Effect of Mineral and Bio-fertilizer Application on Growth and Yield of Wheat *Triticum aestivum* L.

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Abstract

Field experiment was conducted during 2012- 2013 in silt loam soil at experimental station of Crops Department, Abu-Ghraib, State Board of Agricultural Researches in Baghdad, Iraq to study the effect of bio-fertilizers and NP levels on some agronomic traits and yield components of wheat by three bacterial genera (*Azotobacter sp.*, *Azospirillum sp.* and *Pseudomonas sp.*) isolated from rhizosphere soil and *Zea mays* roots to study their effects singly or in combination with NP levels on growth and yield of wheat *Triticum aestivum* L. cultivar IPA 99. The experimental design was a completely randomized block design with three replications for each treatment .The results showed that the combination of bio-fertilizer (*A. chroococcum*, *A. brasilense* and *P. fluorescens*) and 100% NP significantly affected on some agronomic traits and yield components, plant height (99.2 cm), flag leaf area (80.56 cm^2), flag leaf chlorophyll content (55.05 SPAD), number of tillers.$plant^{-1}$(28.4), biomass fresh and dry weight (266.89, 85.89 gm) and all these results affected positively on biological yield (20.967 $ton.h^{-1}$), spike length (13.2 cm), number of spikes.m^{-2} (548.30), average weight of 1000 grains (33.02 gm) and grain yield (6.403 $ton.h^{-1}$). Laboratory experiment results revealed that there was no antagonism between the isolated bacteria (*A. chroococcum*, *A. brasilense* and *P. fluorescens*) therefore they were used as bio-fertilizer.

Keywords: bio-fertilizers. *Azotobacter sp*., *Azospirillum sp.* and *Pseudomonas sp*. wheat.

تأثير الأسمدة المعدنية والحيوية في نمو وحاصل نبات الحنطة *Triticum aestivum* L.

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الخلاصة

نفذت التجربة الحقلية اثناء الموسم الشتوي 2012 – 2013 في تربة مزيجيه طينية غرينية في حقل تجارب قسم المحاصيل الحقلية التابع للهيئة العامة للبحوث الزراعية في ابوغريب, بغداد, العراق بهدف دراسة تأثير الأسمدة الحيوية في بعض صفات نمو وحاصل الحنطة باستخدام ثلاثة أجناس من البكتريا وهي (*Azospirillum sp., Peudomonas sp. Azotobacter sp*.) معزوله من تربة منطقة الرايزوسفير و جذور نبات الذرة لدراسة تأثيرها بصورة منفردة أو مجتمعه و بالتداخل مع التسميد الكيميائي في نمو و حاصل نبات الحنطة (*Triticum aestivum* L.) للصنف إباء 99 (IPA 99). وقد استعمل تصميم القطاعات الكاملة المعشاة بترتيب الألواح المنشقة بثلاثة مكررات. وقد أظهرت نتائج الدراسة الحقلية تفوق معنوي للمعاملة *A. chroococcum* + *A. brasilense* + *P. fluorescens* مع 100% (NP) في إعطاء أعلى متوسط

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في أطوال النباتات (99.2 cm) ومساحة ورقة العلم ($80.56\ cm^2$) ومحتوى الكلوروفيل في ورقة العلم (55.05 SPAD) وعدد الاشطاء. نبات$^{-1}$(28.4) والوزن الرطب والجاف للمجموعين الجذري والخضري (266.89, 85.89 gm) على التوالي والذي انعكس ايجابيا في زيادة الحاصل البيولوجي (20.967 ton.h^{-1}). كما تميزت المعاملة السابقة في إعطاء أعلى متوسط للحاصل ومكوناته إذ بلغ متوسط طول السنبلة (13.2 cm) وعدد السنابل.م$^{-2}$ (548.30) ومعدل وزن 1000 بذرة (33.02 gm) وحاصل الحبوب ton.h^{-1} (6.403). بينت نتائج الدراسه المختبرية عدم وجود تضاد بين الأجناس المعزولة *A. chroococcum* و *P. fluorescens* و *A. brasilense* مما يؤهلها للاستعمال ضمن توليفة السماد الحيوي.

