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Evaluation of Elemental Sulphur Application with *Rhizobia* Inoculation on Peanut Yield and its Quality Grown in Sandy Soil at Egypt

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TWO field experiments were conducted during the summer of two successive seasons of 2011 and 2012, to identify the response of peanut (*Arachis hypogaea* L., cv. Giza 5) to soil application of elemental sulphur (at rate of 15, 30 and 45 Kg/fed before sowing) and Rhizobia (*Bradyrhizobium japonicum*) inoculation, individually or in combination, for determination of the yield criteria, pods yield, 100 seeds yield, seeds & straw yield and shilling percentage at harvest, as well as seed oil, oil yield, protein content and protein yield. The associated amelioration in plant macro and micronutrient contents and uptake, and soil biological activities (nodulation status, nitrogenase, dehydrogenase, CO_2 evolution and total bacterial counts) were assessed in this study. The obtained results indicated that *Rhizobium* inoculation or S addition alleviated the adverse effect of soil nutrient deficiency and caused significant increases in all the studied parameters of peanut and soil. *Rhizobium* inoculation individually caused insignificant increases in all the studied parameters over S addition solely at 45 kg s/fed. Joint addition of sulphur at all rates with *Rhizobium* inoculation, gave high significant increases in all prior studied parameters and soil biological parameters at 50 and 80 days after peanut sowing followed by the individual treatments.

Keywords: Peanut, Sulphur, *Rhizobium* inoculation, Biological activity of soil and sandy soil.

Peanut, (*Arachis hypogaea* L.) is one of the most important crops in Egypt for both exportation and locally consumption. Due to its high nutritive value, peanut seeds are used as a source of dietary protein (25-30%), oil production for industrial purposes (more than 40%), human consumption and animal feeding. Also, it improves soil properties by increasing organic matter and nitrogen content (Khalifa *et al.,* 2013). Increasing peanut production for local consumption and export abroad could be achieved by cultural practices and management as well as chosen the proper planting (Abd El-Maksoud, 2008).

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Biotic and abiotic fertilization were of great importance for increasing peanut quality and productivity.

Sulphur is one of the most macronutrients for the plant, so it plays an important role in growth and development of plants being a constituent of three amino acids viz., methionine, cysteine and cystine. Sulphur is also needed for the synthesis of other metabolites like co-enzyme A, biotin, thiamin (Vitamin B1) and glutathione, besides its role in the synthesis of chlorophyll and improves nodulation in legumes. Due to continuous use of high grade of S free fertilizers and intensive cropping, its deficiency has been reported as hidden hunger in many crops, especially pulses. Ahmad and Abdin (2000) demonstrated that high S fertilization increases chlorophyll, and protein contents in fully expanded upper leaves of *Brassica juncea* L. (mustard) and *Brassica campestris* L., which implies a better photosynthetic activity in comparison with plants grown without sulphur.

Sulphur is usually required by legumes for protein synthesis as a constituent of three amino acids; cystein, cystine and methionine (ELSaadany and Abd EL_Rasoul, 1999), contributes in the conformation of enzyme protein and some coenzyme A essential for metabolism (Nassar *et al.*, 2006), promotes reproductive development and nitrogen fixation and is called a master nutrient for oil seed production (El-Hamzawi, 2000). Sulphur also increases sugar content of seed (El-Sayed, 2006) and favorable increases translocation of carbohydrates through hydrolyzing more glycosides (Azer *et al.*, 2003). The botanical requirement for sulfur equals or exceeds the requirement for phosphorus. It is an essential nutrient for plant growth, root nodule formation of legumes, and immunity and defense systems. Sulfur deficiency has become widespread in many countries (Ceccotti, 1996; Zhao *et al.*, 1999 and Blake-Kalff, 2000).

Use of Microorganisms as biotic fertilizer, which can either fix atmospheric nitrogen, solubilize phosphate, synthesis of growth promoting substances or by enhancing the decomposition of plant residues to release vital nutrients and increase humic content of soil, will be environmentally begin approach for nutrient management and ecosystem function (Wu *et al.*, 2005). Of the well-known biofertilizeres are the microbial inoculants, which applied to seeds or soil in order to increase soil fertility and plant growth. Zahran (1999) reported that, biological Nitrogen fixation represent the major source of N input in agricultural soils including those of arid regions. The major N-fixing systems are the symbiotic systems, which play a significant role in improving soil fertility and productivity of low N-soils.

The *Rhizobium*-legume symbioses have received most attention and have been examined extensively. Atta *et al.* (2003) and Mohsen and Saeed (2005) reported that, *Rhizobium* inoculation significantly increased number of pods/plant, seed number/pod, seeds weight/pod, seeds weight/plant, 100-seed weight as well as seed and straw yield, N, P, K, Mn and Zn uptake of legume plants. These microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity (Kloepper, 2003). In a few cases, this effect has been suggested to involve solubilization of otherwise unavailable mineral nutrients (Badawi *et al.,* 2011). In soil, both macro- and micronutrients undergo a complex dynamic equilibrium of solubilization and insolubilization that is greatly influenced by the soil pH and microflora and that ultimately affects their accessibility to plant roots for absorption (Kandil *et al.,* 2008). Rhizobia are widely used in agriculture for crop improvement because of their ability to fix atmospheric nitrogen. Inoculation of legumes with many selective rhizobia lead to increments in seed yield and nitrogen content (Mekhemar *et al*., 2005).

