

BIOFERTILIZER, SULPHUR AND INORGANIC PHOSPHORUS AND THEIR EFFECT ON MAIZE PLANTS GROWN IN CALCAREOUS SOIL

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ABSTRACT

A field experiment was conducted in two seasons (2005 and 2006) on sandy clay loam calcareous soil at Alexandria Governorate, Egypt to investigate the role of phosphate dissolving bacteria (PDB), *Aspergillus niger* and *Bacillus subtilis* either they applied individually or mixed together as main treatments, 100 and 200 kg fed⁻¹ elemental sulphur as sub treatments, 6.5 or 13 kg P as sub sub treatments applied to maize plant. Evaluation of tested treatments was performed through some yield parameters, i.e. fresh and dry weight of shoots and roots, length of cobs (cm), 100 grain weight (g), dry weight of grains (ton fed⁻¹), dry weight of stover (ton fed⁻¹) and harvest index were considered. Colonization, phosphatase activity, NPK % and some soil properties after maize harvesting were also determined. Results revealed that mixed inoculation with 100 kg S and 13 kg P fed⁻¹ gave the highest NPK % in maize plants and in their grain and in their stover yields. Phosphate dissolving bacteria and *Aspergillus niger* as phosphate dissolvers were superior to *B. subtilis* but their activities increased by combination to all of them. Low P rate increased its efficiency by microbial inoculation and S application but the recommended P rate caused a significant increase in its effect on all plant parameters. It could be concluded that a substantial amount of inorganic P fertilizer could be saved by inoculation, which in turn minimizes the production costs. Generally, to obtain high quality and quantity of maize yield under calcareous soil without harmful degradation of soil, it could be recommended the use of mixed biofertilizer in combination with 100 kg S and 13 kg P fed⁻¹.

Keywords: Calcareous soil, Phosphate dissolving bacteria (PDB), *Aspergillus niger*, *B. subtilis*, S and P application rates, Maize.

INTRODUCTION

Phosphorus is a major limiting factor for crop production in many tropical and subtropical soils (Norman et al., 1995). As a result of high (P) fixation and /or nutrient mining in traditional land use systems, phosphorus is the most widely occurring nutrient deficiency in cereal systems around the world. For this reason, crops are supplied with inorganic (P) fertilizers. However, excess (P) added to crops may cause environmental and economic problems (Withers et al., 2001). Concepcion and Delgado (2005) studied P forms by sequential chemical fraction in soils typical of Mediterranean areas, establishing correlations between P fractions and soil properties. The ratio of the P fraction, which includes more labile P forms (essentially adsorbed) to combine with non-organic P fractions was negatively correlated with soil pH and positively correlated with the portion of combined Fe fractions related to poorly crystalline oxides. Phosphorus (P) and potassium (K) as well as nitrogen (N) are the major nutrients, for plant nutrition. In calcareous soils where pH is alkaline and CaCO₃ is dominated, plants suffer low availability of P and K would cause more serious problems

its yield components. The plot area was 3.0m x3.5m (10.5m²). The soil had received organic farmyard manure (FYM) at the rate of 10 m³ fed⁻¹. Representative soil samples from the experimental field were collected from the upper 30 cm layer to determine the physical and chemical soil properties as described by Black et al. (1965). The results obtained are presented in Table (1).

Table (1): Mechanical and some chemical properties of soil used Soil Particle size distribution

Coarse sand %	Fine sand %	Silt %	Clay %	Texture	CaCO ₃ %
20.79	43.12	20.67	15.24	Sandy clay loam	33.50

II- Soil chemical analyses

EC dSm ⁻¹	pH 1:2.5	CEC Cmole kg ⁻¹ soil	Cations (m mol/L)				Anions (m mol/L)				Total N %	Avail. P ppm	Avail. K ppm
			Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	CO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻			
2.00	8.15	21.3	6.70	4.08	9.20	0.63	-	3.33	7.21	10.7	0.04	10.7	355.0

Sulphur as elemental sulphur was added according the suggested treatments, while, phosphorus as calcium superphosphate (6.5%P) was added basically during soil preparation before sowing according to the planned design. Maize grains inoculated or uninoculated with the bacterial cell suspension that was previously prepared in the presence of 16% Arabic gum as adhesive agent, air dried and then sowed. While for the uninoculated treatments, seeds were similarly treated with the physiological solution without bacterial cells. Ammonium nitrate (33%N) and potassium sulphate (41.5 % K) were added at the rate of 60 kg N fed⁻¹ and 40 kg K fed⁻¹, respectively, each in two equal split doses on 4th and 18th July for all plots and then irrigated every 15 days until September 16th. Mature crop was harvested on September 26th where yields of ears, grains and stover were recorded. Rhizosphere soil samples were taken in July, August and September to determine the microbial counts, root volume of maize plants (Giovannetti and Mosse, 1980) and some soil properties.

Microbial population in maize plant rhizosphere:

The dilution plate count technique was used for enumeration of the microbial community maize rhizosphere plant. Total colony count was made on Bunt and Rovira (1955) modified soil extract agar medium and then incubated at 30°C for 7 days. Total phosphate dissolving bacteria was determined on modified Bunt and Rovira (Abd El-Hafez, 1966) agar medium. *B. subtilis* count in soil samples was done using King's medium (King et al., 1954). Soil phosphatase activity was determined according to Tabtabai and Bremner (1969). All counts were related to dry weight of soil samples at 105 °C. The NPK contents were determined according to Chapman and Pratt (1961).

Obtained results were statistically analyzed following Snedecor and Cochran (1976). All data presented tables and figures are a mean value of two successive seasons of 2005 and 2006.

RESULTS AND DISCUSSION

Microbial counts in sandy clay loam soil containing high amount of CaCO_3 are very low. The cultivation of these soils stimulates the propagation of bacteria and fungi (Azazy et al., 1988). The microbial determination of soil used showed low densities of total colony counts, PDB and *B. subtilis* counts (at the begging) before inoculation (initial counts), which recorded 40×10^4 , 35×10^3 and 4.0×10^3 , respectively, maize grains were inoculated by soaking in liquid culture with either sole or combined inoculum of PDB, (10^8 cells ml^{-1} approximately) and *A. niger*, *B. subtilis* (10^8 cells ml^{-1} approximately). It could be seen from Figure (1) that mixed inoculation with PDB, *A. niger* and *B. subtilis* recorded the highest number of total count bacteria after 30 days from sowing (in July) with So Po and S_2P_2 (7.97 log count) then decreased gradually after 60 & 90 reached to (6.9 & 5.7 log counts) and (6.19 & 6.05 log counts) respectively.

After 60 days in August the highest log count was recorded to PDB inoculation (8.00) with S_2Po followed by SoP_1 (7.97) and S_2P_2 (7.95) then decreased again at 90 day in September to reach (7.3, 6.98 and 6.95), respectively. It is of worth to notice that the total bacterial count remained higher in the inoculated treatments than the corresponding uninoculated ones till the end of the experiment. These results agreed with Falik and Okon (1996), Soliman et al. (2003) and Hanna et al. (2005) who reported that PDB as biofertilizer for cereal crops increased P availability to the cultivated plants.

As indicated in Figure (2) there is a pronounced increase in total PDB counts with SoP_1 inoculated either with PDB and *A. niger* (6.1, 7.6 & 7.4) in July, August and September, respectively, or with mixed inoculation (6.08, 5.6 & 4.6) in July, August and September, respectively. However, (Po) had recorded the lowest values of PDB counts in all treatments, this was probably due to low level of P, which plays an important role in the activation of the indigenous or induced the soil microorganisms. In case of *B. subtilis* inoculation, phosphate dissolving bacteria count was higher in July with SoPo and S_1Po than those recorded by PDB inoculation. Also, it was noticed that the uninoculated treatment recorded high number of PDB than the mixed inoculation with P_2 , P_1 , which recorded (4.92, 4.88 and 4.6) for uninoculated SoP_2 against (4.81, 4.7, 3.7) for mixed inoculation and (5.1, 3.88, 4.6), (4.84, 4.5, 3.5) for S_1P_2 , respectively.