Introduction:

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world terms of area harvested, production and nutrition; as it supplies about 19% of the calories and 21% of the protein to the world's changing dietary patterns, the demand for wheat by 2050 is expected to increase by 31% over the 683 million tons consumed in 2008 [1, 2]. For optimum plant growth, nutrients must be available in sufficient and balanced quantities. The most important constraint limiting crop yield in developing nation worldwide is soil infertility. Soil fertility can be restored effectively through adopting the concept of integrated soil fertility management (ISFM) encompassing a strategy for nutrient management-based on natural resource conservation. Micro-organism function is in long duration causing improvement of the soil fertility, it maintains the natural habitat of the soil and increases crop yield by 20-30% [3].

"Bio-fertilizer" is a substance which contains living microorganisms applied to seed, plant surfaces or soil. Bio-fertilizers add nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus and stimulating plant growth through the synthesis of growth promoting substances. Bio-fertilizers can be expected to reduce the use of chemical fertilizer and pesticides. The micro-organisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers healthy plants could be grown while enhancing the sustainability and the health of soil . Also, bio-fertilizers are eco-friendly organic agro-input and more cost effective than chemical fertilizers. Bio-fertilizers like *Rhizobium sp., Azotobacter sp., Azospirillum sp.* and blue green algae (BGA) are in use since long time ago. *Rhizobium* sp. inoculants are used for leguminous crops. *Azotobacter sp*. can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. *Azospirillum sp.* Inoculants are recommended mainly for sorghum, millets, maize, sugarcane and wheat [4].

Increasing nitrogen levels significantly increased plant height, number of spike/m^2 , spike length, number of grains/spike, 1000 kernel weight, straw grain yield and harvest index [5]. [6] Indicated that the interaction between bio-fertilizer treatments and nitrogen levels had significantly effect on biological yield, grain yield and straw yield. Thus ,the aim was to study the effect of bio-fertilizer (*A. chroococcum*, *A. brasilense* and *P. fluorescens*) and mineral fertilizer (NP) in different levels (0, 25, 50, 75 and 100% of recommended dose) on some growth and yield in order to determine the suitable limiting for wheat crop yield.

Materials and Methods:

Isolation and identification of *bacteria Azotobacter sp*., Azospirillum *sp*. and *Pseudomonas sp*.

Soil was taken at 10-15cm depth supplied from 3 random places of maize field in Abu-Ghraib, samples which contained root of *Zea mays* were air dried to be used for bacterial isolation.

Azotobacter sp. was isolated from soil samples according to [7] then morphological and several bio-chemical tests were done according to [8,9].

Azospirillum sp. was isolated from *Zea mays* roots according to [10]. After purification series bio-chemical and morphological tests were done according to [9,11-14].

Pseudomonas sp. was isolated from soil rhizo-spheric particles according to [15]. Then after the purification and several bio-chemical and morphological tests were done according to [16,17].

Antagonism test:

Antagonism effect was determined according to [18]. Petri dishes which contained 10ml of Nutrient agar [9] inoculated with 7 days old culture from each isolated bacteria. Dishes were incubated at 25±2ºC for 7 days.

Preparation of inoculums and seed inoculation:

Inoculums preparation was completed by growing the selected strains of bacteria in 250 ml conical flask having 100 ml nutrient broth by incubating at 28±1ºC in the orbital shaking incubator with 100 rpm for three days to attain uniform cell density (10^8-10^9 CFU.ml^{-1}). The seeds of wheat were inoculated by mixing with sterilized peat containing sugar solution 10% and Arabic gum with three-day-old inoculums of respective strain. Seed for control were mixed with sterilized peat containing sterilized broth, solution of sugar and Arabic gum. Inoculated seed were air dried under shade for 6-8 h before sowing [19].

Field experiment:

Field experiment was carried out at the farm fields of state board of agriculture research, crop department in Abu-Ghraib. Before executing the experiments field soil was examined for various physical-chemical characteristics by using standard protocols. The inoculated seeds of wheat were used in combination with different levels of NP (0, 25, 50, 75 and 100%) of recommended dose (P_2O_5 100 Kg.h^{-1} and Urea 200 Kg.h^{-1}). This experiment was designed in randomized complete block with three repeats for each treatment and the plot area was (2×3 m^2). Seeds were planted by hand irrigated with canal water at 10 December 2012. Growth parameters was taken after 120 days from sowing at flowering stage while yield parameters were taken at harvest ,8 may 2013.

