Egypt. J. of Appl. Sci., 33 (1) 2018 *17-40*

EFFECT OF FERTILIZATION SOME SULPHUER SOURCES ON SOIL FERTILITY AND COWPEA PRODUCTIVITY UNDER SALINE SOIL CONDITIONS

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Key Words: Bio inoculation, sulphur sources, cowpea, saline soil.

ABSTRACT

Two field experiments were conducted at El-Qantra Shark (Ismailia Governorate, Egypt) during summer 2016 and 2017 seasons to evaluate the influence of soil application of sulphur from different sources *i.e.* agricultural sulphur (AS), gypsum (G) and calcium sulphate (CS) in four rates 0 , 0.2, 0.3 and 0.4 ton fed.$^{-1}$ for AS and 0, 2, 3 and 4 ton fed^{-1} for G and CS as control, low rate, medium rate and high rate, respectively solely or inoculation with *Thiobacillus* sp. strain on inhibitory the hazardous effects of soil salinity stress as well as vegetative growth, yield and its quality of cowpea (*Vigna unguiculate* L. c.v. Kafr El-Sheikh) as well as some chemical characteristics of the experiment soil after harvest. Yield and yield components as well as macro and micronutrient contents and uptake of cowpea seeds were increased as a result of applied different sulphur sources and rates and/ or bio inoculation with *Thiobacillus* and their combinations. Seed protein content and protein yield as well as total chlorophyll and free amino acids were increased significantly and proline content was decreased as affected by the treatments. Fertilized treatments decreased values of soil pH and EC and increased soil available N, P, K and S as well as Fe, Mn and Zn content after harvest. The superior treatment was observed when calcium sulphate inoculated with *Thiobacillus*, especially at the highest rate of (4 ton fed^{-1}), which gave highest values for all variables under study and increases in seed yield /fed., was about 57.52 and 40.21 % than that plants which untreated the 1st and 2nd seasons, respectively.

INRTODUCTION

Cowpea is a seed legume food that plays a critical role in the life of millions of people in Africa. The seed contains between 200-250g protein kg^{-1}, about twice the protein content of most cereals. The crop can fix about 100 kg N fed^{-1} and make available about 27 kg Nfed^{-1}for succeeding crops grown in rotation with it **(Aikins and Afuakwa, 2008).**

Sulphure and bio- fertilizers, added alone and in combination, are an important means of plant nutrition, particularly in saline soils. Attention has therefore been focused on addition combinations of mineral and bio fertilizers, such as sulphure (S) as a technique to overcome the adverse effects of soil salinity on growing plants. Sulphure is the fourth major essential nutrient element after N, P and K plays an important role , not only in growth and development of higher plants and increase stress tolerance in plants **(Nazar *et al* ., 2011). Kineber *et al.* (2004)** indicated that the application of S led to decreased of soil pH value by the oxidation of S to sulphate through various species of soil pH improve the availability of micronutrients (Fe, Mn and Zn) and improvise the chemical properties of alkaline soil. **Ashraf and Mostafa (2012)** found that the N, P and K concentration in pea plants were increase with treated by sulpure compared with control under saline soil. As with S improved the chemical properties of soils because it increased activity of microorganisms which increase the nutrient cycling and the availability of absorbed nutrients by plant roots.

Gypsum is soil amendment important for improved sodic soil and soil salinity. Gypsum has a calcium content of 23 % and 19 % sulphur. The calcium in the applied gypsum enables sodium displacement on the cation exchange capacity of the soil. The increase quantity of calcium is required thus it is a mass action process **(Gelderman *et al*., 2004).** Gypsum (Ca_2SO_4 $2H_2O$) is low cost, and used for sodic soil reclamation and to achieved sulfur fertilizer **(Jaggard and Zhao, 2011).**

Jyub *et al*. (2007) indicated that the application of different sulphure sources i.e. (Gypsum, Agriculture sulphure and ammonium sulphate) led to decreased of soil pH slowly from (8.50 – 7.50) for soil treated with gypsum ; followed by sulphur (8.50 – 7.70) and ammonium sulphate (8.5- 7.80 compared with control (8.50 – 8.20) after 20 weeks. **Stamford *et al.* (2004)** found that the application of high amount of gypsum to sodic soil was redacted soil pH.

The bio-fertilizer (Sulphur –Oxidizing bacteria *Acidithiobacillus* with elemental sulfur produced sulfuric acid from gypsum application the H^+ released by the acid may contributed decisively to reduce soil pH in sodic soils **(Stamford *et al*., 2007).** Sulfur – Oxidizing bacteria is promoted the availability of elemental sulfur in soil and solubilization of the otherwise –unavailable soil phosphorus **(El-Tarabily *et al*., 2006).**

The aim of this study was to evaluate the sulphur sources of three sources (Gypsum, Agriculture sulphur and ammonium sulphate) combined with or without biofertilizer (*Thiobacillus* strain)on saline soil fertility and Cowpea productivity and quality.

MATERIALS AND METHODS

Two field experiments were conducted at El-Qantra Shark, Ismalia Governorate , Egypt, during two successive summer seasons (2016) and (2017), to evaluate some sulphure sources and its effect on improve soil salinity properties and cowpea productivity and quality. Local seed variety (V*igna unguiculata L.)* cv. Kafr El-Sheikh was used.

The main physical and chemical properties of soil were presented in Table 1 which was determined according to **Cottenie *et al.* (1982) and Page *et al.* (1982)**.

Table (1). Some physical and chemical properties of soil under study.

Particle size distribution (%)				Texture	O.M (%)	$CaCO_3$ (%)		
Coarse sand	Fine sand	Silt	Clay					
5.49	70.00	8.13	16.38	Sandy loam	0.62	12.85		
pH (1:2.5)	EC (dSm^{-1})	Cations (meq^{-1})				Anions (meq^{-1})		
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	HCO_3^{-}	Cl^{-}	SO_4^{--}
8.25	10.85	14.62	25.96	67.03	0.89	12.85	55.10	40.55
Macronutrients ($mgkg^{-1}$)				Micronutrients ($mgkg^{-1}$)				
N	P	K	S	Fe	Mn	Zn		
37.26	3.85	185	4.36	6.49	2.63	0.66		

This experiment included 24 treatments, which were the interaction between three soil amendments (agricultural sulphur (AS), gypsum (G) and Calcium sulphate (CS) combined with or without bio-fertilizers .

These treatments were arranged in a split split plot in a complete block design with three replications. Soil amendments were randomly distributed in the main plot; the rates of soil amendment were randomly arranged in the sub plot, while biofertilizer was arranged in sub sub plot.

The area of each experimental plot unit was 4x5m which divided in five rows with 4 m long and wide of 60 cm. Sowing seeds of Cowpea were 10^{th} May 2016 and 2017, respectively. Seeds were inoculated with *Thiobacillus* strain (salt tolerant PGPR) biofertilizer. The biofertilizer was applied at a rate of 100 g for 15 kg seeds wetted with 300 ml of adhesive liquid (Arabic gum). Seeds were thoroughly mixed with the inoculants in the shade, then sown immediately and covered with soil. More biofertilization was added three times at 21, 45 and 65 days through liquid sprays on soil at a rate of 12 L/ fed. Seeds of inoculated or un-inoculated cowpea were sown by hand on the western side of the ridge. Three of seeds were sown in hole with 5 cm depth. After 21 days of sown, the plants of each hole were thinned to one plant. All tillage processes were carried out before sowing. Agricultural sulphur was added at rates of (0, 0.2, 0.3 and 0.4 ton fed^{-1}), while gypsum and calcium sulphate were added at rates of (0, 2, 3, 4 ton $fed.^{-1}$) as control, low, medium and high rates 25 days from sowing. Super phosphate (15.5 % P_2O_5) was applied at rate of 100 kg $fed.^{-1}$ during tillage soil. Urea (46 % N) was applied at rate of 30 kg N $fed.^{-1}$ on three doses after 21, 45 and 65 days from planting. Potassium sulpate (48% K_2O) was applied at rate 75 kg fed^{-1} on two doses after 21 days and 50 days from seeds sowing. Harvest was done on 15 September 2016 and 2017 respectively.

