J. Agric. Sci. Mansoura Univ., 30 (9): 5661 - 5673, 2005

EFFECT OF SULFUR APPLICATION WITH *Thiobacillus* AND *Rhizobium* INOCULATION ON SOIL PROPERTIES AND PEA PLANT PERFORMANCE

Hussein, Kh.A.*; M.B. El-Kotkate and H.H. El-Sebaay****

* Soil & Water Dept., and ** Microbiology Unit, Botany Dept., Fac. of Agric., Al-Azhar Univ., Cairo, Egypt

ABSTRACT

A pot experiment was carried out at the green house of the Faculty of Agriculture, Al-Azhar University, Cairo, Egypt, to study the effect of sulfur application with inoculation with *Thiobacillus* and *Rhizobium* as biofertilization under two methods of sowing; post-plow and dry sowing on soil properties and pea growth. The obtained results suggest that soil amendment with elemental sulfur at as high as 300kg S.fed^{-1} proved to be highly significant in promoting better fertility status that led to a remarkable plant response as plant height and dry matter production as well as better plant chemical composition. Also, inoculation with biofertilizer (symbiotic nitrogen-fixing bacteria; rhizobia, and sulfur-oxidizing bacteria; thiobacilli) proved to be very efficient in the same respect. Applying elemental sulfur together with both bacteria proved to be very effective in lowering pH values of the studied soil rendering most nutrient elements (macro and micro ones) available to plant roots absorption. This eventually enhances plant growth and performance towards desired vegetative growth then better yield of pea plants. This work has also exhibited the positive role of providing enough moisture in the soil rhizosphere. This moisture should be sufficient for good seed germination and survival, and enhanced activity of micro-organisms, as well. This is the case with dry sowing method.

INTRODUCTION

Since sulfur is an essential nutrient element for plant growth, its application was verified in most soils. If it is applied as elemental sulfur, it'll need to be mineralized into sulfuric acid or other forms to be utilized by grown plants. Sulfur mineralization is aided by specific groups of bacteria, which need to be inoculated either onto the seed or the soil. In addition, rhizobia are non-symbiotic atmospheric nitrogen fixers, which increase nitrogen compounds in soil solution for plant use.

Perez *et al.* (1984) observed that inoculation with *Rhizobium* had no significant effect on total biomass or on nodule number/plant, or when given P, S, Zn and Ca alone, either.

Lopez *et al.* (1985) showed that sulfur application increased plant sulfur concentration in uninoculated plants and increased tissue sulfur content. Highest N_2 fixation by inoculated plants was obtained at 44.8kg sulfur/ha. The addition of 2.5mg S/litre gave the highest total dry matter, acetylene reduction rate, total N content and percentage increase in fixed N_2 in inoculated plants.

Sharma *et al.* (1993) showed that seed and straw yield of *Vigna radiata* maximized with 60kg P/ha applied either with *Rhizobium* or starter nitrogen. Number of nodules/plant increased with up to 90kg P. Nitrogen, P, K and S uptake increased due to *Rhizobium* and P application. Mary and Hao (1995) stated that the elemental sulfur significantly increased soluble salts through the formation of sulfate salts, which are mostly soluble, while it decreased soil pH through the production of sulfuric acid.

Naidu *et al.* (1996) showed that nodule dry weight on the roots of *Vigna radiata* was highest in both years with 60kg S+ inoculation with rhizobium. Seed yield and protein contents were highest with the 40kg S+ seed inoculation with rhizobium.

Without *Rhizobium* inoculation, S, N and Chicken manure (CM) treatments significantly increased seed yield and 100 seed weight (El-Sheiku *et al.* 1997). Also, Saraf *et al.* (1997) stated that mean seed yield was higher with inoculation than without it (1.03t versus 0.88t/ha). Seed yield was highest with 60kg P_2O_5 (1.24t/ha) in both years and with 80kg S (1.14t/ha) in 1992.1993 only.

Matt (1998) stated that the levels of atmospheric S over Western Europe decreased, and more frequent incidents of S deficiency in crops took place. In Ireland more than 30% of soils were found deficient in S. Crop responses to S increase with increasing levels of fertilizer N. Fertilizer P applied at a rate of 40 kg/ha had no significant effect on yield response or uptake of S in herbage. However when applied at a rate of 100 kg/ha yield response and S uptake were significantly reduced.

Steve *et al.* (1998) found that mineralization of soil organic S to sulfate is mainly a microbial process. Mineralization contributes to crop S uptake, particularly in the later phases of crop growth. Most cases of S deficiency have been observed when a large amount of N was applied.

Jim Bauder (2000) reported that the critical level of sulfur extract by $CaCl_2$ was 7.3 to 9 mg S/kg soil, while it reached 0.15% in plant. He also added that sulfur application was the best way to lower soil pH and that it was the best to satisfy plant requirements of sulfur.

