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Effect of Applied Bio-fertilizers, Seaweed Extract and Elemental Sulphur on Productivity of Sunflower Grown in Newly Reclaimed Slightly Saline Soil

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A FIELD experiment was conducted during two successive seasons on a slightly saline sandy clay loam soil calcareous in nature at a newly reclaimed area comprising the desert zone of El-Fayoum district, El-Fayoum Governorate, Egypt, located between latitude 29° 21′ 48″ N longitude 30° 44′ 45″ E. The main target of this study was to identify the positive effects of applied elemental sulphur (*i.e*, at the rates of 0, 200, 350 and 450 kg fed^{-1}, as soil application), seed bio-inoculation (*i.e.,* P-dissolving bacteria of *Bacillus megatherium* var. *phosphaticum*) and seaweed extract (at the rate 1.0 kg fed^{-1} with 400 liter water as foliar application) either solely or combined treatments on sunflower (*Helianthus annuns,* c.v. Hysun 336). Growth criteria, *i.e.,* plant height, number of leaves/plant as well as dry matter weight/plant and leaf content of total chlorophyll were determined after 50 days of sowing. At harvest yield and yield components as head diameter, 100 seeds weight, seeds weight fed^{-1}, seed oil, protein and nutrient contents were also determined. Some soil properties, *i.e.*, soil pH, ECe, ESP and available macro and micronutrient contents were also takes into consideration. Samples of the plants rhizosphere were collected after 50 and 90 days of sowing to determine CO_2 evolution, dehydrogenases activity and total bacterial counts.

Data showed a clear response for studied soil properties as well as soil nutritional status as a result of the applied treatments, particularly those treated with the highest rate of elemental sulphur in combination with bio-fertilizer and foliar seaweed extract. The best and achieved greatest values of growth and nutritional status of plants, particularly at early flowering stage were associated with plants subjected to the combined treatments compared to the other solely ones, which positively reflected on seed yield and its quality (oil and protein content). In general, the values of plant parameters were optimized, for bio-inoculated, with increasing the applied elemental sulphur rate 350 or 450 kg fed^{-1}, with insignificant difference.

The results also, showed that inoculation with P-dissolving bacteria in presence of different elemental sulphur levels combined with foliar seaweed extract encouraged the total bacterial counts, CO_2 evolution and dehydrogenase activity at 50 and 90 days.

So that, it could be recommended that elemental sulphur, seed bio-inoculation with P-dissolving bacteria and seaweed extract as foliar application may be used to alleviate the hazardous effects of saline soil calcareous in nature, on sunflower seed yield and its quality.

Keywords: Alkaline soil, Elemental sulphur, Bio-fertilizer, P-dissolving bacteria and sunflower plant .

Sunflower (*Helianthus annuus* L.) is an important oil seed crop worldwide, and it is an important crop in Mediterranean areas where salinity is an increasing problem (Di *et al*., 2007). Sunflower is moderately sensitive to soil salinity; the promotion of sunflower could be successful to increase the domestic production provided proper cultivars are available which are suitable to different soil and climatic conditions (Khatoon *et al*., 2000). Egyptian desert soils calcareous in nature are generally characterized by alkaline condition, and then available nutrient contents are surveyed as low levels. So, soil fertilization management practices are ones of the most important agro-management factors that affect the yield and its components of the different crops, especially those grown on the newly reclaimed desert soils (Patel and Shelke, 1998).

Sunflower has a relatively high nutrient requirements and most soils which the crop is grown are deficient in one or more nutrients for optimum seed yield, oil and protein content. Phosphorus is needed during the earliest stage of plant growth. Any P deficiency during early growth can great reduce yield potential of tops and seeds. Therefore, the amount of P in sunflower seeds can be important to help seedling establishment and in determining final seed yields (Bolland, 1997).

Many solutions were executed to reduce the previously mentioned problems, out of them using bio-fertilization with P-dissolving bacteria and the application of elemental sulphur to the soil. The later is emphasized by the statement of P availability is a function of either soil pH or $CaCO_3$ content, and the application of sulphur increased the nutrients availability, particularly P and micronutrients in the soil .These findings are in harmony by those outlined by Azer *et al*. (2003) who reported a significant response in seed yield, crude protein and P contents.