Yield Parameters:

Plants samples of 10 plants were taken after 60 days from sowing to determine total chlorophyll as described by **Saric et al . (1967)** and proline content according to methods described **by Bates *et al.* (1973).** Measurements were recorded on 20 plants chosen randomly from each plot for the following characteristics, plant height (cm) which measured from soil surface to top of the plant; pod yield (ton $fed.^{-1}$); seed yield (ton $fed.^{-1}$) and 100-seed weight (g).

Each dry plant sample was separated into straw and pods. Both straw and pods were air –dried and oven dried at $70C^o$ for 72 hrs. then ground and leapt in plastic bags for chemical analysis. 0.4 g of plant sample was digested using H_2SO_4 mixed with $HClO_4$ according to the method described by **Chapman and Pratt (1961).** The plant content of N (%) was determined according to **AOAC (1990).** Protein (%) was estimated by multiplying the nitrogen (%) by 6.25 according to **Hymowitz *et al.* (1972).** P, K, Fe, Mn and Zn concentration were determined in plant using the methods described by **Cottenie *et al.* (1982) and Page *et al.* (1982).**

The obtained data were statically analysis using that the COSTAT program and L.S.D. 5 % was calculated according to **Gomez and Gomez (1984).**

RESULTS AND DISCUSSION

Effect of different sulphur sources and bio inoculation with *Thiobacillus* on some soil properties after cowpea harvest

Soil pH

Results of soil analysis in Table (2) show that values of soil pH in combined data of the two studied seasons was slightly reduced due to the addition of agricultural sulphur (AS), gypsum (G) and calcium sulphate (CS) led to the reduction was pronounced in case of high rate of treatments where the pH slightly decreased from 8.15 to 8.02 for AS, 8.18 to 8.07 for G and 8.13 to 8.01 for CS when inoculation with *Thiobacillus* and decreased from 8.20 to 8.10 for AS, 8.23 to 8.12 for G and 8.20 to 8.10 for CS without inoculation. These results are agreement by **Ayub *et al.* (2007)** who reported that the gypsum reduced soil pH slowly from (8.5–7.5) in about 20 weeks followed by sulphur (8.5–7.7) compared with control (8.5–8.2). The decrease in soil pH could be discussed as follows: calcium ions react with bicarbonate to precipitate calcite ($CaCO_3$) and release protons (H^+) in soil solution which neutralize the hydroxide ions (OH^-) and decrease the soil pH **(Rasouli *et al.*, 2013).** The replacement of sodium by calcium and the formation of neutral salts with $SO4^=$ and a decrease in sodium concentration as a fraction of the cations. Moreover, gypsum solubility is also enhanced as a result of increased ionic strength of solution and the formation of the sodium sulfate ion pair. Besides, large quantities of CO_2 have been evolved during leaching process, some of which would become soluble in soil solution giving carbonic acids, **(Abdel-Fattah, 2012).** Also, **Nasef *et al.* (2009)** reported that the applied of bio-fertilizer resulted in reduction of soil pH due to various acids (amino acids such as glycine and cysteine as well as humic acid) or acid forming compounds and active microorganisms released from the addition of bio-fertilizer

Table (2). Effect of different sulphur sources and bio inoculation with *Thiobacillus* on soil pH and EC after cowpea harvest

Sulphur source (S)	Sulphur rate (R), ton fed^{-1}	pH (1:2.5)		EC (dSm^{-1})		
		Bio –fertilizer (B)				
		With	Without	With	Without	Mean
Agricultural sulphur (AS)	Control (0)	8.15	8.20	7.56	8.56	8.06
	Low (٠.٢)	8.10	8.16	6.25	8.22	7.24
	Medium (٠.٣)	8.06	8.12	5.49	7.92	6.71
	High (٠.٤)	8.02	8.10	4.38	7.53	5.96
Mean				**5.92**	**8.06**	**6.99 b**
Gypsum (G)	Control (0)	8.18	8.23	8.95	9.25	9.10
	Low (٢)	8.13	8.17	7.55	8.98	8.27
	Medium (٣)	8.10	8.13	6.85	8.46	7.66
	High (٤)	8.07	8.12	5.68	7.98	6.83
Mean				**7.26**	**8.67**	**7.96 a**
Calcium sulphate (CS)	Control (0)	8.13	8.20	7.22	8.27	7.75
	Low (٢)	8.09	8.15	6.14	7.85	7.00
	Medium (٣)	8.05	8.13	5.30	7.44	6.37
	High (٤)	8.01	8.10	4.20	6.89	5.55
Mean				**5.72**	**7.61**	**6.66 c**
Mean of bio				**6.30b**	**8.11a**	
Mean of sulphur rate	control			**8.30 a**		
	Low			**7.50 b**		
	Medium			**6.91 c**		
	High			**6.11 d**		
F-test				**S:** R:** B:** SxR: ns**		
				SxB: ** RxB: SxRxB: ns**		

Because nitrification of ammonium is an acid forming reaction, the net effect will be a lowered pH. Also, the positive relationship between soil and bio-fertilizers in reduces the hazards of soil salinity and enriches nutrients in soil **(Rashed, 2006).**These results are in a harmony with those obtained by **Ahmed *et al.*(2006) and Sabir *et al*. (2007).** With respect to sulphur materials, **Poraas *et al.* (2009)** indicated that the use of the acidic sulphur materials such as mineral sulphur had very negligible influence on reduce the pH. **Farook and Khan, (2010)** stated that, the use of sulfidic materials decreased soil pH by 0.1 to 0.2 pH units compared with the initial soils.

Soil salinity (EC)

As for soil salinity, the obtained data in table (2) indicate also that application of the different sulphure sources caused an appreciated reduction in the EC values. However, the different amendments caused a clear decline in the EC values with increasing addition rates. The effect is more pronounced due to the addition of high rate of CS with bio inoculation treatment and the EC value 4.20 dS m^{-1} was recorded compared with EC value of control (9.25 dS m^{-1}) and gave 54.6% rate of depression.

The efficiencies of sulphure sources in decreasing soil EC arranged as follow: G > AS > CS and high > medium > low > control for sulphur rate.

Residual available N, P and K macronutrients

Table 3 reveals that the application of different sulphur sources and rates increased the concentrations of available nitrogen, phosphorus, potassium and sulphur in the soil compared with the control for all rates under study especially when the sulphur inoculated with *Thiobacillus*. In this regard, **El-Kouny (2009)** pointed out that application of elemental sulphur increased total N and availability of P and K in soil sample as compared with the control. The plots under calcium sulphate treatment showed the maximum accumulation of available N, P and K, especially at the high rate. Highest soil available N, P and K contents for combined data (50.3, 4.23 and 220 mg kg^{-1}), respectively were obtained due to calcium sulphate treatment while, it was 6.55 mg kg^{-1} for available S due to addition of high rate of agricultural sulphur treatment. This was found true under inoculation with *Thiobacillus*.

