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Effect of Unconventional Phosphorus Sources and Phosphate Solubilizing Bacteria on Fractions of Phosphorus in a Calcareous Soil Cultivated with Wheat Plants

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Author's contribution

The sole authoress designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

A pot experiment was conducted at the experimental farm of Faculty of Agriculture, Ain Shams University, Egypt. To study the effect of recycling fish bone and bone meal as well as rock phosphate in a comparison with the ordinary superphosphate on the fractionation and availability of phosphorus in a calcareous soil. These treatments combined with or without adding specific bacteria. Wheat plants were grown under these treatments and their combinations to evaluate the residual effect of such treatments compared to the control (without any treatment) under different physiological stages of grown plants. Generally, the non-traditional phosphorus sources could succeed to substitute the ordinary superphosphate, as a source of phosphorus for soil and wheat plants, especially with addition of specific P-dissolving bacteria. The treatments of ½ OSP+FB and FB alone with adding P solubilizing bacteria were the best, which gave an acceptable results for the availability and fractions of P in the studied calcareous soil, more than giving a vigor plant growth compared with the other treatments. It means that recycling house wastes such as these unconventional P sources can replace with the traditional P sources without any contamination to the soil and growing plants with heavy elements, considering them environmentally friendly.

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Keywords: Ordinary superphosphate; rock phosphate; fish bone; bone meal; phosphate solubilizing bacteria; calcareous soil; phosphorus fractionation; wheat plants.

1. INTRODUCTION

As an essential element of plant nutrition, phosphorus (P) plays an important role in the agricultural sustainable development and ecosystem balance. In calcareous soils, precipitation of insoluble Ca phosphates is believed to be a major factor in the loss of availability of applied P [1]. Only 10-20% of the P applied through fertilization in calcareous soils is taken up by plants in the year of application because the majority of applied P is rapidly fixed or precipitated into poorly available forms [2]. The P dissolved from fertilizers undergoes various reactions with the inorganic and organic constituents of the soil resulting in products with varying degrees of solubility. Soil P is found in several chemical forms including soluble and exchangeable, organic bound, Ca-bound, Fe-Al-bound and residual phases. These phases are remarkably different in bioavailability, mobility and chemical behavior, thus play different roles in soil fertility [3]. Information about these fractions may shed some light in understanding of the P sinks and sources in soil and are essential for an efficient P management in agricultural fields.

Application of chemical P fertilizer like ordinary superphosphate is an effective method to increase yields, but is costly and may also lead to environmental problems such as presenting a serious risk of cadmium accumulation in soil [4]. Also, rock phosphates are derived from ancient marine deposits with total phosphate content around 30% while its available phosphate content ranges from 1 to 2% [5]. Important unconventional P sources which are environmentally safe include fish bone and bone meal. Fish bone is, like most animal by-products, rich in N and P. According to [6], it contains about 10% N, along with about 6% P. It is most frequently used as a feed additive, but can be used as a fertilizer. Bone meal contains about 27% total phosphate, and nearly all of it is available; other reports showed contents of P between 11-22% as P_2O_5. There is a great deal of confusion about the phosphate of bone meal because much of it is sold as a feed additive [7]. As a slow-release fertilizer, bone meal is primarily used as a source of P and protein. The N-P-K ratio of bone meal is generally 4-12-0, though some steamed bone meals have N-P-K of 1-13-0. Bone meal is also an excellent source of Ca, but does not provide enough N to plants.

Microbial fertilization is the vital implementation in sustainable agricultural systems to reduce environmental pollution and natural resource deterioration. Symbiotic and asymbiotic nitrogen-fixing bacteria, as well as the bacteria that are able to increase P solubility and intake by plants, are commonly used for microbial fertilization purposes [8]. *Bacillus megaterium* var. *phosphaticum*, which is considered a rhizobacteria, can exert a positive effect on plant growth through solubilization of inorganic phosphate and mineralization of organic phosphate, which make P available to plants [9].

Wheat (*Triticum aestivum* L.) is the first major and staple food crop in Egypt. Over 30% of the caloric intake comes from wheat flour products. However, the total production of wheat is still so far from the country requirements by about 40 to 50%, which is covered through importation [10]. Wheat plants can grow in calcareous soils and their root exudates can improve P availability in these soils.

