

Effect of bio fertilization with p-dissolving bacteria, rock phosphate, sulphur and organic manure in comparison with superphosphate on wheat (*triticum aestivum*) productivity and soil fertility.

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Abstract

Two field trials were conducted in two successive growing seasons (2012/2013 and 2013/2014) at the Experimental Farm of Mallawy Agricultural Research Station (ARC), Minia Governorate, Egypt to study the effect of biofertilization using P- dissolving *Bacillus megaterium*, P- applied at 43kg ha^{-1} as rock phosphate (RP), farmyard manure (FYM) at 24 Mg ha^{-1} , S at 240kg ha^{-1} individually or in different combinations, as well as superphosphate at 16 or 32kg ha^{-1} on wheat. Yield of grains, straw, and grain+ straw increased by addition of such treatment. Uptake of N, P and K increased by different magnitudes. Highest positive effect was by biofertilization combined with RP, FYM and S giving effect rather similar to that of the high P rate as superphosphate.

Key Words: bacteria, rock phosphate, sulphur , organic manure

Introduction

Under the Egyptian conditions, fixation and precipitation of applied soluble P in soil causes low efficiency of P fertilizer. Some soil microorganism can dissolve insoluble phosphate in soil, thus supplying the grown plants with available phosphate (Saad and Hammad, 1998). Rock phosphate may be economically effective if applied in combination with application of phosphate- dissolving inoculation specialized organisms such as the *Bacillus megaterium* bacteria (Haynes, 1992; and Abou El-Salehein and Ahmed, 1998; Montenegro and Zepata, 2002 and Ali et al 2012).

Most Egyptian soil contains too little phosphorus to sustain high yields of crops and phosphates are the second fertilizers after nitrogen used for application to crops. Soluble phosphate is subjected to precipitation insoluble forms and crops may use only a little portion of applied soluble P. Response of wheat to soluble phosphorus fertilizer is mainly positive (Mohamed and Saad 2004; Yadav et al 2007; Salton et al 2011; Khan et al 2012 and Gay et al 2013).

Methods for increasing the efficiency of rock phosphate are include the use of biofertilization by inoculation of phosphate solubilizing bacteria, elemental sulphur and organic manure addition (Vessey 2003 ; Habashy 2005 and Ismail et al 2014 B). Drever and Vance (1994) reported that rock phosphate can be solubilized under the influence of acidity. Addition of sulphur leads to formation of acidic acid and this makes soil condition more favorable for plant growth, including the availability of phosphorus (Deluca et al, 1989; Jaggi et al, 2005 and Kacar and Katkat, 2007).

The present study assesses the effectiveness of biofertilization with and without addition of rock phosphate under conditions of organic manure and / or sulphur addition on wheat crop.

Materials and Methods

Two field experiments were conducted in two successive growing seasons (2012/2013 and 2013/2014) at the Experimental Farm of Malawy Agricultural Research Station, Malawy Town, Minia Governorate, Egypt to study the effect of rock phosphate along with phosphorine inoculation with *B. megaterium* as a P- dissolving biofertilizer. In addition, farmyard manure and sulphur application were included two rates of superphosphate were added. The crop was wheat (*Trilicum aestivum*, cv). The soil was a clay (Table 1 shows soil properties). The design of the experiment was a randomized complete blocks, four replicates.

The treatments were as follows:

Rock phosphate application of 300kg Rock phosphate /fed.

Bacterial inoculation:

Seeds of wheat were inoculated with *Bacillus megaterium* bacteria supplied by the Department of Microbiology, Soil, Water and Environmental Institute.

Organic manure application:

Farmyard manure was used at 24 Mg (megagram) per hectare and Table 2 shows main properties of the manure used in the experiment (2012-2013 and 2013-2014 seasons).

Table 1. Physical and chemical characteristics of soils of the experiment of 2012-2013 and 2013-2014 seasons.

Soil characteristic	2012/2013	2013/2014
Particle size distribution		
- Coarse sand %	2.65	3.01
- Fine sand %	10.24	12.98
- Silt %	35.46	31.90
- Clay %	51.65	52.11
Soil texture	Clay	Clay
pH (1:2.5) soil water suspension	8.1	8.0
EC dSm ⁻¹ (soil paste extract)	1.22	1.30
Organic mater gkg ⁻¹	14.1	13.9
Soluble cations and anions (mmolel⁻¹)		
- K ⁺	1.26	1.13
- Na ⁺	0.38	0.42
- Ca ⁺⁺	5.35	6.11
- Mg ⁺⁺	4.64	4.57
- Co ³⁻⁻	0.00	0.00
- HCo ³⁻	3.75	3.11
- Cl ⁻	3.60	3.76
- SO ⁴⁻⁻	4.28	5.36
Soil available N (mgkg ⁻¹)	30	25
Soil available P (mgkg ⁻¹)	10	9
Soil available K (mgkg ⁻¹)	170	183

Table 2. Chemical properties of the Farmyard Manure .

