

## Soil Solarization and Inoculation with Sulphur Oxidizing Bacteria and Their Effects on Some Soil Properties

Hala H Gomah\*, Mahmoud SM, El-Rewainy HM and Abdrabou MR

Soil and Water Department, Faculty of Agricultural, Assiut University, Egypt

### Abstract

Two greenhouse pot experiments (clayey and sandy soil) were conducted in order to evaluate the effects of solarization (covering the soil with transparent plastic sheets), inoculation with sulfur oxidizing bacteria SOB (isolated *Thiobacillus*), addition of filter mud cake FMC (one of the sugar industry wastes, Nagaa Hammady Sugar Factory, Egypt) as a source of organic matter and elemental sulfur on some sand and clay soil properties.

In both soils, the temperature of solarized soil was always higher than the nonsolarized one with an average of 6°C at 8:00 a.m. and 14°C at 4:00 p.m. which resulted in a reduction in organic matter percentage (OM%). Both FMC and S addition had great effects on increasing soil total soluble salts compared to the increase that resulted from either solarization or SOB inoculation. The effect of elemental sulfur addition on decreasing soil pH was higher than the other treatments in clay soil, while FMC addition was the most effective treatment in sandy soil. The highest increase in available S was always found when soils were treated with elemental sulfur. Each of the treatments increased the available P in both soils; however the most effective treatment was FMC addition. Soluble  $\text{Ca}^{+2} + \text{Mg}^{+2}$  and  $\text{K}^+$  were always increased due to each of the treatments. The highest increase in soluble  $\text{Na}^+$  was due to increasing soil temperature by solarization compared to the other treatments.

**Keywords:** Soil solarization; Sulphur oxidizing bacteria; Soil properties; Greenhouse experiments

### Introduction

The term 'soil solarization' or simply 'solarization' refers to the solar heating of moist soil by covering it with clear plastic sheets during periods of high solar radiation. Soil solarization induced changes in soil physical and chemical properties, such as soil structure [1,2], water potential [3], soil pH and EC [4]. Solarization speeds up the breakdown of organic material in the soil resulting in release of soluble nutrients, such as calcium ( $\text{Ca}^{+2}$ ), magnesium ( $\text{Mg}^{+2}$ ), potassium ( $\text{K}^+$ ), and fulvic acid, making them more available to plants [5].

The main reasons for assigning *thiobacilli* as the dominate role in sulfur oxidation process in soils are: Chemolithotrophic sulfur oxidizing bacteria will oxidize reduced forms of sulfur since this is the only or preferred way of obtaining energy [6]. Sulfur oxidation occurred more rapidly when inoculated sulfur used [7]. However, the oxidation of reduced sulfur in soils usually regarded as a microbial process, although some non-biological oxidation of the elemental does occur [8]. Sulfur oxidation is in fact, a chemical process, where tetrathionate accumulate at 45°C in a fertile loam soil, a result suggesting that thermophilic or abiotic oxidation of elemental sulfur to  $\text{S}_4\text{O}_6$  was occurring [6].

The most important factors governing the rate of sulfur oxidation are temperature and water potential [9], variation in particles size [10], manufacturing processes and additives [11], soil fertility [12].

Sulfur oxidation and nutrient availability may increase with the addition of organic matter to sulfur amended soils that contains low amount of organic matter [13]. Soil pH decreased from 8.1 to 6.7 by the application of WS 90 (wetable S) at a rate of 1000 Kg S  $\text{h}^{-1}$  compared with the control [14]. Sulfur application considerably increased the EC and the soluble sulfate in calcareous soils with little differences between different rates of application [15] showed that. In some calcareous soil, S-application increased the chemically available P from native soil apatite or added rock phosphate [16], whereas in others it increased available P only when P-fertilizer was added to the soil but soil P was

not affected [17]. Our experiment was achieved to indicate the changes of the some soil properties as a result of solarization and/or inoculation with sulfur oxidizing bacteria along with sulfur and filter mud cake addition.

### Materials and Methods

Two greenhouse experiments (clayey soil and sandy soil) were conducted at Soils and Water Department in black polyethylene bags 25-30 cm high, 20 cm diameter filled with 5 kg soil. The soil was irrigated and lifted for 48 hours to reach field capacity then, covered by colorless transparent polyethylene sheets. The soil was kept moist near field capacity across the experiment and water was added as the soil needed. Soil temperature was measured daily at 8:00 a.m. and 4.00 p.m. at 8 cm depth in nonmulched and mulched soils using soil thermometers. Eight weeks after the termination of solarization period the soil samples were taken with 2.5 cm diameter soil tube, air-dried and stored in plastic bags for chemical analysis [18].

### Isolation of *Thiobacillus* strain

The isolate used in the present study is *Thiobacillus* spp. The *Thiobacillus* strain was isolated from composite sample of clayey soil of Assiut Experimental Farm which cultivated with faba bean plants. Starkey's medium was used for the isolation and subsequent cultivation have the following composition  $\text{NH}_4\text{Cl}$  0.2 g,  $\text{KH}_2\text{PO}_4$  0.4 g,  $\text{MgCl}_2$  0.1 g,

\*Corresponding author: Hala H Gomah, Soil and Water Department, Faculty of Agricultural, Assiut University, Egypt 71526, Tel: 00201096955337; Fax: 2331384088(+2); E-mail: [Halagomaa71@hotmail.com](mailto:Halagomaa71@hotmail.com)

Received June 26, 2014; Accepted July 17, 2014; Published July 24, 2014

Citation: Gomah HH, Mahmoud SM, El-Rewainy HM, Abdrabou MR (2014) Soil Solarization and Inoculation with Sulphur Oxidizing Bacteria and Their Effects on Some Soil Properties. J Microbial Biochem Technol S3: 005. doi:10.4172/1948-5948.S3-005

Copyright: © 2014 Gomah HH, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

FeCl<sub>2</sub> trace, NaHCO<sub>3</sub>, 1.0 g and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 5.0 g dissolved in 1 L distil water [19]. The medium was sterilized at 15 pounds pressure for fifteen minutes, the thiosulfate and carbonate were sterilized, separately, and then they were added by means of sterile pipettes. The initial reaction of this medium was about pH=8.6 to 9.6. Ten grams of soil were added to 200 ml of the sterilized liquid medium in a 250 ml Erlenmeyer flask and shaken on rotary shaker for 1 hr then incubated at 30-34°C for 15 days. One ml aliquots of the suspension was spread on the surface of the Starkey's solid agar medium in Petri plates and the plates were incubated at 30-34°C for 10 days. After incubation, the developing *Thiobacillus* colonies were repeatedly purified by single colony isolation and the purity of the selected isolate was then tested microscopically before studying their characteristics.

