Efficiency of P and K biofertilizers in utilizing soil and rock P and K by sorghum (*Sorghum bicolor*) grown on a light clay *torrifluvent* soil.

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Abstract

Two greenhouse pot experiments were done on biofertilizers P (B*. megaterium*), experiment 1(***Expt. 1***) and biofertilizer K (***B. circulans***), experiment 2 (***Expt.2***). Rock sources were rock phosphate for P (RP) and potassium feldspars for K (RK).The design was a randomized complete block, factorial, 3 replicates. Addition of biofertilizer (B_1) and no addition of it (B_0) were tested with the rock source (A_1) or without it (A_0) ,with 4 treatment combinations of : neither (B_0A_0), bio only (B_1A_0) , rock only(B_0A_1) and both combined (B_1A_1). RP (150 g P kg^{-1}) and RK (152 g K kg^{-1}) were used at rates (mg kg^{-1} soil) of 300 mg P , and 240 mg K during soil preparation. Inoculation was done through seeds as well as through soil Increases in yield of whole plant (shoots+roots) growth (47 days) were 14.0% (B_1A_0) , 28.7% (B_0A_1) and 31.1% ((B_1A_1) in ***Expt.1*** and 45.9, 63.6 and 75.1% respectively for ***Expt. 2***. Respective increases in uptake were 28.9, 42.3 and 71.2% for N , 113 , 131 and 162% for P , and 9.3 , 17.8 and 29.3% for K in ***Expt. 1***; 12.7, 10.4 and 47.5 % for N , 42.6, 36.9 and 57.8 % for P and 35.5, 40.2 and 85.0% for K in ***Expt. 2***. The highest nutrient uptake increases upon applying the biofertilizer + the rock source occurred in P for ***Expt. 1*** and in K for ***Expt. 2*** .Available nutrients after end of growth in ***Expt. 1*** increased by 75.0 and 50.0 and 77.4% respectively for N ; 26.2% , 1.3 and 56.4 % for P , but no increase for K. In ***Expt. 2*** ,no increase occurred to N , and the only increase in P (14.5%) was by the biofertilizer + the rock source ; increases for K were 4.6 , 42.4 and 48.1 % respectively.

Keywords: biofertilizers, rock P, sorghum, light clay soil

Introduction

In conventional agriculture there are two kinds of chemical materials used to enhance crop production: fertilizers and pesticide. Chemical fertilizers are plant food while chemical pesticides are its medicine. However being soluble chemicals, their excessive application could harm the environment through pollution which calls for rationalization and safe soil fertility management. Organic and biological fertilization, separately or combined may provide an answer, though with no spectacular results (**Abdel-Salam, 2014**). On the other hand, using natural rock sources of nutrients bearing nutrients such as P and K assisted by inoculation with special microorganisms that enhance solubilization of those nutrients may a practical venture. Soil microorganisms are very important in performing operations on nutrient cycling, as well as promoting plant health and growth **(Han et al., 2007 and Bhardwaj et al., 2014)**. Plant growth-promoting rhizobacteria (PGPR) are groups of beneficial microorganisms which carry out operations leading to providing plant nutrients to roots of plants as well as providing them with some substances for pest resistance **(Persello-Cartieaux et al., 2003, Kennedy et al., 2004 , Nelson 2004 , Sahoo et al. 2013 and Sahoo et al. 2014)**.

Micro-organisms dissolving P such as ***Bacillus megaterium*** (**Sharma et al., 2013.**), and those dissolving K as ***Bacillus circulans*** (Sheng **and He 2006**, **Megali et al. 2013. and Parmar and Sindhu, 2013)** may be used combined with rock sources to provide plant with P and K. The use of inocula of these microorganisms to inoculate seed and soils is referred to as biofertilization . Biofertilizers which are specialized in solubilizing P and K are manufactured and marketed under different commercial brand names. Their use is hoped to participate in having sustainable agriculture which is environmentally friendly. Clean Bio-fertilizers are low cost, renewable sources and may help in decreasing dependence on chemical fertilizers. The ***B. megaterium*** bacteria were used to solubilize (dissolve) insoluble phosphates through their decomposing of organic matter forming organic acids that cause insoluble phosphate to dissolve (Aziz **et al. 2012**). Biofertilization using P-dissolvers were applied ,with positive response, in field experiments on maize (**Abdel-Salam et al. 2012)** , faba beans (**Abdel-Salam et al. 2014**) and sunflower (**Abdel-Salam et al 2015**).The ***B. circulans*** bacteria are used to solubilize potassium of the K-bearing minerals by excreting organic acids which dissolve K and/or chelate silicon ions releasing K into soil solution **(Basak and Biswas 2008 , Basak and Biswas 2010 , and Megali et al. 2013).** The current study involves experiments using microorganisms to provide plant (Sorghum ***bicolor***) in presence or absence of P and K applied in rock forms (rock phosphate and potassium feldspars).