Table 1-Physical-chemical features of field soil

Features	Unit	Value
Sand	g. kg^{-1} soil	204
Silt	g. kg^{-1} soil	320
Clay	g. kg^{-1} soil	476
pH		7.7
EC	dS m^{-1}	1.93
Available nitrogen	mg. kg^{-1} soil	14.58
Available phosphorus	mg. kg^{-1} soil	24.36
Extractable potassium	mg. kg^{-1} soil	375.16

Table 2-Field treatments

Treatments	Bio-fertilizers	NP levels	Treatments	Bio-fertilizers	NP levels
T1	0	0%	T21	A+B	50%
T2	A	0%	T22	A+C	50%
T3	B	0%	T23	B+C	50%
T4	C	0%	T24	A+B+C	50%
T5	A+B	0%	T25	0	75%
T6	A+C	0%	T26	A	75%

T7	B+C	0%	T27	B	75%
T8	A+B+C	0%	T28	C	75%
T9	0	25%	T29	A+B	75%
T10	A	25%	T30	A+C	75%
T11	B	25%	T31	B+C	75%
T12	C	25%	T32	A+B+C	75%
T13	A+B	25%	T33	0	100%
T14	A+C	25%	T34	A	100%
T15	B+C	25%	T35	B	100%
T16	A+B+C	25%	T36	C	100%
T17	0	50%	T37	A+B	100%
T18	A	50%	T38	A+C	100%
T19	B	50%	T39	B+C	100%
T20	C	50%	T40	A+B+C	100%

A = *Azospirillum brasilense* B = *Azotobacter chroococcum* C = *Pseudomonas fluorescence*

Agronomic traits:

Leaf area (cm^2), Plant height (cm), Tillers number per plant, Chlorophyll content (by using Minolta SPAD 502), Biomass fresh and dry weight ($gm.plant^{-1}$).

Yield and yield components:

Numbers of spikes per m2, spike length (cm), 1000 grains weight, grain yield (g/m2) were calculated then converted to $t.ha^{-1}$, Biological yield ($t.ha^{-1}$) and Harvest index (HI).

Statistical analysis:

Statistical analysis was carried out by using Genestat computer program with 0.05 significance level (95% confidence).

Results and Discussion:

The antagonisms test:

Rhizo-sphere is a rich habitat of micro-organisms and should be explored for obtaining potential PGPR, which can be useful in developing bio-inoculants for enhancement of growth and yield of crop plants. Bacterial inoculations improved wheat agronomic traits and yield. In this study three genus of free living bacteria were isolated, purified and identified by series of bio-chemical tests. *Azotobacter* isolate were found to be viscous, opague, raised, convex, glistening, and smooth on sucrose mineral salt agar. Moreover, its ability for utilization of carbon source further confirmed the isolate to be *A. chroococcum* as reported by earlier workers [20-22]. The morphological results for *Azospirillum* (table 3) were found to be gram negative vibriod shape with vibratory movement. Besides, it's ability to convert N-free malate semi-solid medium color from blue to green with subsurface white pellicles growth, in addition to positive results of catalase and oxidase test further confirmed the isolate to be *Azospirillum brasilense* as reported by [22-24]. According to table (3) results revealed the bio-chemical results and the identification tests of *Pseudomonas* isolate which was found to be green-yellow fluorescent color on King's B medium, gram negative, rod shape, gelatin liquefaction and starch hydrolysis further confirmed the isolate to be *Pseudomonas fluorescens* as reported by earlier workers [25-27]. The results revealed that there were no antagonisms noticed between the isolated bacteria after 7 days of incubation at 25±2ºC.

Effect of bio-fertilizers and NP levels on some agronomic traits of wheat plants under field conditions:

Data presented in table (3) revealed that average plant height was significantly increased up to 7.40% by application of bio-fertilizers *A. brasilense* (A) + *A. chroococcum* (B) + *P. fluorescens* (C) bacteria. On the other hand, application of NP at 100% significantly stimulated plant height 7.85%. The interaction between bio-fertilizers and NP showed significantly effect on plant height. However, minimum plant height (85.6cm) was recorded in control treatment, while maximum plant height (99.2cm) was recorded in treatment of A+B+C bacteria with 75 and 100% NP levels.

Table 3 summarized the effect of bio-fertilizers with chemical fertilizer on flag leaf area. The application of bio-fertilizers A+B+C bacteria was significantly increased average flag leaf area up to 18.55%, while application of 100% NP significantly increased it up to 18.96%. Interaction between bio-fertilizers and NP showed significant effect on flag leaf area. Maximum flag leaf area (80.56cm^2) was recorded in treatment of A+B+C bacteria with 100% NP compared with the minimum number which was recorded (54.51cm^2) in control treatment.