Chemical property	2012/2013 season	2013/2014 season
EC (1:5)	6.70	6.50
Organic matter gkg ⁻¹	147	178
C/N ratio	14/1	13/1
Total N (gkg ⁻¹)	7.3	7.9
Total P (gkg ⁻¹)	2.1	2.6
Total K (gkg ⁻¹)	6.6	7.2

The thirteen different treatments are as follows:

- 1- Not treated
- 2- 43kg P as Rock phosphate h⁻¹.
- 3- Biofertilization as Inoculation with phosphate, dissolving bacteria (PDB).
- 4- Organic manure (24Mg h⁻¹) (Mg: megagram=10⁶g).
- 5- Sulphur treatment (240kg S h⁻¹) (pulverized sulphur)
- 6- P as rock phosphate + Biofertilization
- 7- P as rock phosphate + farmyard manure "FYM" (24Mg h⁻¹).
- 8- P as rock phosphate + sulphur.
- 9- P as rock phosphate + Biofertilization + FYM.
- 10- P as rock phosphate + Biofertilization + sulphur.
- 11- P as rock phosphate + Biofertilization +FYM + sulphur.
- 12- P as calcium superphosphate (16kg h⁻¹) "P₁".
- 13- P as calcium superphosphate (32kg h⁻¹) "P₂".

The rock phosphate contained 60 g Pkg⁻¹

The wheat (*Triticum aestivum*, V) Beni Suef 1 was sown during the third week of November 2012/2013 and 2013/2014 at rate of 144 kg seeds h⁻¹. The previous crop was maize in both growing

seasons. The field was surface irrigated with Nile water.

Inorganic nitrogen fertilizer was applied to at the recommended rate of 180 kg Nh⁻¹ as urea (460gN kg⁻¹) added in two equal doses. The first was before the first irrigation and the second was before the second irrigation. Inorganic Potassium was applied to at the recommended rate of 520 kg h⁻¹ as potassium sulphate (400 gK kg⁻¹) applied with the first dose of nitrogen fertilizer. Following 75 days of planting, samples of 10 wheat plants were randomly chosen from each plot, dried in the oven at 70°C to determine dry weight/plant and content of N, P and K.

The crop was harvested during the first week of May in the two seasons. The wheat grain and straw yields were determined. Plants were cut at 2-3 cm above the soil surface, each plot separated into grains and straw. The wheat grains and straw and recorded. Samples were ground and sieved to pass through 0.5 mm screen, and analysed for N, P and K. Soil samples of surface layer (0-30) were collected from each plot after the harvested and analyzed for N, P and K. Total N was determined in plant material

using the (Chapman and Pratt 1978) which P was determined in plant digests using the ascorbic acid method and K was determined by the flame photometer.

Results and Discussion

- Growth parameters:

Data in Table 3 reveal that there was significant increase due to application of rock phosphate, phosphorine, farmyard manure, sulphur and superphosphate on plant. Treatment of applying

biofertilizer in combination with FYM, rock-P and S caused an increase of 43.9% in the first season and 59.3% in the second season. These results indicate the high efficiency of phosphorus dissolving bacteria in mobilizing rock phosphate (Kucey et al, 1989 and Kumar et al, 2009). Farmyard manure must have produced organic acids during its decomposition which would dissolve rock phosphate (Awaad et al, 2000 and Jat et al, 2000). Sulphur application would lead to formation of sulphuric acids which help in dissolution of rock phosphate (Deluca et al, 1989, Ceccotti, 1996 and Marzihi et al 2010).

Table 3. Effect of rock phosphate along with phosphorine, organic manure and sulfur as well as mineral phosphorus fertilization on growth characters.

