

NPK BIOFERTILIZERS ON SORGHUM (*SORGHUM BICOLOR*) GROWN ON A LIGHT CLAY TORRIFLUVENT SOIL AND INTERACTIONS AMONG THEM.

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A.brasilense *Sorghum bicolor*

ABSTRACT

A pot experiment was carried with biofertilizers of N₂-fixers (*A. chroococcum*+*A.brasilense*), P-dissolvers (*Bacillus megaterium*) and K-dissolvers (*Bacillus circulans*) for sorghum (*Sorghum bicolor*) grown on a light clay *torrifluvent* soil. Different combinations of such N₁, P₁ and K₁ biofertilizers were compared with the N₀P₀K₀ non-addition which gave 15.2 g pot⁻¹. All additions giving one or more or all of the 3 biofertilizers caused positive response. Ranges of % increase were: 63 (N₁P₀K₀) to 81(N₁P₁K₀) for yield; 63 (N₀P₀K₁) to 140 (N₁P₁K₀) for N uptake; 88 (N₁P₀K₁) to 224 (N₁P₁K₁) for P uptake and 69 (N₀P₁K₀) to 130 (N₀P₀K₁) for K uptake. When given singly (solely), the percentage increase caused by any of the 3 biofertilizers was higher than when given in presence of any or both of the others (i.e. interaction effects). For yield, increases of 63, 67 and 65 % occurred due to a sole application of N, P and K biofertilizers respectively. Main (average) increases were 13, 14 and 12 % for each biofertilizer respectively (irrespective of presence or absence of the others). The average increase by one was greater in absence of each of the others, and generally non effective in presence of the other. Similar patterns occurred regarding uptake of N, P and K. The interactions among the 3 biofertilizers were evident. An indication of competition among the micro-organisms could have taken place. Practical implications indicate that biofertilizers could be used to decrease total dependence on chemical fertilizers.

1. INTRODUCTION

In conventional agriculture chemical fertilizers are used to enhance plant growth and increase crop yields. Their excessive application could harm the environment. Thus less of their application and more of organic and biological fertilization can help to decrease the harmful effect. Introduction of beneficial microorganisms into soil in a capacity

of **biofertilization** could increase plant growth by providing nutrients in available forms (Han et al., 2006, Abdel-Salam et al. 2012, Abdel-Salam 2014 , Sahoo et al. , 2014 and Abdel-Salam et al. 2015). However , increased crop production by application of biofertilizers alone may not be considerable so as to depend on them alone (Abdel-Salam 2014) . Besides providing nutrients in available forms , biofertilizers can produce beneficial substances such as the plant hormones indole acetic acid 'IAA', gibberellins 'GA' and cytokinins 'CK' (Abdel-Fattah et al., 2013 and Chi et al., 2010). Biofertilizers were reported to enhance plant tolerance to stress and resistance to pathogens (Chi, et al., 2010 , Thamer et al., 2011 and Sahoo et al., 2013). Inocula of microorganisms used as biofertilizers include (a): **N₂-fixing micro-organisms** as *Azotobacter* and *Azospirillum* species (Bhardwaj et al 2014 , Sahoo et al., 2013, Sahoo et al. 2014 and Saikia et al., 2013) (b) phosphorus solubilizing micro-organisms such as *Bacillus megaterium* (Sharma et al., 2013.) , and (c) potassium solubilising microorganisms as *Bacillus circulans* (Sheng and He 2006 , Megadi et al. 2010. and Priyanka and Sindhu 2013). *Azotobacter* bacteria are free living non-photosynthetic aerobic N₂- fixing bacteria which activate nitrogen cycle in the rhizosphere (Sahoo et al 2013) , and produce vitamins as thiamine and riboflavin (Revillas et al. 2000) and hormones as indole acetic acid "IAA", gibberellic acids "GA" and cytokinins "CK" (Abdel-Fattah et al., 2013). *Azotobacters* were used to biofertilize many crops including wheat, oat, barley mustard, sesame, rice, linseeds, sunflower, castor, maize, sorghum, cotton, jute, sugar beets, sugarcane, tobacco, tea, coffee, rubber and coconuts ; with yield increases of 2 to 45% in vegetables, 9 to 24% in sugarcane, and up to 31 % maize, sorghum and mustard (Wani et al., 2013). Mali and Bodhankar (2009) mentioned that *Azotobacter chroococcum* produce antifungal and phytohormone substances . *Azospirillum* bacteria are other free-living N₂-fixers which produce regulating substances , gibberellins, ethylene and auxins (Bashan et al., 2004 and Perrig et al., 2007) which thrive in wet soils (Bashan et al., 2004 , Gholami et al. 2009) and can be effective in biofertilizing many crops (Saikia et al. 2013 and Sahoo et al., 2014) .*B. megaterium* bacteria are used to solubilize (dissolve) insoluble phosphate while decomposing organic matter and forming organic acids (Aziz et al. 2012). Biofertilization using N₂fixers, P-dissolvers were applied on maize (Abdel-Salam et al 2012) , faba beans (Abdel-Salam et al 2014) and sunflower (Abdel-