Microscopic examination of the isolate grown on Starkey's broth and agar media at age of 24 hrs and 5 day old were made for the determination of cell morphology (gram reaction dimension and motility). Also, colony characteristics (size, form, shape, surface, elevation, consistency, color and edge) were examined on the strain. The composition of broth Starkey's medium, routinely used for growing and testing morphological and physiological characteristics of the isolate e.g., diverse growth temperature requirements of this isolate, determine the optimum growth temperature, capability of use organic substrate (yeast, glucose) and denitrification as follows:

**Growth temperature requirements and determining the optimum temperature of *Thiobacillus* spp. isolate:** Five sterilized tubs (contained 9 ml of Starkey's liquid medium inoculated with 1 ml of 5 day old isolated culture) were used in this test. The tubes were incubated at 4, 25, 30, 34, 45 and 50°C. After 5-days the positive tubes were counted [20].

**Using (glucose and yeast extract) by isolated *Thiobacillus*:** Starkey's liquid medium used for this test and modified by adding D-Glucose or Yeast Extract at concentration of 5 g/L. The glucose and yeast extract prepared at this concentration and sterilized separately then, added by sterilized pipettes. Five sterilized tubs (contained 9 ml of Starkey's glucose or Yeast Extract liquid medium) was inoculated with 1 ml of 5 days old culture of the isolated *Thiobacillus*, and the tubes were incubated for 5 days at 34°C, then the positive tubes were counted [20].

**Growth curve characteristics:** A growth curve was made for *Thiobacillus* isolate grown on a sterilized 200 ml of Starkey liquid medium dispensed in a 250 ml capacity Erlenmeyer flask (five replicate used with the same preparation medium). The medium was inoculated with 2 ml of 5 days-old culture of the strain, and the inoculated flasks were incubated for 5 days at 30-34°C. 10 ml samples of the developing cultures were taken periodically for optical density readings. The optical density was measured at wavelength 600 nm using Spectronic 20, Bauch and Lomb colorimeter [20].

The experimental design was factorial experiment with four factors arranged as split plots on the basis of Randomized Complete Block Design with four replications. Two levels of solarization (solarization and nonsolarization) were allocated to the main plots, and two levels of filter mud cake (with 2% filter mud cake and without filter mud cake) were assigned as subplots, two level of elemental sulfur (with 1 ton/ feddan, elemental sulfur and without elemental sulfur) were assigned as sub-subplots, and two levels of inoculation with sulfur-oxidizing bacteria (with inoculation and without inoculation) were assigned as sub-sub-subplots. Bacterial inoculums were prepared as Starkey's liquid inoculums contained 10<sup>9</sup> viable cells/ml, determined on plates

of Starke's agar medium). Supper phosphate fertilizer was added to the hole unites of all the experiments at a rate of 1 ton/feddan in pots and laboratory experiments and 500 kg/feddan at field experiment.

The clay soil had a clayey texture (48, 29 and 23% clay, silt and sand) with 2.5% CaCO<sub>3</sub>, 1.3 dS m<sup>-1</sup> EC (1:1), 7.6 pH (1:1 suspension) and 1.3% OM. Whereas the sandy soil had a sandy texture (3, 8 and 89% clay, silt and sand, respectively) with 15.2% CaCO<sub>3</sub>, 1.7 dS m<sup>-1</sup> EC (1:1), 8.3 pH (1:1 suspension) and 0.5% OM. The used Filter Mud Cake (FMC) contained 70.74% organic matter, pH (1:10 suspension) 6.75, EC (at 25°C and 1:10 extract) 4.00 ds/m, total phosphorus (P<sub>2</sub>O<sub>5</sub>) 1.78 %, total potassium (K<sub>2</sub>O) 0.47 %, total sodium (Na<sub>2</sub>O) 0.16 %, total calcium (CaO) 0.94 %, total magnesium (MgO) 0.12 %, total sulfur (S) 2.95 %, total Fe 169.6 ppm, total Mn 297.0 ppm, total Zn 259.3 ppm and total Cu 196.9 ppm.

Total soluble salts were measured in 1:1 soil extracts by the electric conductivity method (EC) [21]. Calcium and magnesium were titrated by versene [22], while sodium and potassium were measured by flamephotometere [21]. Organic matter content was determined using Walkely-Black method, following [22]. Soil pH was measured in a 1:1 (soil:water) suspensions using a glass electrode [21]. Available Phosphorus was determined calorimetrically using the chlorostanous phosphomolybdic acid method according to [21]. Available sulfur in soil samples was extracted using 500 ppm P of KH<sub>2</sub>PO<sub>4</sub>. Also, sulfur was determined in soil extracts and FMC digests by turbidity method. Soil texture was carried out using the pipette method [23].

## Results and Discussion

### Morphological, cultural characteristics and growth curve of isolated *Thiobacillus* strain:

Results of the tested morphological and cultural characteristics of *Thiobacillus* isolated strain show that cells shape were small, gram-negative, single rods, 0.5-4.0 µm in length and 0.5<sup>-1</sup> in diameter, produce no spores and non-motile. Tables 1 and 2, respectively, indicate some cultural characteristics and the physiological characteristic determined for the isolated *Thiobacillus* strain in comparison to known *Thiobacillus* species.

Cultural characteristics of the isolate *Thiobacillus* strain indicate that on agar thiosulfate+glucose medium the isolate produce circular, convex, yellow and viscid colonies 1-3 mm in diameter with entire edge. Whereas, in the thiosulfate agar medium without glucose the colonies color was white and its size was 0.5-0.7 mm in diameter but with the same other previous characteristics (in the thiosulfate+glucose agar medium) (Table 1).