Treatments	Dry weight /plant(g)	
	Season (1)	Season (2)
Control	1.73	1.18
R.P.	2.07	1.45
B.	1.96	1.25
F.Y.M.	2.19	1.66
S.	1.98	1.34
R.P.+B.	2.20	1.58
R.P.+F.Y.M.	2.21	1.70
R.P.+S.	2.12	1.64
R.P.+B.+F.Y.M	2.46	1.82
R.P.+B.+S.	2.26	1.76
R.P.+B.+F.Y.M+S.	2.49	1.88
50%R.R.P	2.35	1.55
100%R.R.P.	2.51	1.86
L.S.D 0.05	0.075	0.083

RP: rock phosphate B: biofertilizer
 FYM: farmyard manure S: sulfur
 RRP: recommended rate of phosphorus

-Yield:

Biofertilization, FYM as well as S addition caused increases in grain yields. Increases due to these treatments in the first season were 11.20, 21.10 and 18.39% respectively (Table 5). Similar trends were obtained in the second season. These increases are manifestations of the positive effect of these treatments on growth parameters such as number of spikes m⁻² and number of grains spike⁻¹.

Application of rock phosphate increased grain, straw and biological yields by 17.04, 9.52 and 11.34 % respectively (Table 5) in the first season and 25.97, 17.39 and 20.29% respectively in the second season. Positive effects on wheat growth and yield components before in Tables 3 and 4. These results are in agreement with those obtained by (Haynes 1992 and Abou- Hassien et al 2002).

Addition of biofertilizer + rock phosphate +FYM + sulphur increases grain, straw and grain +straw yields by 37.22, 14.29 and 21.65% respectively in the first season; and 17.24, 4.35 and 9.26% in the second season. These results indicate the combined effect which decrease soil pH that make partial acidulation and consequently dissolution of rock

phosphate (Singh and Reddy 2011 and Ismail et al 2014 A). Biofertilization in combination with rock phosphate and FYM with S increased yield equal to yields of the high rate of superphosphate.

- N, P and K uptake (Table 6-8)

Rock phosphate application increased N, P and K uptake by grains and straw. Increases of total N, P and K due to rock phosphate were 15.0, 42.5 and 11.8% respectively in the first season, and 18.5, 54.6 and 18.2 in the second season. These results agree with those obtained by Correa et al (2005) and Abd El- Hafeez et al (2013).

Biofertilization increased N, P and K uptake by 13.6, 38.3 and 7.5% respectively in the first season and 21.5, 46.1 and 17.0% in the second one. These results are in harmony with those obtained by (Attia and Badr El Din 1999 and Khalid 2004). Biofertilization combined with rock phosphate enhanced the effect of rock phosphate indicating increased solubility of P (Singh et al 1994 and Zabihhi et al 2011).

Table 4. Effect of rock phosphate along with phosphorine, organic manure and sulfure as well as mineral phosphorus fertilization on grain, straw and biological yields.

Treatments	grain yield (Mg ha ⁻¹)		straw yield (Mg ha ⁻¹)		Grain+ straw yield (Mg ha ⁻¹)	
	Season (1)	Season (2)	Season (1)	Season (2)	Season (1)	Season (2)
	Control	7.96	5.49	15.12	11.04	23.28
RP	9.32	6.92	16.56	12.96	25.92	19.92
B.	8.85	6.71	15.84	12.96	24.24	19.44
FYM	9.64	6.81	16.56	13.44	26.40	19.94
S.	9.42	6.67	16.32	13.20	24.96	19.9*2
RP+B	9.32	7.10	16.56	12.96	25.92	20.16
RP+FYM	9.92	7.10	17.28	13.20	27.36	19.92
RP+S	9.57	6.99	17.76	12.96	26.40	19.92
RP+B+FYM	10.25	7.35	16.80	13.92	26.88	20.64
RP+B+S	9.14	7.81	17.28	13.44	26.64	19.20
RP+B+FYM+S	10.92	7.53	17.28	13.92	28.32	21.66
SP	9.17	6.99	15.84	12.24	24.96	19.44
SP	11.03	7.49	17.04	13.68	28.32	21.36
L.S.D	0.40	0.53	0.50	0.48	0.86	1.12

See Foot Notes of Table (3)

Table 5. Effect of rock phosphate along with phosphorine, organic manure and sulfure as well as mineral phosphorus fertilization on N, P and K uptake in grains.

