilization) gave the lowest average of 83.25 cm, with percentage increase of 27.53%. This result in the rise of the stem may be attributed to this through adjusting the pH and the readiness of the elements necessary for growth, elongation and cell division, in addition to the role of sulfur in the formation of amino acids that are important in the occurrence of vital processes and its entry into the process of photosynthesis. Therefore, these substances worked to increase the nodes in the stem and the formation of the longest internodes. Then the stem elongated (Table 1), and this resulted in the formation of the ear in a high place in the stem

It was also noted from the same table that the treatment of adding bacteria T1 gave the highest average of the ear height, reaching 96.24 cm, compared to the treatment without adding T0, which gave the lowest average of 89.24 cm, with percentage increase of 7.84%. Perhaps it is due to the effect resulting from the increase in the number of microorganisms of the thiobacillus type, which oxidizes sulfur and releases hydrogen ions, which reduces the degree of reaction and thus increases the readiness of absorb nutrients [19].



The results table (2) showed that spraying with nano-zinc had a significant effect on the ear height, as the Zn2 treatment gave the highest average of 97.54 cm, compared to the treatment without adding Zn0, which gave the lowest average of 87.51 cm, with percentage increase of 11.46%. This may be due to the availability of nutrients, including zinc, necessary for the growth and development of the plant, which stimulated the division and elongation of cells and thus led to the length of one Internode and the number of Internodes in the plant, which was reflected positively in the height of the plant (Table 1), which explains the reason for the height of the ear.

Table (2): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on the ear height of maize crop (cm).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)		
		T0	T1			
S0	Zn 0	79.20	84.67	81.93		
	Zn 1	75.00	89.00	82.00		
	Zn 2	82.00	89.63	85.82		
S1	Zn 0	80.33	90.50	85.42		
	Zn 1	87.33	93.00	90.17		
	Zn 2	89.60	92.00	90.80		
S2	Zn 0	93.33	97.00	95.17		
	Zn 1	104.67	110.00	107.33		
	Zn 2	111.67	120.33	116.00		
				Means (S)		
Interaction (S*T)	S0	78.73	87.77	83.25		
	S1	85.76	91.83	88.79		
	S2	103.22	109.11	106.17		
				Means (Zn)		
Interaction (Zn*T)	Zn0	84.29	90.72	87.51		
	Zn1	89.00	97.33	93.17		
	Zn2	94.42	100.66	97.54		
Means (T)		89.24	96.24			
L.S.D. 0.05						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
2.05	2.05	1.68	3.56	N.S	N.S	5.04

The same table shows that there are no significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T). The results also showed that there are significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), where the S2Zn2 treatment recorded the highest average (116.00 C) compared to the S0Zn0 treatment, which gave the lowest average (81.93 C). The results also



showed that there were no significant differences in the binary interaction between thiobacillus bacteria and nano-zinc (T^*Zn). The table also shows that there are significant differences in the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S^*T^*Zn), where treatment $S2T1Zn2$ gave the highest average ear height (120.33 cm) compared to treatment $S0T0Zn1$, which gave the lowest average (75.00 cm).

Leaf area (cm^2)

The results table (3) showed a significant effect when adding levels of agricultural sulfur (S) on leaf area, as treatment S2 gave the highest average of 6766 cm^2 , while the comparison treatment S0 (without fertilization) gave the lowest average of 5864 cm^2 , with percentage increase of 15.38%. Perhaps the increase in one leaf area unit is due to the formation of dissolved sulfates, the main source of agricultural sulfur after its decomposition and absorption by the root, as it participates in many of the nutritional processes necessary to increase the shoot [20]. Sulfur contributes to stimulating leaf growth and development, which increases the number and size of leaves and improves leaf growth and development, thus increasing the leaf surface available for photosynthesis and absorption of nutrients from the surrounding environment [21].