Figure (3) indicate that mixed inoculation increased the *B. subtilis* counts than those in the other individual inoculated treatments. Mixed inoculation gave higher numbers being 7.65 in August with S_2P_2 and 7.58 with S_2P_1 compared to *B. subtilis* inoculation only that recorded 6.21 and 6.0 with the same treatments and time. While, *B. subtilis* inoculation recorded higher values due to S_1Po treatment than the other treatments (5.89, 7.0 and 5.9) in July, August and September, respectively against (5.72, 5.18 and 5.56), (5.15, 6.95 and 5.78) for mixed and PDB inoculations, respectively, at same time and treatment.



Fig (1): Changes in total bacterial count in soil maize rhizosphere plant through different planting periods of maize (a log. c.f.u.g⁻¹ oven dried soil initial total colony in soil was (5.6) log cycle. (S₀, S₁, S₂ are 0, 100 and 200 kg S fed⁻¹ and P₀, P₁, P₂ are 0, 6.5 and 13 kg P fed⁻¹).

The final counts of all estimated microorganism groups were still higher than those of initial in all inoculated treatments over those belong to the uninoculated ones. However, the use of biofertilizer either mixed together or individually in addition of half dose of inorganic P 6.5 kg fed⁻¹ (P₁) combined with the organic FYM, which enhanced the growth of most important microorganism groups in maize rhizosphere plants and in the presence of sulphur, which causes the decreasing in soil pH and hence plays a role in the availability of macro and micro nutrients in soil. These results are similar to those obtained by Misra (2003) and Sequera and Ramirez (2003). The positive effect of PDB combined with *A. niger* as phosphate solvers and acid producers on counts of different microbial populations may be referred to the improvement in conditions of the maize rhizosphere plants especially in presence of high amount of CaCO₃ and pH 8.5 in such soil. Results are also in harmony with those obtained by Abdalha et al., (1984). The stimulative in microbial population may be due to the association effect of PDB, *A. niger* and *B. subtilis* either as mixed or single inoculation in maize rhizosphere plants (Mahmoud, 2000).

Phosphatases are considered key enzymes in the phosphorus cycling in soil (Dick and Tabatabai, 1984). Variation in phosphatase activity apart from indicating changes in the quantity and quality of soil phosphate substrates (Rao and Tarafdar, 1992). Table (2) illustrates the changes with time of phosphatase activities as affected by inoculation and application of S and P. However, the highest activities values of phosphatase in soil were recorded by PDB and *A. niger* combined with full dose of P (P₂) 494 μmol P in July and increased during the growth season to reach 1375 and 1450 μmol in August and September, respectively, followed by *Bacillus* inoculation attained with full dose of P than mixed and uninoculated treatments.

Table (2): Changes with time of phosphatase activities μ mole P g⁻¹h⁻¹ in calcareous soils as affected by sulphur, phosphorus and biofertilizer and maize cultivation.

Sulphur (S)	So			S ₁			S ₂		
	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂
Phosphorus (P)									
Microbial inoculation (M)									
PDB & <i>A. niger</i>									
July	250	386	479	247	400	457	299	396	494
August	1010	1250	1360	1101	1237	1372	1025	1240	1375
September	860	1301	1450	791	1310	1400	810	1211	1440
<i>B. subtilis</i>									
July	211	348	344	209	383	301	206	339	399
August	401	740	919	420	739	899	416	760	902
September	302	591	701	301	580	715	300	585	670
Mixed inoculation									
July	179	260	266	196	272	297	190	290	289
August	470	687	708	479	690	712	465	688	717
September	201	510	802	200	510	809	199	516	800
Uninoculated									
July	113	248	270	119	240	148	116	263	163
August	398	501	780	301	499	781	311	510	769
September	100	261	401	110	270	400	120	268	389

So, S₁, S₂ are 0, 100 and 200 kg S fed⁻¹
 P₀, P₁, P₂ are 0, 6.5 and 13 kg P fed⁻¹

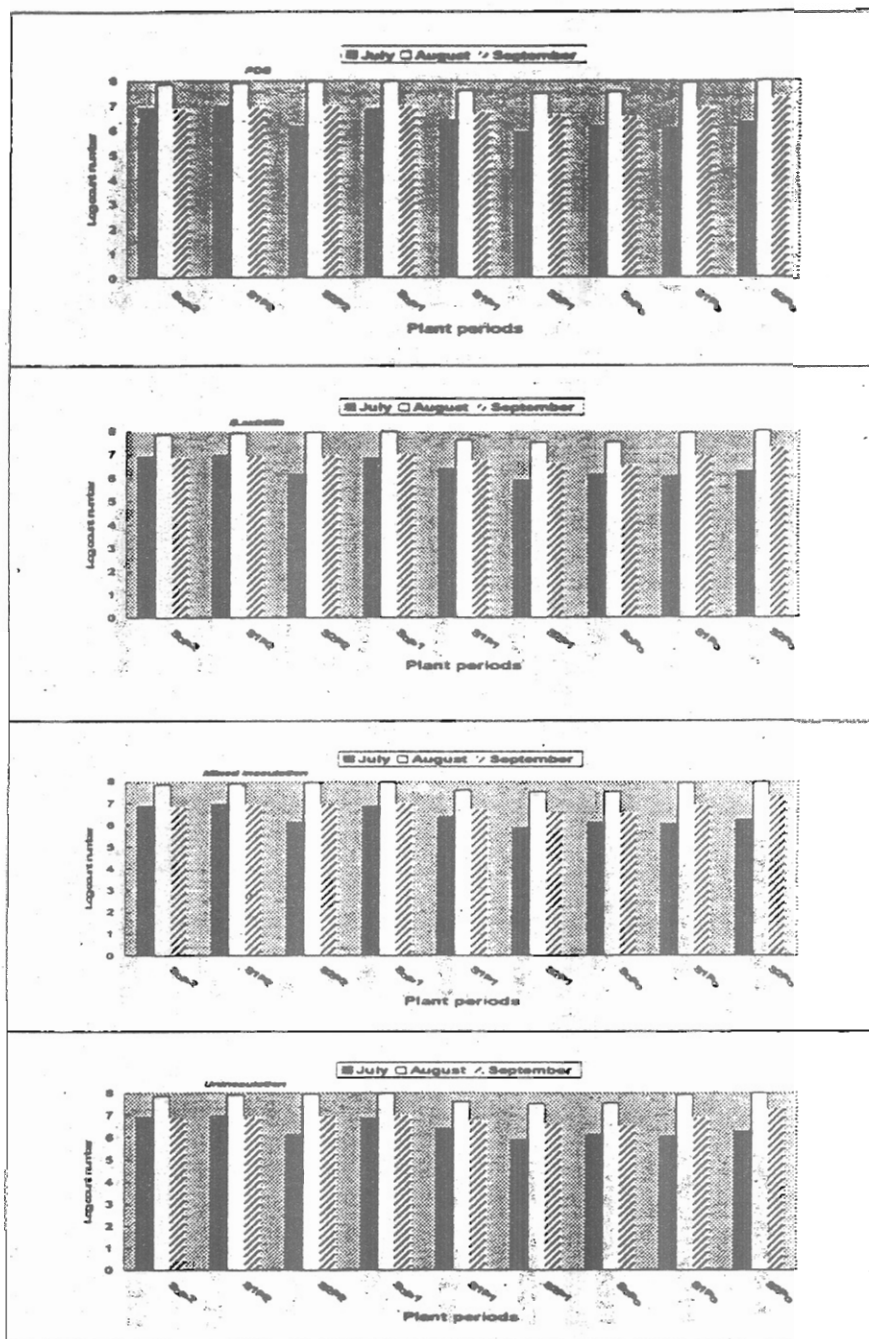


Fig (2): Changes in total phosphate dissolving bacteria count in soil maize rhizosphere plant through different planting periods of maize (log. c.f.u.g⁻¹ oven dried soil initial T PDB in soil was (4.54) log cycle.