Thus, the main objective of this study is to evaluate the effects of different traditional and non-traditional sources of P with or without phosphate solubilizing bacteria and their combinations, on the availability and forms of P in calcareous soil cultivated with wheat plant at different physiological stages.

2. MATERIALS AND METHODS

The current study was carried out in winter season of 2014 under greenhouse conditions at Faculty of Agriculture, Ain Shams University, Qalubia governorate, Egypt. The climatic data at the studied site during the winter season of 2014 are shown in Fig. 1.

2.1 The Pot Experiment

A pot experiment was conducted in a calcareous soil, *Typic Torripsamments*, sandy soil from El-Bustan at the West side of Cairo-Alexandria desert road, about 47 km South of Alexandria, Egypt. Representative soil samples were collected from the surface layers (0-20 cm), air dried and ground to pass through a 2 mm sieve. Some physical and chemical characteristics of the studied soil before cultivation were determined according to the standard methods outlined by [11] and the results obtained are presented in Table 1. Each polythene lined pot (18 cm in diameter and 15 cm in height) was packed uniformly with 3 kg of the investigated soil. Eighteen treatments were applied as follows:

1- Control (without P) – with *Bacillus megaterium* bacterial inoculation
2- Control (without P) – without bacterial inoculation
3- Ordinary superphosphate (OSP) – with *Bacillus megaterium* bacterial inoculation
4- OSP – without bacterial inoculation
5- Rock phosphate (RP) – with *Bacillus megaterium* bacterial inoculation
6- RP – without bacterial inoculation
7- Fish bone (FB) – with *Bacillus megaterium* bacterial inoculation
8- FB – without bacterial inoculation
9- Bone meal (BM) – with *Bacillus megaterium* bacterial inoculation
10- BM – without bacterial inoculation
11- ½ OSP+FB – with *Bacillus megaterium* bacterial inoculation
12- ½ OSP+FB – without bacterial inoculation
13- ½ OSP+BM – with *Bacillus megaterium* bacterial inoculation
14- ½ OSP+BM – without bacterial inoculation
15- ½ RP+FB – with *Bacillus megaterium* bacterial inoculation
16- ½ RP+FB – without bacterial inoculation
17- ½ RP+BM – with *Bacillus megaterium* bacterial inoculation
18- ½ RP+BM – without bacterial inoculation