This investigation was carried out to identify the response of peanut (*Arachis hypogaea* L, cv. Giza 5) to sulphur fertilization at rate of (15, 30 and 45 Kg/fed before sowing) and Rhizobia (*Bradyrhizobium japonicum*) inoculation, individually or in combination, under sandy soil conditions for determination of the yield criteria, pods yield, 100 seeds yield, seeds & straw yield and shilling percentage at harvest, as well as seed oil, oil yield, protein content and protein yield. The associated amelioration in plant macro and micronutrient contents and uptake, nodulation status (number and dry weight of nodules/plant), nitrogenase, dehydrogenase, CO_2 evolution and total bacterial counts were taking into consideration in this study. By which enable the peanut to tolerate nutrient deficiency and survive, not only by completing its life cycle, but also, by appropriating to be marketing.

Material and Methods

Layout of experimental field

A field experiment was carried out in sandy soil at Ismailia Agricultural Research Station, Ismailia Governorate, Egypt, during the two successive seasons of 2011 and 2012, to identify the response of peanut (*Arachis hypogaea* L, cv. Giza 5) to sulphur and Rhizobia (*Bradyrhizobium japonicum*) inoculation, individually or in combination. The experimental design was of randomized complete block with four replicates, where the area of each plot was 10.5 m^2. The soil samples (0-30 cm) were taken before the performance of the experiment, where some physical and chemical properties were determined using the standard methods according to Black (1965) and Page *et al*. (1982).

Rhizobia (*Bradyrhizobium japonicum*) were provided by biofertilizer production unit, Soils, Water and Environment Research Institute, Agriculture Research Center (ARC), Giza, Egypt. *Rhizobium* was cultured in yeast mannitol broth medium (Vincent, 1970), incubated at 28°C for three days on a rotary shaker until early log phase to ensure population density of 4×10^9 cfu / mL culture. Vermiculite supplemented with 10 % Irish peat was packed into polyethylene bags (300 g carrier per bag), then sealed and sterilized with gamma irradiation (5.0×10^6 rads). *Rhizobium* culture was injected into the carrier to 60% of the maxima water holding capacity. Rhizobia

(Bradyrhizobium japonicum), a N-fixing bacteria, was used as inoculum, where peanut seeds were mixed gently with inoculant at a rate of 300 g carrier per 60 kg seed, prior to sowing using arabic gum solution (16%) as an adhesive agent.

Elemental sulphur was conducted as 15 Kg S/fed., 30 Kg S/fed and 45 Kg S/fed, singly or in combination with Rhizobia (*Bradyrhizobium japonicum*) inoculation. As sulphur metal is insoluble in water, so, after application, it should be intensively incorporated into soil, where it is oxidized by soil microorganisms to form SO_4^{2-} to be available for plants (Stroehlein and Pennington, 1986).

All plots received basal doses of phosphorus fertilizer during soil tillage before peanut seeds sowing, at the rate of 30 Kg P_2O_5/fed as calcium superphosphate (15% P_2O_5). The potassium fertilizer was added at the rate of 48 kg /fed as potassium sulphate (48% K_2O), as recommended dose after 35 days from sowing. All tested treatments received 30 Kg N/fed in the form of ammonium nitrate (33.5% N) in two equal doses, one after thinning and the other after one month from sowing.

Seeds of peanut (*Arachis hypogaea* L) cultivar were provided by Field Crops Research Institute, ARC, Giza, Egypt. Seeds of peanut were inoculated as investigated above and planted on 4 and 9th of May for the first and second agricultural seasons, respectively, at a rate 60 Kg/fed. Two seeds were in hill and 20 cm spacing, after emergency, plants were thinned to one plant per hill. Other field practices were followed in the usual manner for peanut cultivation. Plants were grown till maturity and harvested. The area of each sample was 1 m^2. The harvest dates were on 6 and 9th of September for the first and second seasons, respectively. At harvest (120 days after sowing), agronomic trials were as follows: 100-seeds weight, Pods yield, seeds yield, straw yield and shilling %.

Methods of analyses

For chemical determinations, plants were fine powdered after harvest; wet digestion for dry material was carried out according to Chpman and Pratt (1961). Nitrogen percentage was determined in peanut seeds by micro Kjeldal method and Seed crude protein percentage was calculated by multiplying N% by 6.25 as described by AOAC (1990). Phosphorus and potassium percentages were estimated in peanut seeds according to AOAC (1990). Oil percentage in seeds was determined by Soxlit apparatus and petroleum ether as an organic solvent as described by AOAC (1990). The atomic absorption spectrophotometer was used to determine Zn, Mn, Fe, and Cu in seeds according to the method described by AOAC (1990).

Nodulation status, total bacterial counts and activities of some enzymes

After 50 and 80 days from sowing, four plants and their surrounded soil rhizosphere were selected randomly from each treatment and used to determine the following parameters:- Nodulation status and nitrogenase enzyme: Nodules were separated carefully from roots of each plant, counted and weighed, g/plant; used for nitrogenase (N_2-ase) assay, then dried and weighed. Nitrogenase enzyme (N_2-ase) activity of nodules was assayed by the acetylene reduction assay (Hardy *et al*., 1973). Total bacterial counts were determined in peanut rhizosphere soil according to Holm and Jenson (1972) by plate count technique using soil extract agar medium. Dehydrogenase (DHA) enzyme activity of rhizosphere plants was also determined by the method described by Casida *et al*. (1964). CO_2 evolution in peanut rhizosphere soil was estimated according to Allen (1959).

All data obtained were statistically analyzed according to Gomez and Gomez (1984).

Results and Discussion

The data obtained was mean of both growth seasons.

General view on the experimental soil

Initial state of the experimental soil is shown in Table 1, which indicates that sandy soil is characterized by sandy textural grade, with a low content of organic matter and low SP%. The available macro- and micronutrient contents of soil under consideration are lower than the critical limits. Accordingly, the studied soil is suffering from deficient in plant nutrients.

TABLE 1. Physical and chemical properties of the experimental soil.