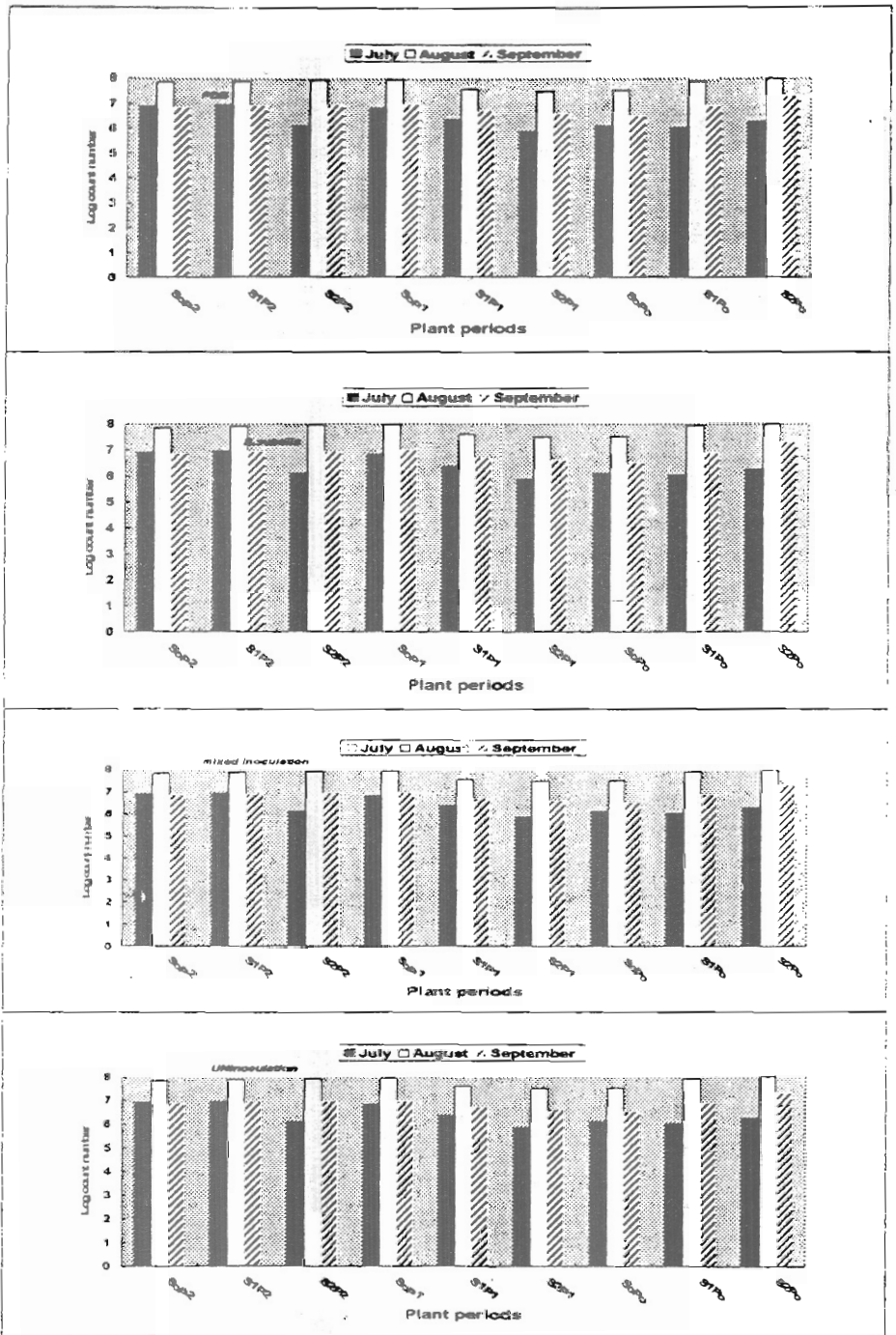


Fig (3): Changes in *B. subtilis* count in soil maize rhizosphere plant through different planting periods of maize (log. c.f.u.g⁻¹ oven dried soil initial *B. subtilis* in soil was (3.6) log cycle.

The present results show that phosphatase activity in maize rhizosphere plants was enhanced concurrent with the growth of plants and these results are in harmony with Goerge et al. (2002) who noted that phosphatase activity in maize rhizosphere plants was increased due to the inoculation with the agroforestry bacterial species.

Data in Table (3) indicate that the heaviest fresh and dry shoots due to the application of biofertilizers combined with different rates of applied S and P. The values were (116.09, 75.24 & 55.25) for fresh weight of shoots for *Bacillus*, mixed inoculation and PDB and *A. niger*, respectively, in July against 87.9 for uninoculated attained with SoP_2 at the same time, then increased to reach (451.27, 435.05 and 451.27) in August for the same treatments against (245.9) for uninoculated treatment, while the values of dry weight of shoots were (24.41, 14.71 & 11.44) for the same treatments in July against 19.14 for uninoculated, then increased to reach (101.79, 109.07 & 76.07) in August and 151.92 for uninoculated. These results were in agreement with Mahfouz (2003) who demonstrated that inoculating the plants with biofertilizers resulted in the heaviest fresh and dry weights of them. It is clearly noticed here that uninoculated plants recorded higher fresh and dry weights than those given by the plants inoculated with PDB in some cases. This may be due to the presence of recommended dose of P, which is essential macronutrient for plant, and may be also referred to maize cultivars which use P more efficiently. El-Kherbawy et al. (2007) demonstrated that the magnitude of response to P fertilization varied markedly among varieties.

Results recorded in Table (3) reveal that fresh and dry weights of roots had the same trend of shoots. The highest values of fresh weight recorded were (18.33, 17.98, 17.8 & 13.14) for *Bacillus* $So P_2$, mixed inoculation $So P_2$, uninoculated S_1P_2 & PDB S_1P_1 , respectively, in July, while the lowest values were (7.78, 7.21, 6.41 & 5.12) for fresh weight of root of PDB $So P_1$, *Bacillus* S_1Po , mixed inoculation $So Po$ & uninoculated S_1P_1 , respectively, and (1.69, 1.49, 1.43 & 1.47) of the same abovementioned treatments.

Data in Table (4) find out that, inoculating the plant with *Bacillus* combined with inorganic P (SoP_2) gave the highest root volume mean values (15.3, 44.0, 95.0 ml^3) in both tested seasons due to July, August and September, respectively, in the absence of sulphur followed by PDB (13.0, 40.0 & 55.3) than mixed inoculation, which recorded its high values of root volume at the end of growing season in September as follows (84.3, 79.7 and 75.0) for S_1P_1 , SoP_2 and S_1P_2 , respectively.

Table (3): Effect of Sulphur, phosphorus and biofertilizer on fresh and dry weight of shoot and root of maize cultivated in calcareous soil

