

Maize (*Zea mays*) biofertilization with *B. megaterium* and chemo-fertilization with rock or soluble P: Available N,P,K,Fe,Mn,Zn and Cu residual in soil after harvest.

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ABSTRACT

Application of P-biofertilizer (*Bacillus megaterium*) and P-chemical fertilizers (rock phosphate and superphosphate) with and without organic farmyard manure was studied in a 3-factor field experiment on maize (*Zea mays*). Factors being P-biofertilizer(B), P-chemical fertilizer(P) and organic manure(M). Treatments being: B₀ (no-biofertilizer), and B₁ (P-dissolving *B. megaterium* bacteria), P₀ (no P), P₁ (rock phosphate 70 kg P ha⁻¹) and P₂ (calcium superphosphate 35 kg P ha⁻¹), M₀ (no manure), M₁ (24 Mg ha⁻¹) and M₂ (48 Mg ha⁻¹). Treatment not receiving any material gave 3.62 Mg grains ha⁻¹. Applying any or more of the materials increased grain yield by as low as 1% by B₀P₀M₁ to 13% by B₀P₀M₂, up to 87% by B₁P₂M₀ and as high as 129% by B₁P₂M₂. Increase main effects were: B = 40.4%, P = 25.6% for P₁ and 20.0% for P₂; although P₂ surpassed P₁ in presence of the biofertilizer which enhanced soluble P-fertilizer. M = 5.1% for M₁ and 20.0% for M₂ effective in presence or in absence of the biofertilizer. N,P,K,Fe,Mn,Zn and Cu residual in soil after harvest increased were by up to 38, 84, 42, 112, 269, 78, and 83% for each respectively; and highest available contents were 91, 9.8, 308, 4.94, 1.55, 1.51 and 0.42 mg kg⁻¹ for each respectively.

Key words : Biofertilizer, *B. megaterium*, *Zea mays*, Rock P., Organic manure.

Introduction

Biofertilizers are preparations of living cells or latent cells of microorganisms which are applied to the soil-plant system and function actively causing plant nutrients to be available to plant roots (Wu *et al.*, 2005). They can have significant effect in enhancing crop productivity through. There are biofertilizers of microorganisms' inocula specialized for availability of certain plant nutrient(s). Microorganisms used for increasing P availability in soil include *Bacillus megaterium* bacteria (Matson *et al.*, 1977 and Bakhshandeh and, 2015). Soluble phosphate fertilizers are usually used for P application to crops. Despite phosphorus being widely and abundantly present in soils as inorganic and organic forms, many soils have it mainly in insoluble (or difficultly soluble) forms, even following its application as soluble phosphates (Miller and Donahue, 1997), since it is transformed into insoluble forms (fixed) in most soils particularly calcareous and heavy clay soils due to different mechanisms (Di *et al.*, 2013). For these reasons persistent application of soluble forms of orthophosphates is practiced by farmers.

Application of biofertilizers in agriculture, including P-dissolvers, could be practically applicable under field conditions for many crops (Abdel-Salam, 2014) including maize (Abdel-Salam *et al.*, 2012), faba beans (Abdel-Salam *et al.*, 2014) and sunflower (Abdel-Salam *et al.*, 2015). Biofertilizers such as phosphate dissolving bacteria (e.g. *B. megaterium*) would help in satisfying, partly at least, the needs of P to crops (Attia and Badr, 1999). The micro-organisms of the biofertilizers need nutrients and organic substrates in order to live and function actively in the soil, therefore there must be enough organic substances in the soil for their needs

(Berger *et al.*, 2013 and Abdel-Salam *et al.*, 2016). P-dissolving bacteria *Enterobacter agglomerans* were reported (Laheurte and Berthelin, 1988) to consume maize root exudates and promote plant growth. Phosphorus dissolving microorganisms release metabolites which include organic acids that dissolve insoluble phosphates and/or chelate cations bound them (Sagoe *et al.*, 1998). Chuanqing and Weiyi (2004) reported that 7 days after applying *B. megaterium* in suspension cultures, volatile and non-volatile organic acids were detected in the culture including lactic, succinic, malic, citric and acetic acids; all of which can dissolve and/or chelate metal ions, thus releasing P from phosphate rocks and causing solubilization of added rock phosphate. Hameeda *et al.*, (2008) inoculated maize seeds using 4 different P-dissolvers of *Enterobacter asburiae*, *E. cloacae*, *Serratia marcescens* and *Pseudomonas* sp and grew the crop in a field experiment on a P-difficient clay. They obtained grain yield increases of up to 85%. Veruil *et al.*, (2014) used *Pseudomonas tolaasi* and *P. korensis* inoculants on maize seeds and obtained up to 44% increase in grain yield; other traits were affected positively.