The Fruit Research and Extension Center (2004) stated that elemental sulfur was often recommended (50 lb S per 1000 sq. ft) to lower pH values and increase nutrient availability to plants.

The aims of this work were to study the effect of applying two levels of elemental sulfur with or without inoculation with *Thiobacillus* and/or *Rhizobium* on soil properties and the response of pea crop to these treatments.

MATERIAL AND METHODS

A pot experiment was conducted at wire-screen house of the Faculty of Agric., Al-Azhar University in Cairo, in March 2002. Thirty-cm diameter earthen pots were cleaned and seven kilograms of 2mm-sieved soil were put in each experimental pot. Elemental sulfur (99.8%) was applied to the soil in each pot at the rate of 0.1g/kg soil for the first level of sulfur application (100kg S/Fed), while 0.3g S/kg soil for the second (300kg S/Fed). Super phosphate was added at 15kg/fed unified to all pots. The applied sulfur and super phosphate were thoroughly mixed with the soil in each pot. Nitrogen was added prior to sowing as a starter dose at 30kg N/fed unified to all pots.

Applied treatments included the following: (1) Without sulfur addition or bacterial inoculation (control). (2) Inoculation with *Rhizobium* alone. (3)

Inoculation with *Thiobacillus* alone. (4) Elemental sulfur at 100kg/fed. (5) *Thiobacillus* and elemental sulfur at 100kg/fed. (6) *Rhizobium* and elemental sulfur at 100kg/fed. (7) *Rhizobium, Thiobacillus* and elemental sulfur at 100kg/fed. (8) Elemental sulfur at 300kg/fed alone. (9) *Thiobacillus* and elemental sulfur at 300kg/fed. (10) *Rhizobium* and elemental sulfur at 300kg/fed. And (11) *Rhizobium, Thiobacillus* and elemental sulfur at 300kg/fed.

All treatments were carried out under two sowing methods namely post-plough sowing and dry sowing. For the first sowing treatment, half of the pots was irrigated 12 days prior to pea sowing to simulate post-plough planting, while the other half was irrigated immediately after sowing to simulate dry sowing conditions.

Each of these treatments was assigned four pots as replicates. The pots were arranged in a completely randomized experimental design. Statistical analysis of variance of the obtained data pertaining to different measurements was achieved according to **Snedecor and Cochran (1967)**. Mean values of the different treatments will be differentiated according to **Duncan (1984).** This makes the total number of treatments reaches 22 treatments (88 pots).

Five pea seeds, variety Master B, were sown into the soil in each pot. Inoculation with *Thiobacillus* bacteria was achieved by adding 20ml of a liquid culture medium containing $1.8x10^6$ $cfu.ml^{-1}$. *Rhizobium* bacteria were inoculated into the soil at the time of sowing by adding 20ml of liquid culture containing (mean viable counts) $x10^6$ $cfu.ml^{-1}$.

Sulfur oxidizing bacteria (*Thiobacillus*) were isolated from the studied soil according to (Starkey, 1966) and their sulfur oxidizing efficiencies were measured according to the American Public Health Association (1975). The most efficient isolates were cultured on Starkey liquid medium for seven days. Rhizobium bacteria were isolated from true nodules formed on the roots of pea plants that were sown earlier for this purpose under sterilized conditions. Then, the bacteria were propagated on the yeast extract manitol agar according to Allen (1961). Rhizobium growth was purified and assured according to Gram's staining and microscopic examination for the very distinguished form of *Rhizobium* bacteria. Rhizobium bacteria were grown on YEM liquid medium for 72hrs.

Microbial rhizosphere activities were studied for all treatments after 20, 45 and 60 days from sowing using plate dilution frequency method on suitable media for counting total bacteria (spore formers and non-spore formers), actinomycetes, sulfur oxidizing bacteria (*Thiobacilli*) and fungi population.

Soil mechanical analysis was achieved on samples prior to sowing and soil texture was defined according to Piper (1950). Regarding soil chemical analysis. Soil pH and EC and soluble cations and anions were achieved according to Jackson (1973), organic matter and CEC to Page *et al.* (1982), available P to Olsen *et al.* (1965), and available N to Onken and Sunderman (1977).. The obtained physical and chemical analyses of the studied soil are depicted in Table 1.

Table 1: Soil physical and chemical properties.