Sulphur is the fourth major nutrient in crop production. Elemental S has been used for many years in the reclamation and improvement of sodic and calcareous soils (Wassif *et al*., 1993). More attention has been given to sulphur application to soils due to its favorable effects in promoting nutrient availability in soils (Saleh, 2001). Application of sulphur as amendment for alkaline and/or calcareous soils has received little attention, as an inhibitor for ammonia volatilization (Abdou *et al.*, 2011). The nutrient availability of soils can be increased with the application of S. Thus, there is a growing interest in S applications to improve availability of nutrients and overcome nutrient deficiencies in both alkaline and calcareous soils (Neilsen *et al*., 1993). Because of the high cost and adverse effects of commercial fertilizers especially N, P and K fertilizers, use of natural sources, micronutrients and S may be used as a nutrient and soil acidifier and S fertilizer has recently gained importance in agricultural production (Atilgan *et al*., 2008). An individual application of S or combined with Fe dropped soil pH in calcareous soil (Abbaspour *et al*., 2004) but application of S combined with N fertilizer significantly increased the availability of micronutrients (Erdal *et al*., 2004). Sulfur is a constituent of the amino acids cysteine and methionine and part of proteins that plays an important role in the synthesis of vitamins and chlorophyll in the cell (Marschner, 1995 and Kacar & Katkat, 2007). As a result of S deficiency, plants show retarded growth (Motior *et al*., 2011) and reduction in yield and quality. The efficacy of S to satisfy the S demand of crops depends, however, on speed and magnitude of its oxidation to H_2SO_4 which is taken up by plants (Yang *et al*., 2010 and Abdou *et al.,* 2011).

The microbial strain such as *B.megatherium* is one of the most important agents to sustain P availability in the treated soil. These strain agents are the primary substances controlling the enhanced plant growth, absorption nutrients and photosynthesis process (Makovacki and Milic, 2001). Moreover, seeds inoculation with bio-fertilizer is economically important as it resulted in reducing the needs of N and P fertilizers and improving the crop yield. Azzam and Omran (2005) found that bio-fertilization improved plant growth characters and seed yield of sunflower plants.

Seaweed extract contained macro nutrients, trace elements, organic substances like amino acids and plant growth regulators such as auxin, cytokinin and gibberellins. They are particularly suitable content (Chapman and Chapman, 1980). Verkleij (1992) stated that application of seaweed extract enhanced the water retention capacity of soil. Seaweed extracts are known to enhance seed germination and plant growth (Thirumaran *et al*., 2009). They have been also shown to increase crop yield, improve growth and induce resistance to frost, fungal and insect attack and increase nutrient uptake from soil. The beneficial effect of seaweed extract on terrestrial plants includes improving the overall growth, yield and the ability to with stand adverse environmental conditions (Asirselvin *et al*., 2004). Abdel-Mawgoud *et al.* (2010) seaweeds extract was extracts obtained from seaweeds have gained importance as foliar sprays for several crops because the extract contains growth promoting hormones (cytokinins), micronutrients (Fe, Cu, Zn, Co, Mo, Mn and Ni), vitamins and amino acids.

The current work aimed to evaluate the integrated effect of bio-fertilization with *Bacillus megatherium*(PDB) potential and elemental sulphur and foliar application of seaweed extract on properties of nutritional status of a newly reclaimed soil calcareous in nature besides the effect of applied treatments on yield and yield components of sunflower.

Material and Methods

To achieve the aforementioned target, a field experiment was conducted during two successive growing seasons on a slightly saline-sodic sandy clay loam soil calcareous in nature at a newly reclaimed area comprising the desert zone of El-Fayoum Governorate, Egypt, located between latitude 29° 21′ 48″ N and longitude 30° 44′ 45″E.The main physic-chemical properties and nutritional status of the soil are presented in Table 1 (Page *et al.*, 1982).

TABLE 1. Some physical and chemical properties of the experimental soil.

Soil characteristics	Value	Soil characteristics	Value
Particle size distribution%:		Soluble cations (soil paste $mmol_c L^{-1}$):	
Sand	51.0	Ca^{2+}	15.3
Silt	35.0	Mg^{2+}	10.7
Clay	13.7	Na^{+}	28.2
Textural class Sandy clay loam		K^{+}	0.6
Soil chemical properties:		Soluble anions (soil paste $mmol_c L^{-1}$):	
pH 1:2.5 (soil water suspension)	8.15	CO_3^{2-}	0.00
$CaCO_3$ %	14.0	HCO_3^{-}	3.0
Organic carbon %	0.99	Cl^{-}	36.1
ECe (dS m^{-1}, soil paste extract)	5.48	SO_4^{2-}	15.7
		ESP	15.7

Available nutrients mg kg^{-1}							
N	P	K	S	Fe	Mn	Zn	Cu
37.15	4.07	201.35	7.94	4.23	0.80	0.65	0.38

The applied treatments comprised different rates of elemental sulphur, *i.e.*, 0, 200, 350, 450 kg fed^{-1}, which were thoroughly mixed with the topsoil during soil preparation, 21 days before sowing followed by light irrigation. Sunflower seeds were inoculated with *Bacillus megatherium* (PDB) which was obtained from the Dept., Agric . Microbiol., Soil,Water and Environ. Res. Inst. (SWERI), Agric. Res. Center (ARC), Giza, Egypt on the same day of sowing. The inoculation was executed by mixing sunflower seeds with heavy cell suspention of (PDB) (with about 10^9 cfu ml^{-1}). The applied rate of foliar seaweed extract was at 1.0 kg fed^{-1} with 400 L of water. Analysis of seaweed extract used is illustrated in Table 2. Experimental plots (10.5 m^2) were arranged in split split plot design with three replicates for each treatment (Gomez and Gomez, 1984).