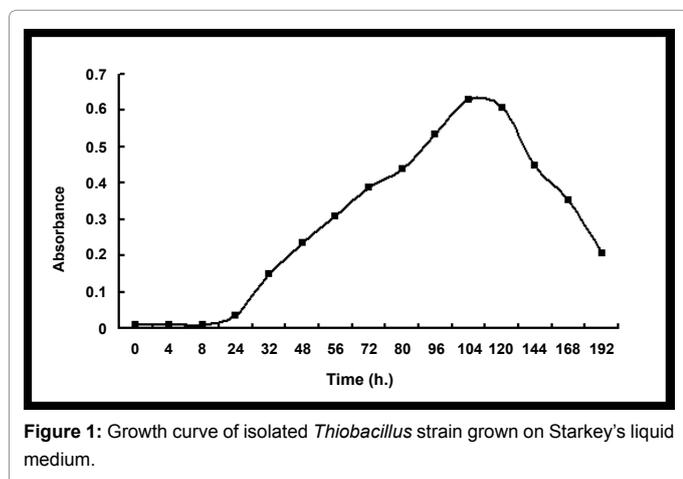
The physiological characteristics of the isolate *Thiobacillus* strain in comparison to known *Thiobacillus* species according to [24,25] indicate

Colony characteristic	Thiosulfate medium+glucose	Thiosulfate medium
Form	Circular	circular
Shape	convex	convex
Surface	smooth	smooth
Elevation	raised	raised
Size	1-3 mm	0.5-0.7 mm
Color	yellow	white
Consistency	viscoide	viscoide
Edge	entire	entire

**Table 1:** Cultural characteristic of isolated *Thiobacillus* strain on Thiosulfate medium with or without glucose.

Test	Isolated <i>Thiobacillus</i>	<i>T.nonellus</i>	<i>T.versutus</i>	<i>T.delicatus</i>	<i>T.thioparus</i>
Motility	-	-	+	-	+
Formation of surface pellicle of sulfur particle on thiosulfate medium	+	+	-	+	+
Growth as chemolithotroph	-	-	-	-	+
Anaerobic growth and denitrification with thiosulfate	-	-	-	+	-
Growth on nutrient agar	+	+	+	-	-
Growth on thiosulfate agar	+	+	+	+	+
Mixotrophic growth with thiosulfate +glucose	+	+	+	+	-
Mixotrophic growth with thiosulfate + yeast extract	+	+	-	+	-
Autotrophic growth with sulfur	-	-	-	+	+
Optimum temperature	30	25-30	30-35	30-35	28°C
Optimum Ph	7	7.5-7.9	7.5-7.9	5.5-6.0	6.5-7.2
Final pH when grown on thiosulfate medium	5	5.0-5.5	5.8-6.5	2.5-3.5	3.5-4.0
Proposed species	<i>Thiobacillus novellus</i>				

**Table 2:** Physiological Characteristic determined the isolated *Thiobacillus* strain in comparison to known *Thiobacillus* species (Bergey's 1989).



**Figure 1:** Growth curve of isolated *Thiobacillus* strain grown on Starkey's liquid medium.

that there are four species of *Thiobacillus* are too closely to our isolate. These species are *T. thioparus*, *T. delicatus*, *T. versutus*, *T. novellus*.

*T. thioparus* is strictly chemolithotroph and autotrophic bacteria. The lowest pH produced anaerobically in thiosulfate medium is 3.5-4.0. *T. delicatus* can grow anaerobically and the lowest pH produce in thiosulfate medium is 2.5-3.5. *T. versutus* had ability to motile by means of polar flagella and do not form surface pellicle of sulfur particles. The lowest pH produced in thiosulfate medium is 5.8-6.5. *T. novellus* can grow mixotrophically on thiosulfate+glucose medium, it is facultative chemolithotrophic bacteria, the final pH when grown on thiosulfate medium is 5.0-5.5, cannot grown anaerobically, nonmotile and it can form a pellicle of sulfur particles on thiosulfate medium. Though, according to these physiological characteristics we can say that the proposal species of the present isolate is *T. novellus* where the present isolate have the same characteristics of *Thiobacillus novellus* (Table 2).

The growth curve, drawn for *Thiobacillus* isolated strain in Starkey's liquid medium is shown in Figure 1. Optical density was measured, by Spectrophotometer where the turbidity is related to both the cell numbers and the sulfur particles that produce with the growth of bacteria. Therefore, both the numbers of cells and the amount of sulfur particles could be used as a function for growth of bacteria. A lag phase of 24 hrs was recorded for the isolated strain, after which cell numbers increased exponentially with the time. As indicated from the measurement of optical density of Starkey's liquid media, the maximum total growth for the strains occurred after 104 hrs from inoculation.

### Effect of solarization on soil temperature

Curves in Figure 2 show that the weekly mean of temperature in both covered soils were generally greater than those in uncovered soils with an average of 6 and 14°C at 8:00 a.m. and 4:00 p.m., respectively, overall the experimental period. After the fifth week, which always recorded the lowest temperatures, gradual increase in the temperature was recorded in all cases. The temperature was fluctuating due to the fluctuation of the ambient air temperature. These findings are in accordance with those reported by [24,25].

### Effect of solarization, FMC and S application, and inoculation with sulfur-oxidizing bacteria on some soil properties

**Main effect:** Data in Tables 3 and 4 summarize the main effects of solarization, application of Filter Mud Cake (FMC), elemental sulfur (S) and inoculation with Sulfur-Oxidizing Bacteria (SOB) on some properties of clay and sandy soils, respectively. Data show the noticeable significant effects of solarization, S and FMC addition on most of the discussed soil parameters. Data indicate that solarization of clay soil caused a highly significant reduction in soil organic matter percentage by about 23% and 30% in clay and sandy soils, respectively, compared to that of nonsolarized treatments. This was due to the increase of soil temperature which was higher in sandy soil than in clay soil and resulted in rapid decomposition of organic matter. The most obvious result was the significant increase in soil organic matter content by about 73% (from 1.21% to 2.09%) and 213% (from 0.3% to 0.94%) in clay (Table 3) and sandy soil (Table 4), respectively, received FMC over the untreated soil. While the addition of sulfur decreased OM content in sandy soil by 10.6%, it had no effect in clay soil. The inoculation with SOB almost had no effect on soil OM decomposition in both soils.