Soil physical properties		Soil chemical properties			
Coarse sand %	10.60	pH	8.40	Fe, ppm	0.68
Fine sand %	65.82	$CaCO_3$ %	0.43	Mn, ppm	1.56
Silt %	10.65	O.M %	0.15	Zn, ppm	0.85
Clay %	12.93	Av. N ppm	1.81	Cu, ppm	0.60
Textural class	Loamy Sand	Av. P ppm	0.27	CEC (meq/100g soil)	10.00
Saturation, %	16.4	EC, $dS.m^{-1}$	4.50		
Cations	($meq.l^{-1}$)	Anions		($meq.l^{-1}$)	
Na^+	2.10	CO_3^{--}		0.00	
K^+	0.20	HCO_3^-		0.91	
Ca^{++}	1.48	Cl^-		2.47	
Mg^{++}	0.68	SO_4^{--}		1.08	

Regarding plant analysis, plant samples were oven dried at 60°C to preserve nitrogenous compounds and ground. A sample was wet-digested by Kjeldahl method according to Chapman and Pratt (1961) using sulfuric acid and hydrogen peroxide to determine both N and P. Nitrogen was measured by titration of ammonia against standard HCl, while P was determined colourimetrically using spectrophotometer.

After sixty-three days from sowing, plant height, root length, number of nodules on the roots, number of pods per pot, dry weight of roots per pot, shoot dry weight per pot, and pod weight per pot, as well as seed yield per pot were all recorded per treatment. Available N, P, Fe, Mn, Zn, and Cu were traced in the studied soil to recognize the effect of different treatments on their status in plant under the condition of both methods of cultivation and different levels of sulfur application, and inoculation with both sulfur-oxidizing and nitrogen-fixing bacteria as well. Available Fe, Mn, Zn, and Cu were determined using atomic absorption spectrophotometer according to Lindsay and Norvel (1984).

Microbial activities in the rhizosphere of different treatments were determined by plate dilution frequency method on specific media. Total bacterial counts (sporolated and non-sporolated) were achieved on soil yeast extract agar medium (Mahmoud *et al.*, 1964). Actinomycetes population was assessed on inorganic-salts starch agar medium (Szabo, 1974). *Thiobacillus* (sulfur oidizing bacteria) was assayed according to Stainer (1958), while fungal population was assayed on potato dextrose agar medium. These analyses were done on rhizosphere soil after 20, 45 and 60 days after sowing.

RESULTS AND DISCUSSION

Data in Table 2 reveal that bacterial activity was significantly prominent when elemental sulfur took place, especially at the level of 300kg $S.fed^{-1}$ (1 fed=0.42ha), simultaneously with inoculation with both rhizobium and sulfur-oxidizing bacteria (treatment no. 11). This held true at all times of sampling. It can be referred to the ability of rhizobium to fix atmospheric nitrogen and provide the plants with some of its nitrogen requirements. In-addition, sulfur-oxidizing bacteria (*Thiobacillus thioxidance*) produce acidic effect into the rhizosphere, which, in turn, renders more organic materials and nutrient elements available for microbial activity.

Increasing the application rate of elemental sulfur to the soil could significantly promote greater bacterial activity in the rhizosphere of pea plants. This agrees with the finding of **Lopez *et al.* (1985).** They showed that highest N_2 fixation by inoculated plants was obtained when sulfur application increased to 44.8kg sulfur/ha.

Table 2. Bacterial counts ($x10^6$ cfu/ml) in the rhizosphere at different growth stages of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria.

Serial*	Sowing after plowing			Dry sowing		
	Days after sowing					
	20	45	60	20	45	60
1	22 f**	28 f	32 f	29 h	33 h	39 h
2	31 d	35 e	41 e	37 f	41 g	47 g
3	20 f	30 f	41 e	31 g	38 g	48 g
4	31 d	41 d	53 d	38 e	49 f	57 f
5	25 e	41 d	55 d	60 c	68 d	70 e
6	60 c	75 b	80 c	65 b	81 b	90 b
7	68 b	78 b	91 a	85 a	95 a	99 a
8	60 c	65 c	78 c	65 b	75 c	80 c
9	62 c	68 c	79 c	50 e	55 e	65 e
10	70 b	75 b	85 b	55 d	65 d	78 d
11	85 a	89 a	95 a	88 a	98 a	99 a

* (1) Without bacterial inoculation or sulfur addition (control), (2) Inoculation with *Rhizobium* alone, (3) Inoculation with *Thiobacillus* alone, (4) Elemental sulfur at 100kg/fed alone, (5) *Thiobacillus* and elemental sulfur at 100kg/fed, (6) *Rhizobium* and elemental sulfur at 100kg/fed, (7) *Rhizobium, Thiobacillus* and elemental sulfur at 100kg/fed, (8) Elemental sulfur at 300kg/fed alone, (9) *Thiobacillus* and elemental sulfur at 300kg/fed, (10) *Rhizobium* and elemental sulfur at 300kg/fed, and (11) *Rhizobium, Thiobacillus* and elemental sulfur at 100kg/fed.

** Mean values sharing an alphabet are not significantly different at 5% level.

Generally, all applied treatments surpassed the control treatment in bringing more bacterial activity into the rhizosphere of pea plants. But, inoculation with rhizobium alone was the least effective in promoting the bacterial activity at all sampling times and under the conditions of both types of sowing conditions. This coincides with the results of **Perez *et al.* (1984)**. They observed that inoculation with *Rhizobium* alone had no significant effect on total biomass or on nodule number/plant. They recommended the application of some other plant nutrients such as P, S, Zn and Ca.