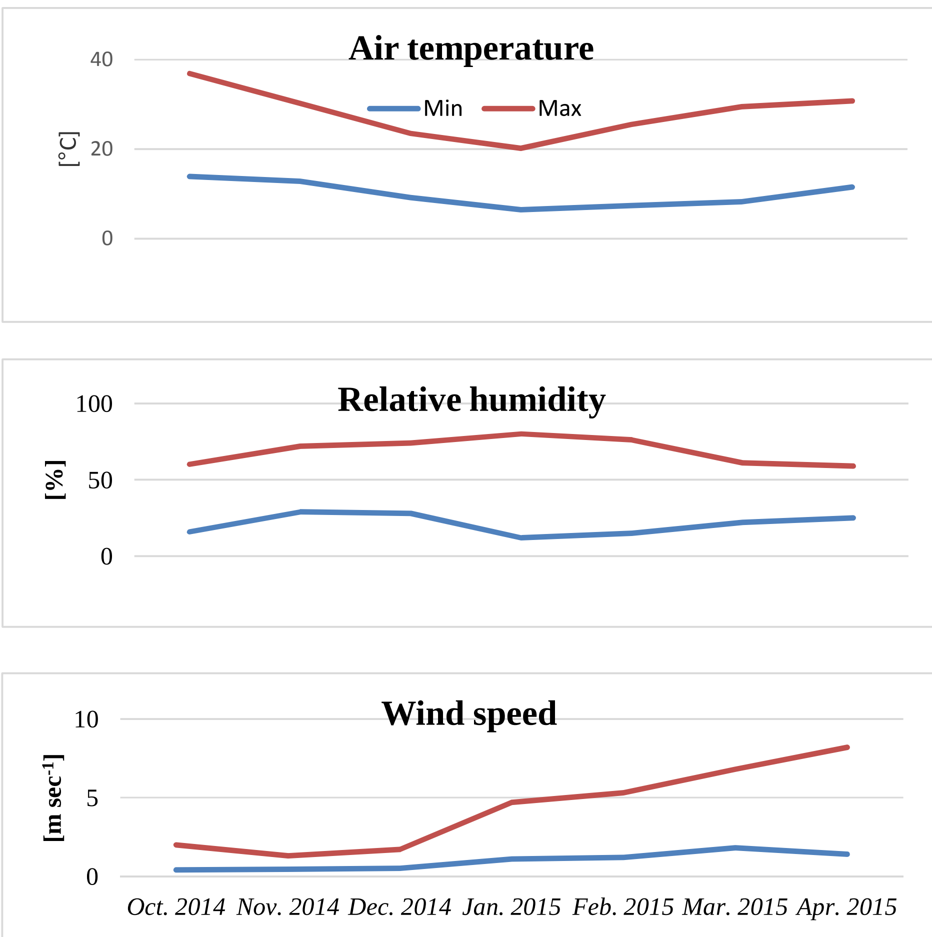

Fig. 1. Climatic data at the studied site during the winter season of 2014

The treatments were applied 15 days before plant cultivation and mixed well with the studied soil at a rate of 8 g/kg soil (equal to 8 ton/fed/15 cm depth), as recommended by the Egyptian Ministry of Agriculture for wheat cultivation. Fish bone and bone meal were collected from fish and meat shops, respectively, air dried and ground well to pass through a 2 mm sieve. The used P sources in this study were subjected to some chemical analyses as shown in Table 2. Tap water was used for irrigation to keep the moisture of the soil at the field capacity till the end of the experimental work.

Wheat grains (*Triticum aestivum* L. cv. Sakha 94) were cultivated on 17th of November 2014. After 2 weeks from germination, the plants were thinned to 3 ones per pot. Depending on the treatment, after 4 days from grains cultivation, 20 ml/pot of *Bacillus megaterium* var. *phosphaticum* bacterial suspension were added, supplied by the Department of Microbiology, Faculty of Agriculture, Ain Shams University. The fertilization of N and K were applied in form of $(NH_4)_2SO_4$ and K_2SO_4, at doses of 1 g/pot (equal to 0.33 ton/fed/15 cm) and ½ g/pot (equal to 0.17 ton/fed/15 cm), respectively, in two batches the first one at the vegetative growth stage and the other one before the expulsion of spikes stage. Plants were harvested on the first of April 2015, prepared and kept for some macro and micro nutrients determinations.

Soil in the investigated pots was sampled 4 times: (i) after grains germination (after 14 days from cultivation), (ii) at the vegetative growth stage (after 40 days from cultivation), (iii) before the expulsion of spikes stage (after 75 days from cultivation), and (iv) at harvest of wheat plants (after 135 days from cultivation). The collected samples were air dried, crushed, sieved through a 2 mm sieve and stored at 4℃ till analyzed.

2.2 Methods of Analysis

Soil available P extracted by Olsen extract (0.5 M $NaHCO_3$ at pH 8.5) and total P of wet digested soil using H_2SO_4/H_2O_2 mixture were spectrophotometrically determined according to [12].

Sequential extraction of P forms in the soil was performed with (i) 0.1 N NaOH to determine the non-occluded Al- and Fe-bound P, (ii) 1 M NaCl and citrate-bicarbonate (CB) to determine P sorbed by carbonates during the preceding NaOH extraction, (iii) citrate-bicarbonate-dithionite (CBD) to determine P occluded within Fe oxides and hydrous oxides, and (iv) 1 N HCl to determine Ca-bound P, according to the method described by [11].

Table 1. Some physical and chemical characteristics of the surface layer of the experimental soil (0-20 cm) before plant cultivation

Particle size distribution, %		**Soluble cations, mmol$_c$ L^{-1}**	
Sand	96.2	Ca^{2+}	7.06
Silt	2.70	Mg^{2+}	4.22
Clay	1.10	Na^{+}	1.10
Textural class	Sand	K^{+}	0.36
FC, %	8.10	**Soluble anions, mmol$_c$ L^{-1}**	
WP, %	2.50	CO_3^{2-}	0.00
SP, %	19.5	HCO_3^{-}	3.19
$CaCO_3$ fractions, %		Cl^{-}	2.35
Coarse sand	9.51	SO_4^{2-}	3.12
Fine sand	3.02	**Total macro nutrients, %**	
Silt	3.34	N	0.42
Clay	2.63	P	0.01
$CaCO_3$, g kg^{-1}	185	K	0.02
OM, g kg^{-1}	1.30	**Available macro nutrients, µg g^{-1}**	
CEC, cmol$_c$ kg^{-1}	10.5	N	3800
pH (1:2.5 soil:water suspension)	8.26	P	1.00
EC$_e$, dS m^{-1}	0.71	K	140

