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ENHANCING THE FERTILIZER VALUE OF ROCK PHOSPHATE THROUGH SOIL AMIENDEMENTS AND PHOSPHATE DISSOLVING BACTERIA FOR GROWING FABA BEAN IN CALCAREOUS SOILS

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ABSTRACT

A two years field experiments were carried out on a private farm at Al-Shaarawy village, Al- Bostan district (30°43' 25.14' N 30° 17' 23.94' E elevation 14 m),Al - Behaira Governorate, Egypt; during the two successive winter seasons of 2010 / 2011 and 2011 / 2012. The aim of this study was to evaluate the combinations of rock phosphate (RP), at the rate of 22.5 kg P_2O_5/fed.) with elemental sulphur (S,200kg/fed.), compost(OM, 20 $m^3 fed^{-1}$) or seed inoculation with phosphate dissolving bacteria (PDB) to enhance the availability of phosphorus from rock phosphate and their effects on yield of faba bean plants (*Vicia faba* L.) in comparison with 22.5 kg P_2O_5 kg fed^{-1} as single supper phosphate (SSP). Results showed that application of RP only or RP + S + PDB showed a negative effect on yield and N, P and K contents of faba bean plants, but faba bean yield was greatly improved when the applied RP was mixed with compost (OM). Since any combination including compost showed a significant increase in faba bean yield over the combination without compost. It means the importance of compost to ameliorate RP properties. Moreover, the effect of RP combined with Compost was greatly improved when combined with S and/or PDB and the combination of RP + compost + S + PDB recorded the highest increases in seed and straw yields (41% and 42%) as well as N,P and K content of faba bean plants.

INTRODUCTION

Phosphorus is an essential nutrient for plant that is applied to soil as inorganic phosphates. Much of these phosphates used as fertilizer is immobilized after application and becomes unavailable to plants (Singh and Kapoor, 1994).

Under Egyptian soil conditions, phosphorus availability in soil is governed by many factors (pH and soil contents of $CaCO_3$, organic matter and clay). In spite of the considerable addition of P to these soils, phosphorus availability decreases sharply in a short period after application (Miller *et al.*, 1990). They added that under alkaline soil conditions, the available phosphorus in the added fertilizer is transformed rapidly to tri-calcium phosphate, which is unavailable to the plants. The direct application of apatite instead of phosphate fertilizers is not suitable, especially in soils with a high pH. However, using materials with acidic effect such as sulphur, OM and sulphuric acid and/or rock phosphate combined with phosphate dissolving bacteria (PDB) such as *Pseudomonas, Azospirillum, Burkholderia, Bacillus, Enterobacter, Rhizobium, Erwinia, Serratia, Alcaligenes, Arthrobacter, Acinetobacter and Flavobacteriu*, which can produce some organic acids, will release phosphorous from rock phosphate and can replace P-fertilizers

(Rodríguez *et al.,* 1999) Gluconic acid was reported as the most frequent agent for mineral phosphate solubilization produced by *Pseudomonas* sp. (Illmer *et al.*, 1992) and *Erwinia herbicola* (Liu *et al.*, 1992).

Therefore, rock phosphate is a good source of phosphorus if it is combined with compost and powdered sulphur with phosphate dissolving bacteria (Lotfollahi *et al.,* 2001). Addition of compost to the soils improves their physical, chemical and biological properties, which influence the growth and development of plants. Also, organic acids produced from decomposition of organic matter help to dissolve the rock phosphate and increase the availability of phosphorus. Subehia (2001) found that the use of rock phosphate in conjunction with different composts was similar to the use of superphosphate. Sulphur oxidation in soils is an effective process in the reclamation of sodic soils besides providing the sulphur needs for plants. More importantly, this process will lower the soil pH of resulting in an increased activity of some plant nutrients near the root zone and consequently cause an improvement in the yield and quality of agricultural crops. Kumar *et al.* (1992) reported the superiority of rock phosphate + sulphur compared with rock- phosphate alone in increasing macro-and micronutrients in soils and decreasing soil pH, which may be due to the oxidation of sulphur to sulphuric acid. El-Sayed (1999) revealed that Phosphate dissolving bacteria (PDB) play an important role in releasing P from rock, tri-calcium or other difficult P-forms through the production of organic and inorganic acids, as well as CO_2. These substances convert the insoluble forms of P into soluble ones. PDB also affect other nutrients rather than phosphorus. For example, Nassar *et al.* (2000) reported that faba bean seeds inoculated with PDB increased number of total bacteria generally and PDB in particular in the rhizosphere zone, number of nodules and released ammonia from bound complex nitrogen compounds.