Soil characteristics	Value	Soil characteristics	Value
Particle size distribution%:		***[1]EC (dS m^{-1}, at 25ºC)**	**0.61**
Coarse sand	71.35	*Soluble ions (soil paste m molc L^{-1}):*	
Fine sand	20.21	Ca^{2+}	1.74
Silt	2.54	Mg^{2+}	1.65
Clay	6.08	Na^{+}	2.12
Textural class	Sandy soil	K^{+}	0.52
Ca CO_3 %	1.05	CO_3^{2-}	0.00
pH (1:2.5 soil water suspension)	8.09	HCO_3^{-}	2.05
O. M. (%)	0.38	Cl^{-}	1.72
Organic-C %	0.24	SO_4^{2-}	2.53
Total counts of bacteria	5.2×10^4	Saturation percent (S.P. %)	22.3
Total counts of fungi	3.4×10^4	*[2]ESP %	4.92

Available nutrients (mg kg^{-1})

N	P	K	S	Fe	Mn	Zn
42.83	4.88	410	7.44	5.13	1.01	0.85

*[1]EC:- Electrical conductivity, *[2]ESP:- Exchangeable sodium percentage.

Response of some soil properties and available nutrients to the applied treatments

A. Some soil properties

In respective of elemental sulphur, data in Table 2 showed a clearly response of some soil properties, *i.e.,* pH, EC (Electrical conductivity) and ESP (Exchangeable sodium percentage) to the applied treatments, particularly those treated with the highest rates of elemental sulphur of 30 or 45 kg fed^{-1}, with insignificant differences. That was true, since elemental sulphur can oxidized by many soil microorganisms forming sulphuric acid, leading to frequent reactions with chemical compounds resulting from the microbial activity of Rhizobia *(Bradyrhizobium japonicum)* itself, consequently such acidic media led to lowering soil pH value. Also, the created sulphuric acid reacts with the native soil $CaCO_3$ and resulting in $CaSO_4$. The latter can be ionized to Ca^{2+} and SO_4^{2-}, which was also reduced soil pH. These results are in agreement with those obtained by (Awadalla *et al.,* 2003).

TABLE 2. Effect of sulphur and Rhizobia inoculation on ameliorating some soil properties.

Treatments	**Sulphur rate (Kg/fed)**	**Soil properties**		
		pH	**EC (dS m-^{1})**	**ESP %**
UnInocutation	Control	8.22	5.34	15.45
	15	7.85	5.04	14.05
	30	7.34	4.56	11.00
	45	7.32	4.51	10.80
Inocutation	Control	8.20	5.21	15.22
	15	7.78	4.56	11.52
	30	7.25	3.60	10.70
	45	7.21	3.41	9.30
LSD at 0.05		--	0.45	1.32

[1]EC:- Electrical conductivity,[2]ESP:- Exchangeable sodium percentage.

On the other hand, the released soluble ions of Ca^{2+} can be improved soil aggregation, due to a Ca^{2+} partial substitution by exchangeable Na^+ that enhancing the coagulation of Na-separated clay particles and leading to reduce ESP value, which encouraging the formation of small clay domains. Such clay domains are coated with soil humified organic substances, and then forming coarse pores that are increased soil permeability and accelerating leaching of a pronounced content of excess soluble salts, and then reducing EC value. The effective role of microbial activity in combination with applied elemental sulphur for ameliorating soil properties could be interpreted according to many opinion outlined by Bacilio *et al*. (2003), Shaban and Omar (2006) and Ashmay *et al*. (2008) who reported that, rhizobial strains produce several phytohormones (*i.e.,* indole acetic acid and cytokinins) and organic acid. Such products simultaneously improving soil structure, *i.e.,* increasing aggregate stability and drainable pores. Consequently, these created conductive pores enhancing the leaching process of soluble salts through irrigation fraction.

B. Soil available nutrients

In general, the obtained data presented in Table 3 showed that, the beneficial effect of the applied treatments, particularly elemental sulphur at the applied rates of 30 or 45 kg fed^{-1}, with insignificant differences. That was commonly achieved by lowering soil pH and in turn encouraging the availability of plant essential nutrients, especially phosphorus and sulphur as macronutrients as well as Fe, Mn, Zn and Cu as micronutrients.

The superiority of combined effect of added elemental sulphur as soil application and bio-fertilizer as Rhizobia *(Bradyrhizobium japonicum)* for the noticeable increment in soil available nutrient contents could be attributed to the pronounced decreases in the values of soil pH, EC and ESP vs the favorable amelioration in soil biological conditions that encouraging the released nutrients from soil native sources in the available forms, as well as easier mobility towards plant roots, and in turn their uptake by plants. In addition, the application of elemental sulphur tend to accelerate the released active inorganic acid (H_2SO_4) that leads to controlling soil availability and mobility of nutrients, which are more sensitive to the undesirable effects of alkaline soil media. Consequently, the applied elemental sulphur to the soil plays an important role for its nutritional status, whether be under demand as strategic storehouse for unavailable native nutrients. In this connection, Mohammed, (2004) interpreted the integrated role of applied elemental sulphur plus bio-fertilizer (Rhizobia), which resulted in more pronounced nutrients availability in the soil, on the basis of lowering soil pH and microbial activity that enhances the solubilization of nutrient from the native and added sources. Moreover, such prevailing conditions enhance the slow release of nutrients during the mineralization processes as well as minimizing their possible lose by leaching. These finding are also in agreement with Kaplan *et al*. (2005), who reported that, a potential strategy to enhance nutrients availability is the lowering soil pH that can be achieved through application of acid-producing fertilizers like sulphure-containing materials.

TABLE 3. Effect of sulphur and Rhizobia inoculation on soil availability of some nutrient contents.