Salam et al 2015) with positive response .The *B. circulans* bacteria are used to solubilize potassium in K-bearing minerals soils by excreting organic acids to dissolve K and/or chelate silicon ions releasing K into soil solution (Basak, and Biswas 2009 & 2010 , Megadi et al. 2010, and Priyanka, and Sindhu 2013). The current study aims at assessing biofertilization by three kinds of microorganisms: N₂-fixers , P-dissolver bacteria (PDB) and K-dissolver bacteria (KDB) on sorghum.

2 . MATERIALS AND METHODS

A pot experiment was carried out under greenhouse to study the effect of inoculation with biofertilizers providing N, P and K for sorghum (*Sorghum bicolor*) grown on a light clay *torrifuvent* soil taken from an arable field in Toukh, Kalubiya (Table 1).The design was a randomized complete block, factorial (3 replicates) with 3 factors as follows:(1)N-bio-fertilization: none (N₀) and N₂-fixers *Azotobacter chroococcum* + *Azospirillum brasilense* (N₁) ; (2)P-bio-fertilization: none(P₀)and PDB *Bacillus megaterium* (P₁) and (3) K-biofertilization: none (K₀) and KDB *Bacillus circulans* . Thus there were 8 treatments (2 N x 2 P x2 K). Eight seeds were sown per pot (Pot of 3 kg capacity). Seven days after seeding, emerged seedlings were thinned to 5 per pot. Watering of pots was by tap water every day so as to reach about 85% of the soil moisture retention capacity. After 47 days, plants were cut above the soil surface, weighed, then oven-dried at 70°C till near constant weight. Roots of plants were extricated from the pots , weighed then dried as done with the above-soil surface parts. Plant samples were wet-digested by a concentrated H₂SO₄/ HClO₄ mixture for determination of N, P and K. Soil and plant analyses were done according to methods by Chapman and Pratt (1961) and Black et al. (1965).

Table 1: Physical and chemical characteristics of the soils used in the study.

Soil property				Notes: 1.Extractants of available nutrients :N (KCl) ; K (NH ₄ AOc) ; P (NaHCO ₃) . 2. I / C : light clay (according to international soil texture triangle) . 3.EC of paste extract.		
Particle size distribution%		pH:(1:2.5 soil water)	7.8			
Sand	56.5 %	EC(dSm ⁻¹)	2.13			
Silt	26.8 %	CaCO ₃	31.5			
Clay	16.7 %	OM (g kg ⁻¹)	0.24			
Texture	IC	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

3 . RESULTS AND DISSCUSION.

Since the experiment is a factorial , the non-treated treatment (i.e. the treatment given no biofertilizers) is referred to as the "no-biofertilizer treatment" or "the non-treated treatment", and not referred to as "the **control**" treatment, since the term **control** is used in the **simple one-factor experiments** (i.e. non-factorial experiments). Factorial experiments should be statistically analyzed as **such** and results should be tabulated **appropriately** exhibiting the **main** as well as the **interaction** effects . The main effect of each factor will be mentioned, and if there is (are) interaction effect(s), further elaboration on results would be done.

3.1 Yield (Table 2):

3.1.1. Shoots dry matter yield:

Application of any or more than one biofertilizer caused increases in yield. The non-treated plants showed 15.24 g pot⁻¹ ,increased by 55.3% (N₁P₁K₁) to 75.4% (N₀P₁K₁) indicating a positive effect of bio-fertilization. Higher yield obtained by the treatment given P and K over that given N , P and K may indicate a competition between the N₂-fixer and the two other biofertilizers. (Mohammadi and Sohrabi 2012). **Effect of the N-biofertilizer** was an increase averaging 9.7 % (main effect). The positive effect of N was most prominent where no P-dissolvers were present giving as much as 29.6% increase, but no significant increase in presence of P-dissolvers. The positive effect was also marked where no K-dissolvers were present giving 24.8%, but no response given in their presence. Therefore N₂-fixers gave their highest response in absence of any one of the other two biofertilizers. This shows a 2-factor significant interaction caused by each of P-dissolvers and K-dissolvers affecting the response to N₂-fixers. Besides, a 3-factor interaction occurred when no response to the N₂-fixers was obtained under conditions of a combined presence of P- + K-dissolvers ; but 64.9% increase by N₂-fixers in absence of the other two together. **Effect of the P-biofertilizer** shows an average increase of 10.2% , and the increase was particularly where no N₂-fixers were present averaging 30.2% compared with no increase in their presence. There was 24.8% increase in absence of K-dissolvers but no response in their presence A 3-factor interaction is shown when the 65.0% increase obtained by P-dissolvers in absence of both N₂-fixers and K-dissolvers are compared with no increase in presence of N₂-fixers+K-dissolvers.