Inoculation of biofertilizers in combination with limited rates of rock phosphate or soluble superphosphate may prove practical in sustainable agriculture (Singhal *et al.*, 2012). The current study aims at assessing biofertilization of maize involving P-dissolving biofertilizer (PDB) with or without rock phosphate and soluble phosphate and the effect of organic manure.

Materials and Methods

This investigation concerns assessment of using biofertilizers involving PDB (*Bacillus megaterium* var. *phosphaticum*) to enhance response of maize to

rock phosphate, ordinary superphosphate and farmyard manure (FYM).

Moshtohor area, El-Qalubia Governorate, Egypt during 2014season . The crop was maize "*Zea Mays L.*" Hi-tec 20/31.The soil was a heavy clay *torrifluent* (Table 1), irrigated with Nile water (Table 2).Properties of fertilizers are in Tables 3 and 4.

3.1. The field experiment:

The field experiment was conducted at the Faculty of agriculture Moshtohor located at

Table 1. Properties of the soil of 0-30-cm soil of the experimental field of the Faculty of Agriculture Moshtohor Qalubia .

Property	Value	Property	Value
Particle size distribution		Soluble ions (mmolcL-1)	
Sand %	12.7	Ca ^{Y+}	4.7
Silt %	36.7	Mg ²⁺	2.2
Clay %	50.6	Na ⁺	6.8
Texture class	Heavy Clay*	K ⁺	0.3
Saturation percent (%)	65.2	HCO ₃ ⁻	1.3
Moisture parameters		Cl ⁻	8.2
Field capacity (% w/w)	41.2	SO ₄ ²⁻	4.5
Wilting point (% w/w)	22.3	Available NPK&Total P (mg kg ⁻¹)	
Available water(% w/w)	18.9	Available N	75
Chemical properties		„ P	8
Organic matter (g kg ⁻¹)	13.1	„ K	200
CaCO ₃ (g kg ⁻¹)	21.0	Total P	460
pH(1:25 w:v soil/ water)	8.2	* According to the International Soil Texture Triangle (Moeys, 2016).	
EC “paste extract” (dSm ⁻¹)	1.51	** Extracts are : KCl (for N) ; DTPA and NH ₄ HCO ₃ . (P,K).	
CEC (cmolc kg ⁻¹)	28.7		

Table 2. Properties of the Nile irrigation water (used for irrigating the maize crop).

pH	EC dSm ⁻¹	Soluble ions (mmolc L ⁻¹)							SAR
		Ca ²⁺	Mg ²⁺	Na ⁺	K ⁺	HCO ₃ ⁻	Cl ⁻	SO ₄ ²⁻	
7.20	0.56	1.90	1.10	2.00	0.99	1.21	3.02	1.76	1.33

SAR: Sodium adsorption ratio.

Table 3. Analysis of Ca-superphosphate (CSP), and rock phosphate (RP)

Source	P (g kg ⁻¹)	Contents of total micronutrients (mg kg ⁻¹)			
		Fe	Mn	Zn	Cu
CSP	65	26	147	180	4
RP	130	4088	254	793	11

Table 4. Aggregates diameter(mm) of superphosphate (CSP)and rock phosphate(RP).

Source	Aggregate diameter(mm)						
	10-2	2-1	1-0.5	0.5-0.25	0.25-0.125	0.125-0.063	<0.063
CSP	24.33	9.20	18.45	30.26	14.85	2.71	0.20
RP	1.14	0.62	2.70	17.12	43.56	30.55	4.31

Table 5. Properties of the organic manure used in the study.

Property	Value	Total nutrient	Value
EC dS/m (1: 5 water extract)	8.2	N (g kg ⁻¹)	7.7
pH (1: 5 water extract)	7.23	P (g kg ⁻¹)	10.2
Organic matter (g kg ⁻¹)	310	K (g kg ⁻¹)	12.1
Organic carbon g (kg ⁻¹)	182	Fe (g kg ⁻¹)	3.22
C/N ratio	23.6	Mn (mg kg ⁻¹)	152
Moisture content (g kg ⁻¹)	14.4	Zn (mg kg ⁻¹)	110

3.2. The Experiment:

The experiment was conducted in a randomized complete block (RCB) design, factorial, in 3 replicates, The 3 factors are bio-fertilization, P-fertilization and manuring.

3.2.1. Factors and their treatments are :

I. Bio-fertilization: 2 treatments i.e. B₀: no biofertilization; B₁: PDB biofertilization.

II. P-fertilization: 3 treatment i.e. P₀: no P-fertilization ; P₁: RP at 70kg P ha⁻¹ and P₂ SP at 35 kg P ha⁻¹ (RP rate being 2 times SP in view of insolubility of the former).