Residual available Fe, Mn and Zn micronutrients

Data in Table (4), show that the concentration of available Fe, Mn and Zn followed the same trend of that observed for macronutrients hence, application of AS, G and CS treatments at different rates especially under inoculation with *Thiobacillus* were increased the concentration of available Fe, Mn and Zn in the soil compared with the control. In this regard, **Khan *et al.* (2007)** reported that application of sulfidic materials was effective in enhancing the release of essential plant nutrients into the growing media, which are very essential for crop production in poor soils. The highest soil available Fe, Mn and Zn contents for combined data (9.12, 3.06 and 0.85 mg kg^{-1}), respectively were obtained due to CS + bio inoculation treatment at high rate of addition.

Table (3) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on macronutrients availability in soil after harvest

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Available macronutrients (mg kg^{-1}) N			P			K			S		
		Bio addition (B)											
		With	without	Mean	With	without	Mean	With	without	Mean	With	Without	Mean
AS	Control	40.2	38.1	39.2	3.98	3.94	3.96	192	189	191	5.28	4.95	5.12
	Low	43.2	41.2	42.2	4.09	4.03	4.06	198	193	196	6.38	5.07	5.73
	Medium	47.3	43.2	45.2	4.13	4.07	4.10	205	197	201	6.45	5.12	5.79
	High	49.3	43.7	46.5	4.17	4.09	4.13	210	200	205	6.55	5.20	5.88
Mean		**45.0**	**41.5**	**43.3 b**	**4.09**	**4.03**	**4.06**	**201**	**195**	**198**	**6.17**	**5.09**	**5.63**
G	Control	39.3	38.7	39.0	3.95	3.89	3.92	189	188	189	5.26	4.85	5.06
	Low	41.3	39.2	40.2	4.06	3.97	4.02	193	190	192	5.32	4.93	5.13
	Medium	44.6	39.9	42.2	4.09	4.01	4.05	196	192	194	5.36	5.04	5.20
	High	46.6	41.2	43.9	4.12	4.05	4.09	198	195	197	5.40	5.10	5.25
Mean		**42.9**	**39.7**	**41.3 c**	**4.06**	**3.98**	**4.02**	**194**	**191**	**193**	**5.34**	**4.98**	**5.16**
CS	Control	40.1	39.6	39.8	4.01	3.98	4.00	195	191	193	5.32	5.06	5.19
	Low	44.6	42.0	43.3	4.12	4.08	4.10	203	196	200	5.50	5.14	5.32
	Medium	49.0	44.5	46.7	4.18	4.12	4.15	215	205	210	5.75	5.22	5.49
	High	50.3	46.4	48.4	4.23	4.17	4.20	220	209	215	5.80	5.36	5.58
Mean		**46.0**	**43.1**	**44.6 a**	**4.14**	**4.09**	**4.11**	**208**	**200**	**204**	**5.59**	**5.20**	**5.39**
Mean of bio		**44.6 a**	**41.4 b**		**4.10**	**4.03**		**201 a**	**195 b**		**5.71 a**	**5.09 b**	
Mean of sulphur rate	**control**	**39.3 d**			**3.96**			**191 b**			**5.12 b**		
	Low	**41.9 c**			**4.06**			**196 ab**			**5.39 a**		
	Medium	**44.7 b**			**4.10**			**202 ab**			**5.49 a**		
	High	**46.2 a**			**4.14**			**205 a**			**5.57 a**		
F-test		S:** R:** B:** SxR: **			S:ns R:ns B:ns SxR:ns			S:ns R:* B:* SxR:ns			S:ns R:** B:** SxR:ns		
		SxB:ns RxB:**SxRxB: **			SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns			SxB:** RxB:ns SxRxB:ns		

Table (4) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on micronutrients availability in soil after harvest

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Available micronutrients (mg kg^{-1})								
		Fe			Mn			Zn		
		Bio addition (B)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
AS	Control	7.56	6.69	7.13	2.87	2.65	2.76	0.71	0.68	0.70
	Low	8.14	7.25	7.70	2.93	2.69	2.81	0.74	0.71	0.73
	Medium	8.39	7.65	8.02	2.98	2.73	2.86	0.78	0.74	0.76
	High	9.00	7.85	8.43	3.04	2.78	2.91	0.81	0.76	0.79
Mean		**8.27**	**7.36**	**7.82 b**	**2.96**	**2.71**	**2.83**	**0.76**	**0.72**	**0.74**
G	Control	7.95	7.19	7.57	2.90	2.67	2.79	0.72	0.69	0.71
	Low	8.65	7.36	8.01	2.97	2.73	2.85	0.76	0.73	0.75
	Medium	8.88	7.89	8.39	3.02	2.77	2.90	0.80	0.77	0.79
	High	9.20	8.32	8.76	3.07	2.82	2.95	0.87	0.79	0.83
Mean		**8.67**	**7.69**	**8.18 a**	**2.99**	**2.75**	**2.87**	**0.79**	**0.75**	**0.77**
CS	Control	7.84	7.15	7.50	2.89	2.65	2.77	0.73	0.69	0.71
	Low	8.44	7.33	7.89	2.95	2.70	2.83	0.75	0.72	0.74
	Medium	8.75	7.82	8.29	2.99	2.75	2.87	0.82	0.75	0.79
	High	9.12	8.10	8.61	3.06	2.78	2.92	0.85	0.78	0.82
Mean		**8.54**	**7.60**	**8.07 a**	**2.97**	**2.72**	**2.85**	**0.79**	**0.74**	**0.76**
Mean of bio		**8.49 a**	**7.55 b**		**2.97 a**	**2.73 b**		**0.78 a**	**0.74 b**	
Mean of sulphur rate	control	**7.40 d**			**2.77**			**0.70 b**		
	Low	**7.86 c**			**2.83**			**0.74 b**		
	Medium	**8.23 b**			**2.87**			**0.78 ab**		
	High	**8.60 a**			**2.93**			**0.81 a**		
F-test		S:** R:** B:** SxR: ns			S:ns R:ns B:** SxR: ns			S:ns R:** B:** SxR: ns		
		SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns		

Effect of different sulphur sources and bio inoculation with *Thiobacillus* on total proline, chlorophyll and total free amino acid contents.

Table 5 show that proline content values in fresh weight of leaves significantly decreased by application of different sulphur sources and rates especially with bio addition of *Thiobacillus*. The differences were significant within the treatments. Increasing under salinity stress than the inoculated plants with bioferilizers and high rate of sulphur fertilizer might be caused by the induction or activation of proline syntheses from glutamate or decrease in its utilization in protein syntheses or enhancement in protein turnover. Thus, proline may be the major source of energy and nitrogen during immediate post stress metabolism and accumulated proline apparently supplies energy for growth and survival, thereby inducing salinity tolerance **(Gad 2005).**

As for total chlorophyll and total free amino acids content, data reveal that values more significantly increase duo to the addition of treatments. The difference between the sulphur sources and rates were significant. The highest chlorophyll content of 51.6 mg g^{-1} fresh weight of leaves and total free amino acids 32.2 µg g^{-1} dwt were obtained due to the application treatment of CS at high rate + biofertilization representing an increases of 70.9 and 57.8 %, respectively.