Table 2. N, P and K contents as percentages (%) of the studied P sources

P source	N	P	K
OSP	0.10	27.0	0.30
RP	0.04	18.0	0.10
FB	8.30	6.00	0.17
BM	1.78	14.3	n.d*
½ OSP+FB	8.40	19.0	0.24
½ OSP+BM	1.82	28.5	0.17
½ RP+FB	8.32	11.2	0.19
½ RP+BM	1.79	22.0	0.04

OSP: Ordinary superphosphate, RP: Rock phosphate, FB: Fish bone and BM: Bone meal.
**n.d means not detected*

The harvested plants were separated into roots, shoots and grains, dried at 70℃ in an air forced oven for 48 h, and then digested by H_2SO_4/H_2O_2 mixture according to the method described by [13]. Total N in the plant digests being determined using Kjeldahl method. Total P was determined using Spectrophotometer according to [12]. Total K in plant was determined using Flame photometer as described by [14].

2.3 Experimental Design and Statistical Analysis

The experiment was designed in a completely randomized design and each treatment was replicated three times. Data were statistically analyzed using the analysis of variance adopting a SAS software package at $P \leq 0.05$ [15].

3. RESULTS AND DISCUSSION

3.1 Chemically Available P in the Experimental Soil

Data in Table 3 showed that all treatments significantly increased the available P content of soil during the studied physiological stages. The increases in the presence of specific bacteria were more obvious than without adding these bacteria. The highest effect was achieved due to application of OSP followed by ½ OSP+FB then ½ OSP+BM. Available P in the soil increased till the vegetative growth stage and then decreased after 75 days from plant cultivation till the end of the experiment (after 135 days from cultivation). This may be due to high P content of the applied treatments (Table 2) which should be reflected on the available amount in the studied soil. In addition, *Bacillus megaterium* increased solubilization of P in both the applied treatments and the soil, consequently, available P content increased. However, the decrease in available P in the soil after 75 days from plant cultivation might be attributed to penetration and adsorption of P into the porous solids [16] as well as precipitation of Ca-P minerals during the period of the experiment, in addition of plant uptake and its requirements depending on its physiological stage. Sharma et al. [17] reported that an adequate supply of P during early phases of plant development is important for laying down the primordia of plant reproductive parts. In their study, [18] found that much of the P applied as mono calcium phosphate to Australian soils was converted rapidly to relatively insoluble forms after 4 weeks and only 10% of added P remained available (90% fixed) for plant. Also, [19] found a decrease of 30% in extractable P from amended soils after 45 days from incubation due to amend of humic substances (HS) and vermin-compost. While in our results, a decrease of around 46% in available P from amended soil after 75 days indicates the positive effect of the applied treatments on P availability. Han et al. [20] found that although RP is not readily available to a plant because the minerals are released slowly and its use as fertilizer often causes insignificant yield increases of current crop, adding P solubilizing bacteria being favorable and increased soluble P available to plant growth. This may be due to that specific bacteria synthesize organic acids solubilize P as reported by [9].

3.2 Phosphorus Fractions in El-Bustan Calcareous Soil

Data in Table 4 showed that, in the P amended or non-amended soil (the control treatment), with or without adding specific bacteria, P distribution followed the descending order: HCl-P (Ca-bound P)>CB-P (P sorbed by carbonates)>CBD-P (P occluded within Fe oxides and hydrous oxides)>NaOH-P (non-occluded Al- and Fe-bound P). On average the sum of P fractions of non-amended soil (control) with adding specific bacteria was 1.0% more than that of without adding P solubilizing bacteria. Similarly, sum values of P fractions of the soil treated with *Bacillus megaterium* var. *phosphaticum* were higher by 3.0 and 2.0% under rock phosphate (RP) and fish bone (FB), but 4.0% under ordinary superphosphate (OSP), bone meal (BM), ½ OSP+FB, ½ OSP+BM, ½ RP+FB and ½ RP+BM treatments, respectively. These sorts of P fractions agreed with those obtained by [21] in calcareous soil at El-Nobaria, Egypt, who indicated that the most of applied P was associated with poorly soluble Ca-P; this is expected under calcareous soil conditions. Also, he showed that P sorbed by carbonates came in the second order and related with Ca-P, followed by P occluded within Fe oxides and hydrous oxides, and lastly with a small amount followed by Al-P and Fe-P which can probably be used by plants.