Sulphur (S)	S ₀						S ₁						S ₂					
	P ₀		P ₁		P ₂		P ₀		P ₁		P ₂		P ₀		P ₁		P ₂	
	sh	r	sh	r	sh	r	sh	r	sh	r	sh	r	sh	r	sh	r	sh	r
Phosphorus (P)																		
Microbial inoculation (M)	a - Fresh weight																	
a-PDB & <i>A. niger</i>	45.24	8.25	48.59	7.78	41.67	11.75	34.62	8.91	55.25	13.14	49.4	10.4	53.24	11.83	43.69	9.47	47.4	11.52
July	285.73	37.61	313.23	40.39	277.93	61.15	358.77	37.18	268.75	52.53	311.49	48.66	390.96	47.88	291.21	23.1	216.93	26.06
August		23.07		24.37		22.87		16.55		25.02		37.87		38.13		18.49		22.21
September																		
b- <i>B. subtilis</i>	37.4	9.48	42.02	8.55	116.09	18.33	21.42	7.21	32.11	8.76	66.25	13.23	27.12	9.15	52.3	11.96	45.19	11.55
July	389.26	42.77	261.7	38.25	451.27	71.54	273.36	49.55	208.24	59.85	255.3	26.99	407.83	57.44	286.04	32.98	187.67	14.6
August		18.61		39.1		90.97		35.22		35.4		34.57		23.8		35.96		52.92
September																		
c-Mixed inoculation	22.29	6.41	30.79	6.63	59.92	17.98	54.34	10.84	46.74	10.05	73.24	14.17	66.15	16.60	40.75	10.61	51.65	11.98
July	33.89	239.42	31.17	388.69	58.37	270.78	47.92	261.55	29.08	435.05	55.87	192.07	28.45	319.98	44.43	388.07	57.32	
August	274.64	47.00	20.56		48.69		55.97		42.44		47.04		33.97		52.14		19.79	
September																		
d-Uninoculated	60.60	10.93	54.22	9.58	87.9	16.78	44.85	12.9	37.54	5.12	73.22	17.80	37.24	7.61	58.2	8.33	61.84	13.63
July	260.46	31.68	276.78	59.00	245.9	30.63	263.91	30.8	330.28	41.31	160.79	21.15	357.35	54.04	343.33	60.40	227.50	26.72
August		28.76		17.74		24.42		28.76		30.32		20.40		38.84		19.99		20.93
September																		
	b - Dry weight																	
a-PDB & <i>A. niger</i>	10.14	1.72	10.61	1.69	8.61	2.40	6.53	1.77	10.33	2.67	10.74	2.31	11.44	2.70	10.37	2.17	10.23	2.25
July	44.57	6.10	28.87	10.90	54.72	17.15	78.36	9.06	43.52	10.40	66.10	9.68	76.07	11.01	35.19	4.56	59.08	4.81
August		17.0		18.76		15.29		13.36		16.87		28.98		20.15		14.12		15.01
September																		
b- <i>B. subtilis</i>	8.03	2.16	9.83	1.95	24.41	3.88	5.47	1.49	8.03	1.68	14.33	3.32	5.26	2.05	11.27	2.22	12.93	2.48
July	74.85	10.25	48.92	7.22	101.79	25.16	54.33	14.61	64.95	11.47	54.25	6.05	98.84	15.30	53.16	8.59	28.73	4.06
August		14.43		17.61		50.42		28.06		24.93		30.32		18.50		33.97		40.20
September																		
c-Mixed inoculation	5.88	1.48	6.45	1.54	11.59	3.24	10.75	2.40	8.49	1.91	14.71	2.88	13.51	3.15	9.03	2.11	9.56	2.10
July	75.36	7.34	40.16	6.35	92.16	18.67	55.85	14.23	65.30	8.22	109.07	12.89	44.59	10.45	63.75	11.11	78.31	12.42
August		34.06		14.56		30.50		26.19		26.92		37.50		33.05		43.26		13.89
September																		
d-Uninoculated	11.18	2.22	10.47	1.75	19.14	2.54	9.64	2.47	8.46	1.37	16.05	2.85	6.80	1.71	10.47	1.90	11.87	2.12
July	48.06	8.19	78.33	15.94	51.92	9.11	56.28	8.27	64.33	9.31	44.63	4.29	84.93	13.49	79.08	12.00	56.61	6.68
August		22.06		16.20		19.37		23.45		15.40		14.19		28.83		10.60		12.48
September																		

S₀, S₁, S₂ are 0, 100 and 200 kg S fed⁻¹

P₀, P₁, P₂ are 0, 6.5 and 13 kg P fed⁻¹

Sh= shoot

r= root

Table (4): Effect of Sulphur, phosphorus and biofertilizer on root volume (ml³) of maize cultivated in calcareous soil

Sulphur (S)	S ₀			S ₁			S ₂		
Phosphorus (P)	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂
Microbial inoculation (M)									
PDB & <i>A. niger</i>									
July	8.7	8.0	10.7	8.0	13.0	11.7	11.0	9.3	9.7
August	18.7	26.0	40.0	23.0	35.0	28.7	28.3	12.0	13.7
September	55.3	42.0	44.5	44.3	47.7	65.3	55.0	48.3	60.0
<i>B. subtilis</i>									
July	10.7	10.0	15.3	6.3	7.3	12.7	8.3	11.7	11.7
August	23.7	22.7	44.0	28.7	29.3	19.0	34.3	19.7	16.0
September	28.3	44.3	95.0	39.5	56.7	53.7	40.3	55.7	58.7
Mixed inoculation									
July	7.3	7.0	7.0	11.3	8.7	9.3	12.7	9.7	10.0
August	16.0	18.3	30.7	23.3	18.7	36.0	20.0	24.0	38.8
September	62.3	41.3	79.7	71.0	84.3	75.0	54.7	68.3	42.3
Uninoculated									
July	10.0	7.3	12.0	7.0	6.7	11.3	6.7	9.7	9.7
August	19.7	38.5	19.5	18.7	19.0	21.5	28.3	34.7	14.7
September	60.0	45.7	52.0	43.0	48.7	45.0	60.3	39.3	45.3

So, S₁, S₂ are 0, 100 and 200 kg S fed⁻¹
 Po, P₁, P₂ are 0, 6.5 and 13 kg P fed⁻¹

Bacillus subtilis recorded the lowest values of root volume in the absence of P in July (6.3 ml³) with S₁P₀ (28.3 ml³) with SoP₀ in September. However, the inoculation of the plants with biofertilizers in presence or absence of different doses of nitrogen and phosphorus chemical fertilizers increased the root volume compared to uninoculated plants. These results are in harmony with Badran et al. (2002&2003) who indicated that inoculation of anise plants with biofertilizers either in the presence or absence of inorganic N & P fertilizers increased significantly the number of branches compared to the uninoculated ones.

The effect of S, P and biofertilizers on some yield components of maize cultivated in calcareous soil are illustrated in Table (5). Results showed that gradual increases in different parameters were observed when different levels of P were applied. Increasing P₂O₅ levels, lead to significant increase in values of the tested maize parameters. Owing to the interaction between levels of P₂O₅ and bacterial inoculation, results also indicated that full dose of P (P₂) in combination with mixed inoculation recorded (21.9, 23.5, 21.0 cm) with So, S₁, S₂, respectively, for cobs lengths followed by PDB and *A. niger* (21.8, 23.0, 19.5 cm) with the same treatments of sulphur.

One hundred-grain weight (g) as an indicator for grain filling had the same trend of cobs length and recorded (37.6, 39.2, 39.9 g) for mixed inoculation followed by (34.8, 36.3, 39.8 g) for PDB & *A. niger*. It was noticed that uninoculated maize plants had recorded higher values than those obtained due to *B. subtilis* inoculation either in absence of S₀ or with half dose (S₁). Results also showed that PDB & *A. niger* as phosphate solubilizers or the mixed inoculation increased 100-grain weight significantly. Mixed inoculation was superior to PDB & *A. niger* alone. Data are confirmed by the work of Mukherjee and Bagde (2004) on rice.

Table (5): Effect of Sulphur, phosphorus and biofertilizer on some yield components of maize cultivated in calcareous soil

Sulphur (S)	So			S ₁			S ₂		
	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂
Microbial inoculation (M)	Length of cobs (cm)								
a- PDB & <i>A. niger</i>	20.0	21.0	21.8	20.7	21.5	23.0	16.0	19.5	19.5
<i>B. subtilis</i>	18.0	18.7	20.7	20.8	20.7	22.7	17.4	17.8	18.0
Mixed inoculation	19.5	20.3	21.9	22.5	22.2	23.5	18.0	19.8	21.0
d-Uninoculated	18.3	19.5	20.2	20.5	20.3	21.8	17.2	17.5	19.2
	100-grain weight (g)								
a-PDB & <i>A. niger</i>	35.2	34.7	34.8	38.0	36.1	36.3	37.7	38.9	39.8
b- <i>B. subtilis</i>	30.4	32.7	33.0	33.6	35.2	36.6	37.6	38.9	40.9
c-Mixed inoculation	36.5	38.1	37.6	37.9	38.4	39.2	39.4	40.5	39.9
d-Uninoculated	33.5	33.7	33.6	34.1	34.4	38.4	34.3	36.9	38.4
	Dry weight of grains (ton fed ⁻¹)								
a-PDB	2.70	2.76	2.81	2.96	3.18	3.38	2.56	3.04	3.27
b- <i>B. subtilis</i>	2.28	2.63	2.80	2.40	3.10	3.21	2.43	2.93	3.06
c-Mixed inoculation	2.81	3.01	2.87	3.34	3.52	3.83	3.08	3.11	3.29
d-Uninoculated	2.21	2.55	2.72	2.40	2.58	2.73	2.44	2.73	2.84
	Dry weight of stover (ton fed ⁻¹)								
a-PDB & <i>A. niger</i>	3.93	4.12	4.41	4.90	5.30	5.80	3.87	4.07	4.31
b- <i>B. subtilis</i>	3.57	3.65	4.11	4.23	4.74	5.40	3.50	4.00	4.05
c-Mixed inoculation	5.01	5.39	5.62	6.00	6.68	7.80	4.04	5.00	5.41
d-Uninoculated	3.39	3.72	3.88	4.10	4.34	4.97	3.18	3.36	3.52
	Harvest index of maize								
a-PDB & <i>A. niger</i>	40.71	40.11	38.97	37.68	37.49	36.86	39.78	42.73	43.11
b- <i>B. subtilis</i>	39.02	41.95	40.32	36.24	39.53	37.27	41.00	42.34	43.18
c-Mixed inoculation	35.85	35.95	33.89	35.21	34.54	32.90	43.25	38.35	38.25
d-Uninoculated	39.43	40.64	41.22	36.90	36.94	35.51	43.33	44.80	44.66
L.S.D. at 0.05 levels									
	M	S	P	M x S	M x P	S x P	M x S x P		
1- Length of cobs (cm)	0.84	0.75	0.59	n.s	n.s	n.s	n.s		
2- 10 grain weight (g)	1.16	1.36	0.93	n.s	n.s	n.s	n.s		
3- dry weight of grains ton fed ⁻¹	0.15	0.10	0.10	0.21	0.20	0.17	0.34		
4- dry weight of straw ton fed ⁻¹	0.09	0.09	0.08	0.18	0.15	0.13	0.26		
5- harvest index	-	0.56	0.47	1.12	0.94	0.81	1.36		