The same table also showed that the treatment of adding bacteria T1 gave the highest average of 7260 cm^2 , compared to the treatment control (T0), which gave the lowest average of 5314 cm^2 , with percentage increase of 36.62%. The reason is due to the role of Thiobacillus bacteria in enhancing the availability of sulfur in the soil, which is extremely important for the growth and development of maize (Table 1, 2). They contribute to converting organic sulfur compounds into mineral forms that are easier for the plant to absorb, thus improving the absorption of nutrients and enhancing plant vitality. This It is consistent with the findings of [22]. In addition, its participation in the decomposition of organic materials supports soil fertility and nutrient cycling, and thus these nutrients are translated into increased cell division and reproduction, which contributes to increasing the efficiency of the shoot [23].

The results table (3) showed that spraying with nano-zinc had a significant effect on unit leaf area. The Zn1 treatment gave the highest average of 6413 cm^2 , and it did not differ significantly from the Zn2 treatment, which gave an average of 6334 cm^2 , respectively, compared to the treatment without adding Zn0, which gave an average of 6114 cm^2 . An increase of 4.89% and 3.59%, respectively. The reason for this increase may be due to increased concentrations of nano-zinc and activating a number of enzymes and its inclusion in the formation of the amino acid tryptophan, which is important in cell elongation, the formation of energy compounds, and the formation of RNA and DNA necessary for cell division, and this in turn increases the activity of water absorption. And nutrients, which is reflected positively in increasing the leaf area of plants [24]. In addition to the distinction of nanotechnology with the size of its small particles and their equal distribution in nanometers, this effectively and quickly affects the growth and development of plant stages. Also, due to the extremely small nanoparticles, zinc is allowed to move through cellular membranes. This result is consistent with what was found by [18].



The same table shows that there are significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T), where the S2T1 treatment recorded the highest average of 7930 cm², compared to the S0T0 treatment, which gave the lowest average of 5092 cm². The results also showed that there were significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), as the S2Zn1 treatment recorded the highest average, and it did not differ significantly from S2Zn2, S1Zn2, and S0Zn1, which amounted 6949, 6876, 6730, and 6660 cm², respectively, compared to the S0Zn2 treatment, which gave the lowest average of 5395 cm². It is clear from the table that there are no significant differences between thiobacillus bacteria and nano-zinc (T*Zn). The table also shows that there are significant differences in the triple interaction coefficients for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where the S2T1Zn1 treatment gave the highest average, which did not differ significantly from S2T1Zn2, S1T1Zn2, S0T1Zn1, and S2T1Zn0 amounted 8180, 7982, 7730, 7677 and 7630 cm², respectively compared to Treatment S0T0Zn0 gave the lowest average of 4695 cm².

Table (3): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on the leaf area of maize crop (cm²).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)		
		T0	T1			
S 0	Zn 0	4695	6378	5536		
	Zn 1	5643	7677	6660		
	Zn 2	4938	5852	5395		
S 1	Zn 0	5125	7540	6332		
	Zn 1	4889	6371	5630		
	Zn 2	5730	7730	6730		
S 2	Zn 0	5317	7630	6473		
	Zn 1	5718	8180	6949		
	Zn 2	5769	7982	6876		
				Means (S)		
Interaction (S*T)	S0	5092	6636	5864		
	S1	5248	7214	6231		
	S2	5601	7930	6766		
				Means (Zn)		
Interaction (Zn*T)	Zn0	5046	7182	6114		
	Zn1	5416	7409	6413		
	Zn2	5479	7188	6334		
Means (T)		5314	7260			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Z*T
230.0	230.0	187.8	398.4	325.3	N.S	563.4



Total Chlorophyll (mg g^{-1})

The results of table (4) showed significant differences when adding levels of agricultural sulfur (S) in the total chlorophyll characteristic, as treatment S2 recorded the highest average (2.572 mg g^{-1}), while the comparison treatment S0 (without fertilization) recorded the lowest average (1.446 mg g^{-1}).with percentage increase of 77.86%. The increase in chlorophyll content may be attributed to the increased availability of macro- and micronutrients, as shown in (Table 1, 3), which can contribute to increasing the production of chlorophyll (the green pigment) in plants, which is responsible for the process of photosynthesis in plants, and thus can enhance photosynthesis activity and increase chlorophyll production in plants [25], or perhaps the increase is due to the element sulfur as it is one of the most important components of the porphyrin ring which is considered one of the basic parts of chloroplasts[26]. Therefore, it contributes directly or indirectly to increasing the activity of the photosynthesis process, which reflects positively on the plant's chlorophyll content [27].