III. Organic manuring: 3 treatments i.e. M₀: no manure ; M₁: FYM at 24Mg ha⁻¹ M₂: FYM at 48 Mg ha⁻¹. All plots received N (250 kgNha⁻¹ as urea “460 g N kg⁻¹”) and K (100 kgKha⁻¹ as K-sulphate 400 g K kg⁻¹”). Main properties of the manure are in Table 5. Plot size was 10.5 m². Thus total number of treatment combinations=2x3x3= 18. Treatment codes are shown in Table 4. The total overall number of the experimental plots was 54. The crop was harvested 120 days after seeding. Samples of soil and plant were taken after harvest for analysis. Soil samples were air dried while plant samples were oven-dried at 70°C for 24 h. All plots received N and K fertilizers. Manure, P and K were applied during land preparation while N was added in 2 equal splits with 1st and 2nd irrigations.

3.4. Soil, plant and water analyses:

Analyses of soil water and plant were done by methods cited by USDA (1954), Chapman and Pratt, (1961), Page et al., (1982) and Klute (1986). Available N was extracted by KCl, available P was extracted by Na-bicarbonate and available K was extracted by ammonium acetate. Measurement of P in extract was done calorimetrically by the ascorbic acid method of Murphy and Riley (1962) while that of K was by flame photometry. Measurement of available micronutrients were done by spectrophotometry,

Results And Discussion

Maize grain yield (Table 6):

Grain yield was increased by application of any or more of the biofertilizer (B), chemical fertilizers (P) or the manure (M). The lowest grain yield of 3.62 Mgha⁻¹, increased by a very slight non-significant 1% due to the low rate of manure M₁ (B₀P₀M₀), but to a significant increases starting from 12.7 by the high rate of manure M₂ (B₀P₀M₂) to as high as 129% due to biofertilization combined with superphosphate and the high rate of manure i.e. B₁P₂M₂. The second highest yield surpassed the lowest by 87% was given by biofertilization combined with superphosphate i.e. B₁P₂M₀. The considerable high increase given by PDB and the SP when combined with manure demonstrates enhancement of PDB to the positive effect of

superphosphate. Abdel-Salam (2014) mentioned that biofertilization could result in increased yields reaching practical levels under some field conditions, and that in some field trials on maize under salinity conditions Abdel-salam et al., (2012) obtained maize grain yield increases of up to 30% by P-biofertilization. Hameeda et al (2008) reported up to 85% yield increase in maize grain under field conditions. EL-Assiouty and Abo-sedera (2005) reported increased seed yield of spinach (*Spinacia oleracea*) by biofertilization. Bakhshandeh, et al.,(2015) obtained increased yield in rice grains upon biofertilization with different strains of PDB bacteria. Laheurte and Berthelin (1988) reported that PDB *Enterobacter agglomerans* benefit from exudates by maize roots and showed high efficiency in dissolving insoluble P.

I- Response to biofertilization:

The main effect of the biofertilization shows 40.4% average increase, The increase was most marked where no P-fertilizer was present (54.0%) or in the presence of SP (50.4%), more than in presence of RP (21.4%). On the other hand, the increases were more in presence of manure (45%) in presence of M₁ and 39.5% in presence of M₂ than in absence of manure (36.7%). Yazdani et al.(2012) obtained marked yield increase upon applying PDB to maize.

II- Response to P application:

The main effect shows 20.0 and 25.6 % increases due to RP and SP respectively. However, the RP source surpassed the SP one where no biofertilization was done (37.6% increase by RP and 27.5% increase by SP). On the other hand SP surpassed RP in the presence of the biofertilizer (only 8.4 % increase by RP, and 24.6% increase by SP) indicating that the P-dissolving bacteria augmented the positive effect of SP. Biofertilization using *Bacillus megaterium* phosphate dissolving bacterial inocula was noted by Chuan-ying et al., (2014) to have a high positive effect particularly in soils of high total P.

III- Response to organic manure:

The main effect of organic manure treatments shows a progressive increase with increased rate of manure application with averages of 5.1 and 20.0% due to M₁ and M₂ respectively. The manure was equally effective in presence as well as in absence of the biofertilizer. In absence of P, the high manure rate was effective (18.3% increase) whereas the low rate was not effective. In presence of RP both manure rates were effective (13.4 and 27.9% increases by M₁ and M₂ respectively), but in presence of SP it was the high manure rate which gave a positive response. This shows a high efficiency of manure application where rock phosphate is used. Enujike (2013) obtained up to 300% increase in grain yield of maize grown on a

loamy soil by the application of 30 Mg organic manure ha⁻¹.

Available N after maize harvest mg kg⁻¹ soil (Table 6):

Available N was increased by biofertilization combined with P-and organic manure. The 66.0 mg N kg⁻¹ soil, given by the non-treated B₀P₀M₀ increased by up to 38.3% due to the B₁P₀M₂ treatment; indicating a positive cumulative effect of biofertilization with manure and P application.