Therefore, the aim of this study is to evaluate the efficiency of phosphate dissolving bacteria inoculation (PDB), sulphur and compost in releasing phosphorus from rock phosphate and its effect on vegetative growth, chemical composition and yield of faba bean plants as a p-responsive crop.

MATERIALS AND METHODS

A two years field experiments were carried out on a private farm at Al-Shaarawy village, Al- Bostan district (30°43' 25.14' N 30° 17' 23.94' E elevation 14 m), Al - Behaira Governorate, Egypt; during the two successive winter seasons of 2010 / 2011 and 2011 / 2012. Soil samples were collected, prior to tillage, from surface layer (0-20 cm) for physical and chemical analyses (Page *et al.,* 1982), (Table 1).

Furthermore, Organic manure compost was analyzed according to Cottenie *et al.* (1982) and Page *et al.* (1982) (Table 2).

Each experiment included nine treatments arranged in complete randomized block design with three replicates. Seeds of faba bean (*Vicia faba* L.) were sown in hills spaced 20 cm apart on both sides of the ridge, under drip irrigation system.

Table(1):Some physical and chemical properties of the experimental soil

Characters	Value
Particle size distribution %	
Sand	65.18
Silt	18.51
Clay	16.31
Textural class	Sandy loam
Chemical analysis	
$CaCO_3$ (%)	22.30
pH (1 : 2.5, soil : water, suspension)	8.15
EC (dS /m, 1 : 5 soil :water extract)	0.53
soluble ions (meq / 100 g soil)	
Ca^{++}	0.37
Mg^{++}	0.16
Na^{+}	0.58
K^{+}	0.08
CO_3^{--}	0.00
HCO_3^{-}	0.30
Cl^{-}	0.59
SO_4^{--}	0.30
Organic matter (%)	0.26
Organic carbon (%)	0.15
Total N (%)	0.04
Total P (%)	0.12
Total K (%)	1.21
Available contents (mg/kg soil)	
N	11.7
P	3.32
K	70.0
Fe	3.61
Zn	0.52
Mn	0.44

Table (2): Some properties of the used compost

Property	Content
Moisture content (%)	13.00
EC 1:10 (dS m^{-1})	3.80
pH (1 : 10)	7.50
Total N (%)	0.78
Total P (%)	0.52
Total K (%)	1.68
C/N ratio	22.70:1
Organic matter (%)	30.55
Organic carbon (%)	17.71

For all treatments, (Super phosphate and Rock phosphate) and sulphur were applied during seed bed preparation. While compost was uniformly incorporated into the soil layer of 20 cm depth with power tiller two weeks before planting. After thinning, all experimental plots received 20 kg N fed^{-1} as a starter doze in the form of ammonium sulphate (20.6% N) as well as 24 kg K_2O fed^{-1} in the form of potassium sulphate (48% K_2O). For the treatments that received PDB bacteria inoculation, faba bean seeds were coated with the bacterial inoculum containing *Bacillus megaterium* vr, phosphaticum that brought from the Biological Nitrogen Fixation Unit, Soils Water & Environ. Res. Inst., Agric. Res. Center, Giza, Egypt.