Treatments	**Sulphur rate (Kg/fed)**	**Soil available nutrient content (mg kg^{-1} soil)**							
		Macronutrients				**Micronutrients**			
		N	P	K	S	Fe	Mn	Zn	Cu
UnInocutation	Control	35.61	3.59	191.1	6.73	3.93	0.98	0.82	0.47
	15	41.95	4.13	201.4	8.58	4.50	1.34	1.09	0.70
	30	46.10	4.47	207.0	10.85	4.78	1.61	1.23	0.81
	45	47.15	4.77	209.2	10.98	5.95	1.66	1.29	0.85
Inocutation	Control	37.18	5.98	199.4	8.02	4.75	1.10	1.01	0.76
	15	56.19	6.74	223.3	9.34	6.13	1.34	1.44	1.17
	30	65.54	7.53	236.9	12.76	7.00	2.49	1.79	1.47
	45	66.19	8.19	241.7	12.93	7.13	2.61	1.92	1.56
LSD at 0.05		2.11	0.98	5.80	0.45	0.23	0.11	0.05	0.04

C- Yield and yield components

Data in Table 4 showed that, using any rate of sulphur fertilizer have an alleviating effect on deleterious effect caused by nutrients deficiency, resulting in a significant increase in yield and yield components of peanut.

TABLE 4. Effect of sulphur and Rhizobia inoculation on yield and yield components of peanut plant.

Treatments	**Sulphur rate (Kg/fed)**	**100-seed weight (g)**	**Pods yield (Kg/ fed)**	**Seed yield (Kg/fed)**	**Straw yield (Kg/fed)**	**Shilling (%)**
Uninoculation	Control	74.40	1375	928	1710	63.06
	15	77.15	1469	991	1837	65.34
	30	78.63	1496	1013	1888	66.69
	45	80.70	1520	1032	1858	67.58
Inoclatation	Control	81.27	1546	1063	1990	64.44
	15	85.44	1571	1100	2009	66.11
	30	88.45	1603	1136	2038	67.12
	45	90.71	1611	1161	2065	68.34
LSD at 0.05		1.43	78.05	41.07	81.35	1.44

The rate of 45 Kg S/fed was the most effective one, where the relative increases in 100-seed weights and pods yield reached 8.47, likewise, 8.60 compared to the control. The positive effect of sulphur as a fertilizer, may be due to lowering soil pH and increasing nutrients availability through its oxidation by soil microorganisms to sulphuric acid or sulphate rises and exerts a positive effective on soil characters (Table 1), which reflected on the crop yield and its biochemical characters (El-Hamzawi, 2001, Azer *et al.*, 2003 and Nassar, 2007). Sharma and Gupta (1991) found that, the increases in biomass yield with higher S doses may be due to the positive response of plant to applied S, which promotes vegetative growth, starch and seed formation. Also, the increased supply of photosynthates to peanut pods would likely provide an opportunity for seeds to grow to their full size with an obvious increase in seed yield. The results are in accordance with those obtained by Ahmed *et al.* (2011).

Data of Table 4 cleared that, *Rhizobium* inoculation resulted in further promotion of the estimated parameters for both growing seasons as compared with the control, since the percentage of increases in seed and straw yield rose to 14.55% compared to control treatment. In this respect, Sprent and Faria (1988) revealed that, *Rhizobium* is a major group of heterotrophic N_2-fixing organism which invades roots of legumes. Ishac (1988) found that, *Rhizobium leguminosarum* had the effect of fixing nitrogen with leguminous plants. Abdel-Aziz *et al*. (1989) ascribed such effect to N_2-fixation and production of growth promoting substances. Similar results were obtained by Habib *et al*. (2010).

It could be concluded from data of Table 4 that, Rhizobia inoculation even singly took an action and gave the highest yield and its attributes, when it combined with sulphur, the yield was magnified till 45 Kg S/fed, the yield and its attributes were significantly surpassed. This was true for both growing seasons. These results were agreed with those obtained by Abd EL-Fattah and Arisha (2000), who attributes the positive response of plants to the favorable effect of Rhizobia on plant growth, nitrogen fixation, number of pods/plant and seed yield.

In fact, plant growth promoting rhizobacteria have been shown to greatly improve the productivity and quality of many legumes, when they inoculated with rhizobia (Mekhemar *et al*., 2007; Abdel-Wahab *et al*., 2008; Kandil *et al.*, 2008; Dileep-Kumar *et al*., 2001; Vessey and Buss, 2002). Rhizobacteria produced a lot of promoter substances such as auxin, vitamins B group and flavonoids like substances resulting in promotion of initiation and performance of nodulation as well as creation of more infection sites on the hairs and epidermis (Parmar and Dadarwal, 1999; Gage and Margolin, 2000 and Verma *et al.,* 2010).

Nutritional status

Macronutrients content and uptake

The presented data in Table 5 showed that, amendment of soil with S with different rates owing to a significant improvement in macronutrients concentration and uptake in peanut seeds throughout the two growing seasons, as compared with the control treatment. However, dose of 45 Kg S/fed exceeded the other doses in ameliorating the deleterious effect of nutrients deficiency on the concentrations and uptake of essential elements in peanut tissues, grown under such severe conditions. The relative increases in N concentrations and uptake reached 15.67% compared to control treatment. The positive effect of S may be due to decreasing soil pH and increased nutrients uptake and availability to experimental plant (Azer *et al*., 2003; Cui and Wang, 2005).