Effect of the K-biofertilizer shows an average 11.9% increase. The increase was particularly in absence of N₂-fixers giving 27.1% as compared with no effect in their presence. The increase was also in absence of P-dissolvers (26.6%) , and no effect in their presence. The 3-factor interaction is shown when in absence of both N₂-fixers and P-dissolvers; the K-dissolvers caused a considerable 61.4% increase. In combined presence of N₂-fixers+P-dissolvers, the K-dissolvers were not effective.

Table 2 : Effectiveness of NPK biofertilization on sorghum (*Sorghum bicolor*): Dry weight of plant parts (gpot⁻¹)

Bioferts. N&P		Biofert. K								
N	P	K0	K1	Mean	K0	K1	Mean			
Shoots					Roots					
N0	P0	15.24	24.61	19.92	2.80	4.76	3.78			
	P1	25.14	26.73	25.94	4.99	5.86	5.42			
	Mean	20.19	25.67	22.93	3.89	5.31	4.60			
N1	P0	25.13	26.51	25.82	4.70	5.22	4.96			
	P1	25.25	23.70	24.47	6.42	6.57	6.49			
	Mean	25.19	25.10	25.15	5.56	5.89	5.73			
G. mean		22.69	25.39		4.73	5.60				
		Means of P			Means of P					
P0		20.19	25.56	22.87	3.75	4.99	4.37			
P1		25.19	25.22	25.20	5.70	6.22	5.96			
LSD 0.05		N= 1.06 P= 1.06 K= 1.06 NP= 1.49 NK= 1.49 PK= 1.49 NPK=2.11			N= 0.45 P= 0.45 K= 0.45 NP= ns NK= 0.64 PK= ns NPK= ns					
whole plant										
N0	P0	18.04	29.83	23.94	Notes Biofertilizer inocula:- N:Azotobacter chroococcum + Azospirillum brasilense. P:Bacillus megaterium K: Bacillus circulans Application: Seed inoculation+ soil addition.					
	P1	30.12	31.66	30.89						
	Mean	24.08	30.75	27.42						
N1	P0	29.37	31.72	30.55						
	P1	32.59	30.27	31.43						
	Mean	30.98	30.99	30.99						
G. mean		27.53	30.87							
		Means of P								
P0		23.71	30.78	27.24						
P1		31.36	30.97	31.16						
LSD 0.05		N= 1.23 P=1.23 K=1.23 NP=1.74 NK= 1.74 PK=1.74 NPK=2.46								

3.1.2. Roots dry matter yield:

The lowest root yield of 2.80 g pot⁻¹ was by the non-treated. Plants supplied with one or more of the biofertilizers gave more yields, ranging from 70.0 % (N₀P₀K₁) to 134.6% (N₁P₁K₁) indicating a cumulative effect of the 3 biofertilizers (**Mohammadi and Sohrabi 2012**).

Effect of the N biofertilizer was a 24.6 % average. Presence or absence of P-dissolvers did not affect the response to N₂-fixers (i.e. no

interaction), but the increase was in absence of K-dissolvers (42.9%) than the increase in their presence (10.9%).

Effect of the P-biofertilizer shows 36.4% average increase. The increase occurred under all conditions of the other biofertilizers (i.e. no interaction).

Effect of the K-biofertilizer shows 18.4% average increase. The increase occurred particularly in absence of N₂-fixers giving 36.5% as compared with a non-significant effect in presence of N₂-fixers. On the other hand, the positive effect of K occurred irrespective of presence or absence of P (i.e. no interaction caused by P-dissolvers). There was no 3-factor interaction. Positive effect by biofertilization is extended to all plant parts (Sharma et al., 2013)

3.1.3. Shoots+ Roots"(whole plant) dry matter yield .

The lowest yield was 18.04 g pot⁻¹ given by the non-treated. Increases ranged from 62.8% (N₁P₀K₀) to 80.6 % (N₁P₁K₀), indicating the importance of N and P nutrition in particular for increased plant growth. Each of N₁P₀K₁ and N₀P₁K₁ gave yields significantly not different from that given by N₁P₁K₀. This indicates that it is sufficient to apply any two of the three biofertilizers and obtain marked increase in plant growth. Therefore the beneficial effect of the three biofertilizers lies in increasing and enhancing plant growth in general terms (Gholami, et al., 2009 ,Bhardwaj et al., 2014 and Sahoo et al. , 2014).

Effect of the N- biofertilizer was an average 13.0 % increase. The increase was 27.8% in absence of P dissolvers and non-existent in their presence. It was 28.7% in absence of K dissolvers but non-existent in their presence. The 3-factor interaction shows that in absence of both P and K dissolvers, the increase by the N₂-fixers was a considerable 63% compared with no effect given by them in presence of "K+P" dissolvers together.