The two sowing methods varied significantly. In dry sowing, seeds received enough moisture to sustain germination and microbial activities as well. Under post-plow sowing, seeds find less soil moisture contents that may not be enough for good germination. This is supported by the results of Steve *et al.* (1998). They found that mineralization of soil organic S to sulfate is mainly a microbial process, which is sensitive to soil moisture content.

Nearly the same trends with bacterial activity in the rhizosphere of pea plants grown under the conditions of two patterns of sowing were also found in the case of actinomycetes (Table 3), fungi (Table 4), and sulfur-oxidizing bacteria (Table 5). In general, bicfertilization with rhizobium and thiobacillus and amendment with elemental sulfur proved to be highly significant in enhancing total microorganisms activities in the rhizosphere of pea plants provided that soil moisture content is abundant enough to support seed germination and micro-organisms activities, as well. Worthy to say is that all these activities provide each other, and plant roots also, with growth promoting elements. They can also exude organic materials that facilitate the solubility of such elements from their reservoirs in the soil complex. In this respect, Lopez *et al.* (1985) showed that sulfur application promoted N_2 fixation, gave the highest total dry matter, acetylene reduction rate, total N content and percentage increase in fixed N_2 in inoculated plants with rhizobium.

Table 3. Actinomycetes counts ($x10^5$ cfu/ml) in the rhizosphere at different growth stages of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria.

Serial*	Sowing after plowing			Dry sowing		
	Days after sowing					
	20	45	60	20	45	60
1	10 g	18 g	41 f	13 g	21 h	45 f
2	15 f	21 f	36 g	25 e	41 f	52 de
3	18 f	21 f	38 fg	20 f	35 g	45 f
4	23 e	42 d	53 c	55 a	65 a	70 a
5	30 d	35 e	41 e	32 d	38 fg	45 f
6	30 d	42 d	48 d	35 d	45 e	55 d
7	40 c	45 cd	57 b	45 bc	48 de	61 c
8	40 c	50 b	60 b	45 bc	50 d	60 c
9	45 b	48 bc	50 c	41 c	49 d	50 e
10	50 a	55 a	65 a	55 a	60 b	65 b
11	45 b	55 a	60 b	48 b	56 c	65 b

* See the note below table (2).

Table 6 shows that pea plant exhibited the greatest significant height at the highest rate of sulfur application (300kg $S.fed^{-1}$) combined with inoculation with both azotobacter and thiobacillus bacteria. The Table reveals also that the higher the sulfur application rate, the better the plant height was. Also, plants showed higher height with dry sowing method than with that after plowing. This confirms the previously mentioned findings regarding the enhancement of soil fertility with the application of elemental sulfur and inoculation with biofertilizers. From another point of view, sulfur-oxidizing bacteria were more effective in enhancing plant response than rhizobium, the symbiotically living nitrogen fixer organism.

Table 4. Fungi counts ($x10^4$ cfu/ml) in the rhizosphere at different growth stages of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria.

Serial*	Sowing after plowing			Dry sowing		
	Days after sowing					
	20	45	60	20	45	60
1	8 d	12 e	17 h	10 d	13 f	18 h
2	10 cd	15 de	21 g	12 d	17 e	25 f
3	11 c	13 de	18 gh	18 b	39 a	41 a
4	18 a	20 c	25 ef	25 a	30 b	35 bc
5	19 a	25 b	30 cd	20 b	27 c	33 cd
6	20 a	33 a	35 b	25 a	30 b	36 b
7	20 a	25 b	28 de	23 a	30 b	32 de
8	14 b	18 cd	22 fg	18 b	20 d	42 a
9	13 bc	16 d	21 g	15 c	17 e	22 g
10	20 a	24 b	31 c	25 a	27 c	30 e
11	20 a	31 a	42 a	18 b	19 de	25 f

* See the note below table (2).

Table 5. Sulfur-oxidizing bacteria counts ($x10^4$ cfu/ml) in the rhizosphere at different growth stages of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria.

Serial*	Sowing after plowing			Dry sowing		
	Days after sowing					
	20	45	60	20	45	60
1	18 e	27 f	42 f	25 e	45 g	55 e
2	19 e	25 f	45 f	27 e	36 h	51 f
3	41 d	51 e	58 e	51 c	55 ef	65 cd
4	45 bc	60 b	68 b	45 d	53 f	70 c
5	43 cd	53 de	63 cd	46 d	55 ef	63 d
6	47 ab	55 cd	67 b	50 c	61 c	68 c
7	50 a	58 bc	59e	60 b	71 b	78 b
8	45 bc	55 cd	60 de	46 d	57 de	63 d
9	48 ab	50 e	60 de	50 c	55 ef	65 cd
10	41 d	56 cd	65 bc	50 c	60 cd	65 cd
11	51 a	68 a	75 a	65 a	78 a	85 a

* See the note below table (2).