I. Response to biofertilization:

Contents of available N due to biofertilization was very slight, most possibly due to removal of high contents of N from soil treated with PDB. The high available N reported by Elkholy et al., (2010) due to PDB was in soils was most probably in midseason of the crop.

II. Response to P application:

There was no significant differences between P fertilizers in the contents of available N in soil after harvest. However there was a moderate increase in available N in soils receiving chemical P where no PDB biofertilizer was applied.

III. Response to organic manure:

The main effect of organic manure was average increases of 7.9% and 30.6% due to P₁ and P₂ respectively. The pattern of such increase occurred in presence as well as in absence of PDB. It also occurred more under no P application. El-Naggar (1996), Gami et al., (2001), Slattery et al., (2002), Saeid (2002), Mahdy (2003), Ahmed (2007), Kavitha and Subramanin (2007) mentioned that composted cattle manure increased soil nutrients.

Table 6. Effect of Bio-, chemical P-fertilization and manure addition on maize grain yield and available N residual in soil after harvest.

Bio-P-fertilizer (B)	P-fertilizer (P)	Manure application (M)							
		M ₀	M ₁	M ₂	Mean	M ₀	M ₁	M ₂	Mean
		Yield (Mg ha ⁻¹)				Available N (mgkg ⁻¹)			
B ₀	P ₀	3.62	3.65	4.08	3.78	66.0	74.0	89.0	76.3
	P ₁	4.45	4.88	6.26	5.20	69.7	74.3	89.0	77.7
	P ₂	4.85	4.59	5.02	4.82	69.0	74.7	90.3	78.0
Mean		4.30	4.38	5.11	4.60	68.2	74.3	89.44	77.3
B ₁	P ₀	5.23	5.82	6.41	5.82	68.7	76.3	91.3	78.8
	P ₁	5.66	6.60	6.67	6.31	69.7	74.3	89.0	77.2
	P ₂	6.78	6.65	8.30	7.25	69.7	72.3	89.7	77.0
Mean		5.88	6.35	7.13	6.46	69.1	73.9	90.0	77.7
G.Mean		5.10	5.36	6.12		68.7	74.1	89.7	
Mean of chemical P-fertilization									
	P ₀	4.43	4.73	5.24	4.80	67.3	75.2	90.2	77.6
	P ₁	5.06	5.74	6.47	5.76	69.7	73.7	89.0	77.4
	P ₂	5.81	5.62	6.66	6.03	69.0	73.5	90.0	77.5
LSD 0.05		B=ns. P=ns. M=1.01			BP=1.43	B=ns. P=ns. M=1.01			BP=1.43
		BM=N.S. PM=1.75			BPM=n.s.	BM=N.S. PM=1.75			BPM=n.s.

Notes: B₀: no biofertilization; B₁: P-dissolving bacteria (PDB) *Bacillus megaterium*-- P₀: no-P addition; P₁: 70kg P ha⁻¹ as rock phosphate (RP); and P₂: 35 kg P ha⁻¹ as calcium superphosphate (SP) - M₀: no manure M₁: 24Mg FYM ha⁻¹; M₂: 48 Mg FYM ha⁻¹ ..Available N extracted by KCl

Available P after maize harvest (Table 7):

Available P was increased by biofertilization combined with P-and organic manure. The 5.33 mg P kg⁻¹ soil, given by the non-treated B₀P₀M₀ increased by up to 83.8% due to the B₁P₂M₂ treatment; indicating a positive cumulative effect of biofertilization with manure and P application.

I. Response to biofertilization:

Although the increase in available P due to biofertilization was not high averaging 8.8%, it was significant. The positive effect of biofertilization was particularly where no fertilizer P was applied. Elkholy et al., (2010) and LinZhao, et al., (2014) determined available P in soil following harvest of maize biofertilized with PDB *Burkholderia* sp. They found that availability P in the

soil increased in the biofertilized treatment and also noted soil pH decreased upon PDB biofertilization

II. Response to P application:

The main effect of P-fertilizers shows that they increased available P by averages of 8.3% and 10.4% due to P₁ and P₂ respectively. The increase was particularly where no PDB were applied. The increase was also where no organic manure was given. Robbins et. al., (2000) stated mineralization of organic P during organic matter decomposition releases P slowly, resulting in more available P in soil.

III. Response to organic manure:

The main effect of P-fertilizers shows that they increased available P by averages of 8.9% and 15.4% due to P₁ and P₂ respectively. The organic

manure was of marked effect only where no chemical P was present. It was also marked where no PDB was given. Jang et al., (1994) and Carrie et al., (2003) reported that compost application increased available P content after maize harvest.