Effect of the P-biofertilizer was an average 14.4 % increase and the increase was particularly significant in absence of N₂-fixers, giving 29.0% whereas no significant effect was given by P-dissolvers in presence of N₂-fixers. Also the increase was 32.3% in absence of K-dissolvers, but no significant effect in their presence. The considerable 67% increase by P-dissolvers in absence of both N₂-fixers and K-dissolvers, compared with the no positive effect in their concurrent presence, reflects a 3-factor interaction. Thus the relative increase caused by the P-dissolvers was highest when given in absence of both N₂-fixers and K-dissolvers.

Effect of the K- biofertilizer was a 12.1 % average increase. The increase was particularly evident, being 27.7%, in absence of N₂-fixers and none in their presence ; the increase was also in absence of P-fixers, being 29.9% but no effect in their presence. The 3-factor interaction is exhibited by the very high 65% increase due to K in the combined absence of both 'N₂-fixers+ P-dissolvers', compared with the no positive effect in their combined presence. The cumulative effect shown by the combination of two or more of the biofertilizers was indicated by the increase given by their association , the interaction effect was shown as each of them when applied singly caused marked positive response than when introduced where the other(s) is (are) already existent. Studies on response of biofertilizers added singly or in combinations showed variable results including cumulative effects and competitive effects (Han et al. 2006 , Abdel-Salam et al. 2012 and 2015)

3.2. N uptake (Table 3)

3.2.1 . N uptake in shoots

The lowest uptake was 154.3 mg N pot⁻¹ by the non-fertilized. Increases by the fertilized ranged from 60.2 % (N₀P₀K₁) to 135% (N₁P₁K₀) indicating a necessity for N and P in the first place to increase N uptake. **Effect of the N-biofertilizer** was an average 36.3% increase and was particularly high , 57.8 %, in absence of P-dissolvers, compared with 21.3 % increase in their presence. The increase was 50.5%, in absence of K-dissolvers and 24.9% in their presence.

Effect of the P-biofertilizer was an average 23.6% increase ; which was high (44.0 %,) in absence of N₂-fixers than (21.3%) in their presence. The increase was also more (44.2%) in absence of N₂-fixers, than (8.2%) in their presence.

Effect of the K-biofertilizer was a 13.3 % average increase. The increase was higher (26.2%) in absence of N₂-fixers, and non-significant in their presence. A 3-factor interaction was shown when relative increases of : 88 , 81 and 60 % were given by each of N, P and K biofertilizers respectively when given under conditions of no presence of any of the others. When given in presence of the others , results were 13 % increase by N-dissolvers, no effect by the P-dissolvers and 7% increase by the K-dissolvers. Such results indicate a cumulation effect and/or a competition effect among the biofertilizers. Results by other researchers (Abdel-Salam et al. 2012 , 2015) indicated a lower yield increase given by a combination of "N₂-fixers+P-dissolvers" compared with a higher increase given by either applied singly

3.2.2 . N uptake in roots

The lowest uptake was by the non-fertilized (31.4 mg N pot⁻¹). Increases ranged from 75.2 % (N₀P₀K₁) to 193% (N₁P₁K₁) indicating the necessity for N, P and K for high increase in N uptake in roots.

The increase by the N biofertilizer averaged 39.2% and occurred in presence as well as in absence of P-dissolvers, i.e. no interaction caused by P-dissolvers to the effect of N₂-fixers ; but the increase was particularly significant in absence of K-dissolvers and non-significant in their presence probably because of the marked positive effect of the K-dissolvers nullifying a positive effect of the N₂-fixers.

Main effect of P-dissolvers shows an increase of 45.7%. The positive effect of P-dissolvers was unaffected by presence or absence of any of either or both of the N₂-fixers or the P-dissolvers (i.e. no interaction of any kind). This indicates that the increase caused by P-dissolvers in N-uptake by roots occurred irrespective of the status of N₂-fixers or K-dissolvers.

Main effect of K-dissolvers was an average 21.1% increase ; a positive effect which was particularly significantly high, 43.1%, in absence of N₂-fixers, and no effect in their presence. There was no significant interaction caused by P-dissolvers; i.e. the increase given by the K- biofertilizer occurred unaffected by the status of the P-biofertilizer.

3.2.3 . N uptake in shoots+roots (whole plant):

The pattern resembled that of the shoots. The lowest N uptake was by the non-fertilized (185.7 mg N pot⁻¹) ,increased by biofertilization to 62.8% (N₀P₀K₁) to 140 % (N₁P₁K₀) which was not significantly different from the 135% obtained by N₁P₁K₀ indicating the necessity for N, P or N,P and K for high increase in N uptake by whole plant.

Effect of the N biofertilizer shows 36.8% on average , the increase was greater in absence of P-dissolvers (54.5%) than in their presence (24.6%).The increase was also greater in absence of K-dissolvers (52.7%)than in their presence (24.6%).

