SIGNIFICANCE OF APPLIED SOME SOIL AMENDMENTS AND PHOSPHATE DISSOLVING BACTERIA FOR ENHANCING THE AVAILABILITY OF PHOSPHATE IN A CALCARIOUS SOIL

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ABSTRACT:

A field experiment was carried out on a Private Farm at Salah El-Din, El-Bostan (30° 43̥ 25° 14̥ N and 30° 17̥ 23° 94̥ E, elevation 14 m), Nobaria, El-Behiera Governorate, Egypt during the two successive growing seasons of 2008/2009 and 2009/2010. The main target of this study was at evaluating the best combination of rock phosphate with sulphur, organic manure (organic compost) and inoculation with phosphate dissolving bacteria to enhance the availability of phosphate from rock phosphate and their effects on yield and its components of broad bean plants (V*icia faba,L*. Luzdo Otono cv.).

The obtained results indicated that plant growth, seed and straw yields as well as nitrogen, phosphorus and potassium contents in various organs of broad bean plants were highly significantly increased due to the applied combined treatments comparing with the solely ones. It was also found that either sulphur application or inoculation with phosphate dissolving bacteria plus rock phosphate had a significant effect on broad bean yield and its quality, with a superior effect for sulphur application which was more effective than the biofertilizer (PDB). The interaction between rock phosphate combined with PDB or sulphur had significant effect on seed yield/fed, weight of 100 seeds and protein yield/fed. The highest values were recorded when broad bean plants were fertilized with rock phosphate combined with PDB plus sulphur and organic manure.

Application of rock phosphate and different soil amendments individually or together increased N, P and K contents in straw and seeds yields of broad bean plants. The highest contents of the studied nutrients were found when the plants were fertilized by rock phosphate and different soil amendments together. Results also showed the important role of organic matter, sulphur and PDB for releasing phosphorus from rock phosphate. So, it could be concluded that, the combination of different applied soil amendments with simultaneous application of rock phosphate, as a natural P-source, found to be possibility for saving significant quantities from the industrialized inorganic phosphate fertilizers.

Key words: Broad bean, organic manure, phosphate dissolving bacteria, soil amendments, sulphur, yield and its components.

INTODUCTION

Broad bean (*icia faba,L.*) is one of the most important legumes in Egypt. It is intensively used by both human and animals in many countries all over the world and considered a cheap diet with high protein and energy. Therefore, efforts for increasing and improving the quality of such vegetable crop must be given importance.

Under Egyptian soil conditions, phosphorus availability in soil is governed by many factors (pH, $CaCO_3$, organic matter and clay contents). In spite of the considerable addition of P to these soils, the level of available phosphorus decreases sharply after a short period from application **(Miller *et al.,* 1990)**. They revealed that under alkaline soil conditions, the available phosphorus in the added fertilizer are rapidly transformed to tricalcium phosphate thus becomes unavailable to the plants. Rock phosphate is the main source for producing phosphate fertilizers, where the total reserve source of rock phosphate in the world is about 128000 million tons. The direct application of apatite instead of phosphate fertilizers is not suitable, especially in the soils with height pH. But however, using the acidic materials such as sulfur, sulfuric acid, etc. or using rock phosphate combined with microorganisms such as (phosphate dissolving bacteria), which can produce some acids , will release phosphorus from rock phosphate and can replace P-fertilizers.

Therefore, rock phosphate is good source of phosphorus if organic matter and powdered sulfur with phosphate dissolving bacteria are added **(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 use of rock phosphate in conjunction with different organic manures was similar to that of superphosphate. Sulfur oxidation in soils is an effective process in the reclamation of sodic soils besides providing the sulfur needs of plants. More importantly , this process will lower the pH of the soil 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 that the superiority of rock phosphate + sulphur as compared with rock-phosphate alone in increasing macro-and micronutrients in soils and decreasing soil pH may be due to the oxidation of sulfur to sulphuric acid. On the other hand, **El-Sayed (1999)** revealed that PDB plays an important role in releasing P from rock, tricalcium or other difficult P-forms through producing organic and inorganic acids, as well as CO_2. These substances convert the insoluble forms of P into soluble ones. PDB also affects other nutrients rather than phosphorus. In this concern, **(Nassar *et al.,* 2000)** reported that seed inoculation with PDB increased number of total bacteria generally and PDB particularly in the rhizosphere zone, number of nodules and released ammonia from bound complex nitrogen compounds.