The treatments were as follow:

1- 22.5 kg fed^{-1} P_2O_5, as single super phosphate (SSP, 15 % P_2O_5), Control.
2- 22.5 kg fed^{-1} P_2O_5, as rock phosphate (RP, 28% total P_2O_5).
3- R P + 200 kg Sulpher (S) fed^{-1}.
4- R P + PDB (Seed inoculation with PDB just before sowing)
5- R.P. + 20 m^3 OM (compost) fed^{-1}.
6- R.P. + PDB + 200 kg S fed^{-1}.
7- R.P. + 200 kg S + 20 m^3 OM fed^{-1}.
8- R.P. + PDB + 20 m^3 OM fed^{-1}.
9- R.P. + PDB + 200 kg S + 20 m^3 OM fed^{-1}.

At maturity, the following characteristics were recorded.

1- Number of pods /plant.
2- Dry weight of seeds /plant (g).
3- 100 - seed weight (g).
4- Seed and straw yields (Kg fed^{-1}).

From each plot, samples of both seeds and straw were collected, oven dried (70°C); ground and wet digested using a 1:1 (*V/V*) mixture of H_2SO_4 and $HClO_4$ acids. In the acid digest N, P and K contents of both straw and seeds were determined. Nitrogen content was determined by Kjeldahl, phosphorus content was determined by a colorimetric method while Potassium content was determined by flame photometer as described by Chapman and Pratt (1961). Seed protein percentage was calculated by multiplying total N percentage by 6.25 and carbohydrate conten was estimated using the methods out lined by A.O.A.C. (1990). All collected data were statistically analyzed according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

3.1. Effect of the applied treatments on faba bean yield

In comparison with SSP, application of RP solely showed negative effect on faba bean yield, while addition of RP in the presence of compost or S or PDB greatly improved the efficiency of RP and the mixture of RP + compost out yielded the control treatment but yield of RP + PDB was still less than that of RP + OM combination, which increased straw and seed yield by 16.05% and 9.97 %, respectively, over the control, (Table 3). It was also noticed that faba bean yields resulted from combined treatment decreased in the following order; RP + OM > RP + S > RP + PDB. The increase in faba bean yields due to mixing OM with RP could be ascribed to the increasing the availability of phosphorus released from RP in soil during the OM decomposition producing organic acids among them gluconic acid which seems to be the most frequent agent of mineral phosphate solubilization. It is reported as the principle organic acid produced by solubilizing bacteria such as *Pseudomonas* sp. (Illmer, 1992). Also OM is considered as a source for all essential macro and micronutrients, which plays a direct role for a meliorating soil hydro-physical properties (i.e., soil aggregation, bulk density, total porosity, aeration , hydraulic conductivity and available water range), soil chemical characteristics (i.e., soil pH, released organic constituents of active groups such as fulvic and humic acids which have the ability to retain the essential plant nutrients in complex and available chelated forms), soil biological conditions ,i.e., a source of energy for the microorganism activities, which enhance releasing necessary nutrients in available forms throughout their mineralization, and in turn soil fertility status i.e. slow release for nutrients which support root development among the different growth stages , that finally leading to higher yield and its components of faba bean plants. Similar results were gained by Salem (2003) and Mohamed and El-Ganaini (2003).

The simulative effect of RP +OM combination was greatly improved by the addition of S or PDB in favor of RP + OM + S which gave significant increases in straw and seed yields over SSP by about 34 and 36.6%, respectively while RP + OM + PDB gave corresponding straw and seed yield increases by 25.4 and 27.7% respectively, meanwhile the combination of RP + S + PDB induced less increases by about 20 and 18.8%, respectively. The positive effect of elemental sulfur combination with RP + OM on faba bean yield may be attributed to the vital role in reducing soil pH, enhancing nutrient uptake, chlorophyll content and photosynthetic rate which reflected on growth and studied characteristics (El- Shamma, 2000 and Ali, 2002). Elemental Sulphur has an important role in the formation of plant protein and some hormones formation as well being necessary for enzymatic action, chlorophyll formation, synthesis of certain amino acids and vitamins. Hence, sulphur helps to have a good vegetative growth leading to have a high yield and increasing the absorption of macro and micronutrients (Salem, 2003 and Salem *et al.,* 2004).