Data also indicate that solarization, S and FMC addition significantly increased the EC in both soils. The increase of EC in clay soil was 16, 55 and 33% while in sandy soil it was 20, 29 and 45% for solarization, S and FMC treatments over the untreated soil, respectively.

Soil pH was significantly decreased by all the discussed treatments and in both soils as appear in Tables 3 and 4. All treatments resulted in a highly significant increase in extractable S having the highest increase in soil treated with elemental S (75% and 78% increase in clay and sandy soil, respectively, compared to the untreated soil). While solarization, FMC and SOB increased the soil S by 24, 45 and 24% in clay soil and 39, 60 and 30% in sandy soil, respectively, compared with the untreated soils.

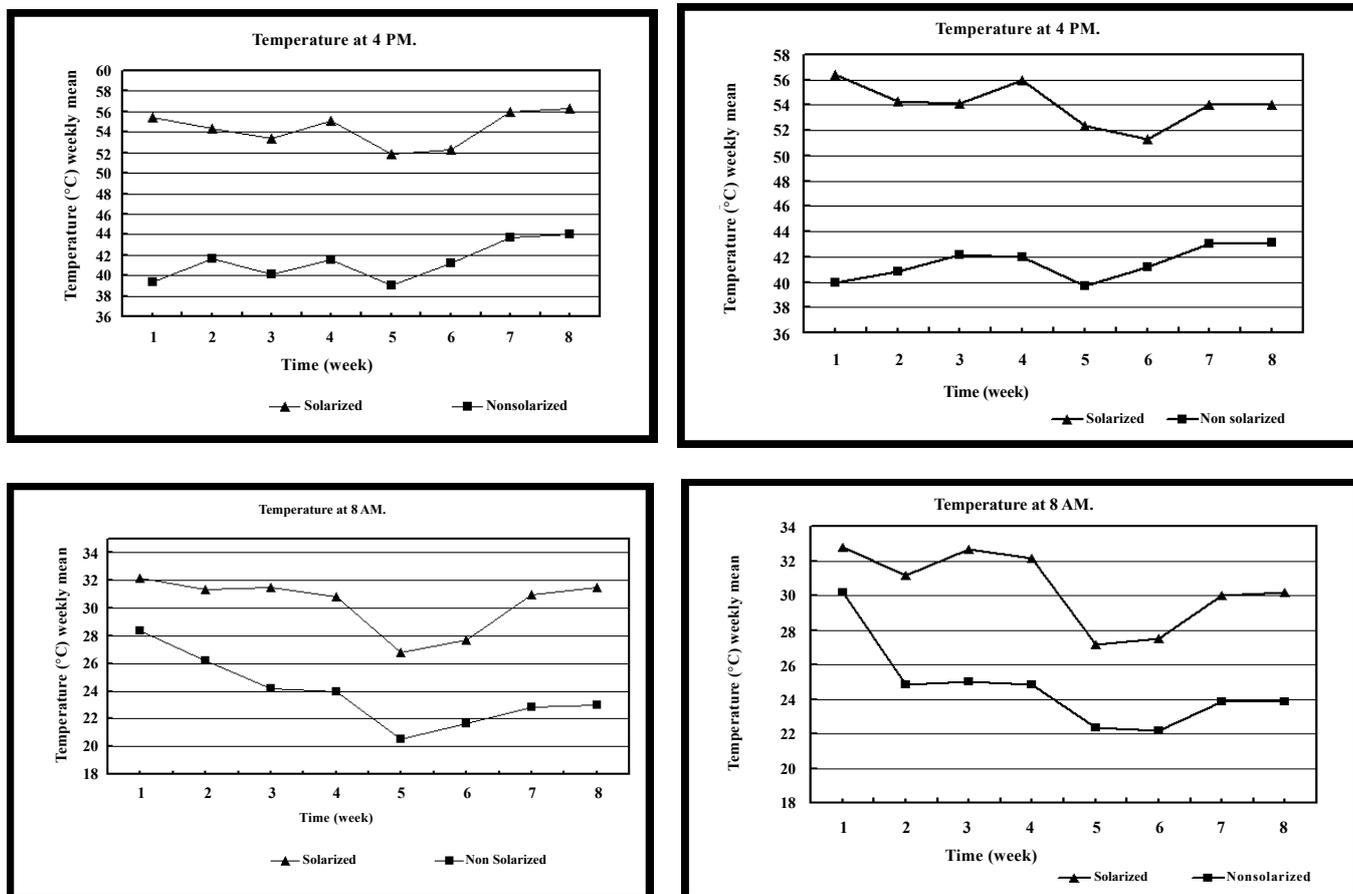


Figure 2: Soil temperature in solarized and nonsolarized clay and sandy soils (at 8:00 a.m. and 4:00 p.m.).

Treatment	OM (%)	EC (ds/cm)	pH	Available S (ppm)	Available P (ppm)	Soluble cations (meq/100 g soil)		
						Ca <sup>2+</sup> +Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
NonSolarized	1.86	9.47	7.69	531	69.4	13.65	1.39	0.038
Solarized	1.44**	10.95*	7.61**	656**	90.88**	18.63**	2.01**	0.048**
- FMC	1.21	8.76	7.66	485	64.43	12.72	1.71	0.037
+FMC	2.09**	11.66	7.63*	702**	95.86**	19.56**	1.69	0.049**
-S	1.68	8	7.72	431	75.5	9.77	1.58	0.039
+S	1.62	12.42**	7.58**	756**	84.78**	22.51**	1.82**	0.047**
Umino	1.59	10.02	7.67	530	72.26	16	1.63	0.041
Ino	1.72	10.39	7.63**	657**	88.02**	16.28	1.77**	0.045

FMC=Filter Mud Cake, S=granuled elemental sulfur, Ino=inoculation with Sulfur-Oxidizing Bacteria (SOB), EC=ds/m at 25°C 1:1 soil extract, pH=1:1 soil suspension, \* =significant (P<0.05) \*\* =high significant (p<0.01).

Table 3: Main effects of solarization, FMC and S addition, and inoculation with SOB on some properties of clay soil.