Dealing with rhizobia inoculation, it led to significant increases in P concentrations and uptake in seeds by 22.19% compared to control. Also, similar trends were found for N & P concentrations and uptake in peanut seeds for both growing seasons. In this context, Dashti *et al.* (1997) suggested that, the promoting mechanism of growth and nitrogen fixing bacteria included direct and indirect effects; the direct one, include an increase in the mobilization of insoluble nutrients followed by enhancement uptake by the plants, production of plant growth regulators and stimulants for plant growth and development; the indirect effect includes positive effect on symbiotic nitrogen fixation by improvement of root nodule number and mass. These results confirmed with (Mohamed *et al.,* 2001 Atta *et al.,* 2003 and Monged *et al*., 2004).

TABLE 5. Effect of sulphur and Rhizobia inoculation on macronutrient content and uptake of peanut seeds.

Treatments	Sulphur rate (Kg/fed)	N		P		K	
		Content (%)	Uptake (Kg/fed)	Content (%)	Uptake (Kg/fed)	Content (%)	Uptake (Kg/fed)
Uninoculation	Control	3.83	35.54	0.392	3.64	0.71	6.59
	15	4.16	41.23	0.428	4.24	0.78	7.73
	30	4.27	43.26	0.440	4.46	0.85	8.61
	45	4.43	45.72	0.467	4.82	0.89	9.18
Inoclatation	Control	4.49	47.73	0.479	5.10	0.90	9.57
	15	4.62	50.82	0.492	5.41	0.95	10.45
	30	4.74	53.85	0.513	5.83	0.96	10.91
	45	4.86	56.42	0.526	6.11	0.98	11.38
LSD at 0.05		0.17	3.22	0.02	0.39	0.03	0.71

With respect to interaction between rhizobia inoculation and sulphur supplements, statistical analysis clarified high significant increase for N, P and K concentrations and uptake in seeds in both growing seasons. The highest impact was inducted under rhizobia inoculation + 45 Kg S/fed addition, since the relative increase in K concentrations rose to 38.03% compared to control treatment. The positive impacts of sulphur may be due to improving soil physical and chemical properties conducted with improvement biological characters and fertility status via rhizobia inoculation. Both complementary actions led to enhancement the availability of most essential nutrients in soil, the plant will accumulate more nutrients to reach the balance between cations and anions, which push the plant to give higher dry matter and longer roots enable more absorption levels, assimilation rates and biochemical processes in whole plant organs (Marchner, 1998).

Micronutrients content and uptake

Data in Table 6 declared that, adding sulphur to soil significantly increased micronutrients content and uptake in seeds in both growing seasons as compared without sulphur application. Application 45 Kg S/fed was the most effective rate than others, where the percentage of increases in Fe and Zn were 15.68 and 19.91%, respectively. That sulphur application rate was produced significant increases for Fe & Zn uptake by about 28.65 and 32.24%, respectively.

Effect of elemental sulphur on the availability of micronutrients was studied by many investigators. They explained the indirect effect of sulphur in soil by its conversion to sulphuric, which has a solvent action for several important micronutrients (Makary, 2002). Yousry *et al.* (1984) found that, DTPA extractable Fe and Mn increased after applying Sulphur. Abd El-Fattah and Hilal (1985) reported that, use of sulphur as soil amendment would in case of Fe, Mn, Zn and Cu deficient soil, increase the availability of those nutrients and evoke a plant response.

TABLE 6. Effect of sulphur and Rhizobia inoculation on micronutrient contents and uptake of peanut seeds.

Treatments	Sulphur rate (Kg/fed)	Fe		Zn		Mn		Cu	
		Content (%)	Uptake (Kg/fed)	Content (%)	Uptake (Kg/fed)	Content (%)	Uuptake (Kg/fed)	Content (%)	Uptake (Kg/fed)
Uninoculation	Control	252.16	234.00	68.31	63.39	73.14	67.87	10.25	9.51
	15	265.96	263.56	74.15	76.48	80.12	79.40	10.91	10.81
	30	277.93	281.54	77.63	78.64	84.59	85.69	11.56	11.71
	45	291.70	301.03	81.23	83.83	88.41	91.24	11.92	12.30
Inoclatation	Control	297.27	316.00	82.66	87.87	91.38	97.14	12.15	12.92
	15	306.27	336.90	87.15	95.87	94.03	103.43	12.35	13.59
	30	316.84	359.93	89.88	102.10	96.38	109.49	12.67	14.50
	45	330.19	383.35	92.14	106.87	98.64	114.52	13.29	15.43
LSD at 0.05		9.02	18.57	3.80	6.26	4.23	7.34	0.40	0.75

Regarding rhizobial inoculation of peanut seeds, results in Table 6 showed a pronounced significant increase in micronutrients content and its uptake in seeds for the two growing seasons. The relative increases in Mn content and uptake was 24.94 % as compared to control. These results are in harmony with those of (Atta *et al*., 2003; Monged *et al*., 2004). Furthermore, the highest content and uptake in peanut seeds were inducted when *Rhizobium* inoculation was associated with sulphur, especially at 45 Kg S/fed for both growing seasons. The relative increase for Cu content was 29.66% as compared to control.

Bio-chemical components of peanut seeds

Results at Table 7 revealed that, crude protein, protein yield, oil percent and oil yield were significantly increases due to sulphur application rate up to 45 Kg S/fed, since the relative increases in crude protein and protein yield reached to 15.66% as compared to S-free application. The positive role of sulphur fertilizer might be due to the fact that, sulphur is an integral part of sulphur-containing amino acids (cystein, cytstine and methionine), hence, improved protein as well as oil synthesis in peanut seeds (Tamak *et al.,* 1997). Likewise, sulphur is usually required by legumes and is called a master nutrient for oil and seed production (Nassar, 2007 and Salimpour *et al.,* 2012). Sulfur is absorbed by plants roots from soil as sulfate and transported as a phosphate ester. Sulfate is reduced to sulfide via sulfite before it is incorporated into cysteine and other organo-sulfur compounds. $SO_4^{2-} \rightarrow SO_3^{2-} \rightarrow H_2S \rightarrow$ cysteine → methionine (Pronk *et al.,* 1990). Ligha and Giri (1999) reported that, increases in oil content by sulphur application might be attributed to involvement of sulphur in the biosynthesis of oil. The higher oil yield by sulphur addition was obviously because of higher seed yield and oil content.