TABLE 2. Some components of seaweed extract used.

Organic Matter %	Potassium (K_2O) %	Nitrogen (N) %	Phosphorus (P_2O_5) %	Free amino acids	Algenic Acid %
45	12	1.5	0.8	20.4 mg kg^{-1}	20
pH	Manitol	Water solubility	Cytokinines Auxins and Gibberellins	Density (g cm^{-3})	Appearance
4.0-5.0	5 %	100 %	600 µg kg^{-1}	0.633	Black powder

All sunflower (*Helianthus annuns,* c.v. Hysun 336) plots received 200 kg fed^{-1} of single superphosphate (15.5% P_2O_5), 200 kg fed^{-1} ammonium nitrate

(33.5% N) and 50 Kg fed^{-1} potassium sulphate (48% K_2O) which incorporated into the top 15 cm of the soil during the preparation of soil cultivation. Hill spacing was 10 cm within the row. Seeds were sown at 3-5 seeds in each hill in the first week of April. Irrigation water was used immediately after sowing, then every one week interval according to agronomic practices in the district. Thinning was carried out at 15 days after sowing to secure two plants per hill on both sides of the ridge.

Three plant samples plot^{-1} were collected 50 days after sowing for determination of the growth criteria plant height, number of leaves plant^{-1} as well as dry weight of plant and leaf chlorophell. At harvest samples were collected for determination of yield components as head diameter, 100 seeds weight, seeds yield fed^{-1}, seed oil & protein and nutrient contents of N, P, K, S, Fe, Mn, Zn and Cu according to (Issam and Sayegh, 2007). Two samples of the rhizosphere plants soil were collected after 50 and 90 days of sowing to determine CO_2 evolution (Shehata, 1972), dehydrogenase activity (Alef, 1995) and total bacterial counts according to Page *et al.* (1982).

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

Results and Discussion

As the obtained results of both successive seasons were not significantly different , their average was taken into consideration.

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

Some soil properties

In respective of elemental sulphur, Data in Table 3 showed a clearly response of some soil properties, *i.e.*, pH, ECe and ESP to the applied treatments, particularly those treated with the highest rates of elemental sulphur, *i.e*., 350 or 450 kg fed^{-1}. That was true, since elemental sulphur can be oxidized by many soil microorganisms and forming sulphuric acid, besides the acidity reacts of the microbial activity of *Bacillus megatherium var. phosphaticum* itself, consequently such acid media led to lowering soil pH. 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).

On the other hand, the released soluble ions of Ca^{2+} can improve 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 ECe 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 many strain produce several phytohormones (*i.e.*, indol 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.

TABLE 3. Effect of applied treatments on some soil properties.

***PDB**	**E-Sulphur (kg fed^{-1})**	**Soil properties**		
		pH	**ECe (dS m^{-1})**	**ESP**
Without seaweed extract				
Un-Inocutation	0	8.22	5.34	15.45
	200	7.85	5.04	14.05
	350	7.34	4.56	11.00
	450	7.32	4.51	10.80
Inocutation	0	8.20	5.21	15.22
	200	7.78	4.56	11.52
	350	7.25	3.60	10.70
	450	7.21	3.41.	10.30
With seaweed extract				
Un-Inocutation	0	8.20	5.21	15.00
	200	7.81	4.81	14.39
	350	7.34	4.12	13.00
	450	7.28	4.00	10.21
Inocutation	0	8.07	5.13	14.93
	200	7.72	4.32	10.80
	350	7.19	3.20	10.13
	450	7.12	3.10	10.01

*PDB= P-dissolving bacteria.

Soil available nutrients

In general, the obtained data presented in Table 4 showed that the beneficial effect of the applied treatments, particularly elemental sulphur at the applied rates of 350 or 450 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 as macronutrients and sulphur 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 P-dissolving bacteria for the noticeable increment in soil available nutrient contents could be attributed to the pronounced decrease in the values of soil pH, ECe 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 easily 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 adverseable effects of alkaline soil media.

TABLE 4 . Effect of applied treatments on soil availability of some nutrient contents.