Therefore, the aim of this study was at evaluating the efficiency of PDB, sulphur and organic manure on releasing phosphorus from rock phosphate and their effects on vegetative growth, chemical composition and yield of broad bean plants.

MATERIALS AND METHODS:

A field experiment was carried out on a Private Farm at Salah El-Din, El-Bostan (30° 43́ 25° 14́ N and 30° 17́ 23° 94́ E, elevation 14 m), Nobaria, El-Behiera Governorate, Egypt during the two successive growing seasons of 2008/2009 and 2009/2010.

Soil samples were taken, before planting from surface layer (0-20cm) for physical and chemical analysis according to **Cottenie *et al.* (1982) and Page *et al.* (1982**) as well as organic manure (compost) were shown in Tables (1 and 2).

Table (1): Some characteristics of the experimental soil.

Soil character	Value	Soil character	Value
Particle size distribution %:		*Chemical analysis of soil water extract 1:5:*	
Sand	65.18	ECe (dSm^{-1})	0.53
Silt	18.51	*Cations (m molc/100 g soil):*	
Clay	16.31	Ca^{++}	0.37
Texture class	Sand loam	Mg^{++}	0.16
Soil pH (1:2.5)*	8.15	Na^{+}	0.58
Organic matter %	0.26	K^{+}	0.08
Organic carbon %	0.15	*Anions (m molc/100 g soil):*	
$CaCO_3$ content %	22.30	CO_3^{--}	0.00
Total N	0.04	HCO_3^{-}	0.30
Total P	0.12	Cl^{-}	0.59
Total K	1.21	SO_4^{--}	0.30

Available macro-micronutrients (mg kg^{-1})					
N	P	K	Fe	Mn	Zn
11.70	3.32	70.00	3.61	0.44	0.52

*1:2.5 w/v soil:water suspension

Table (2): Some characteristics of the used organic manure (compost).

Character	Value
Moisture content %	13.00
EC in dSm^{-1} (1:10 water extract)	3.80
pH (1:10 water suspension)	7.50
Organic matter content %	30.55
Organic carbon %	17.71
Total nitrogen %	0.78
C/N ratio	22.70
Total phosphorus %	0.52
Total potassium %	1.68

Each experiment included nine treatments, which were arranged in complete randomized block design with three replicates. The experimental unit area was 10.5 m^2 consisting of five rows (3.5 m long and 60 cm between row).

The applied treatments were as follow:

1. 150 kg/fed superphosphate (15% P_2O_5), *i.e.*, at a rate of 22.5 kg P_2O_5/fed.
2. 80.36 kg/fed rock phosphate (RP, 28% total P_2O_5), at a rate of 22.5 kg P_2O_5/fed.
3. RP + 200 kg S/fed.
4. RP + PDB, where broad bean seeds were coated just before sowing with the phosphate dissolving bacterial, using Arabic gum (40%) as adhesive agent and sown in hills spaced 20 cm apart on both sides of ridge, after 21 days from sowing, the plants were thinned to two plants per hill.
5. RP + 20 m^3/ fed organic manure (compost).
6. RP + PDB + 200 kg S/fed.
7. RP + 200 kg S/fed + 20 m^3/fed organic manure.

8. RP + PDB + 20 m^3/fed organic manure.
9. **R**P + PDB + 200 kg S/fed + 20 m^3/fed organic manure.

Phosphorus of two sources (superphosphate and rock phosphate) and sulphur were applied during seed bed preparation. Organic manure in the rate of 20 m^3/fed was uniformly incorporated into soil 0-20 cm depth with power tiller two weeks before planting. All the experimental plots received 20 kg N/fed as starter doze in the form of ammonium sulphate (20.6% N). Potassium sulphate (48% K_2O) was added at a rate of 24 kg K_2O/fed after thinning.

Seeds of broad bean (cv. Luzdo otono) were sown on 9th and 11th November in the first and second seasons, respectively, under drip irrigation system. All culture practices were completed according to usual methods being adopted for broad bean plants. At maturity, *i.e.*, 10th and 20th April for the 1st and 2nd seasons, respectively, broad bean plants were harvested and some plant characters such as total weight of green pods/plants (g), number of pods/plant, dry weight of seeds /plant (g), 100 seed weight (g), seed and straw yields (kg/fed) were recorded.