By using different levels of chemical fertilizers with bio-fertilizers data in table (3) showed that average flag leaf chlorophyll content was significantly increased up to 8.08% by application of 100% NP, while bio-fertilizers application A+B+C bacteria increased it significantly up to 9.07%. Flag leaf chlorophyll content revealed significantly effect by the interaction between bio-fertilizers and NP. The lowest SPAD number was recorded (45.41) in the control treatment, while highest SPAD number was recorded (55.05) in treatment of A+B+C bacteria with 100% NP.

Data in table 4 showed the effect for using bio-fertilizers with chemical fertilizers on number of tillers.plant^{-1} under field conditions. Average number of tillers.plant^{-1} was significantly increased up to 40.11% by application of A+B+C bacteria as bio-fertilizers, while application of NP at 100% significantly increased to 67.39%. It was also clear that interaction between bio-fertilizers and NP showed significant effect on number of tillers.plant^{-1}. Minimum number (10.4 tillers.plant^{-1}) was recorded in control treatment, while the maximum number of tillers.plant^{-1} which was recorded in treatment of A+B+C bacteria with 100% NP (28.4 tillers.plant^{-1}).

According to the results presented in table (4) average biomass fresh weight was significantly raised to 53.78% by application of bio-fertilizers A+B+C bacteria, while application of NP at 100% significantly raised it to 28.19%. Biomass fresh weight was of significant impact by interaction between bio-fertilizers and NP fertilizers. However, minimum biomass fresh weight (120.12 gm) was recorded in control treatment. The maximum biomass fresh weight (266.89 gm) was recorded in treatment of A+ B+C bacteria with 100% NP.

Average biomass dry weight which was presented in table 4 increased significantly by application of bio-fertilizers A+B+C bacteria up to 30.83%, however application of NP at 100% significantly raised it to 35.49%. The interaction between bio-fertilizers and NP revealed significant effect on biomass dry weight. Minimum biomass dry weight was recorded in control treatment (48.55 gm), while maximum weight was recorded in treatment of A+B+C bacteria with 100% NP (85.89 gm).

Table 3-Effect of bio-fertilizers and NP levels on some agronomic traits

Plant height (cm)						
NP fertilizer (% of recommended dose)						
Treatment	0	25	50	75	100	Mean
0	85.6**	90.0	90.6	92.2	93.9	90.46
A	89.0	91.4	92.0	92.8	94.0	91.84
B	89.2	94.0	94.4*	95.0*	95.0*	93.52
C	89.0	92.0	93.2	93.4	94.4*	92.40
A+B	90.4	95.6*	96.6*	97.6*	98.2*	95.68
A+C	89.2	91.4	95.0*	95.2*	95.2*	93.20
B+C	89.4	94.2	95.2*	96.4*	97.2*	94.48
A+B+C	90.4	96.2*	96.8*	99.2*	99.2*	97.16
Mean	89.52	93.10	94.22	95.22	96.55	

LSD ≤ 0.05 Bacteria=2.210 NP=1.747 Interaction=4.941						
Flag leaf area (cm^2)						
0	54.51**	62.22	66.10	68.70	73.19	64.94
A	63.63	65.11	68.72	72.01	74.63	68.82
B	66.50	68.23	71.21	75.54*	77.50*	71.79
C	65.25	68.08	69.66	74.38	76.04*	70.68
A+B	65.93	70.30	75.49	77.34*	79.78*	73.76
A+C	62.28	68.82	69.82	74.59	76.44*	70.39
B+C	65.09	69.01	73.20	76.36*	79.32*	72.59
A+B+C	74.52	74.68	75.64*	79.59*	80.56*	76.99
Mean	64.71	68.31	71.23	75.02	76.98	
LSD ≤ 0.05 Bacteria=2.587 NP=2.045 Interaction=5.784						
Flag leaf chlorophyll content (SPAD)						
0	45.41**	47.09	51.54	51.56	53.34*	49.78
A	46.05	50.31	51.63	51.84	54.00*	50.76
B	52.90	52.94	53.50*	53.76*	54.50*	52.32
C	49.29	50.90	52.50	53.62*	54.23*	52.10
A+B	52.80	52.87	53.93*	54.29*	54.90*	53.75
A+C	50.60	51.03	51.99	53.23*	54.45*	52.26
B+C	52.09	52.35	52.76	53.88*	54.86*	53.18
A+B+C	53.40	53.94	54.40*	54.73*	55.05*	54.30
Mean	50.35	51.40	52.72	53.37	54.42	
LSD ≤ 0.05 Bacteria=0.974 NP=0.770 Interaction=2.178						