The same table also shows that the treatment of adding bacteria T1 gave the highest average (2.057 mg g^{-1}) compared to the treatment without adding T0, which gave the lowest average (1.879 mg g^{-1}), with percentage increase of 9.47%. This is attributed to the role of the sulfur-oxidizing bacteria thiobacillus in converting non-mineral sulfur into a mineral ready for absorption, and this is consistent with what was found by [28].

The results table (4) showed that spraying with nano-zinc had a significant effect on the plant's chlorophyll content. The Zn2 treatment gave the highest average (2.236 mg g^{-1}) compared to the Zn0 treatment, which gave the lowest average (1.830 mg g^{-1}), with percentage increase of 22.18%. An improvement in the rate of chlorophyll may occur when increasing the concentration of zinc spray on plants that suffer from a deficiency, including maize, because it is one of the crops that depletes nutrients. Spraying zinc can affect the rate of chlorophyll in plants by activating the enzymes associated with it [29].

The same table shows that there are no significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T). The results also showed that there are significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), where the S2Zn2 treatment recorded the highest average (2.806 mg g^{-1}), while the S0Zn1 treatment recorded the lowest average (1.343 mg g^{-1}). There was also a significant bilateral interaction between thiobacillus bacteria and nano-zinc (T*Zn), as the T1Zn2 treatment was significantly superior in giving it the highest average (2.402 mg g^{-1}), while the T0Zn1 treatment gave the lowest average (1.696 mg g^{-1}). The table also shows that there are significant differences in the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where treatment S2T1Zn2 gave the highest average (2.927 mg g^{-1}) and did not differ significantly from S2T0Zn2, S2t0Zn0, and S1T1Zn2 compared to treatment S0T0Zn0, which gave the lowest average (1.160 mg g^{-1}).

Table (4): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on the character of total chlorophyll of maize crop (mg g⁻¹)

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)		
		T0	T1			
S0	Zn 0	1.160	1.669	1.414		
	Zn 1	1.252	1.434	1.343		
	Zn 2	1.483	1.678	1.580		
S1	Zn 0	1.778	1.440	1.609		
	Zn 1	1.466	1.985	1.726		
	Zn 2	2.039	2.601	2.320		
S2	Zn 0	2.676	2.255	2.465		
	Zn 1	2.369	2.521	2.445		
	Zn 2	2.685	2.927	2.806		
				Means (S)		
Interaction (S*T)	S0	1.299	1.594	1.446		
	S1	1.761	2.009	1.885		
	S2	2.577	2.568	2.572		
				Means (Zn)		
Interaction (Zn*T)	Zn0	1.871	1.788	1.830		
	Zn1	1.696	1.980	1.838		
	Zn2	2.069	2.402	2.236		
Means (T)		1.879	2.057			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.139	0.139	0.113	0.240	N.S	0.196	0.340

Rows number (row ear⁻¹)

The results of table (5) showed a significant effect when adding levels of agricultural sulfur (S) on the number of rows, as treatment S2 gave the highest average of 16.14 row ear⁻¹, while treatment S1 gave the lowest average of 13.26 row ear⁻¹, with percentage increase of 21.71%. The reason is due to the role of sulfur in increasing the readiness of nutrients (Table 1, 3), through acidifying the soil as a result of reducing soil basicity with the help of bacteria that oxidize it. Thus, the readiness of the basic elements that are important in the growth and development of the plant is facilitated, and thus the accumulation of dry matter increases, which reflects positively on this trait [30].

It is also noted from the same table that the treatment of adding bacteria T1 gave the highest average of 14.37 row ear⁻¹, compared to the treatment without adding T0, which gave the lowest average of 13.54 row ear⁻¹, with percentage increase of 6.12%. The



reason for this may be attributed to the role of thiobacillus in secreting enzymes, and with the help of plant roots, with the availability of conditions for growth and decomposition, the efficiency of nutrient absorption by the roots increased, thus increasing the efficiency of the shoot, which was reflected positively in the representation and accumulation of dry matter, and this explains the increase in the number of rows in the ear [4].