Responses observed for plant height were also obtained for root length, number of nodules/plant, fresh weight per plant and dry weight per plant. These parameters showed, more or less, a confirmatory response to the growth conditions. In other words, the significant effect of amending the soil with elemental sulfur supported by the inoculation with biofertilization was confirmed by all measured parameters.

Table 6. Some growth parameters of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria after 63 days from sowing under the conditions of both sowing methods.

No*	Plant height (cm)		Root length (cm)		Nodules/ Plant		Fresh weight (g)		Dry weight (g)	
	Plow	Dry	Plow	Dry	Plow	Dry	Plow	Dry	Plow	Dry
1	25 f	27 h	8 ef	11 bc	2 f	2 e	19 h	26 f	4.7 g	4.5 f
2	40 c	42 c	10 cd	13 ab	4 de	5 d	41 e	43 d	11.4 c	15.8 a
3	39 cd	37 ef	8 ef	9 cd	3 ef	2 e	35 g	38 e	9.8 d	12.4 c
4	10 c	39 cd	8 ef	10bcd	2 f	2 e	46 c	48 c	5.3 f	7.6 e
5	35 e	36 fg	7 ef	8 d	5 cd	5 d	51 a	70 a	11.1 c	12.9 c
6	35 e	34 g	9 ef	11 bc	7 b	9 bc	47 c	56 b	9.1 d	13.2 b
7	37 de	39 cd	10 cd	12 b	6 bc	10 b	38 f	42 d	14.1 a	15.7 a
8	40 c	41 c	10 cd	14 a	2 f	3 e	39 f	46 c	7.4 e	15.8 a
9	45 b	48 b	11 bc	13 ab	5 cd	6 d	43 d	43 d	5.1 f	11.4 d
10	40 c	48 b	12 ab	15 a	7 b	8 c	47 c	48 c	9.8 d	15.2 a
11	49 a	52 a	13 a	15 a	9 a	13 a	49 b	72 a	12.1 b	15.5 a

* See the note below table (2).

Table 7 reflects the soil fertility status in the rhizosphere under the applied treatments. It is clear from the Table that the application of elemental sulfur as a soil amendment and inoculation with biofertilizers could significantly proliferate a prominent fertility status that gave the plants the advantage to be distinguished in all growth parameters measured in this study. The good fertility status was characterized by the highest contents of available nitrogen, phosphorus, iron and manganese, which seemed to be highly suitable for plant growth and development all along its life span.

Table 7. Some soil available nutrients in the rhizosphere of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria after 63 days from sowing under the conditions of both sowing methods.

No*	N, ppm		P, ppm		Fe, ppm		Mn, ppm	
	Plow	Dry	Plow	Dry	Plow	Dry	Plow	Dry
1	161 j	182 j	2.9 i	2.6 h	0.76 i	0.84 i	1.56 j	1.63 i
2	181 ij	200 ij	3.5 hi	4.5 gh	0.80 i	1.00 i	1.75 ij	1.90 hi
3	200 hi	220 hi	4.5 gh	5.5 g	1.10 h	1.31 h	1.92 i	2.00 h
4	211 gh	240 gh	6.1 fg	7.3 f	1.25 gh	1.55 g	2.10 h	2.25 g
5	225 fg	255 fg	7.5 ef	9.0 f	1.36 fg	1.70 fg	2.31 g	2.40 g
6	235 ef	269 ef	9.3 e	11.5 e	1.51 ef	1.85 ef	2.52 f	2.65 f
7	251 de	283 de	11.5 d	13.6 d	1.65 de	2.00 de	2.71 e	2.91 e
8	270 cd	300 d	13.2 cd	18.3 c	1.77 cd	2.15 cd	2.95 d	3.20 d
9	285 bc	331 c	14.7 c	20.0 c	1.90 c	2.30 bc	3.31 c	3.50 c
10	300 b	365 b	16.3 b	23.1 b	2.15 b	2.40 b	3.65 b	3.80 b
11	330 a	395 a	19.2 a	26.0 a	2.40 a	2.60 a	4.11 a	4.55 a

* See the note below table (2).

Table 8 exhibits the plant contents of these elements. It is obvious that plants took advantage of the good fertility status and absorbed enough of these nutrient elements to excel in plant height and other studied plant parameters. This went on the basis of what plant roots found in the rhizosphere could absorb and utilize it as is clear from the Table.

Table 8. Nutrient contents of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria after 63 days from sowing under the conditions of both sowing methods.