Available K after maize harvest (Table 7):

Almost all biofertilized treatments contained more available K than the unfertilized. Lowest available K of 214.5 mg K kg⁻¹ was shown by the B₁P₁M₀ treatment followed by the nonfertilized B₀P₀M₀ which showed 220.3 mg K kg⁻¹. The highest increase was given by the B₁P₂M₂, the biofertilized combined with SP-and manure causing an increase of 44%, indicating a positive cumulative effect of biofertilization+soluble P +manure.

I. Response to biofertilization:

Though the main effect of the biofertilization was small, it was statistically significant. Higher available K caused by biofertilization was more where superphosphate was given. On the other hand the increase occurred whether manure was applied or

Table 7.Effect of Bio-, chemical P-fertilization and manure addition on available P and K residual in soil after harvest

Bio-P-fertilizer (B)	P-fertilizer (P)	Manure application (M)				Mean	M ₀	M ₁	M ₂	Mean
		M ₀	M ₁	M ₂	Mean					
		Available P (mgkg ⁻¹)				Available K (mgkg ⁻¹)				
B ₀	P ₀	5.33	8.27	8.50	7.37	220.3	249.5	301.8	257.2	
	P ₁	7.83	8.40	8.57	8.27	217.3	248.9	304.8	257.0	
	P ₂	8.33	8.40	9.00	8.58	218.4	251.6	306.0	258.7	
Mean		7.16	8.36	8.69	8.07	218.7	240.0	304.2	257.6	
B ₁	P ₀	8.27	8.50	8.70	8.49	218.8	249.2	306.2	258.1	
	P ₁	8.53	8.80	9.40	8.91	214.5	253.4	303.6	257.2	
	P ₂	8.43	8.57	9.80	8.93	223.6	245.9	308.0	262.2	
Mean		8.41	8.62	9.30	8.78	218.9	252.5	305.9	259.1	
G.Mean		7.79	8.49	8.99		218.8	251.3	305.1		
Mean of chemical P-fertilization										
	P ₀	6.80	8.38	8.60	7.93	219.6	249.4	304.0	257.7	
	P ₁	8.18	8.60	8.98	8.59	215.9	251.2	304.2	257.1	
	P ₂	8.38	8.48	9.40	8.76	221.0	253.2	307.0	260.4	
LSD 0.05		B=0.09	P=0.11	M=0.11	BP=0.15	B=1.02	P=1.25	M=1.25	BP=1.77	
		BM=0.15	PM=0.18	BPM=0.26		BM=N.S.	PM=2.17	BPM=3.07		

Notes: B₀ : no biofertilization; B₁: P-dissolving bacteria (*PDB Bacillus megaterium*-- P₀: no-P addition; P₁: 70kg P ha⁻¹ as rock phosphate (RP); and P₂: 35 kg P ha⁻¹ as calcium superphosphate (SP) - M₀: no manure M₁: 24Mg FYM ha⁻¹ ; M₂: 48 Mg FYM ha⁻¹ ..Available P&K extracted by DTPA.

Available Fe after maize harvest (Table 8):

Available Fe was increased by biofertilization combined with P-and organic manure. The 2.33 mg kg⁻¹ soil, given by the non-treated B₀P₀M₀ increased by up to 112% due to the B₁P₂M₂ treatment; indicating a positive cumulative effect of biofertilization +manure + P application.

I.Response to biofertilization:

The main effect of the biofertilization was a significant increase averaging 9.4%. Biofertilization gave more available Fe where P was applied, but not where no P was applied. This may be an outcome of the existence of iron compounds in the P fertilizers.

not. Elkholy et. al., (2010) showed that the available K in the soil increased by PDB.

II. Response to P application:

The main effect of P-fertilizers shows that treatments which received soluble P in particular showed lower available K after harvest. This may indicate a greater uptake of K by plants which received superphosphate due to increased root system caused by applied calcium superphosphate.

III. Response to organic manure:

The main effect of manure addition shows that increases in available K averaging 14.8% and 39.4% due to M₁ and M₂ respectively. The positive effect of manure was in presence as well as in absence of PDB, and also in presence or absence of P sources. Increase in available K due to organic manures was reported by many researchers (El-Naggar, 1996,, Gami et al., 2001,, Slattery et al., 2002,, Saeid 2002, Mahdy 2003, Ahmed, 2007 and Kavitha and Subramanin, 2007).

Where no manure was added, or where low manure was applied, the increase in available Fe was significant; but in presence of the high manure, no difference was shown by the biofertilized treatment in available Fe. This indicates presence of high available Fe in the high rate of organic manure. Gayatri et al., (2007) and LinZhao, et. al., (2014) reported increased available Fe upon applying PDB and Hider and Kong, (2010) mentioned that PDB produce siderophores which solubilize insoluble iron in soils.