Phosphorus availability was significantly increased by all treatments. The most effective treatment was FMC which increased available P to reach 95.86 and 81.61 ppm with an increase of 49 and 72% in clay and sandy soils, respectively. Application of sulfur and inoculation with S-oxidizing bacteria caused a noticeable effect on NaHCO<sub>3</sub>-extractable phosphorus but the amount of available P in these treatments were less than the amount found with FMC and solarization treatments in both soils. Soluble Ca<sup>2+</sup> + Mg<sup>2+</sup> were significantly increased by solarization, FMC and S addition in both soils as shown in Tables 3 and 4 where it reached 22.51 meq/100 g soil with S addition to clay soil and 6.48 meq/100 g with FMC addition to sandy soil. While the inoculation with SOB had no significant effects in both soils. Increases in soluble

mineral nutrients including NH<sub>4</sub>, NO<sub>3</sub>, Phosphorus, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>3+</sup>, Cl<sup>-</sup> and Cu<sup>2+</sup> have been detected in solarized soils in several studies [26] although sometimes inconsistently [27-29], especially for the minor elements [4,30].

Soil solarization was the most effective treatment in increasing soluble Na<sup>+</sup> in both soils, where it reached 2.01 and 5.05 meq/100 g in clay and sandy soils, respectively. Soluble K<sup>+</sup> was significantly increased in both soils under all treatments except SOB inoculation with almost the same value.

#### Interaction Effects

**Clay soil:** The interaction effects of solarization, application of

Treatment	OM (%)	EC (ds/cm)	pH (1:1)	Available S (ppm)	Available P (ppm)	Soluble cations (meq/100 g soil)		
						Ca <sup>2+</sup> +Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
No solarized	0.73	5.91	7.49	503	56.54	5.08	2.73	0.187
Solarized	0.51**	7.12**	7.32**	700**	72.56**	6.38**	5.05**	0.281*
-FMC	0.3	5.25	7.5	463	47.48	4.99	4.45	0.192
+FMC	0.94**	7.63**	7.31**	740**	81.61**	6.48**	3.33**	0.276**
- S	0.66	5.62	7.5	433	57.69	5.16	3.46	0.217
+S	0.59**	7.25**	7.32**	770**	71.41**	6.31**	4.32**	0.251*
Unino	0.62	5.76	7.41	524	57.86	5.7	3.78	0.227
Ino	0.62	6.96**	7.4	680**	71.23**	5.76	4.01	0.241

**Table 4:** Main effects of solarization, FMC and S application, and inoculation with SOB on some properties of sandy soil. FMC=Filter Mud Cake, S=granuled elemental sulfur, Ino=inoculation with sulfur-oxidizing bacteria (SOB), EC=ds/m at 25°C 1:1 soil extract, pH=1:1 soil suspension, \*=significant (P<0.05) \*\*=high significant (p<0.01).

Treatment	OM (%)	EC (ds/cm)	pH 1:1	Available S (ppm)	Available P (ppm)	Soluble cations (meq/100 g soil)			
						Ca <sup>2+</sup> +Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	
Nonsolarizad	-FMC	1.3	7.83	7.72	404	54.67	11.15	1.4	0.033
	+FMC	2.41	11.1	7.66	659	84.13	16.14	1.37	0.043
solarized	-FMC	1.13	9.69	7.6	566	74.18	14.29	2.02	0.041
	+ FMC	1.76	12.21	7.61	745	107.58	22.98	2.01	0.053
LSD 1%	0.17	0.47	0.04	44	NS	0.79	NS	NS	
Nonsolarizad	- S	1.9	8.68	7.76	430	66.81	9.24	1.26	0.036
	+S	1.81	10.25	7.62	633	71.99	18.06	1.51	0.041
solarized	- S	1.34	7.31	7.68	432	84.2	10.3	1.9	0.042
	+S	1.55	14.59	7.53	880	97.57	26.96	2.13	0.052
LSD 1%	NS	NS	NS	37	4.07	0.68	NS	NS	
Nonsolarizad	Unino	1.69	8.94	7.7	468	61.66	13.67	1.35	0.036
	Ino	2.02	9.99	7.67	595	77.14	13.63	1.43	0.04
solarized	Unino	1.48	11.11	7.63	593	82.87	18.34	1.91	0.046
	Ino	1.41	10.8	7.58	719	98.9	18.93	2.12	0.048
LSD 1%	NS	0.26	NS	NS	NS	NS	NS	NS	

**Table 5:** The interaction effects of solarization, FMC and S addition, and inoculation with SOB on some soil properties, clay soil. FMC=filter mud cake, S=granuled elemental sulfur, Ino=inoculation with sulfur-oxidizing bacteria (SOB), EC=ds/m at 25°C 1:1 soil extract, pH=1:1 soil suspension.

Treatment	OM (%)	EC (ds/cm)	pH 1:1	Available S (ppm)	Available P (ppm)	Soluble cations (meq/100 g soil)			
						Ca <sup>2+</sup> +Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	
-FMC	-S	1.17	6.3	7.72	333	56.68	6.13	1.5	0.034
	+S	1.26	11.22	7.6	637	72.18	19.31	1.92	0.04
+FMC	- S	2.11	9.69	7.71	529	94.33	13.41	1.66	0.043
	+S	2.07	13.63	7.55	876	97.38	25.71	1.72	0.053
LSD 1%	NS	0.56	0.03	37	4.07	NS	0.14	NS	
-FMC	Unino	1.16	8.63	7.66	398	58.74	12.54	1.63	0.037
	Ino	1.27	8.89	7.66	572	70.11	12.9	1.79	0.038
+FMC	Unino	2.01	11.42	7.67	662	85.78	19.47	1.64	0.045
	Ino	2.16	11.89	7.59	742	105.93	19.65	1.75	0.051
LSD 1%	NS	0.26	0.04	25	3.26	NS	NS	NS	
-S	Unino	1.57	7.9	7.74	361	66.33	9.56	1.48	0.039
	Ino	1.67	8.09	7.69	501	84.68	9.98	1.68	0.039
+S	Unino	1.6	12.15	7.59	700	78.2	22.45	1.78	0.043
	Ino	1.76	12.69	7.56	813	91.36	22.57	1.86	0.049
LSD 1%	NS	NS	NS	NS	3.26	NS	NS	NS	

**Table 6:** The interaction effects of application of FMC, elemental sulfur, and inoculation with SOB on some properties of clay soil. FMC=filter mud cake, S=granuled elemental sulfur, Ino=inoculation with sulfur-oxidizing bacteria (SOB), EC=ds/m at 25°C 1:1 soil extract, pH=1:1 soil suspension.