*PDB (B)	E-Sulphur(S) (kg fed^{-1})	Soil available nutrient contents (mg kg^{-1} soil)							
		Macronutrients			S	Micronutrients			
		N	P	K		Fe	Mn	Zn	Cu
Without seaweed extract (E)									
Un-Inocutation	0	35.61	3.59	191.1	6.73	3.93	0.98	0.82	0.47
	200	41.95	4.13	201.4	8.58	4.50	1.34	1.09	0.70
	350	46.10	4.47	207.0	10.85	4.78	1.61	1.23	0.81
	450	47.15	4.77	209.2	10.98	5.95	1.66	1.29	0.85
Inocutation	0	37.18	5.98	199.4	8.02	4.75	1.10	1.01	0.76
	200	56.19	6.74	223.3	9.34	6.13	1.34	1.44	1.17
	350	65.54	7.53	236.9	12.76	7.00	2.49	1.79	1.47
	450	66.19	8.19	241.7	12.93	7.13	2.61	1.92	1.56
With seaweed extract									
Un-Inocutation	0	36.17	4.47	196.8	6.93	4.38	1.04	0.86	0.60
	200	47.31	4.89	212.2	9.87	5.43	1.49	1.32	0.94
	350	53.17	5.76	222.3	12.48	5.71	2.03	1.53	1.12
	450	55.91	5.91	224.5	12.70	5.83	2.09	1.59	1.17
Inocutation	0	38.12	7.31	206.0	7.08	4.13	1.07	0.89	0.57
	200	63.94	8.23	243.0	10.62	6.18	2.19	1.64	1.31
	350	74.18	8.88	254.6	13.30	7.66	2.83	2.08	1.81
	450	76.30	9.24	263.9	13.68	8.24	3.01	2.19	1.89
ANOVA									
LSD at 0.05	E	3.40	0.12	3.70	0.14	0.90	0.12	0.02	0.01
	B	2.20	0.10	2.10	0.18	0.12	0.42	0.01	0.02
	S	2.50	0.13	2.52	0.08	0.11	0.31	0.23	0.05
	E×B	3.10	0.14	3.36	0.07	0.09	0.50	0.02	0.01
	E×S	2.20	0.09	4.80	0.14	0.08	0.14	0.06	0.02
	B×S	2.70	0.08	2.30	0.16	0.10	0.32	0.05	0.03
	E×B×S	2.00	0.13	2.41	0.11	0.05	0.11	0.02	0.01

*PDB = P-dissolving bacteria.

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, which resulted in more pronounced nutrients availability in the soil, on the basis of lowering soil pH and microbial activity that enhancing the solublization 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.

Total bacterial count, CO_2 evolution and dehydrogenase activity in rhizosphere soil

Results in Table 5 revealed that inoculation with P-dissolving bacteria in presence of different elemental sulphur levels encouraged the population of bacterial counts. However, the highest count of those microorganisms was attained by the treatment inoculated and companied with foliar seaweed extract. The corresponding high values were 77 & 130 × 10^5 cfu g^{-1} soil with foliar seaweed extract and 45 & 70 × 10^5 cfu g^{-1} soil (total bacterial count) for the treatments without foliar seaweed extract at 50 and 90 days from sowing, respectively.

Data presented in Table 5 indicated that the highest values of both CO_2 evolution and dehydrogenase activity were obtained by the treatment inoculated with P-dissolving bacteria in combination with 450 kg fed^{-1} elemental sulphure in presence of foliar seaweed extract. The corresponding high values were 699.3&788.0 mg CO_2 100g $soil^{-1}$ soil and 288.0 & 546.0 µgTPF. 100 g soil day^{-1} (dehydrogenase activity) at 50 and 90 days from sowing, respectively.

TABLE 5. Effect of applied treatments on microbial activity (total bacterial count, CO_2 evolution and dehydrogenase activity) in rhizospher soil at 50 and 90 days after sowing .

*PDB	E-Sulphur (kg fed^{-1})	Total bacteria × 10^5 cfu g $soil^{-1}$		CO_2 (mg. 100 g $soil^{-1}$)		#DHA (µg TPF.100 g soil day^{-1})	
		days after sowing					
		50	90	50	90	50	90
Without seaweed extract							
Un-Inocutation	0	10	13	120.9	123.9	18.3	86.5
	200	14	22	141.9	159.3	22.1	145.3
	350	21	53	218.6	340.8	28.0	275.6
	450	29	60	253.3	371.2	64.0	261.3
Inocutation	0	12	14	156.9	167.2	30.3	80.0
	200	18	38	186.5	273.3	40.4	124.0
	350	36	57	228.0	404.9	48.6	187.8
	450	45	70	328.0	439.5	112.9	339.5
With seaweed extract							
Un-Inocutation	0	13	18	134.5	161.2	92.0	107.3
	200	18	24	156.7	303.5	112.0	229.3
	350	40	74	230.5	388.3	148.0	272.0
	450	51	92	357.1	466.3	312.3	408.0
Inocutation	0	16	22	151.3	187.2	107.6	138.6
	200	34	50	202.7	422.1	126.5	253.3
	350	48	97	253.2	618.5	180.0	260.6
	450	77	130	699.3	788.0	288.0	546.0