Effect of P shows 27.3% average increase. The increase was greater (44.3%) in absence of N₂-fixers than in their presence (16.3%).

Effect of K shows 14.6% average increase .The increase was particularly significant in absence of N₂-fixers (29.0%) but non-significant effect in their presence; and also in absence of P-dissolvers (32.1%) but non-significant in their presence. There was a 3-factor interaction regarding the response to biofertilization, very much similar to that of the N uptake in shoots. The relative increase caused by each of the biofertilizers when applied singly was high: 88%, 80and 63 % for the N, P and K biofertilizers respectively. However the increase due to application of each biofertilizer when applied under condition of a combined presence of the other two was either very low or negative: 17% , 7% and a negative 3% for the N , P and K biofertilizers respectively. Such results may indicate a cumulation effect and/or a competition between the biofertilizers. Results by other researchers (**Han et al 2006, and Abdel-Salam et al. 2012, 2014 and 2015**) indicated a lower increase in nutrient uptake by applying a combination of "N₂-fixers+P-dissolvers" compared with the increase caused by applying either singly.

Table 3 : Effectiveness of NPK Biofertilization on sorghum (*Sorghum bicolor*):N uptake in plant parts (mgpot⁻¹)

Biofert.		Biofert. K		Mean	Biofert. K		Mean
N	P	K ₀	K ₁		K ₀	K ₁	
shoots					roots		
N ₀	P ₀	154.3	247.3	200.8	31.4	55.0	43.2
	P ₁	279.0	299.4	289.2	55.9	69.7	62.8
	Mean	216.7	273.4	245.0	43.6	62.4	53.0
N ₁	P ₀	290.3	343.5	316.9	59.0	61.1	60.1
	P ₁	362.0	339.7	350.8	83.3	92.0	87.6
	Mean	326.1	341.6	333.9	71.1	76.6	73.8
G. mean		271.4	307.5		57.4	69.5	
		Means of P			Means of P		
P ₀		222.3	295.4	258.8	45.2	58.1	51.6
P ₁		320.5	319.6	320.0	69.6	80.9	75.2
LSD 0.05		N=14.55 P=14.55 K=14.55 NP=20.58 NK=20.58 PK=20.58 NPK=29.10			N=6.02 P=6.02 K=6.02 NP=ns NK=8.51 PK=ns NPK=ns		
whole plant					Notes Biofertilizer inoculum:- N: <i>Azotobacter chroococcum</i> + <i>Azospirillum</i> <i>brasilense</i>. P: <i>Bacillus megaterium</i> K: <i>Bacillus circulans</i> Application: Seed inoculation+ soil addition.		
N ₀	P ₀	185.7	302.3	244.0			
	P ₁	334.9	369.1	352.0			
	Mean	260.3	335.7	328.8			
N ₁	P ₀	349.4	404.6	377.0			
	P ₁	445.3	431.7	438.5			
	Mean	397.4	418.1	407.8			
G. mean		328.9	377.0				
		Means of P					
P ₀		267.6	353.5	310.5			
P ₁		390.1	400.5	395.2			
LSD 0.05		N=20.5 P=20.5 K=20.5 NP=29.4 NK=29.4 PK=29.4 NPK=30.1					

3.3. P uptake (Table 4).

3.3.1. P uptake in shoots.

The lowest uptake was by the non-fertilized (18.12 mg P pot⁻¹). Increases obtained by the fertilized treatments ranged from 70.1 % (N₁P₀K₁) to 237% (N₁P₁K₁) indicating full NPK biofertilization giving highest P uptake in shoots.

Effect of the N-biofertilizer was an average 24.6% with increases occurring in presence as well as in absence of P-dissolvers, but the increase was high, 51.15%, in absence of K-dissolvers and non-effective in their presence.

Effect of the P-biofertilizer was an average 42.4 % increase. The increase was not affected by presence or absence of N₂-fixers, but was much pronounced (51.1%) under conditions of K-dissolvers presence, and relatively less pronounced (32.7%) where no K-dissolvers were present. It seems that the P-dissolvers and the K-dissolvers are more active in presence of each other.

Effect of the K-biofertilizer was 19.8% average increase. The increase was particularly significant in presence of P-dissolvers also it was significant in absence of N-fixers.

The 3-factor interaction regarding the response to biofertilization was evident. The relative increase caused by each of the biofertilizers when applied singly was high: 146%, 121 and 114 % for the N, P and K biofertilizers respectively, a considerable effect of the biofertilizers on the uptake of P in particular. However the increase due to application of each biofertilizer in presence of the others was different. The effect of N-biofertilizer when applied under combined presence of the other two biofertilizers was non-existent (a very slight 2% relative decrease). This may indicate a competitive effect between N-fixers and the combined association of P- and K- dissolvers. On the other hand relative increases by P- and K- biofertilizers when others are present were 46%, and 40% respectively; lower increase compared with their effect singly. This may indicate a cumulation effect by the three biofertilizers.