These results are similar to those obtained by Shah et al. (2001) who reported that P application and bacterial inoculation affect yield of soybean through their effects on phosphorus use efficiency. Abo El-Naga et al. (2005) reported that the highest amount of mung bean seeds and straw yields were obtained by *B. megatherium* inoculation in the presence of K fertilizer at 48 kg fed⁻¹. Also, Mohamed and Abbas (2005) used different nitrogen sources under P-biofertilizer and microelements and they recorded 7 & 12% and 42 & 31.6% increases in faba bean seed and straw yields during both tested successive seasons, respectively.

Harvest index (the percentage of grains to whole plant) as shown in Table (5) reveal that PDB either singly or mixed with *B. subtilis* (mixed inoculation) encouraged shoot growth more than grains where the percentage of grains was reduced significantly in that two treatments than that of uninoculated plants. Sulphur showed an irregular trends 100 kg S (S₁), since it increased the harvest index significantly than that of S₀. It was also narrowed significantly by adding S₂ than both S₀ and S₁. The opposite was true in case of P application; the highest harvest index was obtained the use of S₂ combined with either P₁ or P₂ without inoculation, while the lowest one was of that received S₁P₂ and mixed inoculation. The current results are similar to those of El-Maddah et al. (2005) on wheat. The highest yield of grains and stover resulted from the mixed inoculation S₁P₂. The lowest values of grain yield attained due to the treatment, which didn't receive any addition.

At the end of the growing season, samples of grains and straw were analyzed for their content of N, P and K%. Table (6) show that mixed inoculation followed by *B. subtilis* inoculation resulted in higher quantities of stover N gained at the end of life cycle of maize, which recorded 0.85 to 0.89% for mixed and from 0.83 to 0.88% for *B. subtilis* over the uninoculated from 0.81% to 0.86%, while the PDB and *A. niger* inoculation recorded less values than the other types of inoculation except for the case of the absence of P either with S₁ or S₂ dose of sulphur. Nitrogen content of grains had the same trend and recorded (1.18-1.26) for mixed inoculation and from 1.11 to 1.17% for *B. subtilis*. Also, PDB & *A. niger* had more N content in grains than *B. subtilis* in the absence of P. These results are in full agreement, with El-Shahawi (1995); Soliman et al. (2003) and Mansour et al. (2006) who suggested that in wheat cultivation all inoculated treatments showed significant increases in both grain and straw yields compared to uninoculated treatments irrespective of inorganic N fertilizer levels. Data in Table (6) also revealed that biofertilizer inoculation increased greatly the N&K contents in maize grain and stover with corresponding highest percentages of P due to the use of mixed inoculation that recorded from 0.37 to 0.40% and from 0.28 to 0.36 for grain & stover, respectively, followed by PDB and *A. niger* inoculation.

Consequently, the highest K percentages of (0.79-0.86) and (0.98 to 1.07) in respective to both grain & stover had followed same trend as in P% in response to same treatment.

Table (6): Effect of Sulphur, phosphorus and biofertilizer on N, P, and K in grain and stover of maize cultivated in calcareous soil

Sulphur (S)	S ₀						S ₁						S ₂					
	P ₀		P ₁		P ₂		P ₀		P ₁		P ₂		P ₀		P ₁		P ₂	
Phosphorus (P)	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver	Grain	Sto-ver
Microbial inoculation (M)	Total nitrogen (N%)																	
PDB & <i>A. niger</i>	1.14	0.82	1.13	0.83	1.13	0.82	1.22	0.91	1.16	0.86	1.14	0.85	1.22	0.92	1.17	0.88	1.18	0.86
<i>B. subtilis</i>	1.16	0.84	1.17	0.83	1.16	0.83	1.11	0.85	1.13	0.86	1.12	0.85	1.17	0.86	1.15	0.86	1.17	0.88
Mixed inoculation	1.18	0.85	1.21	0.85	1.20	0.84	1.20	0.88	1.22	0.87	1.23	0.87	1.22	0.88	1.25	0.89	1.26	0.89
d-Uninoculated	1.02	0.81	1.17	0.83	1.15	0.83	1.08	0.85	1.12	0.83	1.18	0.84	1.13	0.82	1.11	0.85	1.20	0.86
	Total phosphorus (P%)																	
PDB & <i>A. niger</i>	0.36	0.32	0.36	0.32	0.37	0.32	0.38	0.33	0.37	0.32	0.37	0.32	0.38	0.33	0.39	0.34	0.39	0.37
<i>B. subtilis</i>	0.36	0.26	0.37	0.29	0.35	0.32	0.35	0.32	0.37	0.30	0.35	0.31	0.36	0.32	0.36	0.32	0.40	0.38
Mixed inoculation	0.37	0.28	0.37	0.30	0.37	0.31	0.37	0.31	0.38	0.31	0.39	0.32	0.38	0.31	0.40	0.33	0.39	0.36
d-Uninoculated	0.34	0.22	0.36	0.22	0.37	0.29	0.34	0.25	0.36	0.29	0.35	0.29	0.35	0.27	0.38	0.30	0.35	0.31
	Total potassium (K%)																	
PDB & <i>A. niger</i>	0.73	1.03	0.70	1.02	0.68	1.03	0.81	1.04	0.76	1.02	0.75	1.06	0.82	1.04	0.78	1.09	0.76	1.12
<i>B. subtilis</i>	0.74	0.99	0.74	1.03	0.75	1.02	0.75	1.04	0.76	1.04	0.75	1.06	0.76	1.03	0.76	1.05	0.78	1.10
Mixed inoculation	0.81	0.98	0.80	1.01	0.79	1.02	0.81	1.03	0.83	1.04	0.82	1.03	0.84	1.05	0.86	1.06	0.86	1.07
d-Uninoculated	0.71	0.83	0.73	0.99	0.73	0.99	0.72	0.90	0.73	1.02	0.74	1.03	0.74	0.93	0.75	1.03	0.76	1.06

S₀, S₁, S₂ are 0, 100 and 200 kg S fed⁻¹P₀, P₁, P₂ are 0, 6.5 and 13 kg P fed⁻¹

Uninoculated treatments had the lowest content of N, P and K% in grain and stover. These results are in harmony with Rashad et al. (2001) who reported that *A. chroococcum*, *B. megatherium* and *B. cerculanse* may produce growth promoting substances such as auxins and stimulating the microbial development, which is reflected on the plant nutrient uptake. Also, Farid (2003) added that *Azorhizobia* and many bacteria strains act as plant growth promoting *rhizobacteria* and improve macronutrients (N, P and K) and water uptake. Shady et al. (2007) suggested that faba bean inoculation with biofertilizer increased greatly the N, P & K contents in grain and straw and its effect was highly significant. Soil microorganisms have enormous potential in providing soil phosphates for plant growth. Phosphorus biofertilizers increased the availability of accumulated phosphates for plant growth by solubilization (Gyaneshwar et al., 1998). In addition, the microorganisms involved in P solubilization as well as better scavenging of soluble P can enhanced plant growth by increasing the efficiency of biological nitrogen fixation, enhancing the availability of other trace elements and by production of plant growth promoting substances (Gyaneshwar et al., 1998).