It was also observed that the combined application of RP + OM + S together with PDB induced the highest faba bean yields. The increases in straw and seed yields reached 41 and 42%, respectively, compared with SSP (control). The studied yield parameters, i.e. no. of pods/plant, seed dry weight/plant and 100-seed weight showed similar trend with above mentioned treatments. The detected increases in faba bean yield as a result of PDB addition to the combination of RP + OM + S may be due to the inoculation with phosphate dissolving bacteria, which solubilized unavailable forms of calcium bound phosphate by excreting organic acids such as gluconic acid, formic, acetic, lactic, propionic, fumaric and succinic, those acids lowering the pH which directly bring in insoluble phosphates in soil into soluble forms (Azer *et al.,* 2003 and Ewais, 2006). There are indications that these bacteria may also produce growth promoting substances such as auxins, gibberellins and cytokinins, which could influence the plant growth by making root able to explore more soil and more zones where phosphate ions were chemically liberated from the RP? Similar phosphate effects of PDB have been obtained by Abd El-Lateef *et al.* (1998) on soybean,El-Sayed (1999) on lentil and Abdo (2003) on mungbean.

Table (3): Effect of different applied treatments on yield, components and some seed chemical constituents of faba bean plants (combined data of two seasons)

Treatnents	Total weight of green pods/ plant (g)	Number of pods/ plant	Dry weight of seed / plant (g)	100-seed weight (g)	Seed yield (Kg/ fed.)	Straw yield (Kg/ fed.)	Seed constituents		
							Seed protein (%)	Total carbohydrates (%)	Seed protein (Kg/ fed.)
SP	388.89	29.67	183.93	172.96	2476.48	2676.80	21.13	51.47	523.28
R.P	326.16	25.33	140..90	147.51	1986.72	2255.68	20.00	45.49	397.34
R.P + S	372.13	28.67	170.22	170.28	2249.60	2458.08	20.88	49.16	469.72
R.P + PDB	365.42	27.33	148.53	151.45	2123.84	2389.28	20.50	46.25	435.39
R.P + OM	396.29	30.67	191.37	174.71	2723.52	3106.40	21.88	52.80	595.91
R.P + PDB + S	418.22	31.67	200.51	175.95	2942.88	3223.50	21.56	51.47	634.48
R.P + S + OM	460.65	34.33	215.00	179.38	3384.32	3597.32	22.56	55.80	763.50
R.P + PDB + OM	449.32	32.67	205.27	177.37	3161.92	3356.16	22.19	53.13	701.63
R.P + PDB +OM+ S	521.23	36.33	226.72	184.45	3516.44	3783.52	22.81	56.47	802.10
L.S.D. at 5 %	20.84	3.70	5.26	3.44	65.68	57.80	0.23	3.76	6.32

SP = Super phosphate
R.P= rock phosphate
OM=compost
ES = Elemental sulphur
PDB =phosphate dissolving bacteria

Table (4): Effect of different applied treatments on total N, P and K content (kg fed^{-1}.) of faba bean plants (combined data of two seasons)

Treatments	Total Nitrogen Content (Kg/ fed.)			Total Phosphorous Content (Kg/ fed.)			Total Potassium Content (Kg/ fed.)		
	Seed	Straw	Total	Seed	Straw	Total	Seed	Straw	Total
SP	83.70	39.35	123.05	9.16	5.89	15.05	14.36	59.42	73.78
R.P	63.58	31.58	95.16	4.17	3.16	7.33	9.93	48.27	58.20
R.P + S	75.14	35.64	110.78	7.87	5.16	13.03	12.37	54.08	66.45
R.P + PDB	69.66	34.41	104.07	6.16	4.30	10.46	11.26	52.09	63.35
R.P + OM	95.32	48.15	143.47	10.62	7.14	17.76	17.70	71.14	88.84
R.P+ PDB + S	101.53	50.93	152.46	13.24	8.06	21.84	18.25	72.53	90.78
R.P + S +OM	122.17	59.19	181.36	16.58	9.69	26.27	23.01	84.30	107.31
R.P+PDB+OM	112.25	54.37	166.62	14.86	8.73	23.59	22.13	81.89	104.02
R.P+PDB+OM + S	128.35	63.94	192.29	19.34	10.59	29.93	25.32	94.21	119.52
L.S.D. at 5 %	1.03	0.73	1.31	0.66	0.70	0.99	0.78	1.39	1.38