From each plot, samples of both seeds and straw were dried, ground and wet digested using a H_2SO_4–$HClO_4$ acid mixture. In the digested solution, nitrogen content of the plant organs (straw and seeds) was determined by Kjeldahl method according to **Chapman and Pratt (1961)**. Phosphorus content of plant organs parts was also determined by colorimetric method as described by **Troug and Mayer (1949).** Potassium content was measured by Flam Photometer as described by **Chapman and Pratt (1961).** Moreover, the biochemical constituents in broad bean seeds such as protein and carbohydrate were estimated using the methods outlined by **A.O.A.C. (1990).** All collected data were statistically analyzed according to **Gomez and Gomez (1984)**.

RESULTS AND DISCUSSION:

I. Effect of the applied treatments on yield and its components:

Results presented in Table (3) showed that total weight of green pods/plant, No. of pods/plant, dry weight of seeds/plant, 100 seeds weight, seed and straw yields as well as seed protein %, protein yield/fed and total carbohydrate % were significantly affected by applied RP in combination with soil amendments (S and organic manure) plus PDB either added as solely treatments or together. However, their greatest values were strictly associated with applied combined treatment of (PDB + compost + S). The corresponding relative increase percentages reached 34.03, 22.45, 23.26, 6.64, 41.99, 41.34, 7.95, 53.28 and 9.71% over the control treatment (SP), respectively. These increases in yield and its components of broad bean plants may be attributed to the increases in both cell division and cell elongation. In this concern, **Marschner (1998)** reported that the increment in vegetative growth characters could explained on the basis that phosphorus plays a major role in protein synthesis and protoplasm formation and may increased the proportion or protoplasm to cell wall with the result of an increased cell size. The simulative effect of P on growth of broad bean plants might be due to the fact that phosphorus is a part of molecular structure of nucleic acids DNA and RNA **(Mengel and Kirkby, 1987)**.

Table (3): Effect of different applied treatments on broad bean yield and its components (combined data of two seasons).

P-source and/or soil amendments	Total weight of green pods/plant, g	Number of pods/plant	Dry weigh of seed/plant, g	100 seed weight, g	Seed yield, kg/fed	Straw yield, kg/fed	Seed constituents		
							Protein %	Total carbohydrate %	Seed protein, kg/fed
SP	388.89	29.67	183.93	172.96	2476.48	2676.80	21.13	51.47	523.28
RP	326.16	25.33	140.90	147.51	1986.72	2255.68	20.00	45.49	397.34
RP+S	372.13	28.67	170.22	170.28	2249.60	2458.08	20.88	49.16	469.72
RP+PDB	365.42	27.33	148.53	151.45	2123.84	2389.28	20.50	46.25	435.39
RP+OM	396.29	30.67	191.37	174.71	2723.52	3106.40	21.88	52.80	595.91
RP+PDB+S	418.22	31.67	200.51	175.95	2942.88	3223.50	21.56	51.47	634.48
RP+S+OM	460.65	34.33	215.00	179.38	3384.32	3597.32	22.56	55.80	763.50
RP+PDB+OM	449.32	32.67	205.27	177.37	3161.92	3356.16	22.19	53.13	701.63
RP+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 0.05	20.84	3.70	5.26	3.44	65.68	57.80	0.23	3.76	6.32

SP=Superphosphate, RP=rock phosphate, S=Sulphur, PDB=Phosphate dissolving bacteria, OM=Organic manure (compost)

In addition phosphorus plays an important role in photosynthesis and respiration, it is also essential for division and development of meristematic tissues. Similar results were reported by **Abdul-Galil *et al.* (2003) and Abdo (2003)**. On the other hand, seed inoculation with the phosphate dissolving bacteria (PDB) caused remarkable increases in most parameters as compared with rock phosphate fertilizer only. These increases may be due to the inoculation with phosphate dissolving bacteria which solubleize unavailable forms of calcium bound phosphate by excreting organic acids such as formic, acetic, lactic, propionic, fumaric and succinic, those acids lowering the pH which directly bring in unsoluble phosphates in soil into soluble forms **(Nassar *et al.*, 2000; Azer *et al.*, 2003 and Ewais, 2006)**. There are indications that theses bacteria may also produce growth promoting substances such as auxins, gibberellins and cytokinins. Such substances could influence the plant growth by making root able to explore more soil and more zones where phosphate ions were chemically liberated from the P- source. These finding are in accordance with those obtained by **Abd El-Lateef *et al.* (1998)** on soybean**, El-Sayed (1999)** on lentil and **Abdo** (2003) on mungbean.