The distribution of P among the different fractions in the untreated calcareous soil showed that the Ca-bound P is the prevalent form, in the presence or absence of P solubilizing bacteria. It represented about 57.1 and 68.9% of the sum of P fractions, respectively. The treatment of ½ RP+FB came in the second order after control with adding bacteria as giving the highest value (51.3%) for the Ca-bound P fraction. While the lowest one (46.9%) was shown by OSP treatment in the presence of bacteria. On the other hand, without adding bacteria, the greatest value (61.1%) was obtained by the treatment of RP while the lowest one (57.2%) was due to OSP treatment.

Phosphorus sorbed by carbonates was the second dominant fraction. On average, it represented 30.0 and 21.2% of the sum of P fractions in the soil with or without adding bacteria, respectively. The amount of Ca-P was found to be related to carbonate content of the studied soil. These results are in agreement with those obtained by [22]. On the other hand, P occluded within Fe oxides and hydrous oxides fraction represented 9.82 and 8.02% of the sum followed by non-occluded Al- and Fe-bound P. It amounted 3.07 and 1.91% of the sum in the studied soil with or without adding P solubilizing bacteria, respectively.

Table 3. Effect of the applied P sources on P availability in El-Bustan calcareous soil ($\mu g\ P\ g^{-1}$ soil), during the physiological stages of growing wheat plants

Treatment	After grains germination (14 days after cultivation)	At the vegetative growth stage (40 days after cultivation)	Before the expulsion of spikes stage (75 days after cultivation)	At plant harvest (135 days after cultivation)
		In the presence of P solubilizing bacteria		
Control (without P)	0.40	1.05	0.69	0.38
OSP	25.4	31.5	17.1	13.7
RP	2.51	2.30	1.42	1.16
FB	1.07	1.62	1.37	1.94
BM	0.70	1.42	1.08	1.47
½ OSP+FB	24.6	31.1	14.7	11.7
½ OSP+BM	24.0	28.4	12.5	7.59
½ RP+FB	1.48	1.88	2.30	1.29
½ RP+BM	1.06	1.53	1.34	1.18
$LSD_{0.05}$	**0.29**	**0. 55**	**0.19**	**0.16**
		In the absence of P solubilizing bacteria		
Control (without P)	0.14	0.18	0.19	0.16
OSP	23.3	23.8	12.0	8.65
RP	0.99	0.98	0.60	0.32
FB	0.62	0.75	1.18	0.89
BM	0.53	0.62	0.80	0.82
½ OSP+FB	20.9	17.9	10.0	5.89
½ OSP+BM	18.2	16.1	7.45	3.22
½ RP+FB	1.08	1.36	1.21	0.91
½ RP+BM	0.63	1.18	0.93	0.68
$LSD_{0.05}$	**0.11**	**0.31**	**0.12**	**0.10**

See footnotes of Table 2

Table 4. Effect of the applied P sources on P fractions in El-Bustan calcareous soil (μg P g^{-1} soil), after harvesting wheat plants

Treatment	Total P	NaOH-P	CB-P	CBD-P	HCl-P
		In the presence of P solubilizing bacteria			
Control (without P)	101	3.09	30.2	9.88	57.4
OSP	110	6.04	37.1	15.3	51.6
RP	106	5.11	35.3	12.4	53.2
FB	103	5.25	33.4	11.9	52.4
BM	106	4.81	36.7	11.4	53.1
½ OSP+FB	108	6.32	34.9	13.5	53.3
½ OSP+BM	112	6.08	39.0	12.4	54.5
½ RP+FB	105	4.91	33.6	12.6	53.9
½ RP+BM	109	4.89	37.8	12.0	54.3
$LSD_{0.05}$	**1.72**	**0.47**	**0.75**	**0.51**	**0.93**
		In the absence of P solubilizing bacteria			
Control (without P)	100	1.92	21.3	8.06	69.2
OSP	106	4.76	27.9	12.9	60.8
RP	103	3.97	27.0	8.98	62.9
FB	101	3.99	26.1	9.15	61.4
BM	102	3.32	28.0	8.86	61.8
½ OSP+FB	104	5.01	26.7	9.90	61.9
½ OSP+BM	108	4.78	31.6	9.34	62.2
½ RP+FB	101	3.95	26.4	9.26	61.4
½ RP+BM	105	3.89	30.2	8.59	62.3
$LSD_{0.05}$	**1.04**	**0.32**	**0.60**	**0.45**	**0.79**