Materials and Methods

Two pot experiments were carried out under greenhouse conditions at the faculty of agriculture of Benha University, during the summer season of 2014 in order to study the effect of applying biofertilizers of

P and K on growth, yield and chemical composition of sorghum (***Sorghum bicolor*** L. Moench; cv. Horas) grown on a light clay ***torrifluvent*** collected from the 0-30-cm surface layer of an arable field in Mitkenana village, Toukh, Kalubiya Governorate, Egypt. The soil was air-dried, crushed, sieved through a 50-mm sieve in order to preserve the natural peds and soil aggregates, thus keeping the soil a well aerated medium for plant growth in pots. The soil was thoroughly mixed and prepared for the experiments. Data of physical and chemical properties of the soil as well as the water used for irrigation are shown in Table 1. Each experiment was designed a randomized complete block, factorial with 3 replicates. Polyvinyl chloride (PVC) pots (each having 30-cm depth and 20-cm diameter to hold 3 kg soil) were used. Where inoculation was performed, seeds were inoculated with the relevant micro-organism inocula immediately before seeding, the soil of the biofertilization treatments were also supplied with the relevant inoculants during preparation (through spraying with inocula suspension). Each of the two biofertilizers was in two forms: peat-moss-mounted, and suspension .The peat-moss forms were commercial products of PHOSPHORIN (for P) containing ***Bacillus megaterium*** , and POTASIUMAG (for K) containing ***Bacillus cerculans***. Both biofertilizers are products of the Egyptian Ministry of Agriculture. The suspension forms were prepared by the Microbiology Department of the Faculty of Agriculture. Seeds were sown (8 seeds pot^{-1}) on Sept. 2nd 2014, then the 7-day old, 3-cm tall transplants were thinned to 5 per pot. Watering was by tap water every day so as to reach about 80% of the soil moisture retention capacity (rather equivalent to field capacity).

Table 1. Properties of the soil and irrigation water used in the study.

Soil properties						
Particle size distribution%		**pH: (1:2.5 soil :water, w:v) =**	**7.8**			
Sand	**56.5**	**EC(dSm^{-1})**	**2.13**			
Silt	**26.8**	**$CaCO_3$ (g kg^{-1})**	**31.5**			
Clay	**16.7**	**OM (g kg^{-1})**	**2.4**			
Texture	***light* clay**	**Soluble ions (mmolc L^{-1})**				
Available (mg kg^{-1})		**Ca^{2+}**	**8.3**	**SO_4^{2-}**	**6.1**	
N	**25**	**Mg^{2+}**	**4.2**	**CO_3^{2-}**	**0.0**	
P	**9**	**Na^+**	**7.6**	**HCO_3^-**	**5.6**	
K	**88**	**K^+**	**0.7**	**Cl^-**	**9.1**	
Irrigation water properties						
EC :	**0.73 dSm^{-1}**			**Ions (mmlc L^{-1})**		
pH	**7.6**	**Ca^{2+} : 3.8**	**Mg^{2+}: 2.3**	**Na^+: 0.9**	**K^+ : 0.1**	**SAR**
Ca^{2+} 3.8	**Mg^{2+} 2.3**	**SO_4^{2-}: 0.6**	**CO_3^{2-} : 0.0**	**HCO^{3-}: 1.9**	**Cl^- : 4.6**	**0.5**

Notes:
1. Extractants of available nutrients: N (KCl); K (NH_4OAc); P ($NaHCO_3$).
2. Texture is according to international soil texture triangle (**Farshad, 1984**)
3. EC of paste extract.