The results table (5) showed that spraying with nano-zinc had a significant effect on the number of cob rows, as the Zn2 treatment gave the highest average of 15.11 row ear⁻¹, and it did not differ significantly from Zn1 compared to the treatment without the addition of Zn0, which gave the lowest average of 12.44 row ear⁻¹. with percentage increase of 21.46%. The superiority of the Zn2 treatment may be attributed to the role of vital zinc in activating enzymes as a cofactor for many enzymes in plants. These enzymes play an important role in metabolic processes such as respiration, glycolysis, protein and amino acid synthesis, and regulating cellular structure, including regulating cell growth, development and division, therefore zinc contributes indirectly to the Increase the representation of dry matter and thus reflects positively on this trait [31].

The same table shows that there are significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T) , where treatment S2T1 recorded the highest average of 16.51 row ear⁻¹and did not differ significantly from S2T0 compared to treatment S0T0, which recorded the lowest average of 11.59 row ear⁻¹. The results also showed that there are significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), as the S2Zn1 treatment recorded the highest average of 15.85 row ear⁻¹, and it did not differ significantly from S1Zn2 compared to the treatment of S1Zn0, which gave the lowest average of 11.46 row ear⁻¹. The results also showed that there was a significant bilateral interaction between thiobacillus bacteria and nano-zinc (T*Zn), as the T1Zn2 treatment excelled by giving it the highest average of 15.69 row ear⁻¹ and did not differ significantly from T0Zn1, while the treatment T0Zn0 gave the lowest average of 11.56 row ear⁻¹. The table also shows that there are significant differences in the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where the treatment S2T1Zn2 gave the highest average plant height, which amounted to 16.23 row ear⁻¹, and it did not differ significantly from S2T1Zn1, S2T0Zn0, S0T1Zn0, S0T1Zn2, S2T0Zn1, and S1T. 0Zn2 And S1T0Zn1 compared to treatment S0T0Zn0, which gave the lowest average of 10.27 row ear⁻¹.

Table (5): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on the number of rows in the ear of maize crop (row ear⁻¹)

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	10.27	15.83	13.05
	Zn 1	11.60	12.33	11.97
	Zn 2	12.90	15.65	14.27



S1	Zn 0	11.22	11.70	11.46		
	Zn 1	14.93	12.50	13.72		
	Zn 2	15.43	14.55	14.99		
S2	Zn 0	15.87	12.47	14.17		
	Zn 1	15.62	16.09	15.85		
	Zn 2	13.27	16.23	14.75		
				Means (S)		
Interaction (S*T)	S0	11.59	15.49	13.54		
	S1	13.63	12.88	13.26		
	S2	15.78	16.51	16.14		
				Means (Zn)		
Interaction (Zn*T)	Zn0	11.56	13.33	12.44		
	Zn1	14.91	14.42	14.67		
	Zn2	14.53	15.69	15.11		
Means (T)		13.54	14.37			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.63	0.63	0.51	1.09	0.89	0.89	1.54

Grains number (grain ear⁻¹)

The results table (6) showed that there was a significant effect when adding levels of agricultural sulfur on the number of grains in the ear, where treatment S2 gave the highest average of 670.0 grain ear⁻¹, while treatment S0 (without fertilization) gave the lowest average of 430.6 grain ear⁻¹, with percentage increase of 55.59%. Perhaps it is due to the role of sulfur as an important element in the formation of essential amino acids, which are among the basic units for the synthesis of proteins. Therefore, it contributes indirectly to the efficiency of vital processes within plant tissues, in addition to its contribution to improving the efficiency of using nutrients (Table 1, 3), which contributes to improving their absorption. This is reflected in an increase in dry matter assimilation as a result of the efficiency of the shoot, and then in an increase in yield and its components [32].

The same table also shows that the treatment of adding bacteria T1 gave the highest average of 500.8 grain ear⁻¹, compared to the treatment without adding (T0), which gave the lowest average of 482.0 grain ear⁻¹, with percentage increase of 3.90%. The reason may be due to the role of biofertilizers, including thiobacillus bacteria, in providing a variety of important nutrients such as nitrogen, phosphorus, potassium, and other nutrients, as well as their role in improving the texture of the soil by modifying or reducing the pH of the soil. It also contributes to increasing the content of organic matter in it and increasing its capacity. It helps retain water and nutrients, which leads to optimal growth conditions that reflect positively on the plant's yield and its components [33].