- See footnotes of Table 2.

- NaOH-P, CB-P, CBD-P and HCl-P means non-occluded Al- and Fe-bound P, P sorbed by carbonates,

- P occluded within Fe oxides and hydrous oxides, and Ca-bound P, respectively

Application of the different amendments increased the P occluded within Fe oxides and hydrous oxides fraction as compared to the control either with or without adding bacteria. The treatment of OSP was the most effective treatment as it is amounted 4.09 and 4.11% increases, while the lowest ones were 0.94% increase compared with the control for the BM treatment with adding bacteria and 0.16% for the treatments of ½ RP+BM without adding bacteria. In addition, ½ OSP+BM caused significant increase in P sorbed by carbonates fraction recording 4.8 and 8.1% over the control treatment with or without adding bacteria, respectively. The lowest ones were obtained by the treatment of ½ RP+FB with adding bacteria recorded 2% increase over the control while ½ OSP+FB without adding bacteria caused 4.6% increase. In the meantime, the treatment of ½ OSP+FB increased the non-occluded Al- and Fe-bound P fraction by about 2.78 and 2.93% over the control with or without adding bacteria, respectively. The lowest values were obtained by the treatment of ½ RP+FB with adding bacteria (1.42% over the control) and the treatment of BM without adding bacteria (1.34%). In this concern, [17] reported that the average P content in soil was about 0.05% (w/w) but only 0.1% of the total P is available to plant because of poor solubility and its fixation in soil. Application of RP to soils with pH higher than 5.5 may not be effective because of reduced solubility. Regarding the BM, it increased soil pH, while OSP and FB decreased it, which should be reflected on the nutrient forms and availability [7].

3.3 Dry Matter Content, Weight of 1000 Grains, Macro and Micro Nutrient Concentrations in Wheat Plants

Data in Table 5 showed the dry weight, weight of 1000 grains, macro nutrients (N, P and K) and micro nutrients (Fe, Mn, Zn and Cu) concentrations in wheat plants cultivated in El-Bustan calcareous soil as affected by the different P sources and treated with *Bacillus megaterium* var. *phosphaticum*. Addition of such amendments to the studied soil improved general plant vigor and encouraged their yields. The highest effect on increasing wheat yield was achieved due to the treatment of ½ OSP+FB followed by FB and OSP applied separately. At the same time, RP, BM and their combinations, plus the treatment of ½ RP+FB caused moderate effect on wheat dry matter yield compared with the control treatment which resulted in the lowest yield. It was featured that the treatment of FB increased soil water holding capacity which is needed in managing the calcareous soils and improving plant growth.

Table 5. Dry weight, weight of 1000 grains, some macro and micro nutrient concentrations of wheat plants cultivated on calcareous soil as affected by the different P sources, in the presence of P solubilizing bacteria