After 47 days plants were cut 1-cm above the soil surface, weighed, then oven-dried at 70^{o}C till near constant weight. Root system of plants was extricated from soil pots following submergence of the "soil/root-system" in water, then after air-drying roots were weighed. Soil and plant samples were analyzed using methods cited by **Black et al (1965)** and **Chapman and Pratt (1961**).

Results and Discussion

Experiment 1: The P-dissolver "PDB" biofertilizer (B_1) and no application of it (B_0) were tested with rock-P "RP" (A_1) or without it (A_0).

Dry matter yield (Table 2)

Application of any or both of PDB biofertilizer (B_1) or rock phosphate "RP" (A_1) increased plant growth. The non-treated (A_0B_0) plants showed shoot growth of 20.67 g pot^{-1} , increased due to addition of fertilizers by 5.3% (B_1A_0) , 15.2% (B_0A_1) and 19.7% ((B_1A_1) indicating a positive effect of bio-fertilization ,particularly when combined with RP application. Combining the rock-P with the P- biofertilizer magnified the latter's positive response through the cumulating (additive) effect. The response of root growth was several times that of the shoots: 103% for the PDB, 147% for the RP and 166% for their combination.

Table 2. Sorgumt dry weight (gpot^{-1}) and NPK uptake (mgpot^{-1}) due to PDB biofertilizer and rock P*

Bio P (B)	Rock P (A)											
	A_0	A_1	mean	A_0	A_1	mean	A_0	A_1	mean	A_0	A_1	mean
	Dry weight			N uptake			P uptake			K uptake		
Shoots												
B_0	20.67	23.81	22.24	236	299	268	17.85	39.64	28.74	456	505	481
B_1	21.76	24.74	23.25	313	374	345	37.18	46.82	42.00	464	546	506
Mean	21.21	24.27		275	337		27.52	43.23		460	526	
LSD 0.05 A= ns B = ns AB= 1.65				A:44.0 B:44.0 AB:ns			A: 2.78 B::2.78 AB:3.92			A: 33.43 B : 33.43 AB: ns		
Roots												
B_0	2.04	5.43	3.74	25.8	73.5	49.7	1.90	5.99	3.95	20.4	56.4	38.41
B_1	4.14	5.04	4.59	60.4	74.1	67.3	4.80	6.18	5.49	56.9	70.2	63.58
Mean	3.09	5.24		43.1	73.8		3.35	6.09		38.7	63.3	
LSD 0.05 :A= 0.64 B = 0.64 AB= 0.91				A:9.1 B:9.1 AB:12.9			A: 0.65 B :0.65 AB: 0.91			A:12.06 B :12.06 AB: ns		
Shoots +Roots												
B_0	22.71	29.24	25.98	261.8	372.5	317.2	19.75	45.63	32.69	476.4	561.4	518.9
B_1	25.90	29.78	27.84	373.4	448.1	410.8	41.98	53.00	47.49	520.9	616.2	588.6
Mean	24.31	29.51		317.6	410.3		30.87	49.32		498.7	588.8	
LSD0.05 :A:1.06 B:1.06 AB:1.49				A: 47.7 B :47.7 AB:ns			A:3.13 B :3.13 AB:4.43			A: 35.2 B =35.2 AB=ns		

*B_0 and B_1 non inoculated and inoculated with P-dissolving *B.megaterium* (PDB); A_0 and A_1 non treated and treated with 300 rock phosphate P kg^{-1} .

The increase by the PDB was more where no RP was present, indicating that biofertilization is at its highest positive effect where no extra P source was present. Increases shown by total plant weight (shoots+roots) were in line with those of shoots and roots , they were 14.2 , 28.7 and 31.1% respectively. Increased availability of P in soil upon inoculation with PDB was reported by **Sharma et al. (2013) ,** and increased plant growth and yields caused by PDB were reported in field experiments by **Abdel-Salam et al. (2012)** on maize and **Abdel-Salam et al. (2014)** on beans**.**

N uptake: Application of either PDB or RP, or both combined, increased N-uptake. The increase was highest where the two fertilizers were combined. Increases in shoots N-uptake were 32.6, 27.7 and 58.5% for B_1A_0 ,B_0A_1 and B_1A_1 respectively. Those for roots N-uptake were much greater: 134, 185 and 187 % respectively. The uptake by "shoots +roots" was in a similar pattern and increases of 28.9, 42.3 and 71.2% due to B_1A_0, B_0A_1 and B_1A_1 respectively. This is a manifestation of the considerable positive effect of PDB , particularly in the root system.