Data presented in Table (7) showed that microbial inoculation hadn't a marked effect on soil salinity. Sulphur application raised soil salinity by 0.64 and 1.05 dS/m upon increasing its rates to 100 and 200 kg fed⁻¹, respectively, as compared with plots. which didn't receive S. The increases in salinity were negligible, where 0.25 dS/m only was the increase due to adding 13kg P fed⁻¹. Sameni and Kasraian (2004) concluded from their work on calcareous soils that EC values were well accorded with applications of sulphur.

Soluble anions were free of carbonates. The dominant anion was sulphate followed by chloride. Bicarbonates were the least in the current findings are in agreement with those obtained by Kalocsal et al. (2003).

Divalent Ca²⁺ and Mg²⁺ were generally higher than monovalents soluble ones i.e. (Na⁺ and K⁺). Calcium dominated Na⁺ in the treatments, which received sulphur. Potassium was found in soil solution regardless of the soil treatments.

It could be seen from Table (8) that soil pH values reveal no significant changes due to inoculation and /or P application rates. The decrease occurred in soil pH values was more pronounced due to the treatments which received sulphur at a rate of 200 kg S fed⁻¹ then those which received sulphur at a rates of 100 kg S fed⁻¹. This emphasizes the important role of sulphur addition in soil acidification due to sulphur oxidation to sulphate. These results are in agreement with Marcano et al. (2003), Kalocsal et al. (2003) and Sameni and Kasraian (2004) who applied elemental S to calcareous soils at rates higher than those used in the current work each other.

The concentrations of available P and K content in soil after crop harvesting also shown in Table (8). Microbial inoculation resulted in significant increases in available P and K than uninoculated treatments. The decreasing order in case of available K was mixed inoculation > PDB & *A. niger* > *B. subtilis* > uninoculated differences among them. Wan and Wong (2004) used PDB and found a significant increase in available P and

attributed that to the decrease of pH to 6.5 and El-Maddah et al. (2005) who explained that P-availability increased due to the effect of PDB.

Sulphur application at a rate of 100 or 200 kg fed⁻¹ increased available P and K significantly. These results are in agreement with Sequera and Ramirez (2003) and Sharma (2003) who found a decrease in pH by adding S that increased available P in soil. Also the higher rate of P (P₂) increased available P in soil over the lower rate (P₁ and P₀) with significant difference between each other. The highest value of available P was obtained by the treatment which received mixed inoculation combined with S₂P₂. The highest for available K was given by PDB & *A. niger* combined with S₂, respectively. The lowest values of them were obtained from uninoculated treatments and didn't received S or P in case of available P and uninoculated treatments combined with P₂ in case of K. Rasal et al. (2004) used *Sordaria fumicola* and *A. terricus* spp. as phosphate solubilizing and sulphur oxidizing fungi and obtained increase in P availability EL-Shahawi (1995) and Solaiman et al. (2003) applied used PDB and *A. niger* inoculation and reported that there were increases in available K in calcareous soils.

Generally, it can be concluded that using biofertilizers play an important role in solubilizing phosphorus, releasing K and fix atmospheric nitrogen in addition to consume dose of inorganic N and P fertilizers. Sulphur inoculated with PDB and *A. niger* is more efficient than gypsum in the displacement of sodium and other cations from the soil complex to leaching solution as indicated by Stamford et al. (2002). A substantial amount of inorganic P fertilizer could be saved by inoculation, which in turn minimizes the production costs. Generally to obtain high quality and quantity of maize yield under such calcareous soil without harmful degradation of soil, it could be recommended the use of mixed biofertilizer in adjacent with 100 kg S fed⁻¹ and 13 kg P fed⁻¹ in maize production.

Table (7): Effect of Sulphur, phosphorus and biofertilizer on EC, SP, Soluble anions and cations in studied soil after maize growth season

Microbial inoculation (M)	Sulphur	Phosphorus	EC	SP	Soluble anions (meq/l)				Soluble cations (meq/l)			
					CO ₃ ²⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺
PDB & <i>A. niger</i>	S ₀	P ₀	1.70	40	-	2.63	5.76	8.64	8.20	3.16	5.25	0.42
		P ₁	1.71	40	-	2.38	6.73	7.99	6.87	4.29	5.21	0.73
		P ₂	2.05	39	-	2.33	8.53	9.47	8.32	4.16	7.80	0.55
	S ₁	P ₀	2.64	39	-	2.75	10.32	13.05	10.88	5.68	8.96	0.60
		P ₁	2.47	38	-	2.83	10.05	12.07	10.32	5.06	8.83	0.74
		P ₂	3.35	39	-	2.50	13.02	18.44	15.53	6.93	10.98	0.52
	S ₂	P ₀	3.07	40	-	2.56	11.24	17.09	13.45	5.63	11.13	0.56
		P ₁	3.35	39	-	2.50	13.03	18.44	15.53	6.93	10.88	0.52
		P ₂	3.30	40	-	2.75	12.56	17.73	15.60	5.66	11.22	0.56
<i>B. subtilis</i>	S ₀	P ₀	1.78	39	-	2.50	7.70	7.65	6.85	4.15	6.29	0.62
		P ₁	1.95	39	-	2.33	7.54	9.88	8.31	4.33	6.49	0.62
		P ₂	2.09	40	-	3.00	8.13	9.83	7.53	4.51	8.46	0.46
	S ₁	P ₀	2.39	38	-	2.58	9.66	11.73	9.98	4.66	8.48	0.85
		P ₁	2.68	38	-	2.50	11.61	12.89	11.20	6.19	8.93	0.69
		P ₂	2.31	39	-	2.50	9.10	11.54	9.25	5.40	8.00	0.49
	S ₂	P ₀	3.15	38	-	2.25	10.68	19.02	14.67	5.36	10.88	1.04
		P ₁	3.01	38	-	2.50	11.55	16.07	13.18	6.03	10.20	0.71
		P ₂	2.93	39	-	1.88	11.16	16.69	12.48	5.50	11.25	0.50
Mixed inoculation	S ₀	P ₀	2.08	38	-	2.67	8.24	10.01	8.27	4.21	8.03	0.50
		P ₁	2.18	39	-	2.75	9.11	10.29	9.00	3.72	8.79	0.64
		P ₂	2.25	39	-	2.50	9.59	10.74	9.15	4.06	8.97	0.65
	S ₁	P ₀	2.77	39	-	2.08	12.83	12.91	12.37	4.03	10.78	0.64
		P ₁	2.60	38	-	2.42	10.96	12.87	10.37	3.98	11.17	0.73
		P ₂	2.68	39	-	2.88	11.17	13.05	11.83	4.79	9.83	0.65
	S ₂	P ₀	2.88	38	-	2.25	11.40	15.35	14.00	4.12	10.47	0.41
		P ₁	3.03	39	-	2.50	12.94	15.18	14.53	3.73	11.71	0.65
		P ₂	3.27	39	-	2.83	13.33	16.86	14.80	5.66	11.81	0.75
uninoculated	S ₀	P ₀	1.87	39	-	2.67	8.72	7.53	6.85	4.30	7.32	0.50
		P ₁	1.86	38	-	2.42	8.09	8.35	6.80	3.50	8.03	0.50
		P ₂	2.48	40	-	2.50	10.93	11.82	10.80	4.70	9.02	0.76
	S ₁	P ₀	2.32	38	-	2.17	7.81	13.30	9.30	4.28	8.92	0.75
		P ₁	2.40	39	-	2.58	8.81	12.97	10.15	5.17	8.06	0.60
		P ₂	3.08	39	-	2.50	12.61	15.74	12.81	5.55	12.00	0.47
	S ₂	P ₀	2.45	39	-	2.67	8.32	13.52	10.32	5.20	8.31	0.67
		P ₁	2.85	40	-	2.50	10.93	11.80	10.80	4.67	9.02	0.77
		P ₂	3.36	40	-	2.38	12.52	18.82	13.98	6.80	12.45	0.52

Table (8): Effect of Sulphur, phosphorus and biofertilizer on soil available P and K and pH values after maize harvesting