Table (5) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on proline, total chlorophyll and total free amino acids content in cowpea leafs

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Proline (µmol g^{-1} lfwt)			Total chlorophyll (mg g^{-1} lfwt)			Total free amino acids (µg g^{-1} dwt)		
		Bio addition (B)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
AS	Control	41.4	50.3	45.9	38.4	32.3	35.3	25.7	20.6	23.1
	Low	26.1	45.6	35.9	43.6	33.7	38.6	29.5	23.0	26.2
	Medium	22.1	38.1	30.1	49.6	35.2	42.4	30.1	25.2	27.6
	High	17.6	35.1	26.4	50.2	36.2	43.2	30.6	26.0	28.3
Mean		26.8	42.3	34.5 b	45.4	34.3	39.9 b	28.9	23.7	26.3 a
G	Control	42.6	52.1	47.3	36.5	30.2	33.3	23.2	20.4	21.8
	Low	30.4	48.3	39.4	40.3	31.6	35.9	25.7	21.4	23.5
	Medium	27.2	40.2	33.7	45.0	33.6	39.3	27.9	22.4	25.1
	High	22.9	37.9	30.4	47.3	34.2	40.7	30.1	22.8	26.5
Mean		30.8	44.6	37.7 a	42.2	32.4	37.3 c	26.7	21.8	24.2 b
CS	Control	40.6	50.3	45.5	39.1	33.9	36.5	26.2	21.0	23.6
	Low	28.1	43.1	35.6	43.2	36.9	40.1	29.7	23.1	26.4
	Medium	20.1	36.2	28.2	47.5	40.0	43.8	31.9	23.6	27.7
	High	15.9	27.0	21.5	51.6	40.9	46.2	32.2	24.2	28.2
Mean		26.2	39.2	32.7 c	45.4	37.9	41.6 a	30.0	23.0	26.5 a
Mean of bio		27.9 b	42.0 a		44.3 a	34.9 b		28.5 a	22.8 b	
Mean of sulphur rate	control	46.2 a			35.1 d			22.8 d		
	Low	36.9 b			38.2 c			25.4 c		
	Medium	30.7 c			41.8 b			26.8 b		
	High	26.1 d			43.4 a			27.6 a		
F-test		S:** R:** B:** SxR: **			S:ns R:** B:** SxR: *			S:** R:** B:** SxR: ns		
		SxB:** RxB:** SxRxB:**			SxB:** RxB:** SxRxB:**			SxB:** RxB:** SxRxB:**		

The treatment of control (without fertilizers) increased proline content over the treatments and gave the highest value (52.1 µmol g^{-1} leaf fresh weight). The increases followed the order: control > low> medium> high rate and followed the pattern of: G > AS > CS for sulphur sources.

Growth and yield components

Data in Tables 6 to 10 shows that growth of cowpea such as plant height and yield such as 100 seed weight, both pod and seed yieldfed^{-1} were significantly affected by the single interaction, duel and triple interactions in both seasons.

Main Effect

Regarding the single effect, data in Table (6) show that treated cowpea plants with calcium sulphate (CS) had significantly increased plant height and 100 seed weight as well as pod and seed yield fed^{-1} in both seasons , without significant differences with agricultural sulphur (AS) for 100 seed weight in the 1st season and pod yield in both seasons.

The increases in seed yield, regarding CS was about 8.24 and 9.83 % than AS in the 1st and 2nd seasons, respectively.

Concerning the effect of sulphur rates, data in the same Table indicate that, application of the high rates of sulphur significantly increased plant height and 100 seed weight as well as, pod and seed yield /fed. than other rates of sulphur in both seasons. The increases in seed yield /fed, regarding the highest rate was about 33.16 and 32.63 % than control (untreated plants with sulphur) in the 1st and 2nd seasons, respectively.

AS for biofertilizer effect, such data in the same Table show that, inoculated cowpea plants with *Thiobacillus* strain had significantly increased plant height, 100 seed weight, pod and seed yield in both seasons causing 16.16 and 14.05% than control (untreated plants biofertilizer) in the 1st and 2nd seasons, respectively for seed yield.

Duel interaction

Data in Tables 7 to 9 indicated that , the effect of interaction between sulphur sources and rates, and between sulpur sources and biofertilizer as well as between sulphur rats and biofertilizer on plant height, 100 seed weight, pod and seed yield in both seasons.

As for the interaction between sulphur sources and rates, the data in Table 7 show that the interaction between sulphur sources and rates had significantly increased plant height, 100 seed weight, pod yield and seed yield /fed in both seasons . The highest values of all abovementioned traits were recorded due to treatment of CS and high rates of sulphur in both seasons giving the increases in seed yield by about 41.62 and 43.19 % than untreated plants in the 1st and 2nd seasons, respectively.

Table (6): Effect of sulphur sources , rates and biofertilizer on plant height (cm) and 100-seed weight (g) as well as pod and seed yields (ton fed.$^{-1}$)

Treatments	Plant height (cm)		100-seed weight		Pod yield		Seed yield	
	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season
	Effect of sulphur sources							
Agricultural sulphur (AS)	33.60	37.94	28.98	32.20	3.123	3.352	1.165	1.230
Gypsum G	30.88	35.20	27.25	30.09	3.066	3.191	1.125	1.167
Calcium sulphate (CS)	39.32	42.54	29.47	32.27	3.217	3.440	1.261	1.351
LSD at 0.05 level	**0.81**	**0.65**	**0.32**	**0.56**	**0.137**	**0.113**	**0.062**	**0.073**
	effect of sulphur rates							
Control	28.70	32.91	26.58	28.14	2.968	3.161	0.986	1.045
Low	33.21	37.86	28.06	30.30	3.085	3.283	1.188	1.260
Medium	37.40	40.38	29.50	32.77	3.205	3.398	1.246	1.306
High	39.10	43.08	30.13	34.86	3.285	3.468	1.313	1.386
LSD at 0.05 level	**0.71**	**0.57**	**0.28**	**0.49**	**0.069**	**0.127**	**0.054**	**0.064**
	effect of biofertilizer							
With	38.95	43.36	31.40	34.99	3.761	3.886	1.272	1.331
Without	30.25	33.76	25.74	28.05	2.510	2.769	1.095	1.167
LSD at 0.05 level	**0.49**	**0.39**	**0.19**	**0.34**	**0.059**	**0.088**	**0.037**	**0.044**

Table (7): Duel effect sulphur sources rates and with or without biofertilizer on plant height (cm) and 100-seed weight (g) as well as pod and seed yields (ton fed-[1])

Treatments		Plant height (cm)		100-seed weight		Pod yield		Seed yield	
		2016 season	2017 season	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season
Sources	**Rates**								
AS	**Control**	29.05	34.10	26.60	27.94	2.985	3.220	0.985	1.065
	Low	31.85	37.34	28.15	30.91	3.040	3.315	1.165	1.240
	Medium	36.05	38.94	30.25	33.56	3.205	3.410	1.220	1.275
	High	37.45	41.37	30.95	36.40	3.265	3.465	1.290	1.340
G	**Control**	27.65	30.97	25.80	27.15	2.910	2.975	0.930	0.975
	Low	29.80	34.18	27.25	29.12	3.005	3.090	1.130	1.180
	Medium	32.55	37.20	27.75	31.34	3.125	3.295	1.185	1.220
	High	33.55	38.44	28.20	32.76	3.225	3.405	1.255	1.295
CS	**Control**	29.40	33.67	27.35	29.35	3.010	3.290	1.045	1.095
	Low	38.00	42.07	28.80	30.87	3.210	3.445	1.270	1.360
	Medium	43.60	44.99	30.50	33.42	3.285	3.490	1.335	1.425
	High	46.30	49.45	31.25	35.44	3.365	3.535	1.395	1.525
LSD at 0.05 level		**1.23**	**0.99**	**0.49**	**0.85**	**0.120**	**0.221**	**0.094**	**0.111**

AS= agricultural sulphur , G= gypsum , GS= calcium sulphate

Concerning the interaction between sulphur sources and biofertilizer, the highest values of plant height, 100 seed weight, and pod as well as seed yield of cowpea were recorded with the interaction between Cs and biofertilizer in both seasons (Table 8).