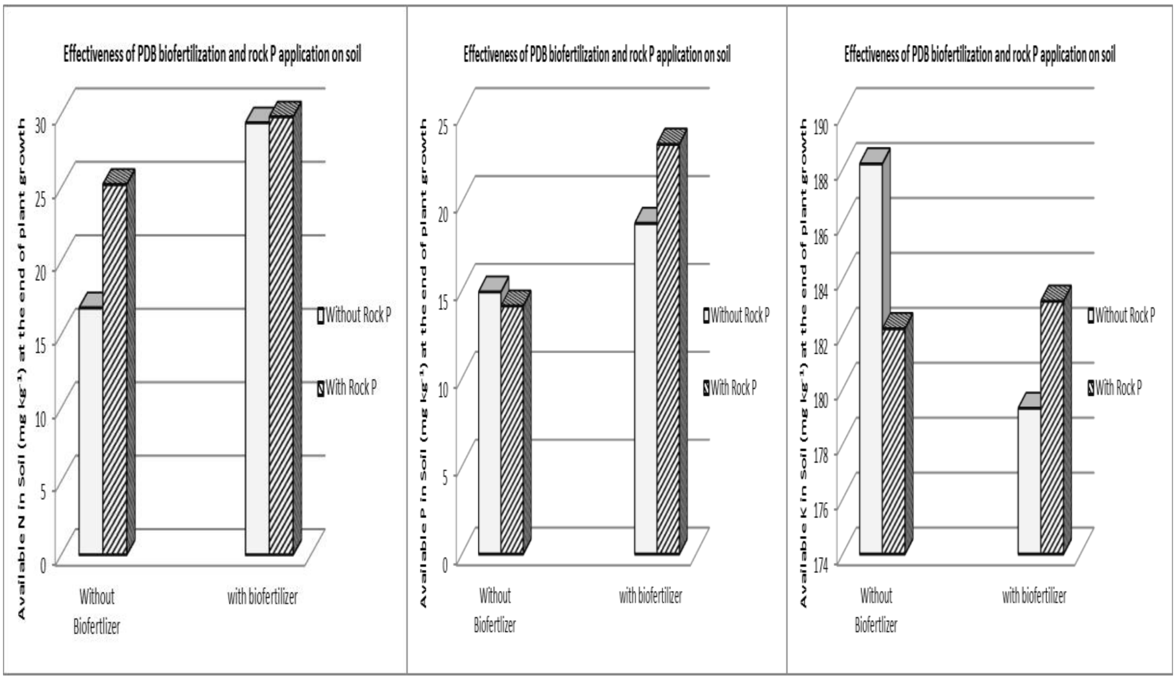
P-uptake: Application of either PDB or RP, or both combined increased P-uptake. Increases were 108% , 122% and 162% due to B_1A_0, B_0A_1 and B_1A_1 respectively for shoots and , 153 215 and 225% respectively for roots, and 113 , 131 and 162% respectively for whole plant. The positive effect of any of PDB or RP on roots P uptake was considerable when any of them was applied in absence of the other.

K-uptake : Application of either PDB or RP , or both combined increased K uptake .Increases due to B_1A_0, B_0A_1 and B_1A_1 were 17.5 , 10.7 and 19.5 % respectively for shoots , 179 , 175 and 244 % respectively for roots, and 9.3 , 17.8 and 29.3% respectively for whole plant. Increased K uptake caused by either PDB or RP occurred under presence or absence of the other.

The uptake of each of N, P and K increased in plant parts conforming with the increase in plant growth as a result of the increase in availability of the P nutrient caused by the P-biofertilizers especially when combined with the mineral P source.