TABLE 7. Effect of sulphur and Rhizobia inoculation on crude protein, protein yield, oil% and oil yield of peanut seeds.

Treatments	Sulphur rate (Kg/fed)	Crude Protein %	Protein yield (Kg/fed.)	Oil %	Oil yield (Kg/fed.)
Uninoculation	Control	23.94	222.16	44.11	409.34
	15	26.00	257.66	46.14	457.25
	30	26.69	270.37	47.31	479.25
	45	27.69	285.76	49.22	507.95
Inoclatation	Control	28.06	298.28	50.12	532.78
	15	28.88	317.68	51.22	563.42
	30	29.63	336.60	53.44	607.08
	45	30.38	352.71	55.22	641.10
LSD at 0.05		1.32	19.48	1.28	29.01

Similarly, *Rhizobium* inoculation produced significant improvement in the estimated parameters for both growing seasons, where the relative increases in oil percent reached 13.63% as compared to control. Concerning the combined treatment with sulphur, data showed that, motivation of *Rhizobium* was highly pronounced, when combined with S fertilizer at rate of 15, 30 and 45 Kg S/fed. The application of *Rhizobium* accompanied with 45 Kg S/fed gave the highest significant increases in all studied parameters, since the relative increases in oil yield rose to 56.62 and 57.87%, at the first and second season, respectively. Regarding to N-fixing bacteria, the important nitrogenase enzymes contains an Fe-Mo-S cluster, is a catalyst that performs the important function of nitrogen fixation, converting atmospheric nitrogen to ammonia that can be used by microorganisms and plants to synthesize proteins, DNA, RNA, alkaloids, and the other organic nitrogen compounds necessary for plant life (Lippard and Berg, 1994). The yield, quality and uptake of nutrients by black gram improved favorably with increasing levels of S as well as in combination with *Rhizobium*, this significant increase in these parameters might be due to better nutritional environment due to *Rhizobium* and S application. Since S is essential for S containing amino acids and plays a vital role in regulating the metabolic and enzymatic process including photosynthesis, respiration and symbiotic N fixation. Whereas the positive effect of *Rhizobium* might be due to its beneficial effect on N fixation and better root development (Kumar and Singh, 2009; Singh *et al*., 2005).

The improvement in plant growth and nutrient uptake upon rhizobial inoculation may be attributed to the several mechanisms such as biological nitrogen fixation (Chanway and Holl, 1991), synthesis of siderophores, compounds that chelate iron from soil, making it available to the plant, (Kloepper *et al.*, 1986 and Verma *et al.,* 2010), solubilizing minerals, or synthesis of plant hormones, such as auxins or gibberellins, (Probanza *et al.*,

2001) or plant hormone regulators, such as 1-aminocycloprophane-1-carbxylate deaminase (Glick, 1995 and Glick *et al*., 1995), an enzyme that decrease endogenous concentrations of ethylene and disease suppression and their coordinated expression were responsible in enhancing plant growth , and nutrient uptake of legumes (Dey *et al*., 2004 and Tilak *et al.*, 2005).

F- Nodulation status and nitrogenase (N_2-ase) activity

Effect of sulphur application and Rhizobia (*Bradyrhizobium japonicum*) individually or in combination as a bio-fertilizer and their impact on nodulation status; number of nodules and its dry weights (mg) per peanut plant and nitrogenase (N_2-ase) activity after 50 and 80 days of sowing were showed in Table 8.

TABLE 8. Effect of applied sulphur and Rhizobia inoculation on nodulation status and nitrogenase (N_2-ase) activity in nodules of peanut roots after 50 and 80 days of sowing.

Treatment	**Sulphur rate (Kg/fed)**	**Nodulation status**				**Nitrogenase assay (n mole C_2H_4 g dry nodules^{-1} hr^{-1})**	
		Number of nodules (Plant^{-1})		**Dry weight of nodules (mg Plant^{-1})**			
		After 50 days	After 80 days	After 50 days	After 80 days	After 50 days	After 80 days
Uninoculation	Control	79	107	114	326	138	1033
	15	88	121	167	388	376	1128
	30	98	134	228	427	593	1232
	45	109	156	289	499	723	1408
Inoclatation	Control	77	105	110	320	141	1028
	15	93	138	201	403	479	1296
	30	112	149	276	509	697	1497
	45	126	171	301	524	771	1581
LSD at 0.05		1.21	1.82	3.08	4.63	4.893	6.109

Rhizobia inoculation improved nodules number and its weighed /plant as well as N_2-ase activity of peanut roots, also enhanced total bacterial counts and dehydrogenase enzyme activity in peanut rhizosphere soil after 50 and 80 days from planting under field conditions compared to uninoculated peanut. These results are in accordance with Massoud *et al*. (2008) who stated that, inoculation with rhizobia induced significant increases in number of nodules/plant, dry weight of nodules/ plant and nitrogenase activity after 75 days from sowing. Also, Akhtar and Siddiqui (2009) showed that, inoculation of Rhizobium prompted significant increases in growth, yield and the number of nodules per root system compared to control plants. The recorded results due to uninoculated plants were acceptable even they were lower than the other inoculated treatments. This behavior could be due to the positive role of native bacteria among several decades ago (Radwan *et al*., 2007).