II. Response to P application:

The main effect of P-fertilizers showed an average increase of 10.5% only with application of the superphosphate . In absence of the biofertilizer , the positive action of the two P-fertilizers was more than in presence of it , and in presence of organic manure, their positive effect was much greater than in the manure’s absence.

III. Response to organic manure:

The main effect of P-fertilizers shows that they increased available Fe by averages of 43.6% and 59.9% due to P₁ and P₂ respectively. In absence of the biofertilizer , the positive action of the two P-fertilizers was more than in its presence , and in presence of organic manure, their positive effect was more than in manure’s absence. Using organic manures many researchers noted increases in available iron (Dahdoh et al., 2001 , Mostafa et al., 2001, Bonde et al., 2004, Modaihsh et al., 2005 and Hammad et al., 2006).

Available Zn after maize harvest (Table 8):

Available Zn was increased by biofertilization combined with P-and organic manure. Lowest available Zn of 0.85 mg Zn kg⁻¹ was shown by the B₀P₁M₀ treatment followed by 0.90 mg Zn kg⁻¹ given by B₁P₁M₀ .The nonfertilized B₀P₀M₀ showed 0.94 mg Zn kg⁻¹ .The highest treatment which showed highest available Zn was that of B₁P₂M₂ treatments which surpassed the non-treated by 78% concerning available Zn after harvest. This reflects the apparent cumulative effect of combining biofertilization with organic manure and p-fertilizers.Treatments receiving

I.Response to biofertilization:

The main effect of the biofertilization was a significant increase averaging 8.0%.The higher available Zn caused by PDB occurred more in presence of manure. Therefore, the PDB activity was enhanced by organic matter. Johnstone and Nolan, (2014) reported that bacteria may produce substances called *zincophores* or *tsinkosphores* which solubilize Zn.

II. Response to P application:

Applying P in either forms caused increase in available Zn only where biofertilization was done. Applying P in either form in presence of no-biofertilization showed a decrease in available Zn; but under biofertilization an increase occurred due to superphosphate in particular. The only P treatment causing increase by manure was the high manure.Hhigh manure must have facilitated solubilization of Zn in soil under the effect of decomposing organic matter (Dahdoh et al.,2001 and Mostafa et al.,2001).

III.Response to organic manure:

The main effect of P-fertilizers shows increases averaging of 39.1% and 55.4% due to M₁ and M₂ respectively. Increases due to manure occurred in presence as well as in absence of biofertilization and also under no p or in presence of each of the P sources.

Dahdoh et al., (2001) , Mostafa et al., (2001 , Bonde et al., (2004) , Modaihsh et al.,(2005) and Hammad et al., (2006) noted a rise in available Zn by manuring.

Table 8. Effect of Bio-, chemical P-fertilization and manure addition on Available Fe and Zn residual in soil after harvest.

Bio-P-fertilizer (B)	P-fertilizer (P)	Manure application (M)							
		M ₀	M ₁	M ₂	Mean	M ₀	M ₁	M ₂	Mean
		Available Fe (mgkg ⁻¹)				Available Zn (mgkg ⁻¹)			
B ₀	P ₀	2.33	3.50	4.29	3.37	0.94	1.28	1.29	1.17
	P ₁	2.70	3.64	4.58	3.64	0.85	1.15	1.23	1.08
	P ₂	2.51	4.44	4.59	3.85	0.92	1.09	1.35	1.12
Mean		2.51	3.86	4.49	3.62	0.90	1.18	1.29	1.12
B ₁	P ₀	3.42	4.46	4.16	4.01	0.93	1.30	1.43	1.22
	P ₁	2.59	3.63	4.49	3.57	0.90	1.23	1.35	1.16
	P ₂	3.37	4.62	4.94	4.31	0.97	1.30	1.51	1.26
Mean		3.13	4.24	4.53	3.96	0.93	1.28	1.43	1.21
G.Mean		2.82	4.05	4.51		0.92	1.28	1.43	
Mean of chemical P-fertilization									
	P ₀	2.88	3.98	4.22	3.69	0.93	1.29	1.36	1.19
	P ₁	2.65	3.64	4.54	3.61	0.87	1.19	1.29	1.12
	P ₂	2.94	4.53	4.77	4.08	0.95	1.20	1.43	1.19
LSD 0.05		B=0.12 BP=0.21 BPM=0.37	P=0.15 BM=0.21	M=0.15 PM=0.26	B=0.03 BM=0.05	P=0.04 PM=0.07	M=n.s. BPM=n.s.	BP=0.05	

Notes: B₀ : no biofertilization; B₁: P-dissolving bacteria (PDB) *Bacillus megaterium*-- P₀: no-P addition; P₁: 70kg P ha⁻¹ as rock phosphate (RP); and P₂: 35 kg P ha⁻¹ as calcium superphosphate (CSP) - M₀: no manure M₁: 24Mg FYM ha⁻¹ ; M₂: 48 Mg FYM ha⁻¹ ..Available Fe and Zn extracted by DTPA.