Available N P and K in soil at end of plant growth (Figure 1)

Contents of available N and P in soil were greater in the treatments which received one or both of the P-fertilizers. Increases in available N were 75.0 and 50.0 and 77.4%. by PDB , RP 50.0 , and their combination respectively . Comparable respective increases regarding available P were 26.2%, 1.3 and 56.4 %. There were no increase in available K shown by the fertilization treatments probably due to high growth with high K uptake of the fertilized treatments causing a decrease in K remaining available in soil.



Available N Available P Available K

Available NPK at end of plant growth (sorghum) as affected by PDB (P-dissolving *B. megaterium*) biofertilization and rock P application ; 300 mg kg^{-1}.

Assessment of response of plant growth and N-P-K in plant and soil to P-biofertilization:

The P biofertilizer must have caused a marked release of insoluble P thus increasing available P as well as other available nutrients leading to increased growth and uptake of nutrients including NPK (**Abdel-Fattah et al., 2013 and Chi et al., 2010**). Solubilization of P would be manefested in greater plant growth and increased uptake of N, P and K nutrients (**Gholami et al, 2009 and Abdel-Salam et al. 2012 and 2015**). The rock phosphate increased plant growth and was supported by PDB inoculation. There are a number of mechanisms involved in P solubilization including a release of complexed P, solubilising insoluble mineral P through dissolution by organic acids, releasing siderophores, protons and hydroxyl ions (**Sharma et al., 2013**). Also, phosphorus dissolving bacteria may form substances such as gluconic and 2-ketogluconic acids which cause phosphate solubilization (**Park et al.,2010**). Production of siderophores, which are specific chelating agents of ferric ions (**Neilands, 1995**) and indole compounds (**Ambrosini et al., 2012**) must have been the cause of solubilizing insoluble phosphate. Application of P-dissolvers increased available N to plants expressed in nutrient uptake and residual available nutrients including P in soil (**Dogan et al., 2011 and Aziz et al. 2012**).

Experiment 2: The K-dissolver "KDB" biofertilizer (B_1) and no application of it (B_0) were tested with rock-K "RK" (A_1) or without it (A_0).

Dry matter yield(Table 3) : Application of any or both of KDB biofertilizer (B_1) or rock-K addition (A_1) increased plant growth. The increases in shoot weight were 45.0, 53.5 and 65.9%, due to B_1A_0, B_0A_1 and B_1A_1 respectively indicating a positive effect of bio-fertilization ,particularly when combined with RK application. Combining the rock-K with the K-biofertilizer magnified the latter's positive response through a cumulating (additive) effect. The response of root growth was several times that of the shoots: 52.8, 135 and 140% for KDB, RK and their combination respectively. Response of weight of whole plant followed a pattern resembling that of the shoot yield. Increases for the abovementioned treatments were 45.9, 63.6 and 75.1% respectively. The increase by the KDB was more where no RK was present, indicating that biofertilization is at its highest where no extra K source was present.

4.3.2. N uptake (Table 3): Application of any or both of KDB biofertilizer (B_1) or rock-K (A_1) increased N uptake. The increases in shoots were 6.1, 1.4and 33.8% due to B_1A_0, B_0A_1 and B_1A_1 respectively indicating highest increase when the biofertilizer was combined RK. The response regarding N uptake showed increases of 71.9, 95.2 and 174 % due to B_1A_0, B_0A_1 and B_1A_1 respectively. Increases for the abovementioned treatments concerning whole plant were 12.7, 10.4 and 47.5 % respectively thus indicating high efficiency of the K biofertilizer when combined with rock K.

4.3.3. P uptake (Table 2): Application of any or both of KDB biofertilizer (B_1) or rock-K (A_1) increased

P uptake by 33.7, 31.0 and 46.1% in shoots due to B_1A_0, B_0A_1 and B_1A_1 respectively. Corresponding increases in roots were 68.9, 154 and 169% for the B_1A_0 B_0A_1 and B_1A_1 respectively, and those in whole plant were 42.6, 36.9 and 57.8 % respectively.