The results table (6) showed that spraying with nano-zinc had a significant effect on the number of grains in the ear. The Zn2 treatment gave the highest average, amounting 527.3 grain ear⁻¹, compared to the treatment without adding (Zn0), which gave the lowest average of 417.1 grain ear⁻¹, with percentage increase of 26.42%. The reason for the increase in the rate of the number of grains in the ear may be due to the effect of foliar nutrition such as zinc, which plays a role in the synthesis of chlorophyll and oxidation and reduction enzymes that are important in the process of photosynthesis and respiration, which causes an increase in the rate of pollination and fertilization and thus positively affects the components of the yield such as the number of grains in the ear. Agreed. These results are with [24].

The same table shows that there are significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T). where treatment S2T1 recorded the highest average of 690.8 grain ear⁻¹, compared to treatment S0T0, which recorded the lowest average of 387.8 grain ear⁻¹. The results also showed that there were significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), as the S2Zn1 treatment recorded the highest average of 589.1 grain ear⁻¹, and it did not differ significantly from S2Zn2 and S2Zn0 compared to the S0Zn1 treatment, which gave the lowest average of 369.4 grain ear⁻¹. There is also a significant bilateral interaction between thiobacillus bacteria and nano-zinc (T*Zn), as the T1Zn2 treatment outperformed significantly by giving it the highest average of 550.8 grain ear⁻¹, while the T0Zn0 treatment gave the lowest average of 387.8 grain ear⁻¹. The table also shows that there are also significant differences for the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where the S2T1Zn2 treatment gave the highest average of 650.5 grain ear⁻¹, which did not differ significantly from S2T0Zn0 compared to the S0T0Zn0 treatment, which gave the lowest average of 287.9 grain ear⁻¹.

Table (6): Effect of agricultural sulfur, thiobacillus bacteria nano-zinc, and their interaction on the number of grains in the ear of maize crop (grain ear⁻¹).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	Zn 0	287.9	460.4	374.1
	Zn 1	389.5	349.3	369.4
	Zn 2	389.8	435.2	412.5
S1	Zn 0	348.0	468.4	408.2
	Zn 1	554.7	438.8	496.4
	Zn 2	539.8	483.1	511.5
S2	Zn 0	639.9	499.9	569.9
	Zn 1	576.9	601.4	589.1
	Zn 2	491.7	650.5	571.1
				Means (S)



Interaction (S*T)	S0	387.8	473.5	430.6		
	S1	596.5	477.5	537.0		
	S2	649.3	690.8	670.0		
				Means (Zn)		
Interaction (Zn*T)	Zn0	387.9	446.4	417.1		
	Zn1	487.3	487.1	487.2		
	Zn2	503.8	550.8	527.3		
Means (T)		482.0	500.8			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
19.69	19.69	16.07	34.10	27.84	27.84	48.22

500-grain weight (g)

Weight is one of the important components of grain yield in maize, as it indicates the accumulation of dry matter in the grain and reflects the efficiency of the source on the one hand and the efficiency of the downstream source on the other hand, and it is affected by growth factors.

The results table (7) showed that there were significant differences in the weight of 500 grains. where treatment S1 gave the highest average of 154.26 g, which did not differ significantly from S0, while treatment S2 gave the lowest average of 149.92 g, with percentage increase of 2.89%. The reason for the superiority of treatments S1 and S0 over S2 in increasing grain weight may be due to the principle of compensation, as the number of rows and grains in the ear decreased in treatments S1 and S0 in (Table 5, 6), which led to a decrease in the yield, which led to the exploitation of the empty space in the ear, and this It agrees with [34], or the reason may be due to the relative decrease in the number of grains in the row, which reduced competition for transported nutrients and thus reflected positively on grain weight. It may be attributed to the addition of agricultural sulfur and its role in the photosynthesis process, thus increasing the activity of vegetative growth characteristics (leaf area and chlorophyll content), which led to an increase in the period required for grain filling, which is reflected in the weight of the grain, through the metabolic processes that occur during the stages of plant growth and development and its storage on The form of nutrients (fats, carbohydrates, and proteins) and their assembly in grains, and this is consistent with the findings of [35].