The increases in seed yield were about 26.30 and 23.40 % than application of AS only in the 1st and 2nd seasons, respectively.

Table (8): Duel effect of sulphur sources and biofertilizer on plant height (cm) and 100-seed weight (g) as well as pod and seed yields (ton fed.$^{-1}$)

Treatments		Plant height (cm)		100-seed weight		Pod yield		Seed yield	
Sources	biofertilizer	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season
AS	With	38.15	42.87	32.32	35.52	3.760	3.915	1.2525	1.330
	Without	29.05	33.01	25.65	28.88	2.487	2.790	1.0775	1.130
G	With	33.80	38.88	29.80	33.87	3.670	3.755	1.2050	1.247
	Without	27.97	31.51	24.70	26.32	2.462	2.627	1.0450	1.087
CS	With	44.92	48.33	32.07	35.58	3.855	3.990	1.3600	1.417
	Without	33.72	36.75	26.87	28.95	2.580	2.890	1.1625	1.285
LSD at 0.05 level		**0.86**	**0.68**	**0.34**	**0.59**	**0.102**	**0.153**	**0.065**	**0.077**

AS= agricultural sulphur , G= gypsum , CS= calcium sulphate

Regarding the interaction between sulphur rates and biofertilizer, data in Table 9 show that, there were significant differences the among the treatments for plant height and 100 seed weight as well as, yield of pod and seeds in both seasons. The interaction between the highest rate of sulphur and biofertilizer recorded the maximum values of plant height, 100 seed weight and pod yield and seed yield in both seasons.

Table (9): Duel effect sulphur sources , rates and biofertilizer on plant height (cm) and 100-seed weight (g) as well as pod and seed yields (ton fed.$^{-1}$)

Treatments		Plant height (cm)		100-seed weight		Pod yield		Seed yield	
		2016 season	2017 season	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season
Sulphur rates	Bio. fertilizer	Effect of sulphur rats x bio							
Control	With	32.06	36.93	28.86	30.51	3.550	3.760	1.023	1.113
	Without	25.33	28.89	24.30	25.78	2.386	2.563	0.950	0.976
Low	With	36.63	42.22	30.80	33.06	3.700	3.860	1.293	1.346
	Without	29.80	33.51	25.33	27.53	2.470	2.706	1.083	1.173
Medium	With	42.53	45.58	32.60	37.16	3.860	3.920	1.350	1.406
	Without	32.26	35.18	26.40	28.38	2.550	2.876	1.143	1.206
High	With	44.60	48.71	33.33	39.22	3.936	4.006	1.423	1.460
	Without	33.60	37.45	26.93	30.51	2.633	2.930	1.203	1.313
LSD at 0.05 level		**0.99**	**0.79**	**0.39**	**0.68**	**0.118**	**0.177**	**0.075**	**0.089**

AS= agricultural sulphur , G= gypsum , GS= calcium sulphate

The increases in seed yield /fed., regarding the CS combined with biofertilizer treated with cowpea was about 49.78 and 49.59 % than untreated plant in the 1st and 2nd seasons, respectively.

The triple interactions

Data in table 10 show that the triple interaction between sulphur sources, rates and biofertilizer had significant effect on plant height, 100 seed weight, pod yield and seed weight in both seasons. Treated cowpea with GS, high rate of sulphur and biofertilizer recorded the highest values of all abovementioned traits than other triple interactions in both seasons. The increases in seed yield was about 69.57 and 70.21 % than that plants which untreated the 1st and 2nd seasons, respectively.

In this regard, Bakry ***et al.*** **(2005)** stated that applying the bacterial fertilizer to soil influenced biological activity in soil leading to improving growth, photosynthesis and dry matter accumulation of the plant. The increase in plant synthetic pigments as a result of bacterial inoculation may be attributed to increases in nitrogen fixed in plants *via* an increase of N_2-ase enzyme activity of bacteria, where nitrogen is a major component of chlorophyll.

The favorable effect of sulphur sources might be attributed to the role of calcium, which is essential for plant as previously mentioned. Also, calcium is essential for many plant functions, some of them are proper cell division and elongation, enzyme activity and metabolism. These results are well supported by the findings of **Sabir *et al.* (2007) and Farook and Khan, (2010).**

Table (10): Triple effect of sulphur sources , rates and biofertilizers on plant height (cm) and 100-seed weight (g) as well as pod and seed yields (ton fed.$^{-1}$)

Treatments			Plant height (cm)		100-seed weight		Pod yield		Seed yield	
Sources	Rates	Bio	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season	2016 season	2017 season
AS	Control	With	32.60	38.76	29.10	30.22	3.580	3.850	1.050	1.190
		Without	25.50	29.45	24.10	25.66	2.390	2.590	0.920	0.940
	Low	With	34.10	41.52	30.90	33.45	3.630	3.880	1.280	1.330
		Without	29.60	33.17	25.40	28.37	2.450	2.750	1.050	1.150
	Medium	With	41.90	43.68	34.30	38.12	3.890	3.940	1.300	1.380
		Without	30.20	34.21	26.20	29.00	2.520	2.880	1.140	1.170
	High	With	44.00	47.52	35.00	40.29	3.940	3.990	1.380	1.420
		Without	30.90	35.22	26.90	32.51	2.590	2.940	1.200	1.260
G	Control	With	30.40	34.85	27.70	29.46	3.450	3.550	0.960	1.030
		Without	24.90	27.10	23.90	24.85	2.370	2.400	0.900	0.920
	Low	With	31.30	36.88	29.90	32.14	3.590	3.730	1.250	1.280
		Without	28.30	31.49	24.60	26.10	2.420	2.450	1.010	1.080
	Medium	With	36.00	40.92	30.50	35.69	3.750	3.790	1.280	1.320
		Without	29.10	33.48	25.00	26.99	2.500	2.800	1.090	1.120
	High	With	37.50	42.88	31.10	38.19	3.890	3.950	1.330	1.360
		Without	29.60	34.00	25.30	27.34	2.560	2.860	1.180	1.230
CS	Control	With	33.20	37.20	29.80	31.85	3.620	3.880	1.060	1.120
		Without	25.60	30.14	24.90	26.85	2.400	2.700	1.030	1.070
	Low	With	44.50	48.26	31.60	33.60	3.880	3.970	1.350	1.430
		Without	31.50	35.88	26.00	28.14	2.540	2.920	1.190	1.290
	Medium	With	49.70	52.14	33.00	37.68	3.940	4.030	1.470	1.520
		Without	37.50	37.85	28.00	29.16	2.630	2.950	1.200	1.330
	High	With	52.30	55.75	33.90	39.20	3.980	4.080	1.560	1.600
LSD at 0.05 level			**1.71**	**1.37**	**0.68**	**1.18**	**0.204**	**0.307**	**0.130**	**0.154**

AS= agricultural sulphur , G= gypsum , GS= calcium sulphate

Seed protein yield

As shown in Table 11 data presents that protein yield of cowpea seeds significantly increased by fertilization. The differences among the factors were significant while there was no significant difference between G and AS also, between high and medium rates. This promoting effect could be clarified the effect of sulphuric materials on enhancing the growth of cowpea and improving the fertility of the studied soil. **Mabrouk (2002)** found that bio-mineral fertilization was more effective in increasing protein content of peanut plants as compared with the individual mineral fertilization. These results are in agreement with those obtained by, **Hussein (2007) and Omran *et al.* (2009).** The maximum value of protein content and protein yield (21.3% and 332 kg fed.$^{-1}$), respectively was recorded in the plants treated with CS at high rate + bio fertilization which recorded 30.7 and 126%, respectively increases over the control treatment (without sulphur addition). Treatments receiving fertilizers followed the order of: CS > AS ≥ G for sulphur source and high ≥ medium > low > control. The interaction effect between the treatment (S, R and B) had in significant effect between them.