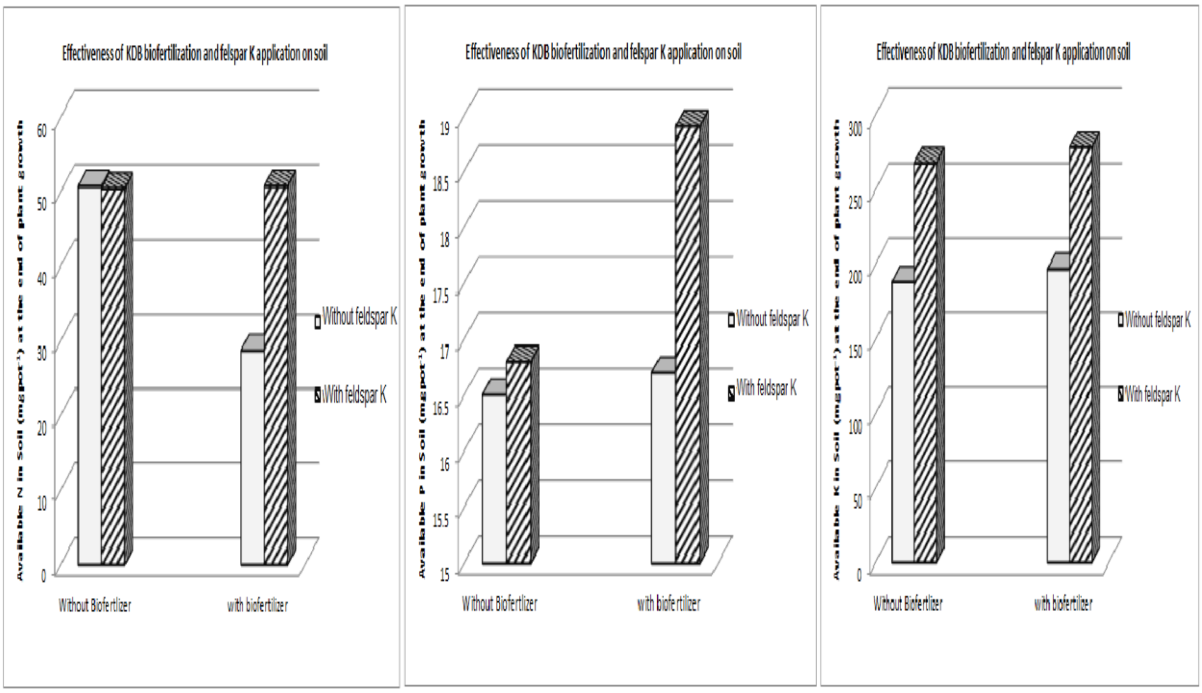
Table 3. Sorgum plant dry weight ($gpot^{-1}$) and NPK uptake($mgpot^{-1}$) due to KDB biofertilizer and rock K*

Bio P (B)	Rock P (A)											
	A_0	A_1	mean	A_0	A_1	mean	A_0	A_1	mean	A_0	A_1	mean
	Dry weight			N uptake			P uptake			K uptake		
						Shoots						
B_0	15.34	23.54	19.44	293	297	295	17.50	22.92	20.21	458	587	522
B_1	22.24	25.45	23.85	311	392	352	23.40	25.57	24.49	617	776	697
Mean	18.79	24.50		302	344		20.45	24.24		538	681	
LSD 0.05 A: 0.78 B: 0.78 AB: ns				A: 10.4 B : 10.4 AB: 14.7			A= 0.99 B : 0.99 AB: 1.41			A= 18.1 B = 18.1 AB= ns		
						Roots						
B_0	2.18	5.13	3.66	31.3	61.1	46.2	1.83	4.65	3.24	19.2	60.3	39.8
B_1	3.33	5.23	4.28	53.8	85.8	69.8	3.09	4.93	4.01	51.7	103.8	79.3
Mean	2.76	5.18		42.6	73.5		2.46	4.79		35.5	83.6	
LSD 0.05 : A:0.64 B : 0.64AB: 0.91				A: 10.1 B :10.1 AB:ns			A= 0.65 B : 0.65 AB: ns			A= 6.4 B = 6.4 AB= 9.1		
						Shoots +Roots						
B_0	17.52	28.76	23.10	324.0	357.8	340.9	19.33	26.47	22.90	477.3	669.1	576.2
B_1	25.57	30.68	28.13	365.2	477.8	421.5	27.57	30.50	29.03	646.8	883.0	764.9
Mean	26.55	29.68		344.6	417.8		23.45	28.48		562.1	776.1	
LSD0.05 :A: 0.64 B : 0.64 AB:0.91				A:14.4 B :14.4 AB:20.29			A=0.93 B = 0.93AB: 1.31			A:24.1 B : 24.1 AB: ns		