Treatment	Dry weight of whole plant (g/pot)	Weight of 1000 grains, g	N	P	K	Fe	Mn	Zn	Cu
				%			$\mu g\ g^{-1}$		
Control (without P)	6.37	13.7							
Root			0.19	0.16	0.17	627	183	11.8	2.80
Shoot			0.22	0.19	0.21	196	95.4	3.36	1.07
Grains			0.90	0.20	0.70	112	41.8	0.96	0.20
OSP	13.2	43.3							
Root			0.32	0.40	0.62	761	237	32.4	9.36
Shoot			0.38	0.51	0.98	332	133	13.0	4.51
Grains			1.14	0.61	1.25	208	79.3	4.73	1.67
RP	10.0	31.7							
Root			0.30	0.28	0.38	750	229	28.7	8.15
Shoot			0.35	0.31	0.51	321	126	11.8	4.02
Grains			1.03	0.40	1.00	193	70.0	3.12	1.28
FB	16.3	46.0							
Root			0.79	0.22	0.53	691	210	20.7	7.13
Shoot			0.98	0.25	0.88	270	114	8.11	3.29
Grains			1.87	0.31	1.12	152	61.7	2.85	1.15
BM	10.0	31.0							
Root			0.39	0.25	0.26	643	191	15.1	3.23
Shoot			0.46	0.29	0.31	213	103	5.75	1.40
Grains			1.23	0.38	0.86	123	44.7	1.93	0.51
½ OSP+FB	16.5	51.3							
Root			0.83	0.29	0.58	723	218	25.1	8.11
Shoot			1.01	0.34	0.93	297	121	9.09	3.87
Grains			1.89	0.43	1.18	178	66.0	3.01	1.23
½ OSP+BM	10.9	34.3							
Root			0.50	0.63	0.42	675	204	19.0	5.34
Shoot			0.61	0.71	0.56	248	110	7.80	2.29
Grains			1.37	0.79	1.06	137	56.5	2.60	0.86
½ RP+FB	10.4	33.3							
Root			0.74	0.23	0.50	705	211	24.5	7.25
Shoot			0.90	0.29	0.81	275	117	8.83	3.29
Grains			1.83	0.35	1.07	162	64.3	2.91	1.09
½ RP+BM	10.1	32.3							
Root			0.43	0.35	0.29	664	198	17.7	4.51
Shoot			0.50	0.44	0.36	227	109	7.33	1.90
Grains			1.30	0.51	0.94	129	52.0	2.36	0.65
$LSD_{0.05}$	**0.29**	**0.94**							
Root			**0.03**	**0.02**	**0.02**	**2.74**	**1.37**	**0.69**	**0.21**
Shoot			**0.07**	**0.04**	**0.06**	**1.48**	**1.04**	**0.40**	**0.17**
Grains			**0.08**	**0.04**	**0.04**	**1.12**	**0.98**	**0.11**	**0.08**

See footnotes of Table 2

Table 6. Dry weight, weight of 1000 grains, some macro and micro nutrient concentrations of wheat plants cultivated on calcareous soil as affected by the different P sources, without adding P solubilizing bacteria

Treatment	Dry weight of whole plant (g/pot)	Weight of 1000 grains, g	N	P	K	Fe	Mn	Zn	Cu
				%			μg g^{-1}		
Control (without P)	5.27	11.7							
Root			0.14	0.12	0.13	540	167	8.19	0.49
Shoot			0.18	0.14	0.15	137	76.7	1.02	0.20
Grains			0.85	0.15	0.50	68.7	32.5	0.39	0.11
OSP	7.77	26.7							
Root			0.21	0.23	0.31	693	206	20.1	5.61
Shoot			0.25	0.27	0.49	275	113	7.88	2.73
Grains			0.95	0.32	0.96	154	59.1	2.73	1.16
RP	6.13	19.0							
Root			0.19	0.18	0.20	681	203	18.8	4.24
Shoot			0.22	0.22	0.25	253	112	7.45	2.29
Grains			0.94	0.25	0.71	140	56.7	2.61	1.03
FB	8.23	27.7							
Root			0.32	0.16	0.24	619	187	16.5	2.87
Shoot			0.37	0.18	0.29	187	98.1	5.56	1.65
Grains			1.14	0.20	0.78	105	43.9	1.91	0.63
BM	5.75	15.0							
Root			0.21	0.17	0.17	556	171	9.84	0.65
Shoot			0.26	0.20	0.23	146	81.0	1.37	0.39
Grains			0.97	0.22	0.61	77.9	36.7	0.56	0.20
½ OSP+FB	8.84	29.3							
Root			0.36	0.20	0.28	653	196	18.0	3.87
Shoot			0.42	0.25	0.33	220	107	7.01	1.94
Grains			1.19	0.27	0.91	129	53.5	2.63	0.86
½ OSP+BM	7.77	25.0							
Root			0.25	0.25	0.22	588	181	13.4	1.38
Shoot			0.29	0.30	0.28	169	93.0	3.71	0.85
Grains			1.02	0.36	0.78	89.5	40.0	0.94	0.39
½ RP+FB	6.44	23.7							
Root			0.28	0.17	0.26	637	194	17.2	3.27
Shoot			0.32	0.19	0.30	202	103	5.69	1.71
Grains			1.09	0.20	0.82	117	49.1	2.00	0.69
½ RP+BM	6.22	21.7							
Root			0.22	0.22	0.19	579	175	12.3	0.93
Shoot			0.29	0.25	0.25	158	83.5	2.65	0.60
Grains			0.98	0.29	0.68	83.8	38.1	0.76	0.23
$LSD_{0.05}$	**0.13**	**0.67**							
Root			**0.02**	**0.01**	**0.02**	**2.51**	**1.14**	**0.44**	**0.09**
Shoot			**0.04**	**0.02**	**0.03**	**1.13**	**0.82**	**0.26**	**0.05**
Grains			**0.05**	**0.03**	**0.04**	**0.98**	**0.50**	**0.07**	**0.03**