Treatments	N –uptake		P – uptake		K – uptake	
	Season (1)	Season (2)	Season (1)	Season (2)	Season (1)	Season (2)
	Control	89.08	62.08	25.68	17.73	46.56
R.P.	106.15	77.52	36.62	25.82	54.50	43.29
B.	100.87	75.79	35.71	25.03	52.65	44.06
F.Y.M.	114.69	85.94	40.82	28.17	64.20	44.76
S.	106.44	76.05	38.95	24.21	54.16	45.75
R.P.+B.	105.21	80.23	37.58	27.21	54.50	43.68
R.P.+F.Y.M.	118.08	90.26	44.88	31.51	65.32	47.37
R.P.+S.	107.08	79	42.50	29.76	52.08	43.03
R.P.+B.+F.Y.M	122.92	93.43	40.27	32.61	88.84	64.51
R.P.+B.+S.	103.20	88.29	41.47	32.18	48.84	50.44
R.P.+B.+F.Y.M+S.	129.98	94.94	47.35	34.92	73.92	51.64
50%R.R.P	102.69	79	38.85	28.32	53.54	44.52
100%R.R.P.	124.58	84.67	48.79	33.26	65.54	49.03
L.S.D	9.88	9.14	4.84	3.43	6.98	5.88

See Foot Notes of Table 3

Table 6. Effect of rock phosphate along with phosphorine, organic manure and sulfure as well as mineral phosphorus fertilization on N, P and K uptake in straw.

Treatments	N –uptake		P – uptake		K – uptake	
	Season (1)	Season (2)	Season (1)	Season (2)	Season (1)	Season (2)
	Control	46.8	33.1	12.1	8.6	214.7
R.P.	53.0	38.9	18.2	15.5	236.8	190.6
B.	53.8	42.7	17.4	14.3	228.3	186.9
F.Y.M.	71.2	61.7	19.8	16.0	261.6	208.1
S.	53.9	43.6	15.3	15.9	221.1	188.7
R.P.+B.	56.4	42.7	23.2	19.5	236.8	186.7
R.P.+F.Y.M.	74.3	55.9	22.4	19.8	269.5	202.1
R.P.+S.	58.5	40.1	23.0	18.0	248.6	188.1
R.P.+B.+F.Y.M	74.3	56.5	25.2	20.9	258.7	215.8
R.P.+B.+S.	57.1	42.9	26.0	21.4	247.1	192
R.P.+B.+F.Y.M+S.	77.7	65.4	22.4	18.0	273.0	218.5
50%R.R.P	52.3	39.3	19	13.4	228.0	176.2
100%R.R.P.	77.7	43.8	23.8	19.0	246.9	203.6
L.S.D	7.1	5.7	3.45	2.4	18.5	20.7

See Foot Notes of Table (3)

Table 7. Effect of rock phosphate along with phosphorine, organic manure and sulfure as well as mineral phosphorus fertilization on total N, P and K uptake.

Treatments	N –uptake (kg ha ⁻¹)		P – uptake (kg ha ⁻¹)		K – uptake (kg ha ⁻¹)	
	Season (1)	Season (2)	Season (1)	Season (2)	Season (1)	Season (2)
	Control	138.3	97.6	40.1	26.7	261.6
R.P.	159.1	115.7	57.2	41.3	292.5	234.6
B.	157.1	118.1	55.5	39.1	281.3	232.1
F.Y.M.	211.8	147.6	63.1	44.5	325.8	254.2
S.	160.2	119.5	56.7	40.3	275.0	232.2
R.P.+B.	161.4	123.1	36.6	46.5	291.7	229.8
R.P.+F.Y.M.	194.7	145.8	67.8	51.5	335.3	249.0
R.P.+S.	165.9	119.3	65.7	47.6	301.8	230.4
R.P.+B.+F.Y.M	199.7	150.1	65.4	53.5	348.1	280.1
R.P.+B.+S.	160.5	131.3	67.7	53.5	295.3	243.2
R.P.+B.+F.Y.M+S	210.1	162.7	72.2	53.1	346.1	269.8
50%R.R.P	157.9	118.3	60.2	42.6	282.4	220.1
100%R.R.P.	201.8	128.4	72.3	53.4	313.1	253.1
L.S.D	16.5	16.1	5.6	4.8	10.5	15.1

See Foot Notes of Table (3)

Application of FYM increased N, P and K increases were 30.1, 57.0 and 24.5% respectively in the first season. This indicates the positive effect of manure (Hassanin 2009, Tahir et al 2011 and Ismail et al 2014 A). Sulphur application increased uptake of N, P and K. Combined sulphur with rock phosphate did not affect N and K uptake composed with rock phosphate alone, while such combined treatment showed higher uptake than with rock phosphate alone-positive effect of S indicates its acidic effect (Muchovej et al 1989).