***B_0 and B_1: non inoculated and inoculated with K-dissolving B. *cerculans* (KDB); A_0 and A_1: non treated and treated with 240 mg feldspar K kg^{-1} .**

4.3.4. K uptake (Table 2): Application of any or both of KDB biofertilizer (B_1) or rock-K (A_1) increased K uptake by 34.7, 28.2 and 69.4% in shoots due to B_1A_0, B_0A_1 and B_1A_1 respectively. Comparable increases for K uptake in roots were 170, 214 and a considerable 441% due to B_1A_0 , B_0A_1 and B_1A_1 respectively , and those for the whole plant were 35.5, 40.2 and 85.0% % respectively.

Available N P and K in soil at end of plant growth (Figure 2): Available N was lowest in the KDB treatment indicating a marked exhaustion of available N . It may indicate a marked use of available N by plant due to increased growth and/or intensive use of available N by the ***B. circulans*** bacteria of the KDB biofertilizer. Combination of the K-biofertilizer with the feldspar K as well as the feldspar-K showed no increase in available N in soil. The pattern with available P revealed no increase except a 14.5%-increase where the two sources were given combined. On The other hand all fertilized treatments showed more available K. Treatments receiving KDB, RK and KDB+RK showed available K exceeding that of the non-treated by 4.6 , 42.4 and 48.1 % respectively . This is a manifestation of the positive effect of KDB and feldspar-K applied singly or combined in increasing available K in soil.



Available N **Available P** **Available K**

Figure 2: Available NPK at end of plant growth (sorghum) as affected by KDB (K-dissolving B.cerculans) biofertilization and rock K application (feldspar K); 240 mg kg^{-1}.

Assessment of response of plant growth and N-P-K in plant and soil
To k-biofertilization:

Biofertilization of K using the potassium-dissolving bacteria "KDB" ***B. Circulans*** caused a release of K from soil as well as from feldspars, therefore increasing available K .The B. *circulans* bacteria were reported by **Abd-El-Fattah et al. (2013) and Chi et al. (2010) to** release K nutrient along with other substances favouring plant growth leading to increased growth and increased uptake of nutrients by a number of crops. Insoluble K was reported to convert into plant available form by acidification formed during activity of *B. circulans* bacteria **(Hinsinger et al. 1996; Sanz Scovino and Rowell 1988; Bakken et al. 1997, 2000)**. The positive effect of feldspar addition without combination with ***B. Cerculans*** indicates its beneficial effect in releasing K **(Harley and Gilkes 2000, and Yao et al. 2003)**, although their effectiveness may be rather low (**Hinsinger et al. 1996; Bolland and Baker 2000 and Harley and Gilkes 2000)**.Fused potassium silicates, which contain $K_2Ca_2Si_2O_7$, has been prepared and used as a slow-release K fertilizers (**Yao et al. 2003**) .

Conclusions and Practical Implicatins

Biofertilizers of bacterial inocula of ***B. megaterium*** (for P fertilization) and ***B. cerculans*** (for K fertilization) can be practical propositions in fertilizing crops and soils in Egypt. Such fertilization can increase the availability of soil as well as rock P and K for plant. Biofertilization technique through seed inoculation in combination with bio fertilizing the soil by through spraying the soil with inocula suspension can cause maximum utilization for availability of rock sources of the two nutrients. On the other hand, biofertilization combined with application with pulverized rock sources of P and K enhances the increase. The increase in sorghum growth could be up to as high as 50% in above-ground plant growth and 150 % in root growth. The practical implications indicate a definite crop yield enhancement obtained by farmers using biofertilizers in combination with rock sources procedure. Such management practice would be environment-friendly as well as cost-effective for Egyptian agriculture.

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