PDB= P-dissolving bacteria. #Initial dehydrogenase activity (DHA) was 3.9 (µg TPF.100 g soil day^{-1}).

Plant parameters as affected by the associated ameliorating soil properties

Plant growth characters

Data presented in Table 6 indicated that the achieved favorable soil conditions due to the applied treatments, particularly the combined ones of elemental sulphur with either bio-fertilizer (P-dissolving bacteria) or seaweed extract, were positively reflected on studied values of sunflower plant growth characters (*i.e.*, the total leaf of chlorophyll, plant height, number of leaves plant^{-1} and dry matter weight plant^{-1}) as compared to the applied solely ones. It can be explained on the basis that the treated soil with elemental sulphur and bio-fertilizer became enriched in the released nutrient contents, especially those of micronutrients, which are involved directly or indirectly in formation of biological components through their roles in the respiratory and photosynthesis mechanisms as well as in the activity of various enzymes (Nassar *et al*., 2002).

Also, the prmotive effect of the applied seaweed treatment on leaf chemical constituents might be attributed to their enhancing effect on the nutritional status of sunflower plants, and then the increase in dry weight that could be attributed to its stimulating effect on vegetative growth and physiological processes. In addition, the increase of total chlorophyll is owing to that the enhanced nutrients uptake plays an important role for stimulating chlorophyll synthesis enzymes, which reflected on formation of chlorophyll molecule (Abdel-Aziz and El-Shafie, 2005).

Further, it could noticed that the obtained values of the studied plant growth characters tended to gradual increases with increasing the applied rates of elemental sulphur from 0 up to 450 kg fed^{-1}, with insignificant difference between 350 and 450 kg fed^{-1} S. Also, it is noteworthy to mention that the applied triple treatment of elemental sulphur as a soil amendment at the highest rate (450 kg fed^{-1}) in combination with bio-fertilizer plus foliar application of seaweed was achieved the greatest values of the tested plant growth characters, with insignificant difference with the applied sulphur rate of 300 kg fed^{-1}. It is also interpreted to have favorable effect of chlorophyll synthesis resulting in more number of leaves with bigger size and higher chlorophyll content. Thus, sulphur helps in increasing the photosynthetic activity of plant (Upasami and Shama, 1986). In addition, combining bio-fertilizer with sulphur that lead to a markedly increase in the tested growth characters, may be due to their outcomes are essentially for certain of protoplasm, and hence producing new cells and new leaves of sunflower plants that lead to a larger leaf area available for photosynthesis and increase dry matter accumulation (Omran and Azzam, 2007). Sangeetha and Thevanathan (2010) reported that application of seaweed extract increase in linear growth of the shoots and roots was associated with a concomitant increase in the number of lateral roots produced, the number of leaves or leaflets produced, increase in leaf area, increase in the chlorophyll a/b and C/N ratio.

TABLE 6. Effect of applied treatments on some plant growth characters 50 day after sowing.

*PDB (B)	E-Sulphur(S) (kg fed^{-1})	Plant height (cm)	No. of leaves plant^{-1}	Leaf chlorophyll (mg g^{-1} fresh weight)	Dry matter weight (g plant^{-1})
Without seaweed extract (E)					
Un-Inocutation	0	76.7	10.9	0.174	7.3
	200	81.3	11.1	0.200	9.9
	350	89.4	13.7	0.217	10.8
	450	90.9	14.0	0.221	11.4
Inocutation	0	73.1	11.5	0.200	9.5
	200	82.0	14.7	0.231	11.2
	350	94.5	15.4	0.275	13.0
	450	97.8	16.5	0.279	13.5
With seaweed extract					
Un-Inocutation	0	87.6	11.2	0.199	8.9
	200	90.0	16.9	0.237	10.7
	350	93.5	17.7	0.296	11.8
	450	96.7	19.2	0.301	12.9
Inocutation	0	81.2	12.0	0.219	10.5
	200	100.1	15.7	0.279	12.7
	350	102.7	18.2	0.307	14.8
	450	107.4	20.1	0.317	15.2
ANOVA					
LSD *at 0.05*	E	2.3	0.9	0.111	0.5
	B	1.5	0.7	0.213	0.4
	S	3.2	0.2	0.211	0.3
	E×B	4.2	0.6	0.114	0.4
	E×S	1.3	0.3	0.200	0.3
	B×S	1.6	0.4	0.231	0.2
	E×B×S	3.1	0.3	0.112	0.4

*PDB= P-dissolving bacteria.