Table (11) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on protein content (%) and protein yield of cowpea seeds

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Protein content			Protein yield		
		Bio addition (B)					
		With	Without	Mean	With	Without	Mean
AS	Control	18.1	16.4	17.2	190	151	170
	Low	20.0	17.2	18.6	256	181	218
	Medium	21.0	17.6	19.3	273	201	237
	High	21.1	17.8	19.5	291	214	252
Mean		**20.0**	**17.2**	18.6	**253**	**186**	**219 b**
G	Control	18.3	16.3	17.3	176	147	161
	Low	19.7	16.9	18.3	246	171	208
	Medium	20.0	17.4	18.7	256	190	223
	High	20.5	17.6	19.1	273	208	240
Mean		**19.6**	**17.1**	**18.3**	**238**	**179**	**208 b**
CS	Control	18.2	16.5	17.3	193	170	181
	Low	20.3	17.1	18.7	274	203	239
	Medium	21.1	17.4	19.3	310	209	259
	High	21.3	17.7	19.5	332	218	275
Mean		**20.2**	**17.2**	**18.7**	**277**	**200**	**239 a**
Mean of bio		**19.9 a**	**17.2 b**		**256 a**	**188 b**	
Mean of sulphur rate	control	**17.3 b**			**171 c**		
	Low	**18.5 a**			**222 b**		
	Medium	**19.1 a**			**240 a**		
	High	**19.3 a**			**256 a**		
F-test		S:ns R:** B:** SxR: ns			S:* R:** B:** SxR: ns		
		SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns		

Macronutrient content and uptake

Data in Tables (12 &13) shows that N, P, K and S uptake by cowpea seeds were increased significantly owing to application of sulphur fertilizers treatments solely or in combination with bio fertilization except for P and S-uptake, the effect of sulphur sources was insignificant. This promoting effect could be related to the supplementary effect of gypsum and sulphur on reducing soil pH, improving soil structure and increasing the availability of nutrients in soil and also, improves the use efficiency of other essential plant nutrients, particularly nitrogen and phosphorus **Mazhar *et al.* (2011).** These results are in a harmony with those obtained by **Ali *et al.* (2008) and Haq *et al.* (2007).** The increase in nutrients is an indication to increased root growth and utilization of N released from bio-fixation. These finding are in agreement with those reported by **Kloepper (2003)** who pointed out that phytohormones produce bacteria which cause pronounced increases for plant root elongation and then uptake of more nutrients *via* the root system, and hence utilization of N as a result bio-inoculation. This is mainly due to the bio-fertilization bacteria playing a dual role in fixation of atmospheric N_2 and producing antimicrobial agents against deleterious rhizosphere bacteria. These results agree with those obtained by **Massoud *et al.* (2004)** who suggested, that inoculation with bacteria increased uptake of N, P and K by pea plants.

Table (12) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on macronutrient content (%) in cowpea seeds

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Macronutrients content (%)											
		N			P			K			S		
		Bio addition (B)											
		With	without	Mean	With	without	Mean	With	without	Mean	With	Without	Mean
AS	Control	2.89	2.62	2.76	0.46	0.37	0.42	2.35	2.24	2.30	0.24	0.16	0.20
	Low	3.20	2.75	2.98	0.49	0.41	0.45	2.39	2.32	2.36	0.28	0.18	0.23
	Medium	3.36	2.81	3.09	0.52	0.45	0.49	2.43	2.38	2.41	0.34	0.20	0.27
	High	3.38	2.85	3.12	0.56	0.47	0.52	2.48	2.41	2.45	0.37	0.26	0.32
Mean		**3.21**	**2.76**	**2.98**	**0.51**	**0.43**	**0.47**	**2.41**	**2.34**	**2.38**	**0.31**	**0.20**	**0.25**
G	Control	2.92	2.61	2.77	0.45	0.35	0.40	2.3	2.22	2.26	0.19	0.14	0.17
	Low	3.15	2.70	2.93	0.47	0.38	0.43	2.37	2.3	2.34	0.21	0.17	0.19
	Medium	3.20	2.78	2.99	0.50	0.42	0.46	2.41	2.34	2.38	0.26	0.19	0.23
	High	3.28	2.82	3.05	0.53	0.44	0.49	2.46	2.38	2.42	0.28	0.24	0.26
Mean		**3.14**	**2.73**	**2.93**	**0.49**	**0.40**	**0.44**	**2.39**	**2.31**	**2.35**	**0.24**	**0.19**	**0.21**
CS	Control	2.91	2.64	2.78	0.47	0.36	0.42	2.37	2.28	2.33	0.25	0.18	0.22
	Low	3.24	2.73	2.99	0.53	0.44	0.49	2.4	2.35	2.38	0.30	0.25	0.28
	Medium	3.37	2.79	3.08	0.59	0.47	0.53	2.46	2.39	2.43	0.37	0.34	0.36
	High	3.40	2.83	3.12	0.61	0.51	0.56	2.49	2.44	2.47	0.40	0.36	0.38
Mean		**3.23**	**2.75**	**2.99**	**0.55**	**0.45**	**0.50**	**2.43**	**2.37**	**2.40**	**0.33**	**0.28**	**0.31**
Mean of bio		**3.19 a**	**2.75 b**		**0.52 a**	**0.43 b**		**2.41**	**2.34**		**0.29 a**	**0.22 b**	
Mean of sulphur rate		**control**	**2.77 b**		**0.41 c**			**2.29**			**0.19 b**		
		Low	**2.96 a**		**0.45 b**			**2.36**			**0.23 b**		
		Medium	**3.05 a**		**0.49 a**			**2.40**			**0.28 a**		
		High	**3.09 a**		**0.52 a**			**2.44**			**0.32 a**		
F-test		S:ns R:** B:** SxR: ns			S:ns R:** B:** SxR:ns			S:ns R:ns B:ns SxR:ns			S:ns R:** B:** SxR:ns		
		SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns		

Table (13) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on macronutrient uptake (kg fed.$^{-1}$) in cowpea seeds