Concerning the interactive effects of treatments, the obtained data showed that the double combined applications being more effective than the single one, while the tri-combinations had the most effect, for enhancing the yield and yield components. Also, the stimulative effect of elemental sulphur application and inoculation with PDB in the presence of rock phosphate on yield and its components may be attributed to the vital role in reducing soil pH, enhancing nutrients uptake, chlorophyll content and photosynthetic rate which reflected on growth and studied characters, **Hewedy (1999), El-Shamma (2000) and Ali (2002).** Sulphur element has important roles in plant protein and some hormones formation as well as it is 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 (**Marschner, 1998; Salem, 2003 and Salem *et al.*, 2004)**. Also, data in Table (3) showed the effect of soil amendments, *i.e.* organic and sulphur on broad bean yield (seed and straw), yield components as well crud protein and carbohydrate (%). In general, the increase in (seed and straw) yield may be due to the addition of organic material which increase the availability of nutrients in soil during the decomposition process and produced CO_2, which plays an important role in increasing phosphorus availability. Also, significantly affected all studies characteristics of broad bean plants, probably due to the applied organic besides it is considered as a source for all essential macro and micronutrients, plays a direct role for a meliorating soil hyrophysical 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 chelate 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.,* low release for nutrients which support root development among the different growth stages , that finally leading to higher yield and its components of broad bean plants. Similar results were gained by **Abdel Aziz *et al.* (1998); Salem (2003) and Mohamed and El-Ganaini (2003)**.

II. Effect of different applied treatments on N, P and K contents of broad bean plant:

The data presented in Table (4) showed difference widely in uptake of N, P and K in broad bean seeds and straw as a result of applied treatments. The highest values of N, P and K contents of broad bean plant organs were recorded from soil received soil amendments combined with rock phosphate as compared with rock phosphate only. Phosphorus uptake was higher in plants fertilized with rock phosphate in combination with bio-fertilizer (PDB) or fertilized with organic manure as compared to those received RP only. That was true, since organic manure and PDB inoculation increased the efficiency of phosphorus released in low phosphate source. This could be attributed to the release of organic acids that can either reduce to pH of the surrounding or directly dissolve the mineral phosphate as a result of anion exchange of $(PO_4)^{-3}$ or due to the chelating property of the organic acid produced by PDB such as acetate, lactate, oxallate, citrate….etc. (**Abd El Latif *et al*., 2005**).

Also, increasing the uptake of N and K by application of PDB can be explained on the basis of depletion of such nutrients for building new tissues (**Nassar *et al.*, 2000**). On the other hand, the positive effect of P application on NPK contents in different parts of broad bean plants can be attributed to increase of the nodual number, size and mass, which in turn increases N_2-fixation by bacteria (**El Koumey *et al.*, 1993**). Moreover, application of P increases its concentration in the vicinity of plant roots and its availability in the soil solution as well as reduces its fixation by soil factors resulting from the introduction of most of P requirements at pre-planting in a limited zone, where the root growth is highly concentrated.

Moreover, **Marschner (1986)** described the enhancing effect of P on K uptake to the energy rich phosphates (in the form ATP) and the close relationship between K-uptake and ATP-ase activity.

Table (4): Effect of different applied treatments on total N, P and k contents in broad bean plants (combined data of two seasons).

P-source and/or soil amendments	Total nitrogen content (kg/fed)			Total phosphorus 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
RP	63.58	31.58	95.16	4.17	3.16	7.33	9.93	48.27	58.20
RP+S	75.14	35.64	110.78	7.87	5.16	13.03	12.37	54.08	66.45
RP+PDB	69.66	34.41	104.07	6.16	4.30	10.46	11.26	52.09	63.35
RP+OM	95.32	48.15	143.47	10.62	7.14	17.76	17.70	71.14	88.84
RP+PDB+S	101.53	50.93	152.46	13.24	8.06	21.84	18.25	72.53	90.78
RP+S+OM	122.17	59.19	181.36	16.58	9.69	26.27	23.01	84.30	107.31
RP+PDB+OM	112.25	54.37	166.62	14.86	8.73	23.59	22.13	81.89	104.02
RP+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 0.05	1.03	0.73	1.31	0.66	0.70	0.99	0.78	1.39	1.38

SP=Superphosphate, RP=rock phosphate, S=Sulphur, PDB=Phosphate dissolving bacteria, OM=Organic manure (compost)