Sulphur (S)	S ₀			S ₁			S ₂		
	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂	P ₀	P ₁	P ₂
Phosphorus (P)									
Microbial inoculation (M)	1- Available P (ppm)								
d- PDB & <i>A. niger</i>	9.0	12.8	13.8	12.3	14.7	19.3	16.2	18.3	21.2
<i>B. subtilis</i>	10.5	13.5	17.3	11.8	15.5	20.4	14.7	19.3	21.3
Mixed inoculation	11.0	12.3	14.8	12.2	15.0	20.3	15.3	15.5	22.3
d-Uninoculated	8.1	10.7	12.3	8.8	11.6	13.4	10.3	11.8	14.0
	2- Available K (ppm)								
a-PDB & <i>A. niger</i>	311	350	356	454	393	318	425	374	260
b- <i>B. subtilis</i>	312	359	245	353	307	315	451	264	251
c-Mixed inoculation	417	354	430	386	356	389	381	417	407
d-Uninoculated	280	223	220	312	276	283	406	237	225
	3- pH values								
a-PDB & <i>A. niger</i>	8.05	8.10	8.07	7.98	7.91	7.86	7.63	7.68	7.68
b- <i>B. subtilis</i>	8.14	8.06	8.13	7.91	7.92	7.89	7.67	7.73	7.83
c-Mixed inoculation	8.07	8.01	8.08	7.84	7.82	7.86	7.76	7.77	7.73
d-Uninoculated	8.09	8.06	8.04	7.91	7.90	7.88	7.69	7.73	7.75
L.S.D. at 0.05 levels									
	M	S	P	M x S	M x P	S x P	M x SxP		
1- Available (P)	0.80	0.35	0.42	0.70	0.84	0.73	1.45		
2- Available (K)	17.05	8.47	5.39	16.94	10.78	9.34	18.67		
3- pH values	n.s	0.04	n.s	0.08	0.07	0.06	0.12		

REFERENCES

- Abdallah, A. R., T. H. El-Dahtory, A. A. Abdel El-Moneim and M. S. A. Safwat (1984). Effect of inoculation with *Bacillus megatherium* var *phosphaticum* and root nodules bacteria on rhizosphere microflora and yield of some leguminous plants. Minia J. Agric. Res. Dvelop., 6:521-539.
- Abdel-Hafez, A. M. (1966). Some studies on acid producing microorganisms in soil rhizosphere with special reference to phosphate dissolvers. Ph.D. Thesis. Fac. Agric. Ain Shams Univ., Cairo, Egypt.
- Abo El-Naga, M. H. M., E. El-Abbas and W. I. A. Saber (2005). Effect of some mechanical sowing methods, *Bacillus megatherium* inoculation and potassium fertilizer rates on the productivity of mung bean crop. Zagazig J. Agric. Res., 32:1727-1742.
- Arshad, M. and W. T. Frankenberger (1998). Plant growth regulating substances in the rhizosphere. Microbial production and functions. Adv. Agron. 62: 46-51.
- Azazy, M. A., Saber, M. S. M. and Boutros, N. B. (1988). The use of biofertilizers and conditions in citrus nurseries in relation to microflora contributing to soil fertility. Egypt. J. Microbiol., 23: 389-402.
- Badran, F. S., F. A. Attia and H. S. Soliman (2002). Effect of combining phosphorene with superphosphate and rock phosphate on seed and oil productivity of *Nigella sativa* L. plants. Proc. of 2nd inter. Conf. Hort. Sci. 10-12 Sept. 2002. Kafr El-Sheikh Tanta Univ., Egypt.
- Badran, F. S., F. A. Attia, E. T. Ahmed and H. S. Sabry (2003). Effect of chemical and biological fertilization on growth, yield and oil production of anise (*Pimpinella anisum* L). II- Effect of NP mineral/biofertilization and micronutrient treatments. First Egyptian Syrian Conference, El-Minia University & Al-Baath University on Agriculture & Food In the Arab World, 8-11 December, 2003. El-Minia Univ., Egypt.
- Black, C. A., D. D. Eins, J. J. White, L. E. Ensminger and F. E. Clark (1965). Methods of Soil Analysis. Amer. Soc. Agron. Inc., Madison, Wisconsin, U.S.A.
- Bunt, J. S. and A. D. Rovira (1955). Microbiological studies of some subantarectic soils. J. Soil Sci., 6:119-128.
- Chapman, H. D. and P. F. Pratt (1961). Methods of Analysis for Soil, Plants and Waters. Univ. Clif. Division of Agric. Science.
- Chen, O. J. and C. F. Chao (2003). Effect of adding acidifying materials to lower soil pH on the behaviors of P in calcareous soils. Taiwanese. J. Agric. Chem.&Food Sci., 41:113-123.
- Concepcion, S. and A. Delgado (2005). Phosphorus fractions and release patterns in typical Mediterranean soils. Soil Sci. Soc. Amr. J., 69: 607-615.
- Dick, W. A. and M. A. Tabatabai (1984). Kinetic parameters of phosphatases in soils and organic waste materials. Soil Sci., 137: 7-15.
- El-Kherbawy, M. I., Y. A. Abdel-Aal, Atiat A. B. Ali and M.A.M. El-Gallad (2007). Phosphorus use efficiency by wheat plants. Egypt J. Appl. Sci., 22: 655 – 669.
- El-Maddah, E. I., M. D. El-Sodany and E. A. Koreish (2005). Effect of *Azotobacter chroococcum* and phosphate solubilizing bacteria on wheat growth, productivity and nutrient availability. J. Agric. Sci. Mansoura Univ., 30: 2895-2908.

- El-Shahawi, A. M. B. (1995). A study on the soil integrated bacterial fertilization. M.Sc. Thesis. Fac. Agric. Dept. Microbiol. El-Mansoura University.
- Falik, E. and Y. Okon (1996). The response of maize (*Zea mays*) to *Azospirillum* inoculation in various types of soils in the field. World J. of Microbiol. and Biotech., 12: 511-515.
- Farid, S. F. B. (2003). Studies on Bio-organic fertilization of wheat under newly reclaimed soils. Ph.D. Thesis. Fac. of Agric., Cairo Univ., Egypt.
- Giovannetti, M. and B. Mosse (1980). An evaluation of techniques for measuring mycorrhizal infection in roots. New Phytol., 84: 489 – 500.
- Goerge, T. S., P. J. Greogry, M. Wood, Read, D. and A. G. Buresh (2002). Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. Soil Boil. & Biochem., 34: 1487-1499.
- Gyaneshwar, P. G. Naresh Kumar and L. J. Parekh (1998). Effect of buffering on the phosphate solubilizing ability of microorganisms. World J. Microbiol. Biotech., 14: 669-673.
- Hanna Mona, M., S. M. A. Kabeel and Darwesh, Fayza M. A. (2005). Effect of organic and biofertilizers on growth, yield and fruit quality of cucumber (*Cucumis sativua* L.) grown under polyethylene low tunnels. J. Agric. Sci., Mansoura Univ., 30: 2827-2841.
- Kalocsai, R.; T. Foldes, R. Schmidt and I. Szakal (2003). Effect of elemental sulphur application and fertilization on the pH and SO_4^{2-} content of soil in an incubation experiment. Agrokemia es Talajtan. 52, 121-132 (c.i. Soil and Fert., 2004, 67: Abst.1473).
- Khavazi, K., H. Besharati, F. Nourgholipour and M. J. Malakouti (2003). Effect of *Thiobacillus* bacteria on increasing phosphorus availability from phosphate rock for corn grown on the calcareous soils of Iran. Proceedings of International Meeting, Kuala Lumpur, Malaysia, 16-20 July, 2001, 280-284.
- King, E.O., M. K. Ward and D. E. Raney (1954). Two simple media for the demonstration of phycocyanin and flourescein. J. Lab. Clin. Med., 44:301-307.
- Lazarovits, G. and S. Norwak (1997). *Rhizobacteria* for improvement of plant growth and establishment. Hort. Sci., 32:188-192.
- Mahfouz, S.A.S.(2003). Effect of biofertilization on growth and oil production of marjoram (*Majorana hortensis* Moench.) plant. Ph.D. Thesis. Fac. Agric., Cairo University.
- Mahmoud, M. K. M. (2000). Studies on biological nitrogen fixation in *Sesbania*. M.Sc. Thesis, Microbiol., Fac. Agric. Mansoura Univ., Mansoura, Egypt.
- Mansour, S. M., Nadia A. A. Ali, W. I. A. Saber and Kh. M. Ghanem (2006). Improvement of growth yield and root colonization of wheat cultivated in salt affected soil inoculated by *Azotobacter* and *Azospirillum* with mineral nitrogenous fertilizer. J. Agric. Sci. Mansoura Univ., 31: 5297-5311.
- Marcano, C.A.E., J. C. Rodriguez and M. Mohammed (2003). The effect of elementary sulphur on the pH and solubility of some nutrients in phosphocoposts. Interciencia, 28: 504-511.
- Misra, S. K. (2003). Effect of sulphur and potassium on yield, nutrient uptake and quality characteristics mycorrhizal and nonmycorrhizal of mustard in Vdic Haplusteps of Kanpur. J. Ind. Soc. Soil Sci., 51: 544-548.
- Mohamed, M. R. and El-El-Abbas (2005). Response of three faba bean cultivars (*Vicia faba* L.) to different nitrogen sources under P-biofertilizer and micronutrients addition. J. Agric. Sci. Mansoura Univ., 30: 8277-8292.