Available Mn after maize harvest (Table 9):

The pattern of change regarding available Mn was very much comparable with that of Fe. Lowest available Mn of 0.42 was that which received superphosphate only, followed by the treatment which received non-treated. Other treatments showed increases in available Mn ranging from 84.8% by each of B₀P₀M₁ and B₀P₁M₁ to as high as 269% by B₀P₁M₂. A cumulative effect of combining high manuring and rock phosphate demonstrate the positive effect of decomposing organic manure in combination with rock phosphate (Dahdoh et al., 2001 and Mostafa et al., 2001).

I. Response to biofertilization:

The main effect of biofertilization was 28.5%. The high response to biofertilization in increasing the contents of available Mn (as done with available Fe) was apparent where no manure or medium manure was added. It was also more evident where any of the two P chemical fertilizers was given, particularly the superphosphate P. Increase in available Mn by PDB bacteria indicates solubilization of insoluble Mn by substances such as siderophores and coproporphyrin III which solubilize insoluble iron in soils (Hider and Kong, 2010, Johnstone and Nolan, 2014).

II. Response to P application:

The two P-fertilizers increased available Mn and the positive effect occurred particularly in

presence of biofertilization. It also occurred in absence of no manure or under the low rate of it.

III. Response to organic manure:

Available Mn was greater in the manured than the non-manured treatments giving averages of 95.7 and 108 % due to M₁ and M₂ respectively. The increase occurred in both non-biofertilized and the bio-fertilized, but more in presence of the biofertilized. Also the increase occurred under no-P as well as under P addition, but more where the rock P was present. Dahdoh et al., (2001, Mostafa et al., (2001), Bonde et al., (2004), Modaihsh et al., (2005) and Hammad et al., (2006) reported increases in available Mn in the soil upon applying organic manures.

Available Cu after maize harvest (Table 9):

Available Cu increased by most treatments which received any one or more of the added fertilizers or manure. The lowest available Cu of 0.23 mg Cu kg⁻¹ was shown by the nonfertilized non manured B₀P₀M₀ treatment as well as each B₀P₁M₀, B₁P₁M₀ and B₁P₂M₀, all of which received no manure. Increases in contents of available Cu after maize harvest ranged between 17.4% by B₀P₂M₀ to 82.3 by each of B₁P₁M₂, B₁P₁M₂ and B₁P₂M₂ of which received manure. This shows the positive effect of manure in particular in increasing available Cu in soil along with other nutrients (Hafez et al., 1992, El-Maghraby, 1996 and. Mekail 1998, Carrie et al., 2003, Bonde et al., 2004, Modaihsh et al., 2005 and Hammad et al., 2006).

Table 9. Effect of Bio-, chemical P-fertilization and manure addition on Available Mn and Cu residual in soil after harvest.

Bio-P-fertilizer (B)	P-fertilizer (P)	Manure application (M)			Mean	M ₀	M ₁	M ₂	Mean
		M ₀	M ₁	M ₂					
		Available Mn (mgkg ⁻¹)			Available Cu (mgkg ⁻¹)				
B ₀	P ₀	0.46	0.85	1.49	0.93	0.23	0.30	0.33	0.28
	P ₁	0.60	1.18	1.55	1.11	0.23	0.29	0.29	0.27
	P ₂	0.42	0.85	1.44	0.90	0.27	0.38	0.35	0.33
Mean		0.49	0.96	1.49	0.98	0.24	0.32	0.32	0.30
B ₁	P ₀	0.64	1.25	1.51	1.14	0.26	0.34	0.33	0.31
	P ₁	0.89	1.46	1.41	1.25	0.23	0.34	0.42	0.33
	P ₂	1.22	1.46	1.50	1.39	0.23	0.42	0.42	0.36
Mean		0.92	1.39	1.48	1.26	0.24	0.37	0.39	0.33
G.Mean		0.71	1.39	1.48		0.24	0.34	0.36	
Mean of chemical P-fertilization									
P ₀		0.55	1.05	1.50	1.03	0.25	0.32	0.33	0.30
P ₁		0.75	1.32	1.48	1.18	0.23	0.32	0.36	0.30
P ₂		0.82	1.15	1.47	1.15	0.25	0.40	0.39	0.34
LSD 0.05		B=0.02	P=0.03	M=0.03	BP=0.04	B=0.01	P=0.02	M=0.02	BP=0.03
		BM=0.04	PM=0.05	BPM=0.07		BM=0.02	PM=0.03	BPM=0.04	

Notes: B₀ : no biofertilization; B₁: P-dissolving bacteria (PDB) *Bacillus megaterium*-- P₀: no-P addition; P₁: 70kg P ha⁻¹ as rock phosphate (RP); and P₂: 35 kg P ha⁻¹ as calcium superphosphate (CSP) - M₀: no manure M₁: 24Mg FYM ha⁻¹; M₂: 48 Mg FYM ha⁻¹ ..Available Mn and Cu extracted by DTPA.