It is also noted from the same table that the treatment of adding bacteria, T1, gave the highest average of 154.64 g, compared to the non-addition treatment, T0, which gave the lowest average of 149.70 g, with an increase rate of 3.29%. Biofertilizers can contribute to increasing grain productivity by improving soil properties and providing essential nutrients to plants. When used correctly, they can improve the efficiency of plants' absorption of nutrients (Tables 1, 3, 4) and enhance their growth and development in general, and thus can have a positive impact. On the components of the outcome [28].



The results table (7) showed that spraying with nano-zinc had a significant effect on grain weight. The Zn2 treatment gave the highest average of 155.53 g, compared to the Zn0 treatment, which gave the lowest average of 149.42 g, with percentage increase of 4.08%. Maize is one of the crops affected by zinc deficiency, and at the same time has a high response when fertilized with it, since zinc has a major role in increasing the formation of chlorophyll and leaf area, thus increasing the process of photosynthesis and respiration and increasing plant activity in absorbing water and nutrients and converting them into dry materials inside the plant, which increases From filling the grains and increasing their weight, in addition to its role in protein metabolism, which contributes to the representation of a larger amount of protein and then its transfer to the grains, and this is consistent with what [36].

The same table shows that there are significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T). As the S0T1 treatment recorded the highest average of 167.46 g, which did not differ significantly from S1T0 compared to the S0T0 treatment, which gave the lowest average of 141.07 g. The results also showed that there are significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), where the S0Zn2 treatment recorded the highest average of 165.88 g, compared to the S2Zn2 treatment, which gave the lowest average of 144.33 g. There is also a significant bilateral interaction between thiobacillus bacteria and nano-zinc (T*Zn), as the T1Zn2 treatment was significantly superior by giving it the highest average of 160.22 g, while the T0Zn1 treatment gave the lowest average of 148.79 g. The table also shows that there are significant differences in the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where treatment S0T1Zn2 gave the highest average of 181.36 g, compared to treatment S2T0Zn2, which gave the lowest average of 131.58 g.

Table (7): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on the weight of 500 grains of maize crop (g).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	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	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	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)
Interaction (S*T)	S0	163.15	141.50	152.32
	S1	141.07	167.46	154.26



	S2	144.89	154.95	149.92		
				Means (Zn)		
Interaction (Zn*T)	Zn0	149.48	149.36	149.42		
	Zn1	148.79	154.34	151.56		
	Zn2	150.84	160.22	155.53		
Means (T)		149.70	154.64			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
3.35	3.35	2.73	5.80	4.74	4.74	8.21

Grain yield (Mg ha⁻¹)

The results table (8) showed that there was a significant difference in grain yield characteristics, as the S2 treatment recorded the highest average of 8.06 Mg ha⁻¹, while the S0 treatment (without fertilization) recorded the lowest average of 5.95 Mg ha⁻¹, with percentage increase of 41.51%. The increase in the average yield in the S2 treatment may be due to the importance of agricultural sulfur in improving the quality of the soil and its chemical composition. Therefore, it increases the availability of nutrients in the soil solution and increases their absorption by the plant, by modifying the degree of soil interaction (pH), in addition to its role as a nutritional element. The plant needs it during the stages of growth and development, as it is involved in the formation of chlorophyll, increasing the green cover exposed to sunlight, and converting the reactions that take place into dry food materials. Thus, this can lead to providing optimal conditions for the growth and development of plants, giving a good shoot, and transferring the products of the photosynthesis process from the sources of their synthesis, to yield components and thus increase grain yield [37]. This is consistent with the findings of [38] that sulfur contributes to the readiness of elements on the one hand, and on the other hand, it participates as a direct nutritional element in vital processes within the plant, due to its participation in the formation of chlorophyll and amino acids.