G- Biological activity of soil

Effect of sulphur application and Rhizobia (*Bradyrhizobium japonicum*) individually or in combination as a biofertilizer and their influence on soil biological activities after 50 and 80 days of sowing were showed in Table 9. Data obtained revealed that, sulphur application and Rhizobia resulted in significant enhancements of dehydrogenase, CO_2 evolution and total bacterial counts over control. The influence of dual application (45 Kg S/fed + Rhizobia) was significantly higher over single S application at the same level. These results was in harmony with those obtained by Mahmoud *et al.* (2007) who reported that, Rhizobia (*Bradyrhizobium japonicum*) combined with organic amendments have more response and enhanced the soil biological activity in terms of increasing total bacterial and cyanobacterial (*S. platensis*) counts, CO_2 evolution, dehydrogenase and nitrogenase activity. It was also reported that, bacteria can be incorporated into soil as organic matter and also as a source of enzymes as they produce extracellular acid and alkaline phosphatases that are active in solution or located in the periplasmatic space of the cell wall. Both biomass exopolysacchraides incorporated into soil, induced a growth promotion of other microorganisms and increased the activity of soil enzymes that participate in the liberation on nutrients required by plants (Caire *et al.,* 2000).

TABLE 9. Effect of applied sulphur and Rhizobia inoculation on the biological activity of soil cultivated by peanut after 50 and 80 days of sowing.

Treatment	Sulphur rate (Kg/fed)	Biological activity of soil					
		Dehydrogenase (μg TPF g dry soil^{-1} day^{-1})		CO_2 evolution (mg 100 g soil^{-1} day^{-1})		Total bacterial counts (10^4 cfu g soil^{-1})	
		After 50 days	After 80 days	After 50 days	After 80 days	After 50 days	After 80 days
Uninoculation	Control	13.5	18.4	8.98	12.80	2.5	2.40
	15	50.6	69.9	11.03	14.76	2.9	2.80
	30	77.5	87.7	12.89	17.66	3.1	3.07
	45	92.6	105.5	14.37	19.60	3.8	3.60
Inoclatation	Control	15.8	20.6	9.07	12.89	2.6	2.50
	15	82.6	104.7	19.12	21.70	40.00	38.50
	30	99.4	122.2	21.03	22.57	66.00	65.00
	45	146.3	176.7	22.81	24.38	90.00	89.10
LSD at 0.05		3.85	4.24	2.07	1.89	1.64	1.61

The current data was in agreement with the findings of Laloknam *et al.* (2006), who reported that, organisms that thrive in hyper saline environments possess specific mechanisms to adjust their internal osmotic pressure. Soil salinity, one of the major abiotic stresses reducing agricultural productivity, affects large terrestrial areas of the world; application of recent developed functional tools for the development of salt-tolerant crops is recommended (Yamaguchi and Blumwald, 2005). One such mechanism is the use of Rhizobia (*Bradyrhizobium japonicum*), which has the ability to accumulate compatible low-molecular weight organic solutes such as glycine and betaine (Kempf and Bremer, 1998).

Root activity and microbial metabolism may serve as sources of CO_2 in soil, the pH value of such soil will be low and the soil becomes acidic. Besides, $NH4^+$ could be created as a result of N-fixing Rhizobia; it undergoes biological transformation in the soil and form acid forming nitrate ions. Similarly, sulphur also produces acid forming sulphate ions through oxidation. Also, ammonium sulphate could be produced in rhizisphere, ultimately low pH. The increased concentration of CO_2, hydrolysis of acid salts and various organic acids increased the total acidity of soil. During organic matter decomposition, humus, organic acids and different acid slats may also be produced. Grover *et al*. (2015) demonstrates that CO_2 influence the richness, composition and structure of soil microbial community and the influence is more on active microbial communities and in the vicinity of roots. High C: N ratio under CO_2 favors nutrient acquisition ability and biological nitrogen fixers.

Sellamuthu and Govindawamy, (2003) reported that bacterial, fungal and actinomycetes population were increased with application of bio-organics and influenced the dehydrogenese activity. The increase in population may be due to presence of humic acid in root zone, which favors the microbial growth in the rhizosphere. The principal direct effects exhibited by humic onto living organisms include an increase in biomass accumulation, nutrient uptake, biosynthesis, antiviral activity (Cacco *et al.*, 2000). The indirect effects are mostly provided by the bio-organic release from organic compounds driven changes in environmental conditions such as bioavailability of some nutrients salts balance, physical, chemical soil properties such as structure of soil, aeration, drainage, water retaining capacity and soil temperature (Hopkins and Stark, 2003). Abou-Zeid and Bakry, (2011) concluded that, bacterial inoculation, generally, enhanced the soil biological activity in terms of increasing microbial counts, CO_2 evolution, dehydrogenase and nitrogenase enzyme activities. This increase of the soil biological activity increased the soil fertility, in turn that is reflected positively on the crop production. These increases may attribute to the N_2-fixing bacteria inoculation promote microbial activity of all another microbes in rhizosphere zone and consequently increased biological activity in soil (Tantawi, 2006). Inoculation of peanut seeds with *Rhizobium* improved nodulation status (Nodules number and dry weight/plant), nitrogenase (N_2-ase) activity of peanut roots and enhanced microbial counts and dehydrogenase (DHA) enzyme activity in rhizosphere soil of peanut after 50 and 80 days from planting compared to the untreated control (Khalifa *et al*., 2013).