0= 0 bacteria A= *A. brasilense* B= *A. chroococcum* C= *P. fluorescens*
*= no significant differences **= control treatment

Table 4-Effect of bio-fertilizers and NP levels on some agronomic traits

Number of tillers.$plant^{-1}$						
NP fertilizer (% of recommended dose)						
Treatment	0	25	50	75	100	Mean
0	10.4**	14.0	17.0	21.0	27.0*	17.90
A	13.6	15.8	17.4	21.8	27.2*	19.16
B	15.8	18.0	20.0	24.0	28.0*	21.16
C	14.4	17.0	18.6	22.0	27.2*	19.84
A+B	20.2	21.2	22.4	26.0*	28.0*	23.56
A+C	17.0	19.0	19.0	23.2	27.2*	21.08
B+C	19.0	20.0	20.0	25.0*	28.0*	22.40
A+B+C	21.6	22.0	26.4*	27.0*	28.4*	25.08
Mean	16.50	18.37	20.10	23.75	27.62	
LSD ≤ 0.05 Bacteria=1.682 NP=1.330 Interaction=3.762						
Biomass fresh weight (g) per plant						
0	120.12**	138.51	148.32	167.86	225.47	160.06
A	161.91	170.17	186.68	197.33	210.28	185.28
B	202.32	215.25	225.97	234.14	250.44	225.62
C	177.70	181.43	194.43	212.34	225.43	198.27
A+B	219.65	223.43	237.34	248.66	258.09	237.48
A+C	182.28	197.94	207.57	220.81	232.43	208.21
B+C	207.97	216.62	226.07	238.29	252.85	228.39
A+B+C	227.13	235.56	246.73	254.46	266.89	246.15

Mean	187.39	197.39	209.14	221.74	240.23	
LSD ≤ 0.05 Bacteria=2.951 NP=2.333 Interaction=6.598						
Biomass dry weight (g) per plant						
0	48.55**	52.90	55.11	66.31	70.49	58.67
A	51.05	54.36	56.95	70.50	71.71	60.91
B	57.08	58.93	69.36	74.72	75.97	67.21
C	51.52	56.62	58.24	72.72	73.77	62.57
A+B	64.46	64.91	77.63*	82.83*	84.57*	74.88
A+C	61.32	62.12	70.69	78.70*	80.82*	70.73
B+C	61.86	63.26	73.35	81.69*	82.87*	72.60
A+B+C	66.20	67.47	79.06*	85.19*	85.89*	76.76
Mean	57.76	60.07	67.55	76.58	78.26	
LSD ≤ 0.05 Bacteria=4.480 NP=3.200 Interaction=9.051						

0= 0 bacteria A= *A. brasilense* B= *A. chroococcum* C= *P. fluorescens*
*= no significant differences **= control treatment

Effect of bio-fertilizers and NP levels on yield and yield components of wheat plants under field conditions:

Table 5 indicated the significant raise in average spike length. By application of bio-fertilizers *A. brasilense* (A) + *A. chroococcum* (B) + *P. fluorescens* (C) bacteria spike length increased up to 11.07%, while application of 100% NP raised averages up to 13.48%. Spike length affected significantly by the interaction between bio-fertilizers and NP. Maximum spike length (13.2 cm) was recorded in bio-fertilizers treatment A+ B+C bacteria with 100% NP, while minimum spike length (10.0 cm) was recorded in control treatment.

Data presented in table 5 exhibited that average number of spike.m^2 was significantly increased by using bio-fertilizers application A+B+C bacteria up to 39.25%, while 100% NP application significantly increased it up to 25.14%. The interaction between bio-fertilizers and NP had significant impact of number of spikes.m^{-2} .The minimum number was (321.00 spikes.m^{-2}) recorded in control treatment and maximum number was (548.30 spikes.m^{-2}) recorded in treatment of A+B+C bacteria with 100% NP.