- Mukherjee-Bhattacharya, R. and U. S. Bagde (2004). New biofertilizer: Fusants of *Azotobacter vineladii* and *Pseudomonas putida* on growth and yield of rice plants. *J. Interacademia*, 8: 146-154.
- Norman, M., C. Reardon and P. Searle (1995). *The Ecology of Tropical Food Crops*. Cambridge University Press, Cambridge, London.
- Olayinka, A. and V. Ailenubhi (2003). Microbial respiration, maize (*Zea mays* L.) growth and nitrogen uptake as affected by cow dung and ammonium sulphate applications. *Nigerian J. Soil Res.*, 4:16-24.
- Rao, A. V. and J. C. Tarafder (1992). Seasonal changes in available phosphorus and different enzyme activities in arid soils. *Ann. Arid Zones*. 31:185-189.
- Rasal, P. H. B. Sangale and K. B. Pawar (2004). Effects of phosphate solubilizing and sulphur oxidizing microorganisms on yield and phosphorus uptake of soybean. *J. of Maharashtra Agric. Univ.*, 29: 51-53.
- Rashad, M. H. ; A. A. Ragab and S. M. Salem (2001). The influence of some *Bradyrhizobium* and *Rhizobium* strains as plant growth promoting *rhizobacteria* on the growth and yield of sorghum (*Sorghum bicolor* L.) plants under drought stress. *Plant Nutrition-Food Security and Sustainability of Agro.5*.
- Rashad, Nahed M.M. (2002). Effect of fertilization on the growth and storability of some aromatic plants. M.Sc. Thesis, Fac. Agric., Kafr El-Sheikh, Tanta University.
- Robles, C. and Berea, J. M. (2004). Response of plant and soil to *Glomus intraradices* and *rhizobacteria* inoculation of maize under intensive cultivation. *Terra*, 22: 59-69.
- Sameni, A. M. and A. Kasraian (2004). Effect of agricultural sulphur on characteristics of different calcareous soils from dry regions of Iran. 1- Disintegration rate of agricultural sulphur and its effect on chemical properties of the soil. *Comm. In Soil Sci. and Plant Analysis*. 35: 1219-1234.
- Sequera, O. and R. Ramirez (2003). Phosphorus, calcium and sulphur availability from acid rock phosphate with sulphuric acid and ammonium thiosulphate. *Interciencia*, 28: 604-610.
- Shady, T. S. M., W. I. A. Saber, S. M. Mansour and Nadia A. A. Ali (2007). Enhancement of nodulation, growth and yield of faba bean inocula with *Rhizobium* and pectinases, cellulases and chitinases producing *Pseudomonas* sp. *Egypt. J. Appl. Sci.*, 22: 105 - 124.
- Shah, P., K. M. Kakar and K. Zado (2001). Phosphorus use efficiency of soybean as affected by phosphorus application and inoculation. In (W.J.Horst. Eds.). *Plant Nutrition- Food Security and Sustainability of Agroecosystems*. Pp 670-671.
- Sharma, S. N. (2003). Effect of phosphate solubilizing bacteria on the efficiency of Mussoorie rock phosphate in rice-wheat cropping system. *Ind. J. Agric. Sci.*, 73: 478-481.
- Singh, J., Deshmukh, M. S. and N. R. Tandulkar (2004). Direct and residual effects of sulphur in cotton-wheat cropping system in sandy loam soil. *Fertilizer News*. 49: 61- 63.
- Snedscor, G. W. and W. G. Cochran (1976). *Statistical Methods* 6th Ed. Iowa State Univ. Press., Amr., Iowa. USA.
- Soliman, B. M., Nadia A. A., Ali and S. M. Ahmed (2003). Response of balady orange trees productivity and some microbial activity to biofertilizer in reclaimed soil. Eleventh Conf. of Microbiol., Cairo. Egypt. Oct.12-14, pp.108-122.

- Stamford, N. P., A. J. N. Silva, A. D. S. Freitas and J. T. Araujo Filho (2002). Effect of sulphur inoculated with *Thiobacillus* on soil salinity and growth of tropical tree legumes. *Bioresource Technol.*, 81: 53-59.
- Tabatabai, M. A. and J. M. Bremner (1969). Use of P-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.*, 1: 301-307.
- Wan, J. H. C. and M. H. Wong (2004). Effects of earthworms activity and P-solubilizing bacteria on P availability in soil. *J. Plant Nutrition and Soil Sci.*, 167: 205-212.
- Withers, P. J. A., A. C. Edwards and R. H. Foy (2001). Phosphorus cycling in UK agriculture and implication for phosphorus loss from soil. *Soil Use and Management*. 17: 139-149.

تأثير التسميد الحيوي والكبريت والفوسفور المعدني على نبات الذرة المزروع في الاراضى الجيرية

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- اجريت هذه التجربة الحقلية لدراسة تأثير التسميد الحيوي بالبكتيريا المذيبة للفوسفات PDB وفطر الاسبرجيليس نيجر *Aspergillus niger* وبكتيريا *Bacillus subtilis* ذات الانشطة الحيوية الهامة في التربة وذلك اما في صورة لقاح منفرد أو خليط فيما بينهم، أو بدون التلقيح في وجود معدلات مختلفة من الكبريت العنصري والفوسفور المعدني على محصول الذرة المزروع في الاراضى الجيرية بقرية أبو مسعود جنوب الاسكندرية وذلك في موسمي ٢٠٠٥-٢٠٠٦. وتم تقييم معاملات التجربة المختلفة عن طريق تقدير العدد الكلي للميكروبات في التربة، العدد الكلي للميكروبات المذيبة للفوسفور وكذلك الاعداد الكلية لميكروبات *B. subtilis*، بالإضافة الى قياس نشاط انزيم الفوسفاتيز في التربة شهريا خلال الموسمين الزراعيين. تم أيضا تقدير بعض الصفات المتعلقة بالنبات كالاوران الرطبة والجافة لكل من المجموع الخضري والجذري، وحجم الجذور، وبعض الصفات المحصولية كطول القولحة، وزن ١٠٠ حبة، اوزان الحبوب والقش. كما تم تقدير النسبة المئوية لكل من N, P, K في الحبوب والقش وكذلك بعض صفات التربة المنزرعة بعد الحصاد.
- وكان من أهم النتائج المتحصل عليها زيادة الاعداد الكلية للمجاميع الميكروبية المختلفة في المعاملات الملقحة عن غير ملقحة، وزيادة نشاط انزيم الفوسفاتيز خاصة في المعاملات الملقحة بمذبيبات الفوسفات أو اللقاح الخليط حتى الحصاد.
- لم تتأثر ملوحة التربة بالمعاملات المختلفة بينما أدت اضافة الكبريت الى خفض pH التربة، كما أدى التلقيح الميكروبي في وجود الكبريت الى زيادة الفسفور الميسر بالترربة ونقص البوتاسيوم الميسر بها.
- كما أدى التلقيح الخليط بالإضافة للكبريت بمعدل ١٠٠ كجم/فدان والفوسفور بمعدل ١٢ كجم/فدان الى أعلى نسبة من النيتروجين والفوسفور والبوتاسيوم في النبات بالإضافة الى أعلى محصول حبوب وحطب الذرة.