The maximum increase percentage was obtained by mixing the soil amendments combined with rock phosphate, which caused increments of 53.35, 111.14 and 76.32% for N, P and K-uptake of broad bean seeds vs 62.49, 79.80 and 58.55% for N, P and K-uptake of broad bean straw as compared with the control treatment (SP). Also, the obtained data showed that the double combined application being more effective than the single one, while the tri-combinations had the most effect, for enhancing the nutrients taken by seeds and straw of broad bean. Such primitives effect of sulphur amendment on nutrients uptake probably due to its important role in reducing pH of the soils, through its oxidation to sulfuric acid by soil microorganisms, and subsequently resulted in solubilization and availability of nutrients to plants. Under the condition of that experiment, it was concluded that application of organic manure to sandy loam soil increased the efficiency of P mineral from RP as a P-source used, in addition to improve the physical and chemical properties of the experimental soil through its ability to adsorb nutrients on active groups or colloidal surfaces. Consequently, increasing the efficiency of nutrients uptake by plant roots are positively reflected on growth and crop productivity. Obtained results are in agreement with those of **Sahu and Jana (2000)** and **Evans *et al.* (2006).**

On conclusion, the addition of soil amendments and bio-fertilizer to sand loamy soil improved chemical and physical soil properties as well as soil available nutrients, where applied soil amendments gave a significantly positive effect than control. The combined treatments of (OM + S + PDB) gave highly significantly increases in yield and nutrient contents of broad bean as compared to the solely ones. The most effective treatment was (RP + S + OM) or (RP + PDB + S + OM) which achieved the highest yield parameters and N, P and K contents of seeds and straw of broad bean.

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أهمية إضافة بعض محسنات التربة والبكتريا المذيبة للفوسفات على تيسر الفوسفات فى تربة جيرية

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معهد بحوث الاراضى والمياه والبيئة – مركز البحوث الزراعية - جيزة – مصر

أجريت تجربة حقلية فى مزرعة خاصة فى قرية صلاح الدين، منطقة البستان، النوبارية، محافظة البحيرة خلال موسمى ٢٠٠٨–٢٠٠٩، ٢٠٠٩-٢٠١٠، بهدف تقييم الاستفادة من التسميد الحيوى والكبريت مع التسميد العضوى على تيسر الفوسفور من الصخر الفوسفاتى وتأثيرهم على محصول الفول الرومى (*Vicia faba,L.* Luzdo Otono cv.) ومكوناته.

وتشير النتائج المتحصل عليها إلى أن المعاملة الملقحة بالبكتريا المذيبة للفوسفات والمسمدة بكل من الكمبوست و الكبريت قد حققت أعلى زيادة معنوية فى نمو النباتات و محصولى البذور والقش وكذلك محتوى النتروجين والفوسفور والبوتاسيوم فى أجزاء النبات المختلفة مقارنة بتلك المعاملات المضاف اليها البكتريا المذيبة للفوسفات أو الكبريت او التسميد العضوى مع التسميد بصخر الفوسفات كل على حدة. كما وجد أن إضافة كلا من الكبريت أو التلقيح الحيوى مع الصخر الفوسفاتى أدى الى زيادة فى المحصول وجودته ولكن يمكن القول أن إضافة الكبريت كان أكثر تأثيرا من المخصب الحيوى (البكتريا المذيبة للفوسفات).

كان للتفاعل بين التسميد الأرضى بصخر الفوسفات مع البكتريا المذيبة للفوسفات أو الكبريت تأثير معنوى على محصول البذور للفدان، وزن ١٠٠ بذرة، محصول البروتين وقد سجلت أعلى قيم لهذه الصفات عند التسميد بصخر الفوسفات + التلقيح بالبكتريا المذيبة للفوسفات + الكبريت + الكمبوست. وقد تسببت إضافة الفوسفات مع مصلحات التربة منفردين أو معا الى زيادة محتوى القش والبذور لنبات الفول الرومى من النيتروجين، الفسفور، البوتاسيوم وكان أعلى محتوى من تلك المغذيات عند تسميد النباتات بالصخر الفوسفاتى ومصلحات التربة معا.

سجلت معاملة التسميد بالصخر الفوسفاتى ومصلحات التربة معا أعلى القيم لجميع القياسات السابق ذكرها، وقد أظهرت النتائج أهمية دور المادة العضوية والكبريت والتسميد الحيوى فى تيسر عنصر الفوسفور من صخر الفوسفات. وأخيرا فإنه يمكن إستنتاج أن إستخدام صخر الفوسفات كمصدر طبيعى للسماد الفوسفاتى مع مصلحات التربة يؤدى إلى توفير كميات معنوية من الاسمدة الفوسفاتية المعدنية المصنعة.