FMC and elemental sulfur, and inoculation with SOB on some clay soil properties is shown in Tables 5 and 6. Although the application of FMC to solarized soil caused a significant effect on increasing soil organic matter percentage up to 1.76%, the increase of OM percentage was much higher by 63% in the nonsolarized soil received the same FMC rate Table 5. Which indicate that solarization decreases the effect of OM addition. Though, solarized soil contained higher EC than the nonsolarized ones, the addition of FMC to any of the soils resulted in more increase in the EC. Which lead to the result that the highest

EC value was found when solarization was combined with FMC addition to the soil. Soil pH was significantly decreased as a result of the interaction between solarization and addition of FMC compared with the unsolarized soil without FMC addition. Available sulfates and phosphate as well as soluble Ca<sup>2+</sup>+Mg<sup>2+</sup> in the soil recorded the highest values in solarized soil amended with FMC.

Solarized soil received elemental sulfur contained less O.M in comparison with nonsolarized soil treated with elemental sulfur, while

Treatment		OM (%)	EC (ds/cm)	pH	Available S (ppm)	Available P (ppm)	Soluble cation (meq/100 g soil)		
							Ca <sup>2+</sup> +Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
NoSolarized	-FMC	0.38	4.55	7.59	373	27.79	4.34	3.22	0.153
	+FMC	1.08	7.28	7.4	633	85.3	5.81	2.24	0.221
Solarized	-FMC	0.22	5.94	7.41	553	67.18	5.63	5.69	0.232
	+FMC	0.81	7.98	7.22	848	77.93	7.14	4.42	0.331
LSD 1%		NS	0.12	NS	15	2.71	NS	NS	NS
NoSolarized.	-S	0.76	4.97	7.51	295	53.53	4.38	2.1	0.17
	+S	0.71	6.85	7.48	711	59.55	5.78	3.37	0.203
Solarized	-S	0.56	6.27	7.48	572	61.85	5.93	4.83	0.265
	+S	0.45	7.66	7.16	829	83.26	6.84	5.28	0.298
LSD 1%		NS	0.19	0.11	11	3.01	0.3	NS	NS
NoSolarized	Unino	0.77	5.11	7.51	453	52.47	5.04	2.7	0.176
	Ino	0.7	6.71	7.48	553	60.61	5.12	2.76	0.197
Solarized	Unino	0.48	6.4	7.32	595	63.26	6.36	4.86	0.278
	Ino	0.54	7.53	7.32	806	81.85	6.41	5.25	0.284
LSD 1%		NS	0.14	NS	11	3.11	NS	NS	NS

**Table 7:** The interaction effects of solarization, FMC and S application, and inoculation with SOB on some properties of sandy soil. FMC=Filter Mud Cake, S=granuled elemental sulfur, Ino=inoculation with sulfur-oxidizing bacteria (SOB), EC=ds/m at 25°C 1:1 soil extract, pH=1:1 soil suspension.

Treatment		OM (%)	EC (ds/m)	PH	Available S (ppm)	Available P (ppm)	Soluble cation (meq/100 g soil)		
							Ca <sup>2+</sup> +Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>
-FMC	-S	0.34	4.48	7.54	322	41.9	4.02	3.84	0.187
	+S	0.26	6.01	7.47	604	53.07	6.01	5.07	0.197
+FMC	-S	0.98	6.76	7.45	545	73.48	6.15	3.09	0.247
	+S	0.91	8.51	7.17	936	89.75	6.86	3.58	0.304
LSD 1%		NS	NS	0.11	11	3.01	0.33	NS	NS
-FMC	Unino	0.31	4.47	7.48	377	42.13	4.65	4.2	0.19
	Ino	0.29	6.02	7.52	549	52.84	5.38	4.71	0.194
+FMC	Unino	0.93	7.04	7.34	670	73.6	5.92	3.36	0.264
	Ino	0.95	8.22	7.28	810	89.63	7.09	3.31	0.287
LSD 1%		NS	0.14	NS	11	3.11	0.35	NS	NS
-S	Unino	0.67	5.12	7.5	363	51.12	4.41	3.41	0.215
	Ino	0.64	6.12	7.49	504	64.26	5.76	3.51	0.22
+S	Unino	0.57	6.39	7.33	685	64.61	6.16	4.15	0.239
	Ino	0.6	8.12	7.31	855	78.21	6.71	4.5	0.262
LSD 1%		NS	0.14	NS	11	NS	NS	NS	NS

**Table 8:** The interaction effects of application of FMC, S, and inoculation with SOB on some sandy soil properties. FMC=Filter Mud Cake, S=granuled elemental sulfur, Ino=inoculation with Sulfur-Oxidizing Bacteria (SOB), EC=ds/m at 25°C 1:1 soil extract, pH=1:1 soil suspension.

it contained more OM when compared with solarized soil without S addition. Mulching soil surface with clear plastic sheets resulted in significant increase in EC, available sulfates, available phosphates and soluble cations (Ca<sup>2+</sup>+Mg<sup>2+</sup>, Na<sup>+</sup> and K<sup>+</sup>) in soil treated with elemental sulfur compared with the soils unsolarized or solarized. Which means that solarization enhanced the effect of S on decreasing the pH and thus increasing the soluble and extractable nutrients.

Addition of FMC to the soil received elemental sulfur led to great increase in soil EC, available sulfates, phosphates, and soluble Ca<sup>2+</sup>+Mg<sup>2+</sup> compared to soil received no FMC with or without sulfur addition (Table 6). The addition of S with or without FMC also increased these values. These facts lead to the enhancement effect of both FMC and S on each other when added together. On the other hand, OM increased by the addition of FMC but not S addition, which led to no significant effect of S on soils with or without FMC addition. The same effect was found in the soils treated with or without FMC and SOB. These results are in agreement with those reported by [31,32].