3.3.2. P uptake in Roots.

The response pattern was rather comparable with that of the P uptake in shoots. The lowest P uptake was that of the non-fertilized ($2.24 \text{ mg P pot}^{-1}$), increased by biofertilization from 141% ($\text{N}_0\text{P}_0\text{K}_1$ or $\text{N}_1\text{P}_0\text{K}_0$) to 375% ($\text{N}_1\text{P}_1\text{K}_1$) indicating full NPK biofertilization giving highest P uptake in roots. **Effect of the N-biofertilizer** was a 44.4% average increase, and that of **the P-biofertilizer** was 74.7 % average increase; and that of **the K-biofertilizer** was a 33.0% average increase. The positive effect caused by each of the three biofertilizers occurred irrespective of the status of the other two fertilizers, i.e. there was no interaction affecting the response of any of them, caused by any or both of the other two. The response to applying each biofertilizer in absence of the others showed increases of 141, 197 and 141% for each of N-, P- and K-biofertilizers when each respectively is applied in absence of the others. The response of applying any of them in presence of the others exhibited increases of 24, 45 and 14% respectively.

3.3.3. P uptake in shoots+roots:

The response pattern resembled that of the P uptake in shoots. The lowest P uptake was that of the non-fertilized ($20.33 \text{ mg P pot}^{-1}$), increased due by 87.7% ($\text{N}_1\text{P}_0\text{K}_1$) to 244% ($\text{N}_1\text{P}_1\text{K}_1$) indicating full NPK biofertilization giving highest P uptake in 'shoots+roots'.

Effect of the N- biofertilizer gave average increase of 21.7% and the increase was comparatively lower (17.1%) in absence of P-dissolvers but higher (24.9%) in their presence. The increase was 47.2% in absence of K-dissolvers and non-significant in their presence.

Effect of the P-biofertilizer was an average 46.7 % increase, and the positive effect was lower 41.6% in absence of N_2 -fixers and greater 51.1% in their presence; indicating a complementary effect of the P-dissolvers and the N_2 -fixers.

Effect of the K-biofertilizer was an average of 27.4 % increase. The increase was more pronounced in absence of N-fixers (53.6%) but little in their presence (9.1 %). The 3-factor interaction regarding the response to biofertilization exhibited increases of 117, 130 and 146 % by applying each of the N, P and K fertilizers singly; the comparable increases when each was applied in presence concurrent existence of the other two were lower: 32, 72 and 28% respectively. This indicates that the increase by applying two biofertilizers combined

rendered any increase caused by adding a third one not as considerable as when it was applied without their presence. This is an interaction which indicates a combination effect and a competition effect among the biofertilizers (Gholami et al 2009 and Abdel-Salam et al. 2012, 2015).

Table 4 : Effectiveness of NPK Biofertilization on sorghum (*Sorghum bicolor*):P uptake in plant parts (mgpot⁻¹)

Biofert.		Biofert. K		Mean	Biofert. K		Mean
N	P	K ₀	K ₁		K ₀	K ₁	
shoots					roots		
N ₀	P ₀	18.12	38.80	28.46	2.24	5.40	3.82
	P ₁	40.02	45.90	42.96	6.67	8.55	7.61
	Mean	29.07	42.35	35.71	4.46	6.98	5.72
N ₁	P ₀	44.62	30.83	37.73	5.40	7.33	6.37
	P ₁	43.23	59.33	51.28	9.69	10.63	10.16
	Mean	43.93	45.08	44.50	7.55	8.98	8.26
G. mean		36.50	43.72		6.00	7.98	
		Means of P			Means of P		
P ₀		31.37	34.82	33.09	3.82	6.37	5.09
P ₁		41.63	52.62	47.12	8.18	9.59	8.89
LSD 0.05		N=2.49 P=2.49 K=2.49 NP= ns NK=3.53 PK=3.53 NPK=4.99			N=0.68 P=0.68 K=0.68 NP=ns NK=ns PK=ns NPK=ns		
whole plant					Notes Biofertilizer inoculum:- N: <i>Azotobacter chroococcum</i> + <i>Azospirillum brasilense</i> . P: <i>Bacillus megaterium</i> K: <i>Bacillus circulans</i> Application: Seed inoculation+ soil addition.		
N ₀	P ₀	20.33	50.00	35.17			
	P ₁	46.67	52.93	49.80			
	Mean	33.50	51.47	42.48			
N ₁	P ₀	44.20	38.17	41.18			
	P ₁	54.43	69.97	62.20			
	Mean	49.32	54.07	51.69			
G. mean		41.41	52.77				
		Means of P					
P ₀		32.27	44.08	38.18			
P ₁		50.55	61.45	56.00			
LSD 0.05		N=2.58 P=2.58 K=2.58 NP=3.65 NK=3.65 PK=3.65 NPK= 5.16					

3.4. K uptake (Table 5):

3.4.1. K uptake in shoots

The lowest K uptake was that of the non-fertilized (323.5 mg K pot⁻¹), increased due to biofertilization by 62.6% (N₀P₁K₁) to 120% ((N₁P₀K₁) indicating that N combined with K is the most effective for having highest K uptake by shoots.