See footnotes of Table 2

The obtained results indicated marked increases in macro nutrient concentrations in the whole wheat plant parts due to treating soil with such treatments, in presence of specific bacteria, especially ½ OSP+FB for N, ½ OSP+BM for P and OSP for K. These results went hand in hand with the contents of such treatments of N, P and K (Table 2). The nutrient concentrations increased from root to shoot to grains which reflect their relatively fast movement from downwards to upwards.

Evaluation of micro nutrients contents in plants is very important to examine how the investigated unconventional phosphorus sources are safe for the studied plants (Table 5). Application of OSP followed by RP to the soil significantly increased the plant concentrations of Fe, Mn, Zn and Cu compared to the control. This may be due to their contents of these elements as impurities. The unconventional P sources and their combinations with OSP and RP came in the second order but were still higher than the control treatment. The results show that micro nutrient concentrations in wheat plants are accumulated in roots> shoots> grains, due to their slow movement. These results agree with those obtained by [23] with wheat plants.

Data in Table 6 showed the dry weight, weight of 1000 grains, macro and micro nutrient concentrations of wheat plants cultivated on El-Bustan calcareous soil as affected by the different applied P amendments, without adding P solubilizing bacteria. The obtained results went hand by hand with those reported in Table 5, although some significant decreases occurred due to absence of P solubilizing bacteria which enhance the nutrient availability in soil and consequently plant uptake beside their effect on promoting plant growth and yield. Erkovan [24] mentioned that *Bacillus megaterium* var. *phosphaticum* released acids into the rhizosphere which could increase the availability of P immobilized in soil and enhance plant growth by increasing the efficiency of uptake of other nutrients.

In general, micro nutrient concentrations in wheat plants reflected the amounts of these elements which are readily available in the cultivated soil, which in turn are highly affected by the kind of amendment applied to this soil, and role of plant roots and micro-organisms as well as their exudates. The obtained data were compared with the potentially toxic levels of trace elements in plants according to [25,26]. They found that 50-250, 30-300, 27-150 and 5-30 $\mu g\ g^{-1}$ for Fe, Mn, Zn and Cu, respectively, were within the normal range, while, 400-1000, 100-400 and 20-100 $\mu g\ g^{-1}$ for Mn, Zn and Cu, respectively, were considered potentially toxic. However, no limiting values were recommended for Fe. Therefore, the studied micro nutrient concentrations in wheat plants at harvest period were within the sufficient range. It is worth to mention that the plants grown on calcareous soil usually suffered from micro nutrients deficiency especially Fe, causing lime-induced chlorosis phenomenon.

4. CONCLUSION

The treatments of ½ OSP+FB and FB alone with adding P solubilizing bacteria gave higher P availability and fractions in the studied calcareous soil, more than giving a vigor plant growth compared with the other treatments in the pot experiment under greenhouse conditions. They featured by increasing their water holding capacity. In addition, they supplied plant with essential elements with suitable ranges. Therefore, it can be recommended that using such unconventional treatments with bio-fertilizers instead of chemical fertilizers; can save money, efforts and secure friendly environment by recycling wastes. In addition, help the soil to make recovery without any contamination with heavy elements such as Cd or causing any strange phenomenon like eutrophication as caused by applying chemical fertilizers in the soil.

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COMPETING INTERESTS

Authoress has declared that no competing interests exist.

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