Egypt . J. of Appl. Sci., 30 (7) 2015 *383-400*

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

Ali A. Abdel-Salam ; Omar H. Elhosainy ; Wissam R. Zahra; Mohamed A. Abdel-Salam and Inas A. Hashem

(Soils and Water Department ,Faculty of Agriculture , Moshtohor, Banha University)

Key Words: Biofertilizers, *B.megaterium ;B.circulans A. chroococcum ; A.brasilense Sorghum bicolor*

ABSTRACT

A pot experiment was carried with biofertilizers of N_2-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 N_1, P_1 and K_1 biofertilizers were compared with the $N_0P_0K_0$ 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 ($N_1P_0K_0$) to 81($N_1P_1K_0$) for yield; 63 ($N_0P_0K_1$) to140 ($N_1P_1K_0$) for N uptake; 88 ($N_1P_0K_1$) to 224 ($N_1P_1K_1$) for P uptake and 69 ($N_0P_1K_0$) to 130 ($N_0P_0K_1$) 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 increases 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): **N_2-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 N_2- 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**). ***Azotobacter***s 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 N_2-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 N_2fixers, 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: N_2-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 ***torrifluvent*** 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 (N_0) and N_2-fixers ***Azotobacter chroococcum*** + ***Azospirillum brasilense*** (N_1) ; (2)P-bio-fertilization: none(P_0)and PDB ***Bacillus megaterium*** (P_1) and (3) K-biofertilization: none (K_0) 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^{o}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 H_2SO_4/ $HClO_4$ 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			
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	***l*C**	**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**

Notes:

1.Extractants of available nutrients :N (KCl) ; K (NH_4AOc) ; P ($NaHCO_3$) .

2. *l* C : light clay (according to international soil texture triangle) .

3.EC of paste extract.

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% ($N_1P_1K_1$) to 75.4% ($N_0P_1K_1$) 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 N_2-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 N_2-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 N_2-fixers. Besides, a 3-factor interaction occurred when no response to the N_2-fixers was obtained under conditions of a combined presence of P- + K-dissolvers ; but 64.9% increase by N_2-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 N_2-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 N_2-fixers and K-dissolvers are compared with no increase in presence of N_2-fixers+K-dissolvers.

Effect of the K-biofertilizer shows an average 11.9% increase. The increase was particularly in absence of N_2-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 N_2-fixers and P-dissolvers; the K-dissolvers caused a considerable 61.4% increase. In combined presence of N_2-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
	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		

Notes
Biofertilizer inocula:-
N:*Azotobacter chroococcum + Azospirillum brasilense*.
P:*Bacillus megaterium*
K: *Bacillus circulans*
Application: Seed inoculation+ soil addition.

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 % ($N_0P_0K_1$) to 134.6% ($N_1P_1K_1$) 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 N_2-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 N_2-fixers giving 36.5% as compared with a non-significant effect in presence of N_2-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% ($N_1P_0K_0$) to 80.6 % ($N_1P_1K_0$), indicating the importance of N and P nutrition in particular for increased plant growth. Each of $N_1P_0K_1$ and $N_0P_1K_1$ gave yields significantly not different from that given by $N_1P_1K_0$.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 N_2-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 N_2-fixers, giving 29.0% whereas no significant effect was given by P-dissolvers in presence of N_2-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 N_2-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 N_2-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 N_2-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 `$N_2$-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 % ($N_0P_0K_1$) to 135% ($N_1P_1K_0$) 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 with21.3 % 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 N_2-fixers than (21.3%) in their presence. The increase was also more (44.2%) in absence of N_2-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 N_2-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 "N_2-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 % ($N_0P_0K_1$) to 193% ($N_1P_1K_1$) 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 N_2-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 N_2-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 N_2-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 N_2-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 N_2-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% ($N_0P_0K_1$) to 140 % ($N_1P_1K_0$) which was not significantly different from the 135% obtained by $N_1P_1K_0$ 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 N_2-fixers than in their presence (16.3%).