Effect of the N biofertilizer gave an average increase of 21.1%, and the increase was more (36.0%) in absence of P-dissolvers than in their presence (9.0%), also more (37.4%) in absence of K-dissolvers, than in their presence (9.8%).

Effect of the P-biofertilizer gave an average increase of 8.7%, and the increase was particularly significant (22.8%) in absence of the N-fixers but non-effective in their presence; it was also particularly significant (27.2%) in absence of the K-dissolvers but non-effective in their presence.

Effect of the K-biofertilizer gave an average increase of 28.2%, and the increase was more (45.0%) in absence of the N₂-fixers than in their presence (15.9%). The increase was more, 48.4%, in absence of P-dissolvers than in their presence (12.3%).

3.4.2. K uptake in roots

The lowest K uptake was that of the non-fertilized (33.9 mg K pot⁻¹), increased 84.4% (N₁P₀K₀) to 345% ((N₁P₁K₁) indicating that combined NPK biofertilization gave the highest K uptake by roots.

Effect of the N biofertilizer gave an average increase of 49.7%, and the increase was 53.2 in absence of P-dissolvers and 47.1% in their presence. The increase was not affected by presence or absence of K-dissolvers.

The P-biofertilizer gave an average increase of 40.1%, and the increase was more (43.4%) in absence of N-fixers than in their presence (33.1%).

The K-biofertilizer gave an average increase of 77.3%, and the increase occurred in absence as well as in presence of N-fixers and also in presence or absence of P-dissolvers or in presence or absence of both together, i.e. no interactions.

3.4.3. K uptake in plant shoots+roots (whole plant)

The lowest K uptake was that of the non-fertilized (357.4 mg K pot⁻¹) increased due to biofertilization by 68.7% (N₀P₁K₀) to 130% ((N₁P₁K₁) indicating that combined NPK biofertilization gave the highest K uptake by shoots+ roots.

Effect of the N biofertilizer gave an average increase of 33.4%, and the increase was 53.0 in absence of P-dissolvers and 18.5% in their presence. However, there was no positive effect of N in absence of K-dissolvers, but an increase of 22.5% occurred in presence of K-dissolvers. The positive effect of N₂-dissolvers on K uptake was prominent 63% in absence of both P- and K-dissolvers but none in their combined presence, exhibiting a 3-factor interaction.

Effect of the P-biofertilizer gave an average increase of 12.2%, and the increase was particularly significant (29.9%) in absence of the N₂-fixers but non-effective in their presence; also the increase was particularly significant (24.9%) in absence of the K-dissolvers but non-effective in their presence.

Effect of the K-biofertilizer gave an average increase of 29.7%, and the increase was more (96.0%) in presence of the N₂-fixers than in their absence (39.0%). **Basak, and Biswas (2009) & (2010)** noticed augmentation effect on K uptake by sudan grass when N₂-fixers and K-dissolvers were used as mixed biofertilizers to the crop. The increase was also greater (37.9%) in absence of P-dissolvers than in their presence

(13.6%). The 3-factor interaction exhibited increases of 97, 69 and 79% due to application of each of the N, P and K biofertilizers singly as compared with comparable lower increases of 17, 19 and 12% respectively when each was applied in a combined presence of the other two. Therefore the increase given by a combined presence of any two biofertilizers must have been marked so that the advent of the third did not cause much increase as when it was applied without their presence. This interaction which indicates that the 2-factor interaction which indicates a less positive effect by one biofertilizer in presence of any of the other two, is accentuated in a combined presence of the two together. (Gholami et al 2009 and Abdel-Salam et al. 2012, 2015).

Table 5 : Effectiveness of NPK Biofertilization on sorghum (*Sorghum bicolor*):K uptake in plant parts (mgpot⁻¹)