Nutrient contents in plant tissue as affected by the applied treatments

The obtained data of the studied macro-(N, P and K), S and micro-nutrients (Fe, Mn, Zn and Cu) contents in the plant tissues of sunflower are presented in Table 7.

The obtained results exhibited pronounced increases for the studied macronutrients, S and micronutrients due to the applied elemental sulphur as a solely treatment, with greatest values when it was combined with both P-dissolving bacteria and foliar seaweed extract. Undoubtedly, the applied solely treatments were usefulness as compared to the combine one for the plant tissue contents of the studied nutrients. That was true, since the nutrients uptake by plants are more related to the released available nutrients and their easily mobility towards the plant roots. Such surpassed effects were more associated with ameliorated soil pH due to the applied elemental sulphur and biological activity and their released activity substances that enhancing nutrients solubilization from both native and added sources, besides the favorable biological conditions that are keeping them in available forms and their mobility for uptake by plant roots. In this concern, Abdallah *et al.* (2010) showed that sulphur deficiency for field grown oilseed rape can reduce nitrogen use efficiency (NUE) and that nitrogen deficiency can also reduce sulphur use efficiency (SUE).

Further, such favored better micronutrient uptakes are in harmony with results obtained by Abdel-Mawgoud *et al.* (2010) who found that the extracts obtained from seaweeds have gained importance as applied for several crops because the extract contains micronutrients (Fe, Cu, Zn, Mn and Mo) and amino acids.

TABLE 7. Effect of applied treatments on nutrient contents in sunflower plants.

*PDB (B)	E-Sulphur (S) (kg fed^{-1})	Macronutrients (%)			S (%)	Micronutrients (mg g^{-1})			
		N	P	K		Fe	Mn	Zn	Cu
Without seaweed extract (E)									
Un-Inocutation	0	1.87	0.164	1.69	0.39	85.0	42.1	17.0	8.2
	200	2.19	0.190	1.78	0.49	120.5	60.3	23.7	13.1
	350	2.39	0.199	1.83	0.60	17.0	79.5	27.3	14.8
	450	2.45	0.210	1.85	0.67	263.5	96.2	28.9	15.6
Inocutation	0	1.97	0.266	1.77	0.46	101.4	48.3	21.7	13.8
	200	2.93	0.295	1.97	0.53	163.5	64.5	32.5	20.0
	350	3.37	0.323	2.08	0.70	244.1	95.3	41.3	28.1
	450	3.44	0.354	2.12	0.74	372.5	101.4	44.5	30.0
With seaweed extract									
Un-Inocutation	0	1.92	0.210	1.75	0.41	94.0	45.2	18.5	13.3
	200	2.49	0.216	1.87	0.53	143.7	77.4	29.5	22.2
	350	2.79	0.521	1.94	0.69	201.0	79.6	34.7	30.0
	450	2.86	0.256	1.98	0.73	307.5	105.6	36.9	32.5
Inocutation	0	2.01	0.313	1.82	0.42	89.3	47.3	19.1	12.9
	200	3.25	0.350	2.13	0.59	162.5	103.4	37.9	34.7
	350	2.85	0.376	2.28	0.73	263.0	134.1	48.5	37.4
	450	3.91	0.390	2.30	0.79	395.2	145.2	51.2	44.3
ANOVA									
LSD *at 0.05*	E	0.23	0.131	0.22	0.01	3.70	4.20	0.90	0.12
	B	0.12	0.111	0.11	0.02	2.10	1.70	1.70	0.22
	S	0.23	0.131	0.10	0.01	2.50	2.50	1.10	0.42
	E×B	0.24	0.121	0.11	0.04	3.11	1.80	1.50	0.64
	E×S	0.10	0.140	0.09	0.01	4.80	3.40	1.00	0.36
	B×S	0.15	0.124	0.10	0.03	2.34	2.90	1.10	0.23
	E×B×S	0.10	0.122	0.13	0.05	2.22	4.10	1.50	0.25

*PDB= P-dissolving bacteria.