Effect of K shows 14.6% average increase .The increase was particularly significant in absence of N_2-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 "N_2-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. N	Biofert. P	Biofert. K: K_0	Biofert. K: K_1	Mean	Biofert. K: K_0	Biofert. K: K_1	Mean
		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		

Biofert. N	Biofert. P	Biofert. K: K_0	Biofert. K: K_1	Mean
		whole plant		
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		

Notes
Biofertilizer inoculum:-
N: *Azotobacter chroococcum + Azospirillum brasilense*.
P: *Bacillus megaterium*
K: *Bacillus circulans*
Application: Seed inoculation+ soil addition.

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 % ($N_1P_0K_1$) to 237% ($N_1P_1K_1$) 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 N_2-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%, 121and 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 mg P pot^{-1}), increased by biofertilization from 141% ($N_0P_0K_1$ or $N_1P_0K_0$) to 375% (($N_1P_1K_1$) 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 mg P pot^{-1}) ,increased due by 87.7% ($N_1P_0K_1$) to 244% (($N_1P_1K_1$) 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%**) 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 pronounce 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		

Biofert.		Biofert. K		Mean
N	P	K_0	K_1	
whole plant				
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		

Notes
Biofertilizer inoculum:-
N: *Azotobacter chroococcum + Azospirillum brasilense*.
P:*Bacillus megaterium*
K: *Bacillus circulans*
Application: Seed inoculation+ soil addition.

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% ($N_0P_1K_1$) to 120% (($N_1P_0K_1$) 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 N_2-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% ($N_1P_0K_0$) to 345% (($N_1P_1K_1$) 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% ($N_0P_1K_0$) to 130% (($N_1P_1K_1$) 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 N_2-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 N_2-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 N_2-fixers than in their absence(39.0%). **Basak, and Biswas (2009)** & **(2010)** noticed augmentation effect on K uptake by sudan grass when N_2-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		

Biofert.		Biofert. K		Mean
N	P	K_0	K_1	
		whole plant		
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		

Notes
Biofertilizer inoculum:-
N: *Azotobacter chroococcum + Azospirillum brasilense*.
P:*Bacillus megaterium*
K: *Bacillus circulans*
Application: Seed inoculation+ soil addition.

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)**

5- REFERENCES.

Abdel-Fattah, D.A.; W.E. Ewedab ; M.S. Zayed and M.K. Hassaneina (2013): Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculants. Ann Agric. Sci., **58:**111-118.

Abdel-Salam, A. A. (2014) . Bio-Organo-fertilization of organic farming**:** Viability & Practicality . 2nd. International Conference Biotechnology Applications in Agriculture, Benha University, Moshtohor and Hurghada, 8-12 Apr. 2014 ,Egypt.

Abdel-Salam , A.A. ; E.H. Noufal ; K. A. Shaban and M.A. Ahmed (2012). Bio-fertilization and efficient productivity of maize grown on a newly reclaimed saline sandy clay soil **.** 1st International Conference Biotechnology Applications in Agriculture, Benha University, Moshtohor and Hurghada, 18-22 Feb. **2012,** Egypt.

Abdel-Salam, A.A. ; T.A. El-Maghraby ; I.M. Farid ; M.A. Abdel-Salam and S.A. El-Shahid (2014). Biofertilization of faba beans (*Vicia faba*) grown on a clay loam *Typic Torrifluvent* soil, using inocula of N_2-fixing *Rhizobium leguminosarum* and P-dissolving *Bacillus megaterium* under field conditions**.** Proc. 2nd Int. Conf. Biotech. Appl. Agric. Vol. 1, Benha Univ., Moshtohor & Hurghada, Egypt, 8-11 Apr. 201**4.**

Abdel-Salam, A. A. ; S. M. Soliman ; Y. G. M. Galal ; W. R. Zahra ; A. A. Moursy and M. A. Hekal (2015). Response of sunflower (Helianthus annuus L.) to N-application and biofertilization with assessment of fertilizer N recovery by ^{15}N versus subtraction methods. J. Nucl. Tech. Appl. Sci., 3(3):157-169.