As in all S treatments, pH always decrease with the addition of S, and this decrease become even more with the addition of FMC. The same effect on pH values were found with SOB treatments combined

with either FMC or S treatments (Table 6). Inoculation with SOB, either with FMC or S treatments, had significant effect on increasing of S, P, Na<sup>+</sup> content and accordingly EC too. This is because of the effect of these bacteria on oxidizing S in soil, which causes the increase of EC. That effect of SOB was enhanced by the application of either FMC or S. On the other hand, the increase in OM, Ca<sup>2+</sup>+Mg<sup>2+</sup> and K<sup>+</sup> content were mainly referred to the addition of FMC and not SOB. While S addition accompanied with SOB resulted in a significant increase in Ca<sup>2+</sup>+Mg<sup>2+</sup> and K<sup>+</sup> content.

**Sandy soil:** Data in Table 7 represent the interaction effects of solarization accompanied with applications of either FMC or elemental sulfur or inoculation with SOB on some properties of sandy soil in pot experiment. Solarization of soil amended with FMC for 8 weeks resulted in significant reduction in soil organic matter content compared with the nonsolarized soil. The same treatment exhibited the highest significant increase in EC values and the lowest value of pH, in comparison with all other treatments. This may be due to the fact that solarization of soil was accompanied with the acceleration of organic matter decomposition which resulted in release of organic acids which in turn reduce soil pH (or increase soil acidity) and increases the solubility of salts.

Data in Table 7 also indicate that both extractable sulfates and soluble cation were increased significantly by solarization of soil with and without FMC treatments. The increase was also found with FMC treatment with or without solarization except for  $\text{Na}^+$  which decreased, but not significantly, with the addition of FMC. Solarization of soil without FMC treatment caused obvious increase in extractable P, the same effect occurred due to the addition of FMC either with or without solarization. However, solarizing the soil treated with FMC decreased the amount of extractable P, which matches the percentage of OM [33].

Solarization of soil always had the same significant effect on decreasing organic matter and pH while increasing EC, S, P and soluble cations when it was combined with either S or SOB treatments. While S addition had highly significant effect on decreasing the pH and on increasing the EC, extractable S, extractable P and soluble cations either with or without solarization. On the other hand, SOB had significant effect on increasing extractable S, P and accordingly EC.

Data in Table 8 summarized the interaction effects of the application of FMC and elemental sulfur, and inoculation with SOB of sandy soil in pot experiment on some soil prosperities. Addition of FMC at a rate of 2% to the sandy soil amended or not amended with sulfur significantly increased OM percentage in the soil compared to those found in soil received no FMC or elemental sulfur. Similar trend was found in regard to Electrical Conductivity (EC). Addition of FMC alongside with sulfur to the soil led to obvious decrease in soil pH from 7.45 to 7.17 (Table 8), this mean that adding elemental sulfur to the soil may be the main reason of reducing soil pH rather than adding FMC. Application of FMC to sulfur amended or un-amended soil led to highly significant increases in both available sulfates and phosphate. Similarly, the concentration of  $\text{Ca}^{+2}+\text{Mg}^{+2}$  and  $\text{K}^+$  in soil extract were greater in soil amended with FMC and sulfur than the un-amended one.

Application of FMC to inoculated or uninoculated soil increased OM percentage and EC in soil. It was noticeable that the application of FMC to inoculated soil scored the lowest value of soil pH. Both available sulfates and phosphates values were greater in soil amended with FMC and inoculated with SOB (Table 8). Soluble  $\text{Ca}^{+2}+\text{Mg}^{+2}$  and  $\text{K}^+$  concentrations reached their highest values in FMC amended soil inoculated with SOB [34].

Though the effect of FMC addition in the presents of either S or SOB, was very clear on decreasing pH and increasing OM, EC, S, P,  $\text{K}^+$  and  $\text{Ca}^{+2}+\text{Mg}^{+2}$ , the effect of either S or SOB was found on increasing EC, S, P,  $\text{K}^+$ , and  $\text{Ca}^{+2}+\text{Mg}^{+2}$  but not on OM. Which indicate that the maximum values found for these parameters were because of the interaction effect between FMC either with S or with SOB. On the contrary, both FMC and S decreased soil pH and thus, their interaction had the highest effect on decreasing pH value to reach 7.17 while it reached 7.28 with the addition of FMC+ SOB.

The interaction between S addition and SOB inoculation resulted in significant increase of EC, S and  $\text{Ca}^{+2}+\text{Mg}^{+2}$  which made the values of these parameters were the highest among the other treatments. On the other hand, the significant increase in  $\text{Na}^+$  and the decrease in pH may be attributed only to S addition and not to SOB inoculation as shown in Table 8.

## Conclusion

Increasing the soil temperature due to solarization resulted in a reduction in OM percentage, while the addition of S had less effect and the incubation of SOB almost had no effect. Both FMC and S addition

had great effects on increasing soil EC compared to the increase that resulted from either solarization or SOB inoculation. The effect of elemental sulfur addition on decreasing soil pH was higher than the other treatments in all clay experiments, while FMC addition was the most effective treatment in sandy soil experiments. Each of the treatments increased the available P in all experiments; however the most effective treatment was FMC addition. Soluble  $\text{Ca}^{+2}+\text{Mg}^{+2}$  and  $\text{K}^+$  were always increasing due to each of the treatments. The highest increase in soluble  $\text{Na}^+$  was due to increasing soil temperature either by solarization or simulation compared to the other treatments [35].