Biofert.		Biofert. K		Mean	Biofert. K		Mean
N	P	K ₀	K ₁		K ₀	K ₁	
shoots					roots		
N ₀	P ₀	323.5	625.4	474.5	33.9	78.9	56.4
	P ₁	539.4	526.0	582.7	63.5	98.2	80.9
	Mean	431.5	625.7	528.6	48.7	88.6	68.6
N ₁	P ₀	578.1	712.9	645.5	62.5	110.3	86.4
	P ₁	607.4	661.6	634.9	87.1	151.0	119.0
	Mean	592.8	687.3	640.0	74.8	130.6	102.7
G. mean		512.1	656.5		61.8	109.6	
		Means of P			Means of P		
P ₀		450.8	669.2	560.0	48.2	94.6	71.4
P ₁		573.4	643.8	608.9	75.3	124.6	100.0
LSD 0.05		N=29.44 P=29.44 K=29.44 NP=41.64 NK=41.64 PK=41.64 NPK=58.88			N=11.32 P=11.32 K=11.32 NP=16.00 NK=ns PK=ns NPK=ns		
whole plant					Notes Biofertilizer inoculum:- N: <i>Azotobacter chroococcum</i> + <i>Azospirillum brasilense</i> . P: <i>Bacillus megaterium</i> K: <i>Bacillus circulans</i> Application: Seed inoculation+ soil addition.		
N ₀	P ₀	357.4	640.7	499.1			
	P ₁	602.9	694.4	648.7			
	Mean	480.2	667.6	573.9			
N ₁	P ₀	704.4	823.2	763.8			
	P ₁	724.2	812.6	768.4			
	Mean	417.3	817.9	766.1			
G. mean		597.2	742.7				
		Means of P					
P ₀		530.9	731.9	631.4			
P ₁		663.6	753.5	708.5			
LSD 0.05		N=29.72 P=29.72 K=29.72 NP=42.02 NK=42.02 PK=42.02 NPK=59.44					

4. IMPLICATIONS AND OVERALL ASSESSMENTS

The increase in the growth of roots and above ground plant parts caused by biofertilizers was associated with an increase in the uptake of the NPK macronutrients. Thus the positive effects of the biofertilizers

were manifested by their positive effects on plant growth (**Chi, et al., 2010 and Bhattacharyya and Jha, 2012**). The response to each of the three biofertilizers was considerably positive particularly when applied solely indicating additive effects by the three biofertilizers when combined (**Han, et al 2006 and Saikia et al 2013**). In some cases there were higher values given by two biofertilizers rather similar to those by the three together. Higher yields entailed higher uptake of nutrients. The indications are that biofertilizers inocula *A. chroococcum* and *A. brasilense* in combination with *B. megaterium* and *B. circulans* may be a practical proposition for supplementary fertilization for crops providing N, P and K nutrients through biological means. (**Dogan et al., 2011 ,Aziz et al. 2012 and Mohammadi and Sohrabi 2012 Abdel-Salam et al. 2012 and Abdel-Salam 2014 and Abdel-Salam et al. 2014**)

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التسميد الحيوي بالنتروجين و الفسفور و البوتاسيوم للذرة الرفيعة النامية في

تربة طين خفيف و التداخلات المختلفة بينهم

علي احمد عبدالسلام ، عمر حسن محمد الحسيني ، و سام رشاد زهره ،

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قسم الاراضي - كلية الزراعة بمشتهر - جامعة بنها

اجريت تجربة اصص استخدام فيها اسمده حيوية هي لقاحات تثبيت النتروجين بكتريا *A. brasilense* + *A. chroococcum* و بكتيريا إذابة الفسفور *B. megatevium* و بكتيريا إذابة البوتاسيوم *B. circulans* . و بتوافقات مختلفة علي نبات الذرة الرفيعة *sorghum bicolor* . زاد وزن النباتات بالتسميد الحيوي ما بين ٦٣ - ٨١ % بتسميد تثبت النتروجين الي تسميد مذيبيات الفسفور معاً و البوتاسيوم. فيما يخص الامتصاص النتروجيني كانت الزيادة من ٦٣% (بمذيبيات البوتاسيوم) الي ٨١% بالتسميد لمثبتات النتروجين مع مذيبيات الفسفور.

وفيما يخص امتصاص النتروجين فكانت الزيادة من ٨٨% المثبتات النتروجين و مذييات البوتاسيوم معاً الي ٢٢٤% بمجموع الاسمدة الحيوية الثلاثة و فيما يخص امتصاص البوتاسيوم زاد التسميد الحيوي من ٦٩% (بمذييات الفسفور) الي ١٣٠% بمذييات البوتاسيوم . كان هناك تداخلات معنوية حيث كانت نسبة الزيادة لأي من الاسمدة الحيوية عالية أكثر حين اضيف السماد الحيوي مفردا و في غير وجود أي من الاسمدة الاخرى. اما في وجود الاسمدة الاخرى سواء وحدها أو مجمعة . فكانت تأثير السماد زيادة نسبة قليلة . كانت الزيادة في حالة التسميد في غياب الاخرين هي ٦٣% لمثبتات النتروجين ٦٧% لمذييات الفسفور و ٦٥% لمذييات البوتاسيوم . اما في وجود كلا الاخرين فكانت الزيادة أقل كثيرا جدا. و تبين دلالات الدراسة امكانية التسميد الحيوي و لكن الي حدود متوسطة في النتائج و انة يمكن استخدام التسميد الحيوي كمكمل للتسميد المعدني.