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Macronutrients uptake (Kg fed.$^{-1}$)											
		N			P			K			S		
		Bio addition (B)											
		With	without	Mean	With	without	Mean	With	without	Mean	With	Without	Mean
AS	Control	30.3	24.1	27.2	4.83	3.40	4.12	24.7	20.6	**22.6**	2.52	1.47	2.00
	Low	41.0	28.9	34.9	6.27	4.31	5.29	30.6	24.4	**27.5**	3.58	1.89	2.74
	Medium	43.7	32.0	37.9	6.76	5.13	5.95	31.6	27.1	**29.4**	4.42	2.28	3.35
	High	46.6	34.2	40.4	7.73	5.64	6.68	34.2	28.9	**31.6**	5.11	3.12	4.11
Mean		**40.4**	**29.8**	**35.1 b**	**6.40**	**4.62**	**5.51**	**30.3**	**25.3**	**27.8 b**	**3.91**	**2.19**	**3.05**
G	Control	28.0	23.5	25.8	4.32	3.15	3.74	22.1	20.0	21.0	1.82	1.26	1.54
	Low	39.4	27.3	33.3	5.88	3.84	4.86	29.6	23.2	26.4	2.63	1.72	2.17
	Medium	41.0	30.3	35.6	6.40	4.58	5.49	30.8	25.5	28.2	3.33	2.07	2.70
	High	43.6	33.3	38.5	7.05	5.19	6.12	32.7	28.1	30.4	3.72	2.83	3.28
Mean		**38.0**	**28.6**	**33.3 b**	**5.91**	**4.19**	**5.05**	**28.8**	**24.2**	**26.5 b**	**2.88**	**1.97**	**2.42**
CS	Control	30.8	27.2	29.0	4.98	3.71	4.35	25.1	23.5	24.3	2.65	1.85	2.25
	Low	43.7	32.5	38.1	7.16	5.24	6.20	32.4	28.0	30.2	4.05	2.98	3.51
	Medium	49.5	33.5	41.5	8.67	5.64	7.16	36.2	28.7	32.4	5.44	4.08	4.76
	High	53.0	34.8	43.9	9.52	6.27	7.89	38.8	30.0	34.4	6.24	4.43	5.33
Mean		**44.3**	**32.0**	**38.1 a**	**7.58**	**5.21**	**6.40**	**33.1**	**27.5**	**30.3 a**	**4.59**	**3.33**	**3.96**
Mean of bio		**40.9 a**	**30.1 b**		**6.63 a**	**4.67 b**		**30.7 a**	**25.7 b**		**3.79 a**	**2.50 b**	
Mean of sulphur rate		**control**	**27.3 c**		**4.07 c**			**22.7 b**			**1.93 d**		
		Low	**35.5 b**		**5.45 b**			**28.0 a**			**2.81 c**		
		Medium	**38.3 ab**		**6.20 ab**			**30.0 a**			**3.60 b**		
		High	**40.9 a**		**6.90 a**			**32.1 a**			**4.24 a**		
F-test		S:* R:** B:** SxR: ns			S:ns R:** B:** SxR:ns			S:** R:** B:** SxR:ns			S:ns R:** B:** SxR:ns		
		SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:ns SxRxB:ns			SxB:** RxB:ns SxRxB:ns		

Highest N, P, K and S-uptake (53.0, 9.52, 38.8 and 6.24 kg fed.$^{-1}$, respectively) in seeds were obtained owing to addition CS at high rate + biofertilization treatment.

Statistical analysis shows that the treatment consisting of calcium sulphate (CS) at high rate + biofertilization was superior for increasing the content and uptake of N, P, K and S as compared to the other treatments. The positive effect was in the ascending order of CS > AS ≥ G for all nutrients under study either for sulphur sources and the effect of sulphur addition rates followed the order: high ≥ medium ≥ low > control for N, and P-uptake; high > medium > low > control for S-uptake. The applications showed insignificant differences among them for K-uptake.

Micronutrients Content

As shown in Table 14. Fe, Mn and Zn-uptake followed the same trend of that for macronutrients uptake. Addition of sulphur fertilizers at different rates solely or in presence of bio inoculation with *Thiobacillus* significantly increased Fe, Mn and Zn uptake compared to the control.

Biofertilization + high rate of CS was most effective for increasing the uptake of Fe, Mn and Zn as compared to the other treatments. These increases may be attributed to the role of microorganisms in improving these micronutrients availability, (Table 14) which was likely attributed to several reasons: 1) reducing the pH of the soil making the nutrients more available; and 2) lowering the redox statues of iron and manganese leading to reduction of higher Fe^{3+}& Mn^{4+} to Fe^{2+} and Mn^{2+} and/or transformation of insoluble chelated forms of micronutrients into more soluble ions **(Castilho *et al.*, 1993).** 3)

Table (14) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on micronutrient content in cowpea seeds

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Micronutrient contents (mg kg^{-1})								
		Fe			Mn			Zn		
		Bio addition (B)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
AS	Control	89.4	79.7	84.6	52.2	48.8	50.5	25.4	18.7	22.1
	Low	94.3	82.6	88.5	56.3	50.2	53.3	25.9	19.0	22.5
	Medium	98.4	85.7	92.1	59.1	53.9	56.5	26.1	18.3	22.2
	High	98.9	88.3	93.6	60.3	56.0	58.2	26.6	18.9	22.8
Mean		**95.3**	**84.1**	**89.7 c**	**57.0**	**52.2 c**	**54.6 c**	**26.0**	**18.7**	**22.4 b**
G	Control	91.2	82.9	87.1	54.2	49.0	51.6	25.9	19.0	22.5
	Low	97.6	91.3	94.5	59.4	52.6	56.0	26.4	19.3	22.9
	Medium	99.2	92.0	95.6	62.1	54.3	58.2	26.7	19.7	23.2
	High	104.0	92.5	98.3	64.6	55.1	59.9	27.0	20.1	23.6
Mean		**98.0**	**89.7**	**93.8 b**	**60.1**	**52.8 b**	**56.4 b**	**26.5**	**19.5**	**23.0 a**
CS	Control	92.9	83.1	88.0	55.3	49.0	52.2	26.1	19.1	22.6
	Low	97.4	93.5	95.5	61.3	53.2	57.3	26.9	19.8	23.4
	Medium	105.0	94.7	99.9	63.3	56.2	59.8	27.3	19.9	23.6
	High	110.0	95.1	102.6	65.1	59.3	62.2	28.0	20.6	24.3
Mean		**101.3**	**91.6**	**96.5 a**	**61.3**	**54.4**	**57.8 a**	**27.1**	**19.9**	**23.5 a**
Mean of bio		**98.2 a**	**88.5 b**		**59.5 a**	**53.1 b**		**26.5 a**	**19.4 b**	
Mean of sulphur rate	control	**86.5 d**			**51.4 d**			**22.4 c**		
	Low	**92.8 c**			**55.5 c**			**22.9 b**		
	Medium	**95.8 b**			**58.2 b**			**23.0 b**		
	High	**98.1 a**			**60.1 a**			**23.5 a**		
F-test		S:** R:** B:** SxR: **			S:ns R:** B:** SxR: **			S:** R:** B:** SxR: ns		
		SxB:** RxB:** SxRxB:**			SxB:** RxB:** SxRxB:**			SxB:ns RxB:ns SxRxB:ns		

Hormonal exudates (such as indol acetic acid, gibberellins and cytokinines) of these micro-organisms can modify root growth (morphology and/or physicology) resulting in more efficient absorption of available nutrients from the soil **(Carietti *et al.*, 1996).**

The positive effect could be related to the S-supplementary as reported by **Kubenkulov *et al.* (2013)** who reported that sulfur and gypsum as comprehensible amendment, which regulate the soil pH and total soluble salts (TSS) for the soda-saline soils. Meanwhile, applied amendments (gypsum and sulfur) accelerated the leaching of Na+ ions from root zone, which seems the main cause to converge the values of pHs, ECe and SAR toward safe limit which improving the availability of nutrients **(Abdelhamid *et al.*, 2013).** The responses percentage to Fe, Mn and Zn uptake by cowpea seeds over control was 135, 131 and 156 %, respectively.