Aziz, G. ; N. Bajsa ; T. Haghjou ; C. Taule ; A. Valverde ; J. Mariano and A. Arias (2012): Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop–pasture rotations in a no-tillage regime in Uruguay. Appl. Soil Ecol., **61:**320-326.

Basak, B. and D.R. Biswas (2009). Influence of potassium solubilizing micro-organism (*Bacillus mucilaginosus*) and waste mica on potassium uptake dynamics by Sudan grass (*Sorghum vulgare* Pers.)grown under two Alfisols. Plant and Soil., 317(1):235-255.

Basak, B. and D.R. Biswas (2010). Co-inoculation of potassium solubilizing and nitrogen fixing bacteria on solubilization of waste mica and their effect on growth promoting and nutrient acquisition by a forage crop Biol. Fertility Soils., 46:641-648.

Bashan, Y. ; G. Holguin and L.E. Bashan (2004): Azospirillum-plant relationships: agricultural, physiological, molecular and environmental advances (1997–2003). Can J. Microbiol., **50:**521-577.

Bhardwaj, D. ; **M.W. Ansari ; R.K. Sahoo and N. Tuteja (2014)**. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb. Cell Factory., 13: 66-72.

Bhattacharyya, P.N. and D.K. Jha (2012) : Plant growth-promoting rhizobacteria (PGPR) : emergence in agriculture. World J. Microbiol. Biotechnol., **28:**1327-1350.

Black, C.A. ; D.D. Evans ; J.L. White ; L.E. Ensminger and F.E. Clark (1965). Methods of soil analysis Agron. Series No., 9: Am. Soc. Agron., WI, USA.

Chapman, H. and P. Pratt (1961). Methods of analysis for soils plants and waters . Division of Agriculture, Univ. of California., USA.

Chi, F. ; P. Yang ; F. Han ; Y. Jing and S. Shen (2010): Proteomic analysis of rice seedlings infected by *Sinorhizobium meliloti* 1021**.** Proteomics., **10:**1861-1874.

Dogan, K. ; Kamail C. I.; G.M. Mustafa and C. Ali (2011) : Effect of different soil tillage methods on rhizobial nodulation, biyomas and nitrogen content of second crop soybean. Afr. J. Microbiol. Res., **5:**3186-3194.

Gholami, A. ; S. Shahsavani and S. Nezarat (2009) . The Effect of Plant Growth Promoting Rhizobacteria (PGPR) on Germination seedling Growth and Yield of Maize**.** Int J Biol Life Sci., **5:**1-8.

Han, H.S. ; S. Supanjani and K.D. Lee (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ., 52(3):130-136

Mali, G.V. and M.G. Bodhankar (2009). Antifungal and phytohormone production potential of Azotobacter chroococcum isolates from groundnut **(***Arachis hypogea* **L.)** rhizosphere**.** Asian J. Exp. Sci., **23:**293-297.

Megadi, V.B. ; P.N. Tallur ; R.S. Hoskeri ; S.I. Mulla and Z. Minnekar (**2010).** Biodegradation of Pendimethalin (herbicide) by *Bacillus circulans* . Ind. J. Biotech., 9:173-177.

Megali, L. ; G. Glauser and S. Rasmann (2013) . Fertilization with beneficial microorganisms decreases tomato defenses against insect pests. Institut National Recherche Agronomique (**INRA)** and Springer-Verlag France. **.**

Mohammad, K. and Y. Sohrabi (2012) : Bacterial biofertilizers for sustainable crop production: A review. J. Agric. Biol. Sci., **7:**307-316.

Perrig, D. ; M.L. Boiero ; Masciarelli, O.A., Penna, C., Ruiz O.A., Cassan, F.D. and Luna, M.V. 2007: Plant-growth promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation**.** Appl. Microbiol. Biotechnol., **75:**1143-1150.