Effect of different applied treatments on NPK contents of broad bean plant

The data presented in Table (4) shows the effect of different combination of ES, OM or PDB with RP on N, P and K contents of faba bean plants, which showed the same trend of faba bean yield. The combination of RP + Om gave a significant increases in N, P and K over SSP by 17.8, 15.9 and 23.2%, respectively, in seeds and 22, 4, 21.2 and 19.7%, respectively, in the straw, while the combination of RP + S or RP + PDB reduced the N, P and K content in both seed and straw. However, the positive effect of RP combined with OM was enhanced by the addition of ES or PDB in favor of RP + OM + S which increased N, P and K content over SSP by 45.9, 81.0 and 60.2% ,respectively, in the seed and 50.4, 64.5 and 41.9%, respectively, in straw. Moreover, the combination of RP + OM + S + PDB recorded the highest increases in N, P and K over SSP by 53.3, 111.1 and 76.3%, respectively, in the seed and 99.0, 97.8 and 76.3%, respectively in the straw. It could be noticed that P content was more affected by the applied treatments in most cases. These results are in agreement with those of Sahu and Jana (2000) and Evans *et al.* (2006).

In conclusion, combined treatments of OM + S + PDB along with RP gave a highly significant increases in yield and nutrient contents of faba bean as compared with the combinations. The most effective treatment was (RP + PDB + S + OM) followed by RP + OM + S, which achieved the highest yield parameters and N, P and K contents of seeds and straw of faba bean .

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تحسين القيمة السمادية لصخر الفوسفات عن طريق بعض محسنات التربة والبكتريا المذيبة للفوسفات وتأثير ذلك على نمو الفول البلدي في الأراضي الجيرية

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أجريت تجربتان حقليتان في قرية الشعراوي – منطقة البستان – محافظة البحيرة ، خلال شتاء موسمي ٢٠١٠/٢٠١١ و٢٠١١/٢٠١٢ ، بغرض دراسة تقيم الخلط بين صخر الفوسفات بمعدل ٢٢,٥ كجم فو٢أ٥ /فدان مع الكبريت بمعدل ٢٠٠ كجم /فدان والكومبوست بمعدل ٢٠ م٣ أو مع التلقيح بالبكتريا المذيبة للفوسفات لتحسين تيسر الفوسفور من الصخر الفوسفاتي وتأثير ذلك على محصول الفول البلدي، بالمقارنة مع السوبر فوسفات الأحادي بمعدل ٢٢,٥ كجم فو٢أ٥.

أظهرت النتائج المتحصل عليها أن استخدام الصخر الفوسفاتي فقط أو مع الكبريت و البكتريا المذيبة للفوسفات (الفوسفورين) أدت الى تأثير سلبي على المحصول و محتوى نباتات الفول البلدي من عناصر النيتروجين والفوسفور والبوتاسيوم ، ولكن كان التأثير إيجابي على محصول الفول إلى حد كبير عندما تم خلط السماد العضوي مع الصخر الفوسفاتي حيث أظهرت هذه المعاملة زيادة كبيرة في محصول الفول البلدى ومحتوى النبات من العناصر الغذائية. وهذا يظهر أهمية خلط الأسمدة العضوية بالصخر الفوسفاتي لتحسين خصائصه، وعلاوة على ذلك، تم تحسين تأثير الصخر الفوسفاتي جنبا إلى جنب مع السماد العضوي بشكل كبير عند تم دمجها مع الكبريت أو البكتريا المذيبة للفوسفات أوالجمع بين السماد العضوي والصخر الفوسفاتي والبكتريا والكبريت حيث سجلت أعلى الزيادات في البذور ومحصول القش (٤١٪ و ٤٢٪)، وكذلك N ، P و K محتوى نباتات الفول البلدي.

قام بتحكيم البحث

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