Biofertilization, manure or sulphur increased the uptake of N, P and K due to the combined effect of biofertilization +manure + rock phosphate+ S caused increases of 32.1, 26.2 and 18.3% over rock phosphate alone in the first season. Same trends were

obtained in the second season. Synergistic activity of biofertilization, FYM and S enhanced N, P and K uptake. These results are in harmony with those obtained by (El- Koumy 2007 and Banerjee et al 2011).

Application of superphosphates increases N, P and K uptake. These results are in agreement with those obtained by (Abd El- Hafeez et al 2013 and Gay et al 2013).

- Soil fertility (Table 8)

Availability of N, P and K in soil after harvesting showed increases due to application of amendment. Increased availability is mostly due to the nutrients release during the course of the experiment (Ali et al 2012). These results agree with those obtained by (Abd El- Lattif 2012).

Table 8. Effect of rock phosphate along with phosphorine, organic manure and sulfure as well as mineral phosphorus fertilization on availability of N, P and K in soil.

Treatments	Soil available N (mgkg ⁻¹)		Soil available P (mgkg ⁻¹)		Soil available K (mgkg ⁻¹)	
	Season (1)	Season (2)	Season (1)	Season (2)	Season (1)	Season (2)
	Control	28.2	26	9.2	9.6	126
R.P.	28.2	26.2	12.4	12.7	128	153
B.	30.6	28	10.9	11	127	152
F.Y.M.	33.3	30.7	13.2	14	140	168
S.	29	26.8	11.8	12	129	153
R.P.+B.	30.8	28.1	14.1	13.9	129	152
R.P.+F.Y.M.	33.1	31	15.6	16	142	169
R.P.+S.	29.3	27	14.7	15	127	152
R.P.+B.+F.Y.M	33.4	33	17	17.6	141	170
R.P.+B.+S.	30.6	28.3	16.7	16.8	130	152
R.P.+B.+F.Y.M+S.	34	32.7	18	18	142	169
50%R.R.P	29.1	26.6	10.6	10.8	129	153
100%R.R.P.	30	27.2	11.2	11.7	130	155
L.S.D	3.9	4	0.58	0.67	7	8

See Foot Notes of Table (3)

Increased availability of P could be arranged by treatments as follows: RP+B+FYM+S> RP+B+FYM> RP+B+S> RP+FYM> RP+S> RP+B> FYM> RP> S> 100%RRP >B>50%RRP. FYM had the greatest effect. The positive effect of FYM reflect its acidifying effect in releasing available P (Gowda et al, 2011). Sulphur must have caused by acidfy increasing available P (Duponnois et al. 2005). **Badr 2006 and Hellal et al (2009)** reported enhancing effects of organic manure in increasing P availability in soil.

Conclusion

The highest grain quality and yield of wheat was obtained by inoculation of seeds with P- dissolving bacteria in combination with 24 Mg FYM, 43kg P as rock phosphate, 240kg S h⁻¹ .

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تأثير التسميد الحيوي مع البكتريا المذيبة للفوسفور وتداخلاته مع صخر الفوسفات والمنشطات العضوية والكيميائية مقارنة بسوبر فوسفات الكالسيوم علي إنتاجية القمح وخصوبة التربة

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**معهد بحوث الاراضى والمياه والبيئه.

- أجريت تجربتان حقليتان بالمزرعة البحثية بمحطة بحوث ملوي ، مركز البحوث الزراعية ، جمهورية مصر العربية خلال موسمي النمو 2013/2012 ، 2014/2013 وذلك من اجل دراسة تأثير التسميد الحيوي باستخدام البكتريا المذيبة للفوسفات مع استخدام صخر الفوسفات بمعدل 43 كجم /هكتار، والماده العضويه بمعدل 24ميجا جرام /هكتار ، والكبريت بمعدل 240كجم/هكتار ،والسوبر فوسفات بمعدل 16، 32كجم/هكتار .
- وقد تبين ان محصول الحبوب والقش والقش +الحيويادت زياده معنويه.
- امتصاص النيتروجين والفوسفور والبوتاسيوم زاد بزيادات مختلفه.
- وجد ان اعلى تأثير ايجابي كان بواسطة التسميد الحيوي مع الصخر والماده العضويه والكبريت اعطت تاثير مساوى مع اعلى معدل من السوبر فوسفات.