No*	N, %		P, %		Fe, ppm		Mn, ppm	
	Plow	Dry	Plow	Dry	Plow	Dry	Plow	Dry
1	1.5 k	1.4 j	0.02 k	0.01 i	80 j	60 j	30 k	20 k
2	1.8 j	1.6 i	0.10 j	0.02 i	90 i	70 i	35 j	25 j
3	2.0 i	1.8 h	0.20 i	0.10 h	100 h	80 h	40 i	30 i
4	2.3 h	2.0 g	0.25 h	0.20 g	110 g	100 g	45 h	35 h
5	2.7 g	2.2 f	0.35 g	0.25 f	115 fg	110 f	50 g	40 g
6	3.0 f	2 2 f	0.40 f	0.31 e	120 f	115 e	60 f	45 f
7	3.2 e	2.5 e	0.45 e	0.39 d	130 e	125 d	70 e	51 e
8	3.6 d	2.9 d	0.50 d	0.42 d	150 d	130 c	80 d	62 d
9	4.0 c	3.1 c	0.55 c	0.49 c	160 c	136 b	85 c	73 c
10	4.3 b	3.6 b	0.60 b	0.55 b	170 b	140 b	90 b	80 b
11	4.6 a	4.0 a	0.70 a	0.60 a	190 a	155 a	105 a	90 a

* See the note below table (2).

Data in Table 9 assures the previously mentioned results in this paper, since the best mineral composition accompanied the combined treatment. This treatment shows that the application of elemental sulfur at the highest rate (300kg $S.fed^{-1}$) combined with inoculation with both bacteria of sulfur oxidation (*Thiobacillus thioxidanse*) and nitrogen fixation (*Rhizobium* sp.) has significantly led to the highest pea plant uptake of nitrogen, phosphorus, iron and manganese.

Generally, plant uptake under the dry sowing condition was significantly greater than that under post-plow sowing. This can be referred to the more appropriate soil moisture content in the rhizosphere under the former. This holds true for each of the four nutrient elements depicted in Table 9. Noteworthy is that the higher rate of sulfur application was significantly better than the lower one in bringing about the highest nutrient uptake. All applied treatments could significantly lead to enhanced nutrient uptake compared with the untreated plants (the control) under both modes of sowing.

This agrees strongly with the conclusion of Sharma *et al.* (1993), who showed that N, P, K and S uptake increased due to *Rhizobium* and P application. This can be interpreted on the basis of that sulfur oxidation proliferates P solubilization through the production of sulfuric acid. This is supported by the report of Mary and Hao (1995). They stated that elemental sulfur decreased soil pH through the production of sulfuric acid.

Table 9. Nutrient uptake (mg/pot) of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria after 63 days from sowing under the conditions of both sowing methods.

No*	N		P		Fe		Mn	
	Plow	Dry	Plow	Dry	Plow	Dry	Plow	Dry
	mg/pot							
1	71 m	63 m	1 e	1 e	0.38m	0.27m	0.14 ij	0.09 j
2	205 i	253 h	11de	3 e	1.03ij	1.11hi	0.40 g	0.40 g
3	196 j	223 l	20 cd	12 de	0.98ij	0.99ij	0.39 g	0.37 gh
4	122 l	152 k	13 d	15 de	0.58l	0.76kl	0.24 hi	0.27 h
5	300 f	284 fg	39 c	32 c	1.28gh	1.42fg	0.56 f	0.52 f
6	273 g	290 f	36 c	41 c	1.09l	1.52ef	0.55 f	0.59 f
7	451 c	393 d	63 b	61 b	1.83d	1.96cd	0.99 c	0.80 e
8	266gh	458 c	37 c	66 b	1.11hi	2.05c	0.59 f	0.98 cd
9	204 i	353 e	28 cd	56 b	0.82jk	1.55ef	0.41 g	0.83 e
10	421 d	547 b	59 b	84 a	1.67de	2.13bc	0.88 de	1.22 b
11	557 b	620 a	85 a	93 a	2.30ab	2.40a	1.27 b	1.40 a

* See the note below table (2).

Considering the data in Table 10 pertaining soil pH and EC values reveals that the application of elemental sulfur alone was significantly not effective in decreasing soil pH or inducing greater soluble salts contents in the soil solution. Neither were sulfur oxidizing bacteria that efficient, nor were nitrogen fixing ones. But, when elemental sulfur was combined with either of the two, soil pH started to drop owing to the production of organic acids or sulfuric acid. The topmost acid production maximized when the three were applied altogether in one treatment.

Table 10. Soil pH, EC, chloride and sodium in the rhizosphere of pea plants as affected by sulfur application rate and/or inoculation with rhizobium and/or sulfur-oxidizing bacteria after 63 days from sowing under the conditions of both sowing methods.