Meanwhile, such favorable effect was extended to the combined treatments at the highest rate of sulphur (450 kg fed^{-1}, being insignificant difference with the sulphur rate of 350 kg fed^{-1}) due to improving soil physical and chemical properties that positively affected the nutrients availability as well as maintaining a suitable soil moisture regime, which showed a pronounced positive effect on the biological activity in soil, Also, the integrated action of the bio-fertilizer and S-transformation to (H_2SO_4), besides the possible released phosphate ion by sulphate ions was, in general, extending parallel close to the corresponding nutrients contents in the plant tissues (El-Tapey and Hassan, 2002). Moreover, the interaction between elemental sulphur and P-dissolving bacteria was significant and the best combination dose was at 450 kg fed^{-1} S with insignificant differences with 350 kg fed^{-1} S, which gave the maximum nutrient content. This confirm the synergism between phosphorus and sulphur (Varavipur *et al.,* 1999).

Yield and its attributes

Data presented in Table 8 revealed that the head diameter, 100 seed weight, seed yield and some parameters of seed quality (oil and protein contents) were substantially improved by the application of elemental sulphur in combination with either P-dissolving bacteria or seaweed extract. That, means the aforementioned best vegetative growth characters were positively reflected on sunflower yield and its attributes and returned on increasing both head diameter, 100 seed weight and seed yield as well as their quality parameters (*i.e.,* seed contents of oil and protein) as shown in Tables 7 and 8.

Regarding to the combination treatments between elemental sulphur, P-dissolving bacteria and seaweed extract application, there was a significant effect at both rates of sulphur 350 and 450 kg fed^{-1} in combination with either P-dissolving bacteria or seaweed extract. These applied treatments enhanced dry matter, seed yield and yield components of sunflower, however, the maximum dry matter weight, head diameter and yield components were achieved at rates of sulphure 350 and 450 kg fed^{-1} in combination with P-dissolving bacteria and seaweed extract.

The highly significant enhancement of sulphur on the oil content of sunflower seed due to responses an essential component in some essential amino acids such as cystein and methionine for protein synthesis (Malhi *et al.*, 2007). Depending upon the presented data in Table 8, oil and protein contents of sunflower seed have been found to be optimized in case of combination with seaweed extract as foliar application. The beneficial effect of seaweed extract on terrestrial plants includes improving the overall growth, yield and the ability to stand with adverse environmental conditions (Asirselvin *et al*., 2004 and El-Said *et al*., 2011). Being greater magnitude of 33.87 and 23.56% was occurred at the combined treatment of sulphur at rate of 450 kg fed^{-1}+ P-dissolving bacteria and seaweed extract for seed content of protein and oil, respectively, mainly due to greater stimulating action of seaweed extract in the synthesis of sulphur containing protein and also localization of micronutrients in protein bodies as discrete particularly in seed as well as higher rate of translocation of them in presence of sulphur from the root to seed via shoot meristem resulting increased transaction and translocation with reduced rate of RNA degradation (Sharma *et al.*, 1990).

TABLE 8 . Effect of applied treatments on yield and its attributes .

*PDB (B)	E-Sulphur(S) (kg fed^{-1})	Head diameter (cm)	Seed yield (ton fed^{-1})	100 seeds weight (g)	Seed oil (kg fed^{-1})	Seed protein (%)
Without seaweed extract (E)						
Un-Inocutation	0	9.47	0.84	3.65	137.4	8.45
	200	10.88	1.09	4.70	148.0	10.93
	350	11.47	1.25	55.4	155.1	12.59
	450	12.70	1.27	5.71	156.9	12.71
Inocutation	0	9.57	0.94	4.75	140.2	9.49
	200	11.14	1.18	5.55	158.6	11.87
	350	13.23	1.35	6.65	170.1	13.50
	450	13.93	1.38	6.60	173.4	13.67
With seaweed extract						
Un-Inocutation	0	8.91	0.92	4.45	139.5	9.24
	200	10.78	1.17	5.35	156.8	11.78
	350	12.81	1.34	5.90	168.9	13.48
	450	14.95	1.39	6.45	171.5	13.55
Inocutation	0	10.30	1.09	5.25	143.1	10.96
	200	12.78	1.28	6.35	166.0	12.84
	350	14.81	1.44	7.40	181.4	14.15
	450	16.89	1.56	7.90	183.7	15.31
ANOVA						
LSD at 0.05	E	1.21	0.02	0.12	1.3	0.09
	B	2.00	0.20	0.13	1.5	0.84
	S	1.64	0.03	0.11	1.6	0.62
	E×B	1.36	0.02	0.11	1.4	0.73
	E×S	1.55	0.05	0.10	1.7	0.64
	B×S	1.64	0.04	0.13	1.3	0.72
	E×B×S	1.54	0.33	0.12	1.2	0.71

*PDB= P-dissolving bacteria

Conclusions

It is noteworthy to mention that data obtained are of the importance in such studied slightly alkaline sandy clay loam soil calcareous in nature, owing to the effective role of the exerted a positive effect on soil fertility status, but also on the different soil properties. Such amelioration in physical, chemical and biological was reflected positively on the sunflower seed yield with high quality as well as chemical constituents of seed (oil and protein). Also, the applied such bio-fertilizer, inorganic soil amendments with foliar seaweed extract had a positive agronomic value due to its capacity to gradually liberate available plant nutrients and to improve soil characteristics with increasing in total bacterial count, CO_2 evolution and soil microbiological activity.