Priyanka, P. and. S.S. Sindhu (2013). Potassium solubilization by rhizosphere bacteria: Influence of nutritional and environmental conditions**.** J. Microbiol. Res., 3(1): 25-31

Revillas, J.J. ; B.Rodelas ; C. Pozo ; M.V. Martinez-Toledo and L.J. Gonzalez (2000): Production of B-Group vitamins by two *Azotobacter* strains with phenolic compounds as sole carbon source under *diazotrophic* and *adiazotrophic* conditions**.** J. Appl. Microbiol., **89:**486-493.

Sahoo, R.K. ; M.W. Ansari ; T.K. Dangar ; S.Mohanty and N. Tuteja (2013). Phenotypic and molecular characterization of efficient nitrogen fixing *Azotobacter* strains of the rice fields. Protoplasma., 251:511-520.

Sahoo, R.K. ; M.W.Ansari ; M.Pradhan ; T.K. Dangar ; S.Mohanty and N. Tuteja (2014). Phenotypic and molecular characterization of efficient native *Azospirillum* strains from rice fields for crop improvement**.** Protoplasma., 4:943-953

Saikia, S.P. ; D. Bora ; A.Goswami ; K.D. Mudoi and A. Gogoi (2013). A review on the role of Azospirillum in the yield improvement of non-leguminous crops**.** Afr. J. Microbiol. Res., **6:**1085-1102.

Sharma, S. B. ; R. Z. Sayyed ; M. H. Trivedi and T.A. Gobi (2013). Phosphate solubilizing microbes: Sustainable approach for managing phosphorus deficiency in agricultural soils. Springer-plus., 2:587-594.

Sheng, X.F. and L.Y. He (2006): Solubilization of potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. Canadian J. Microbiol., 52:66-72.

Thamer, S. ; M.Schädler ; D.Bonte and D.J. Ballhorn (2011). Dual benefit from a belowground symbiosis: nitrogen fixing rhizobia promote growth and defence against a specialist herbivore in a cyanogenic plant**.** Plant Soil., **34:**1209-1219

Wani, S.A. ; S. Chand and T. Ali (2013).Potential use of *Azotobacter chroococcum* in crop production: An overview**.** Curr. Agric. Res. J., **1:**35-38.

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 ،

محمد علي عبدالسلام ، ايناس ابو النصر هاشم

قسم الاراضي – كلية الزراعة بمشتهر – جامعة بنها

اجريت تجربة اصص استخدام فيها اسمده حيوية هي لقاحات تثبيت النتروجين بكتريا A. brasilense + A. cbroococcum 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 مذيبات الفسفور معاً و البوتاسيوم. فيما يخص الامتصاص النتروجيني كانت الزيادة من ٦٣% (بمذيبات البوتاسيوم) الي ٨١% بالتسميد لمثبتات النتروجين مع مذيبات الفسفور.

وفيما يخص امتصاص النتروجين فكانت الزيادة من ٨٨% المثبتات النتروجين و مذيبات البوتاسيوم معاً الي ٢٢٤% بمجموع الاسمدة الحيوية الثلاثة و فيما يخص امتصاص البوتاسيوم زاد التسميد الحيوي من ٦٩% (بمذيبات الفسفور) الي ١٣٠% بمذيبات البوتاسيوم . كان هناك تداخلات معنوية حيث كانت نسبة الزيادة لأي من الاسمدة الحيويه عالية أكثر حين اضيف السماد الحيوي مفردا و في غير وجود أي من الاسمدة الاخري. اما في وجود الاسمدة الاخري سواء وحدها أو مجمعة . فكانت تأثير السماد زيادة نسبة قليلة . كانت الزيادة في حالة التسميد في غياب الاخرين هي ٦٣% لمثبتات النتروجين ٦٧% لمذيبات الفسفور و ٦٥% لمذيبات 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.