Table (15) Effect of different sulphur sources and bio inoculation with *Thiobacillus* on micronutrients uptake (g fed.$^{-1}$) in cowpea seeds

Sulphur source (S)	Sulphur rates (R) ton fed.$^{-1}$	Micronutrients uptake (g fed.$^{-1}$)								
		Fe			Mn			Zn		
		Bio addition (B)								
		With	Without	Mean	With	Without	Mean	With	Without	Mean
AS	Control	93.9	73.3	83.6	54.8	44.9	49.9	26.7	17.2	21.9
	Low	121	86.7	104	72.1	52.7	62.4	33.2	20.0	26.6
	Medium	128	97.7	113	76.8	61.4	69.1	33.9	20.9	27.4
	High	136	106	121	83.2	67.2	75.2	36.7	22.7	29.7
Mean		**120**	**90.9**	**105 b**	**71.7**	**56.6**	**64.1 b**	**32.6**	**20.2**	**26.4 b**
G	Control	87.6	74.6	81.1	52.0	44.1	48.1	24.9	17.1	21.0
	Low	122	92.2	107	74.3	53.1	63.7	33.0	19.5	26.2
	Medium	127	100	114	79.5	59.2	69.3	34.2	21.5	27.8
	High	138	109	124	85.9	65.0	75.5	35.9	23.7	29.8
Mean		**119**	**94.1**	**106 b**	**72.9**	**55.4**	**64.1 b**	**32.0**	**20.4**	**26.2 b**
CS	Control	98.5	85.6	92.0	58.6	50.5	54.5	27.7	19.7	23.7
	Low	131	111	121	82.8	63.3	73.0	36.3	23.6	29.9
	Medium	154	114	134	93.1	67.4	80.2	40.1	23.9	32.0
	High	172	117	144	102	72.9	87.2	43.7	25.3	34.5
Mean		**139**	**107**	**123 a**	**84.0**	**63.5**	**73.8 a**	**36.9**	**23.1**	**30.0 a**
Mean of bio		**126 a**	**97.3 b**		**76.2 a**	**58.5 b**		**33.9 a**	**21.2 b**	
Mean of sulphur rate	**control**	**85.6 d**			**50.8 d**			**22.2 c**		
	Low	**111 c**			**66.4 c**			**27.6 b**		
	Medium	**120 b**			**72.9 b**			**29.1 b**		
	High	**130 a**			**79.3 a**			**31.3 a**		
F-test		S:** R:** B:** SxR: ns			S:** R:** B:** SxR: ns			S:** R:** B:** SxR: ns		
		SxB:ns RxB:ns SxRxB:ns			SxB:ns RxB:* SxRxB:ns			SxB:ns RxB:* SxRxB:ns		

Jena and Kabi, (2012) stated that sulphur application increased Fe, Mn , Zn and Cu uptake by rice plants. Also, significant improvement is usually expected in the use of gypsum on saline soils as sources of Ca and S. **Bello, (2012)** found that the improvement in yield and nutrient content is due to the displacement of sodium by calcium and increase in nutrient use efficiency of rice crop. Sulphur fertilization enhanced the uptake of N, P, K and Zn in the plant. Due to its synergistic effect, the efficiency of these elements is enhanced which results in increased crop productivity.

Application of S fertilizer is useful not only for increasing crop production and quality of the produce but also improves soil conditions for healthy crop. These results are in a harmony with those obtained by **Ahmed *et al.* (2016).**

CONCLUSION

Findings of the present study suggested that application of sulphur is also an effective technology in improving the chemical properties, like pH and EC of salt affected soils and, subsequently, yield attribute of cowpea plants. The sulphur source of calcium sulphate at rate of 4 ton fed.$^{-1}$ in presence of bio inoculation with *Thiobacillus* was superior for amelioration of salt affected soils under study than the other sources and rates, which could also be an effective and suitable alternative amendment for improving the different qualities of salt affected soils and yield of cowpea. Seed inoculation with *Thiobacillus* had the highest performance in yield and seed physical and chemical properties. Therefore, it is recommended that farmers can apply the studied sulphur materials under biofertilization with *Thiobacillus* for increasing the productivity of cowpea crop with good seed quality under saline soil conditions.

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تاثيرالتسميد ببعض مصادر الكبريت على خصوبة التربة الملحية وانتاجية وجودة اللوبيا تحت ظروف الأراضى الملحية

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أقيمت تجربتان حقليتان خلال موسمي صيف 2016 و 2017 م علي أرض ملحية تقع في منطقة القنطرة شرق – محافظة الإسماعيلية – مصر لتقييم تأثير أضافة الكبريت للتربة من مصادر مختلفة وهي الكبريت الزراعي (كب) و الجبس الزراعي وكذلك كبريتات الكالسيوم وذلك بأربع معدلات هي 0 ، 0.2 ، 0.3 و 0.4 طن فدان$^{-1}$ للكبريت الزراعي و 0 ، 2 ، 3 و 4 طن فدان$^{-1}$ لكلاً من الجبس و كبريتات الكالسيوم علي التوالي لتمثل (الكنترول ، المعدل المنخفض ، المعدل المتوسط و المعدل المرتفع) علي التوالي مع أو بدون التلقيح الحيوي بالثيوباسيللس علي تثبيط التأثير الضار للملوحة و رفع كفاءة و جودة أنتاجية اللوبيا صنف كفر الشيخ (*Vigna unguiculate* L. cv. Kafr El-Sheikh) وكذلك امتصاص بعض العناصر الغذائية الكبرى و الصغري وتأثير ذلك علي خصوبة التربة من خلال تحليل التربة بعد الحصاد لتقدير بعض الخواص الكيميائية وبعض العناصر الميسرة الكبري و الصغري بالتربة ويمكن تلخيص أهم النتائج المتحصل عليها كما يلي:

- ➢ أزدادت القيم المتحصل عليها لمحصول اللوبيا ومساهماتة وكذلك لجودة الحبوب ومحتوي وأمتصاص العناصر الكبري و الصغري بواسطة الحبوب كنتيجة لإضافة المصادر و المعدلات المختلفة من الكبريت مع أو بدون التلقيح الحيوي بالثيوباسيللس.
- ➢ أزدادت معنوياً قيم محتوي البروتين ومحصولة وكذلك الكلوروفيل الكلي و الأحماض الأمينية الحرة بينما أنخفضت قيم البرولين المتراكم نتيجة للمعاملات تحت الدراسة.
- ➢ أنخفضت قيم حموضة التربة و التوصيل الكهربي بينما أزدادت العناصر الميسرة الكبري و الصغري بالتربة بعد الحصاد نتيجة لإضافة المعاملات المختلفة تحت الدراسة.

* كانت المعاملة بكبريتات الكالسيوم بالمعدل المرتفع (4 طن فدان$^{-1}$) مع التلقيح الحيوي بالثيوباسيللس هي الأحسن علي الإطلاق والتي أعطت أعلي القيم لجميع القياسات تحت الدراسة مقارنة بباقي المعاملات المستخدمة والتى سجلت زيادة نسبيه فى محصول الحبوب مقدارها 57.52 ، 40.21 % مقارنة بالنباتات غير المعاملة خلال الموسم الاول والثانى على التوالى .