	(pH)		EC (dSm^{-1})		Cl ($meqL^{-1}$)		Na ($meqL^{-1}$)	
No*	Plow	Dry	Plow	Dry	Plow	Dry	Plow	Dry
1	8.4a	8.4a	4.5l	4.5l	2.47a	2.47a	2.1j	2.1j
2	8.2abc	8.3ab	4.7kl	4.6kl	2.21cd	2.35b	2.2ij	2.2ij
3	8.0cde	8.2ab	4.9ijk	4.7kl	2.15de	2.25c	2.3i	2.2ij
4	7,9def	8.1bcd	5.1ghij	4.8jkl	2.01f	2.10e	2.5h	2.3i
5	7.7fg	8.0cde	5.4fgh	5.0hijk	1.90g	2.00f	2.7g	2.5h
6	7.5gh	7.9def	5.6ef	5.2ghi	1.75h	1.95fg	2.7g	2.6gh
7	7.3hi	7.8ef	5.9cde	5.4fgh	1.60l	1.80h	2.9f	2.7g
8	7.1ij	7,7fg	6.1bcd	5.5efg	1.50j	1.72h	3.1de	2.9f
9	6.9jk	7.5gh	6.3bc	5.8def	1.11l	1.60i	3.3bc	3.0ef
10	6.8k	7.3hi	6.5ab	5.9cde	0.90n	1.30k	3.4b	3.2cd
11	6.7k	7.1ij	6.9a	6.3bc	0.60o	1.00m	3.6a	3.3bc

* See the note below table (2).

The Table shows that pH has been significantly dropped with treatment number 11. As a result of different acids formation, their respective salts are formed in the soil solution. This eventually leads to an increase in the electrical conductivity of soil solution as shown in the Table. Luckily, plant roots absorption of nutrient elements mimics the apparent increase in the EC of soil solution. The electrical conductivity is mainly attributed to the soil solution content of sodium and chloride ions as shown in Table 10. This parallels the findings of the Fruit Research and Extension Center (2004), which stated that elemental sulfur was often recommended to lower pH values and increase nutrient availability to plants. The significant drop in soil solution pH can be held responsible for the significant variability in pea uptake of both of iron and manganese as was previously shown in this paper.

In conclusion, soil amendment with elemental sulfur at as high as 300kg $S.fed^{-1}$ proved to be highly significant in promoting better fertility status that led to a remarkable plant response as plant height and dry matter production as well as better plant chemical composition. Also, inoculation with biofertilizer (symbiotic nitrogen-fixing bacteria; rhizobia, and sulfur-oxidizing bacteria; thiobacilli) proved to be very efficient in the same respect. Applying elemental sulfur together with both bacteria proved to be very effective in lowering pH values of the studied soil rendering most nutrient elements (macro and micro ones) available to plant roots absorption. This eventually enhances plant growth and performance towards desired vegetative growth then better yield of pea plants. This work has also exhibited the positive role of providing enough moisture in the soil rhizosphere. This moisture should be sufficient for good seed germination and survival, and enhanced activity of micro-organisms, as well.

REFERENCES

Allen, O.N. (1961). Experiments in Soil. Bacteriology, Purges Publications, Co., Minnisota, USA.

American Public Health Association (1975). Standard methods for the examination of water and wastewater. Washington, D.C.

Chapman, H.D. and P.F. Pratt (1961). Methods of Analysis for Soil, Plant and Waters. Agric. Univ. of California Riverside. USA.

Duncan, D.B. (1984). Multiple range and multiple F test. Biometrics, 11: 1-24.

El-Sheiku, E.A.E and A.a. Elzidany (1997). Effects of *Rhizobium* inoculation, organic and chemical fertilizers on yield and physical properties of Faba bean seeds. Plant Foods for Human Nutrition, 51(2): 137-144.

Jackson, M.L. (1973). Soil Chemical Analysis. Printice Hall, Inc., Englewood Cliffs, N.J. Library of Congress, USA.

Jim, B. (2000). Cereal crop residues and plant nutrients. Montana State Univ. Extension (MSU), Soil and Water Quality Specialist.

Lindsay, W.L. and W.A. Norvell (1978). Development of DTPA soil test for zinc, iron, manganese and copper. Soil Sci. Soc. Am. Proc. 42: 421-428.

Lopez, J.G. (1985). Effect of sulfur on dinitrogen fixation of alfalfa (*Medicago sativa* L.). Dissertation Abstracts International, B, Sciences and Engineering, 46(1): 5B.

Mahmoud, S.A.Z.; M. Abou El-Fadel and M. Kh. El-Mofty (1964). Studies on rhizosphere microflora of a desert plant. Folia microbiologica, 9: 1-8.

Mary, A. R. and W. Hao (1995). An evaluation of composts for landscape soil amendments. Ohio Agricultural Research and Development Center/Ohio State University Extension/Horticulture and Crop Science.

Matt, D. M. (1998). The interaction of applied S with N, P, Se and Mo in soils and on the uptake of these elements by plants. Teagascc, Johnstown Castle, Wexford, Ireland.