I. Response to biofertilization:

The main effect of the biofertilization was a significant increase averaging 10% , and the increase was apparent where P was given , and where manure was added. Upon application of PDB, LinZhao, et al., (2014) noted increases in soil available Cu , and

II. Response to P application:

Available Cu increased by P particularly in presence of manure , and also under conditions of biofertilization.

III. Response to organic manure:

Manure showed average increase of 41.7 and 50.0 % by M₁ and M₂ respectively.. In absence of the biofertilizer , the positive effect P-fertilizers was more than in presence of it , and in presence of organic manure, their positive effect was much greater than in manure's absence.

Conclusion

Applying any or more of PDB biofertilizer of *B . megaterium* , chemical P-fertilizers : rock phosphate "RP" at 70kg P ha⁻¹ or superphosphate "SP" at 35 kg P ha⁻¹ without or with farmyard manure at 24Mg ha⁻¹ or 48 Mgha⁻¹ increased grain maize yield .Non treated plants gave 3.62 Mgha⁻¹ and treated ones gave increases from as low as 1% by the low manure treatment to 13% by the high manure one to a highest increase of 129% by The PDB,SP, high hanure treatment. The second highest increase of 87% was by the PDB , SP treatment. Main effect of biofertilization is an average 40 % increase .Main effect of P : 26% by RP and 20.0 % by SP . The SP was particularly superior to RP in pjsence of PDB..Main e ffect of manure : 5 % and 20 % by the low and high rates respectively. Uptake of N , P and K in grains increased by PDB reaching 40% to more than 100%. Rock phosphate proved of practical use in combination with biolertilization and organic manure for maize under field conditions. N,P,K,Fe,Mn,Zn and Cu residual in soil after harvest increased were by up to , 38, 84, 44, 112, 269, 78, and 83 % for each respectively ; and highest availble contents were 91, 9.8, 308, 4.94, 1.55,1.51 and 0.42 mgkg⁻¹ respectively. .

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التسميد الحيوي بلقاح بكتيريا *B.megaterium* مع التسميد الفوسفاتي الصخري والفوسفاتي الذائب : محتوى العناصر Zn, Mn, Fe, K, P, N و Cu المتبقي في التربة بعد الحصاد

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أجريت تجربة حقلية لدراسة التسميد الحيوي بلقاح بكتيريا بلس ميجاتيريم *B.megaterium* المذيبة للفوسفات مقارنة بالتسميد الكيماوي بصخر الفوسفات وبالسوبر فوسفات مع او بدون التسميد العضوي بالسماد البلدي للذرة الشامية (*Zea mays*) بتصميم قطاعات كاملة العشوائية 3 مكررات تجربة عاملية ذات 3 عوامل هي: تسميد حيوي (بدون وبتسميد حيوي) وتسميد فوسفاتي (بدون وبصخر فوسفاتي 70 كجم فسفور/هـ وبسوبر فوسفات 35 كجم/هـ - ومعدل الفوسفات الصخري ضعف الفوسفات الذائب بالنظر لذويان الثاني) وتسميد عضوي (بدون وبتسميد منخفض 24 ميجاجرام/هـ ومرتفع 48 ميجاجرام/هـ). استجاب المحصول للتسميد بكافة انواعه. بلغ محصول حبوب المعاملة غير المسمدة 3,62 ميجاجرام/هـ ارتفع بنسب تراوحت من 1% بتسميد عضوي منخفض فقط الى 13% بتسميد عضوي مرتفع فقط الى 87% بتسميد حيوي مع سوبر فوسفات الى اقصى زيادة 129% بتسميد حيوي مع سوبر فوسفات مع معدل مرتفع عضوي. ازداد الاثر العام للتسميد الحيوي 40% وللتسميد الفوسفاتي 26% للسوبر فوسفات و 20% للصخر الفوسفاتي. على الرغم من تفوق السوبر فوسفات على الصخر الفوسفاتي عزز التسميد الحيوي الفوسفاتي. والاثر العام للتسميد العضوي 5% للمدل المنخفض و 20% للمعدل المرتفع. وزاد محتوى التسميد للعناصر Zn, Mn, Fe, K, P, N و Cu في التربة بعد الحصاد بالنسب المئوية التالية: 38, 84, 42, 112, 269, 78 و 83% على التوالي. وكان اعلى محتوى لكل منها 91, 9, 8, 308, 4, 94, 1, 55, 0, 42 و 1, 51, مليجرام/كجم على التوالي.