It was also noted from the same table that the treatment of adding bacteria (T1) gave the highest average (7.52 Mg ha⁻¹) compared to the non-addition treatment (T0), which gave the lowest average (6.54 Mg ha⁻¹), with percentage increase of 14.98%. This may be due to the use of sulfur-oxidizing bacteria thiobacillus, which works to improve the soil by providing organic materials and increasing their availability to plants, which led to the continuation of metabolic processes in the formation of carbohydrates and proteins and the regularity of their transport and assembly in grains [4], in addition to its role in increasing sulfur readiness. This is done by converting it from an organic form into a ready-made mineral, thus providing the necessary sulfur in the synthesis of vitamins, proteins, and enzymes that are important in the construction and development of plant stages, which after a series of reactions contributes to increasing grain yield. This result is consistent with [39] who indicated an increase in What occurs when inoculated with bacteria or using biological fertilizers.



The results table (8) showed that spraying with nano-zinc had a significant effect on grain yield, as the Zn1 treatment gave the highest average (8.01 Mg ha⁻¹) and did not differ significantly from Zn2 compared to the no-addition treatment (Zn0), which gave the lowest average (7.12 Mg ha⁻¹), with percentage increase of 12.50%. This is attributed to the role of zinc in reproductive processes (flowering, pollination and fertilization) by stimulating pollen grains and reducing the number of sterile flowers. It also affects the formation of the amino acid tryptophan, which is the main source for building indole acetic acid (IAA), which is directly responsible for the elongation process and is important. In the synthesis of auxin, which positively affects the fruit setting process [40].

The same table shows that there are significant differences in the binary interaction between sulfur and thiobacillus bacteria (S*T), where the S2T1 treatment recorded the highest average (8.59 Mg ha⁻¹) compared to the S0T0 treatment, which gave the lowest average (5.18 Mg ha⁻¹), as shown. Results: There are significant differences in the binary interaction between sulfur and nano-zinc (S*Zn), as the S2Zn2 treatment recorded the highest average (8.02 Mg ha⁻¹), and it did not differ significantly from S2Zn1, S1Zn2, S2Zn0, and S2Zn1 compared to the S0Zn2 treatment, which gave the lowest average (5.60 Mg ha⁻¹). There is also a significant bilateral interaction between thiobacillus bacteria and nano-zinc (T*Zn), as the T1Zn1 treatment was significantly superior by giving it the highest average (8.12 Mg ha⁻¹) and did not differ significantly from T1Zn2, while the T0Zn0 treatment gave the lowest average (6.69 Mg ha⁻¹). The table also shows that there are significant differences in the triple interaction treatments for each of the sulfur fertilizers, thiobacillus bacteria, and nano-zinc (S*T*Zn), where the S2T1Zn2 treatment gave the highest average (8.63 Mg ha⁻¹) and did not differ significantly from the S2T1Zn1, S1T1Zn2, S1T1Zn1, S2T1Zn0, and S2T0Zn2 compared to the treatment. S1T0Zn0, which gave the lowest average (4.85 Mg ha⁻¹).

Table (8): Effect of agricultural sulfur, thiobacillus bacteria and nano-zinc and their interaction on the grain yield trait of maize crop (Mg ha⁻¹).

Sulfur (S)	Nano-zinc (Zn)	Thiobacillus (T)		Interaction (S*Zn)
		T0	T1	
S0	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	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	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)



Interaction (S*T)	S0	5.18	6.72	5.95		
	S1	7.00	7.20	7.10		
	S2	7.53	8.59	8.06		
				Means (Zn)		
Interaction (Zn*T)	Zn0	6.69	7.55	7.12		
	Zn1	7.91	8.12	8.01		
	Zn2	7.76	8.11	7.93		
Means (T)		6.54	7.52			
L.S.D _{0.05}						
S	Zn	T	S*Zn	S*T	Zn*T	S*Zn*T
0.640	0.640	0.523	1.109	0.906	0.906	1.569

It is noted from the results of the study the importance of using agricultural sulfur as a conditioner for Iraqi limestone soils and the necessity of adding thiobacillus bacteria to increase and accelerate the oxidation process, In addition to spraying with nano-zinc, because calcareous soil lacks nutrients, including zinc.

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