Naidu, M.V.S. and Hanuman-Ram (1996). Effect of sulfur and *Rhizobium* inculation on dry matter, grain yield and protein content in green gram (*Vigna mongo* L.). Legume Research, 19(1): 10-14.

Olsen, S.R.; C.J. Cole; F.S. Watanabe and L.A. Dean (1965). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. No. 939, USDA. U.S. Government Printing Office, Washington, DC.

Onken, A.B. and Sunderman, H.D. (1977). Colorimetric determinations of exchangeable ammonium, urea, nitrate, and nitrite in a single soil extract. Agron. J. 69: 49-53.

Page, A.L.; Miller, R.H. and Keeney, D.R. (1982). Methods of soil analysis. Part 2: Chemical and microbiological properties. Second Edition. Agron. J. 9: 2.

Perez, G.J.; R. Borel and F. Beotsch (1984). Priority of elements in mineral nutrition of *Leucaena leucocephala* (Lam) de wit in an acid soil in Costa Rica. Turrialba, 34(1): 91-98.

Piper, C.S. (1950). Soil and Plant Analysis. 1st Ed. Inter. Publishers, New York, USA, pp. 47-79.

Saraf, C.S.; B.G. Shivakumar and R.R. Patil (1997). Effect of phosphorus, sulfur and seed inoculation on performance of chick pea (*Cicer arietinum*). Indian Journal of Agronomy, 42(2): 323-328.

Sharma, Y.M.; P.R. Dikshit; O.R. Turkar and A.M. Sharma (1993). Effect of *Rhizobium* culture with different levels of starter dose of nitrogen and phosphorus on the yield, nutrition and nodulation of summer moong (*Vigna radiata*). Bhartiya Krishi Anusandhan Patrika, 8(2): 106-114.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th ed. The Iowa State Univ. Press, Iowa, USA.

Stainer, R.Y.; M. Donoroff and E.A. Adelberg (1958). Microbiology. McMillan and Co. ed. London.

Starkey, R.L. (1966). Oxidation and reduction of sulphur compounds in soil. Soil Science, 101(4): 297-306.

Steve P. McGrath, Fangjie Zhao, Nicky Riley and Julia Knights (1998). Effects of leaching and mineralization of sulfur on availability to crops. IACR-Rothamsted, Harpenden, Herts AL5 2JQ, United Kingdom

Szabo, I.M. (1974). Microbial comments in a forest-rendzina ecosystem. Academia, Kiado-podapest.

The Fruit Research and Extension Center (2004). Tree Fruit Soil and Nutrition. Ed., Washington State Univ., Pullman, WA 99164 USA

دراسة تأثير إضافة الكبريت والتلقيح المشترك ببكتريا الكبريت (ثيوباسيلس) وبكتريا العقد الجذرية (الريزوبيوم) على خواص التربة ومحصول البسلة

حسين خالد أحمد*، محمد بدوي عمر القطقاط و حسن هداية السباعي****

*** قسم الأراضي والمياه، ** قسم النبات الزراعي- ميكروبيولوجي - كلية الزراعة - جامعة الأزهر، القاهرة**

أقيمت تجربة أصص في مارس ٢٠٠٢ بكلية الزراعة – جامعة الأزهر – القاهرة لدراسة تأثير إضافة الكبريت العنصري مع التلقيح ببكتريا أكسدة الكبريت (الثيوباسيلس) وبكتريا تثبيت الأزوت الجوي (الريزوبيوم) تحت ظروف طريقتي زراعة (الحراثي والعفير) وذلك على خواص التربة ونمو نبات البسلة. أوضحت النتائج المتحصل عليها أن إضافة الكبريت بمعدل ٣٠٠ كجم للفدان أثبتت كفاءة عالية في توفير مستوى خصوبة أرضية أدت إلى استجابة واضحة في ارتفاع النبات وإنتاج المادة الجافة بالإضافة إلى تحسين التركيب الكيماوي للنبات. كما أثبتت بكتريا العقد الجذرية وبكتريا أكسدة الكبريت كفاءة عالية مؤكدة في نفس المضمار. كما وأن إضافة الكبريت مع كلا النوعين من الكائنات الدقيقة قد أدى أيضا إلى تخفيض معنوي في رقم حموضة محلول التربة مما جعل معظم العناصر الغذائية (كبرى أو صغرى) أكثر تيسيرا لجذور النبات وقد نتج عن هذا نمو خضري أفضل تلاه محصول أفضل لنباتات البسلة. وقد أظهر هذا البحث أهمية الدور الإيجابي الذي يلعبه توفير كمية رطوبة ملائمة في منطقة انتشار الجذور تكفي لنمو جيد للبذور واستمرار البادرات الناتجة في حيوية مع ضمان نشاط ميكروبي جيد، وهذا هو الحال مع طريقة الزراعة العفير.