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التاثير التطبيقى للبكتيريا المذيبة للفوسفور ومستخلص الأعشاب البحرية بالاضافة للكبريت المعدنى علي انتاجية عباد الشمس في أرض ملحية مستصلحة حديثا

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أجريت تجربة حقلية علي تربة رملية طينية طميية في نطاق الأراضي المستصلحة حديثا والتي تحتل المنطقة الصحراوية لمحافظة الفيوم – مصر 29° 21′ 48″ N (Latitude) 30° 44′ 45″ E Longitude وهي ذات طبيعة جيرية و بها نسبة من الملوحة البسيطه خلال موسمين زراعين علي محصول عباد الشمس كمحصول زيتي (*Helianthus annuns,* cv Hysun 336) و قد أستهدفت الدراسة معرفة مدي ثأثير التلقيح الحيوي للبذور بواسطه البكتريا المذيبة للفسفور *(Bacillus megatherium* var *phosphaticum*) مع الرش الورقي بمستخلص الأعشاب البحرية بمعدل 1,0 كجم/فدان/400 لتر ماء، مع الاضافة الارضية للكبريت المعدني كمحسن للتربة بمعدل 0، 150، 350، 450 كجم/فدان كمعاملات منفردة أو مشتركة علي قياسات نمو نباتات عباد الشمس (طول النبات، عدد الأوراق، وزن النبات الجاف، الحاله الغذائية، محتوي الكلوروفيل للأوراق) و المحصول (من حيث قطر القرص، وزن 100 بذرة، المحصول الكلي للبذور) مع بعض من قياسات جودة البذور (المحتوي من الزيت و البروتين). كما أخذ في الأعتبار لهذه الدراسة التحسن المصاحب في بعض خواص التربة (رقم الحموضة و درجة التوصيل الكهربي و نسبة الصوديوم المتبادل و المحتوي الميسر من المغذيات الكبري و الصغري) وكذلك النشاط البيولوجي (العدد الكلي للبكتريا و كمية غاز ثاني أكسيد الكربون الناتج عن النشاط البيولوجي و نشاط أنزيم الديهيدروجينيز بالتربة) بعد 50 و90 يوم من الزراعة.

أوضحت النتائج أن هناك تحسن فى صفات التربة و الذي أنعكس بطريقة إيجابية علي القياسات الخضرية لنباتات عباد الشمس وكذلك على محصول البذور و جودتها، حيث كانت أفضل و أعلي القيم المتحصل عليها نتيجة المعاملة المختلطة بين التلقيح الحيوي للبذور مع رش مستخلص الأعشاب البحرية علي الأوراق فى وجود الكبريت المعدني بالمقارنة بالمعاملة المنفردة لأي منها. وبصفة عامة، فأن قيم القياسات النباتية قد تعاظمت بالنسبة للنباتات الملقحة بذورها حيويا و التي تم أضافة مستخلص الأعشاب البحرية إليها بالرش الورقي، بزيادة معدلات الكبريت المستخدم كمحسن معدني للتربة حتي معدل أضافة 350 أو 450 كجم/فدان، بدون فرق معنوي بينهما. هذا و قد أظهرت أيضا علاقة محفزة تمثلت في تلك المعاملة الأكثر ملائمة للحصول علي أعلي محصول و بمحتوي مرتفع لكل من الزيت و البروتين .

كما أظهرت النتائج أن التلقيح الحيوي بالبكتريا المذيبة للفوسفور مع المستويات المختلفة من الكبريت المعدني و مستخلص الأعشاب البحرية عمل علي تشجيع أعداد المستعمرات البكتيرية و كمية غاز ثاني أكسيد الكربون وكذلك نشاط أنزيم الديهيدروجينيز في التربة بمنطقة الجذورعند 50 و 90 يوم من الزراعة.

لذا يمكن التوصية باستخدام التسميد الحيوي بالبكتريا المذيبة للفسفوروالاضافة الورقية لمستخلص الأعشاب البحرية مع اضافة الكبريت المعدني كمحسن للتربة وذلك لتقليل التأثير الضار الناتج عن ملوحة التربه و الطبيعة الجيرية لها والتي قد تؤثر سلبا علي أنتاجية و جودة محصول عباد الشمس .