Conclusion

It could be concluded that, in newly reclaimed sandy soil, the combined treatment of 45 kg S/fed.+*Rhizobium* (*Bradyrhizobium japonicum*) inoculation (300 g carrier per 60 kg seed; with bacterial population density of 4×10^9 cfu / mL culture) was the optimal treatment and effective strategy for improving peanut (*Arachis hypogaea* L, cv. Giza 5) yield and its components. Data indicated that the individual and combined treatments of *Rhizobium* and sulphur showed a positive role on soil characteristics, *i.e*., pH, EC, ESP and available nutrient contents (N, P, K, Fe, Mn and Zn) were increased with increasing the applied *Rhizobium* and sulphur rate. Also, joint application of *Rhizobium* and sulphur resulted in maximum values of soil biological parameters followed by the individual treatments of *Rhizobium* and sulphur, respectively. The enhancements of dehydrogenase, nitrogenase, CO_2 evolution and total bacterial counts over control were relatively higher in all treatments, especially that of rhizobial inoculation. This may be due to, addition of sulphur to soil fertilized by the recommended doses of NPK causes a series of chemical transformations leads to accumulation of organic matter, that alleviates soil characters favor rhizobium growth and activity, which in turn, owe to optimal nitrogen fixation and production of huge beneficial compounds favors total microbial counts and bioactivities, which reflected positively on peanut crop, resulting in significant increases in its yield, yield components and its chemical composition. Finally, reflected positively on biological activities of the treated soil and improvement the quality and quantity of peanut crop.

Recommendation: Application of the dual treatment of 45 kg S/fed + *Rhizobium* (*Bradyrhizobium japonicum*) inoculation (300 g carrier per 60 kg seed; with bacterial population density of 4×10^9 cfu / mL culture) was the optimal treatment and effective strategy for improving peanut (Giza 5) yield and its components in newly reclaimed sandy soil at Egypt, considering the recommended doses of NPK and the usual recommended practices of peanut.

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تأثير إضافة الكبريت و التلقيح بالريزوبيا علي محصول وجودة نبات الفول السوداني النامي في الأراضي الرملية المستصلحة حديثا في مصر

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أجريت تجربتان حقليتان في أرض رملية بمحطة البحوث الزراعية بالإسماعيلية-محافظة الإسماعيلية-مصر، خلال الموسمين ٢٠١١ و ٢٠١٢ م لدراسة مدى استجابة نبات الفول السوداني (*Arachis hypogaea* L) صنف جيزة ٥ للاضافة الارضية للكبريت المعدنى (بمعدل ١٥، ٣٠، و ٤٥ كجم/فدان) والتلقيح بالريزوبيا (*Bradyrhizobium japonicum*) إما في صورة منفردة أو مشتركة.

أظهرت النتائج فاعلية الاضافة الارضية للكبريت المعدنى في تحسين الظروف السيئة الناتجة من نقص التغذية في التربة الرملية. وقد سجل معدل ٤٥ كجم كبريت/فدان كفاءة عالية في إحداث استجابة معنوية في المحصول ومكوناته، حيث وصلت الزيادة النسبية لمحصول البذور إلى ١٢، ١١ مقارنة بالكنترول (تربة غير معاملة بالكبريت). حيث وجدت زيادة معنوية لمحتوى البذور من العناصر الكبرى (نيتروجين-فوسفور-بوتاسيوم) والعناصر الصغرى (حديد-زنك-منجنيز) بالإضافة إلى نسبة البروتين الخام ومحصول البروتين ونسبة الزيت الخام ومحصول الزيت والنسبة المئوية للتقشير.

كما أشارت النتائج إلى كفاءة التلقيح بالريزوبيا في مقاومة الظروف السيئة الناتجة عن نقص العناصر في التربة الرملية وتسببت في إحداث زيادة معنوية في كل النتائج في كل التقديرات السابقة. وأن إضافة التلقيح بالريزوبيا للتربة المحتوي بصورة منفردة أو مشتركة (كبريت معدنى + التلقيح بالريزوبيا) قد أدي إلي زيادة معنوية وموجبة في كل من خواص التربة. وأن التلقيح المنفرد بالريزوبيا دون إضافة الكبريت أدى إلى زيادة غير معنوية في كل القياسات والتقديرات المشار إليها عما سببه الاضافة الارضية للكبريت المعدنى بصورة منفردة عند معدل ٤٥ كجم/فدان، كما تسبب أيضا في إحداث زيادات معنوية في تلك التقديرات والقياسات عن تلك التي أحدثتها إضافة الكبريت بمعدلات أقل من ٤٥ كجم/فدان.

أوضحت نتائج المعاملات المزدوجة أن إضافة الكبريت بالمعدلات المختلفة مع الريزوبيا قد أحدث زيادة معنوية في كل الصفات السابق ذكرها وكانت المعاملة المكونة من ٤٥ كجم كبريت/فدان متحدة مع الريزوبيا هي المعاملة المثلى حيث أحدثت أكبر زيادة معنوية ليس في المحصول ومكوناته وتركيز العناصر الكبرى والصغرى ولكن أيضا في نسبة البروتين الخام ومحصول البروتين بالإضافة إلى نسبة الزيت ومحصول الزيت. كما ادت هذة النتائج إلي تحسين بعض الخواص البيولوجية للتربة (انزيم الديهيدروجينيز و النيتروجينيز وثاني أكسيد الكربون المنطلق من التربة و العدد الكلي من البكتيريا) و التي تفوقت فيها الإضافة المشتركة .

وفى ضوء ما تقدم يوصى البحث باستخدام المعاملة المزدوجة وهي الاضافة الارضية للكبريت المعدنى بمعدل ٤٥ كجم/فدان مشترك مع التلقيح الريزوبي كإستراتيجية فعالة في زيادة إنتاجية الفول السوداني و المنزرع في الأراضي الرملية المستصلحة حديثا في مصر مع الأخذ في الاعتبار الطرق المتبعة في زراعة الفول السوداني واستخدام الجرعات الموصى بها من أسمدة النيتروجين والفوسفور والبوتاسيوم.