## References

1. Katan J and DeVay JE (1991) Soil Solarization. Boca Raton: CRC Press.
2. Thuriés L, Larre-Larrouy MC, Feller C (2000) Influences of organic fertilization and Solarization in a greenhouse on particle size fractions of a Mediterranean sandy soil. Biol Fertile soils 32: 449-457.
3. Zak DR, Homes WE, Mac Donald NW, Pregitzer KS (1999) Soil temperature, matric potential and the kinetics of microbial respiration and nitrogen mineralization. Soil Sci Soc Am J 63: 575-584.
4. Grunzweig JM, Katan J, Ben-Tal Y, Rabinowitch HD (1999) The role of mineral nutrients in the increased growth response of tomato plants in solarized soil. Plant and Soil 206: 21- 27.
5. Ahmed Y, Hameed A, Eslam M (1996) Effect of soil solarization on corn stalk rot. Plant and soil 179: 17-24.
6. Wainwright M (1984) Sulfur oxidation in soils. Adv Agron 37: 349-396.
7. García de la Fuente R, Carrión C, Botella S, Fornes F, Noguera V, et al. (2007) Biological oxidation of elemental sulfur added to three composts from different feedstocks to reduce their pH for horticultural purposes. Bioresour Technol 98: 3561-3569
8. Wainwright M and Killham K (1980) Sulfur oxidation by fusarium solani. Soil Biol Biochem 12: 555-558.
9. Sholeh RD, Lefroy B, Blair GJ (1997) Effect of nutrients and elemental sulfur partial size on elemental sulfur oxidation and the growth of thiobacillus thiooxidans. Aust J Agric Res 48: 497-501.
10. Laisheley EJ, Bryant RD, Kobryn BW, Hyne JB (1986) Microcrystalline structure and surface area of elemental sulfur as factors influencing its oxidation by thiobacilluse albertis. Can J Microbiol 32: 237-242.
11. Lindemann WC, Alburto JJ, Haffner WM, Bono AA (1991) Effect of sulfur source on sulfur oxidation. Soil Sci Soc Am J 55: 85-90.
12. Nor YM and Tabatabai MA (1977) Oxidation of elemental sulfur in soils. Soil Sci Soc Am J 41: 736-741.
13. Cifuentes FR, Lindeman WC (1993) Organic matter stimulation of elemental sulfur oxidation on a calcareous soil. Soil Sci Soc Am J 57: 727-731.
14. Slaton NA, Norman RJ, Gilmour JT (2001) Oxidation rates of commercial elemental sulfur products applied to an alkaline silt loam from Arkansas. Soil Sci Soc Am J 65: 239-243.
15. Modaihsh AS, Al-Mostafa WA, Metwally AI (1989) Effect of elemental sulfur on chemical changes and nutrient availability in calcareous soils. Plant and soil 116: 95-101.
16. Garcia ME, Carloni L (1977) The effect of sulfur on the solubility and forms of phosphorus in soil. Agrochimica 21: 163-169.
17. Gubta VR, Mehla IS (1980) Influence of sulfur on the yield and concentration of Cu, Mn, Fe, and Mo in bersseem (Trifolium alexandrium) grown on two different soils. Plant and soil 56: 229-234.
18. Chen Y, Katan J, Gamliel A, Aviad T (2000) Involvement of soluble organic matter in increases plant growth in solarized soils. Biol Fert Soil 32: 28-34.
19. Starkey RL (1935) Isolation of some bacteria which oxidize thiosulfate. Soil Sci 39: 197-219.
20. Cappuccino JT, Sherman N (1996) Microbiology, Laboratory Manual. 4th ed. pp: 115-128 USA.
21. Jackson ML (1973) Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs. NJ, USA.

22. Baruah TC, Barthakur HP (1997) *A Textbook of soil analysis*. Vikas Publishing House PVT LTD, New Delhi, India.
23. Piper CS (1950) *Soil and Plant Analysis*. 1st. ed. Intrescience Public., New York
24. Streck NA, Schneider FM, Buriol GA (1996) Soil heating by solarization inside plastic greenhouse in Santa Maria, Rio Grande do Sule. *Brazil Agricultural and forest Meteorology* 82: 73-82
25. Gelsomino A, Cacco G (2006) Compositional shifts of bacterial groups in a solarized and amended soil as determined by denaturing gradient gel electrophoresis. *Soil Biology and Biochemistry*. 38: 91-102.
26. Chen Y, Gamlie A, Stapleton JJ, Aviad T (1991) Chemical, physical, and microbial changes related to plant growth in disinfested soils, in *Soil solarization* (J. Katan and J. E. DeVay, Eds.). CRC Press, Boca Raton, Florida. 103-129.
27. Daelemans A (1989) Soil solarization in West-Cameroon: effect on weed control, some chemical properties and pathogens of the soil, in *III international symposium on soil disinfestations* (Vanachter, A., Ed.). *Acta Horticulturae* 255:169-175.
28. Moura MLR and Palminha J (1994) A non-chemical method for the control of *Pyrenochaeta lycopersici* of tomato in the north of Portugal, in *II Symposium on protected cultivation of solanacea in mild winter climates*, Adana, Turkey (Cockshull, K.E., and Y. Tüzel). *Acta Horticulturae* 366: 317-322.
29. Coates-Beckford PL, Cohen JE, Ogle LR, Prendergast CH, Riley DM (1998) Mulching soil to increase yield and manage plant parasitic nematodes in cucumber (*Cucumis sativus* L.) fields: influence of season and plant thickness. *Nematropica* 28: 81-93.
30. Stapleton JJ (1998) Modes of action of solarization and biofumigation, in *Soil solarization and integrated management of soil pests: proceedings of the second conference on soil solarization*, Aleppo, Syria (Stapleton, J. J., J. E. DeVay, and C. L. Elmore, Eds.). *FAO plant production and protection paper* 147: 78-88.
31. Curtin D, Beare MH, McCallum FM (2007) Sulfur in soil and light fraction organic matter as influenced by long-term application of super phosphate. *Soil Biology & Biochemistry* 39: 2547-2554.
32. *Bergey's (1989) Manual of Systematic Bacteriology*. ed .N .R. Kriey and J. G. Holt. vol. I, pp. 1842-1858. Williams and Wilkins , Baltimore, london.
33. Jackson ML (1967) *Soil chemical analysis*. Prentice-Hall, Inc., Englewood Cliffs, New Jersey, U.S.A.
34. Lawrence JR, Germida JJ (1991) Enumeration of sulfur-oxidizing-populations in Saskatchewan agriculture soils. *Can J Soil Sci* 71: 127-136.
35. Page AL, Miller RH, Keeney DR (1984) *Methods of Soil Analysis. Part2- Chemical and Microbiological Properties*. 2ed., USA. pp: 815-830.

This article was originally published in a special issue, **Bacteria: Biochemical Physiology** handled by Editor. Prof. Cheorl-Ho KIM, Sungkyunkwan University, Korea