Application of wheat seeds with bio-fertilizers A+B+C bacteria showed that average 1000 grain weight was significantly increased by 9.46%, while application of 100% NP significantly increased 1000 grains weight by 12.28% (Table 5). 1000 grains weight was significantly affected by the interaction between bio-fertilizers and NP. However, maximum weight of 1000 grains (33.02 gm) was recorded in treatment of A+ B+C bacteria with 100% NP and there were no significant differences between this treatment and treatments of 0, A, B, C, A+B, A+C, B+C bacteria with100% NP and treatments of 0, A, B, C, A+B, A+C, B+C, A+B+C bacteria with 75% NP also treatments of B, C, A+B, A+C, B+C, A+B+C bacteria with 50% NP and treatments of B, A+B, A+C, B+C, A+B+C bacteria with 25%NP. Furthermore minimum1000 grains weight was (24.10 gm) recorded in treatment of zero bacteria and zero NP (control treatment).

Table 5-Effect of Bio-fertilizers and NP levels on yield and yield components

Spike length (cm)						
NP fertilizer (% of recommended dose)						
Treatment	0	25	50	75	100	Mean
0	10.0**	11.0	11.4	11.6	12.0	11.20
A	10.8	11.2	11.6	11.8	12.2	11.52
B	11.4	11.6	12.0	12.2	12.8*	12.00
C	11.0	11.4	11.8	12.0	12.4	11.72
A+B	11.6	11.8	12.4	12.6*	13.0*	12.28
A+C	11.2	11.6	12.0	12.2	12.6*	11.92
B+C	11.4	11.6	12.2	12.4	12.8*	12.08
A+B+C	11.6	12	12.6*	12.8*	13.2*	12.44
Mean	11.125	11.525	12.000	12.200	12.625	

LSD ≤ 0.05 Bacteria=0.3052 NP=0.2413 Interaction=0.6824						
No. of spikes.m^{-2}						
0	321.00**	341.63	366.60	374.80	464.93	373.74
A	329.37	381.63	390.30	439.90	473.30	402.90
B	414.93	433.30	436.63	486.30	503.10	454.85
C	398.30	399.93	424.63	478.30	478.30	435.89
A+B	436.63	459.93	518.30	529.93	539.20	496.80
A+C	399.50	411.63	423.00	483.30	502.00	443.89
B+C	433.30	456.50	474.63	524.93	518.10	481.49
A+B+C	485.00	486.50	539.20	543.30*	548.30*	520.46
Mean	402.25	421.38	446.66	482.56	503.40	
LSD ≤ 0.05 Bacteria=2.948 NP=2.330 Interaction=6.591						
1000 grains weight (g)						
0	24.10**	26.66	28.32	29.22*	33.22*	28.64
A	26.63	28.22	28.76	29.61*	30.69*	28.78
B	29.04	29.47*	30.29*	31.14*	32.21*	30.43
C	28.03	28.35	28.95*	29.62*	29.81*	28.95
A+B	30.01	30.17*	31.14*	31.90*	32.98*	31.24
A+C	29.21	29.56*	30.29*	31.30*	31.75*	30.42
B+C	29.63	29.90*	30.83*	31.39*	32.85*	30.92
A+B+C	30.15	30.31*	31.25*	32.01*	33.02*	31.35
Mean	28.56	29.08	29.98	30.77	32.07	
LSD ≤ 0.05 Bacteria=1.873 NP=1.481 Interaction=4.189						

0= 0 bacteria A= *A. brasilense* B= *A. chroococcum* C= *P. fluorescens*
*= no significant differences **= control treatment

Results presented in table (6) indicated that average of biological yield was significantly increased by application of bio-fertilizers A+B+C bacteria and application of 100% NP up to 16.74% and 20.72%, respectively. Interaction between bio-fertilizers and NP significantly affected biological yield. Maximum biological yield (20.967 ton.h^{-1}) was recorded in treatment of A+ B bacteria with 100% NP while minimum biological yield (14.967 ton.h^{-1}) was recorded in control treatment. Besides there were no significant differences between the greatest value and treatments of B, A+C, B+C, A+B+C bacteria with 100% NP and the treatment of A+B+C bacteria with 75%NP.

Table 6 exhibited that average of grain yield was significantly increased by bio-fertilizers application A+B+C bacteria up to 21.17%, while application of NP at 100% significantly raised grain yield by 44.58%. Grain yield was significantly affected by the combination between bio-fertilizers and NP. The minimum grain yield was (3.387 ton.h^{-1}) recorded in control treatment, while maximum grain yield was (6.403 ton.h^{-1}) recorded in treatment of A+B+C bacteria with 100% NP. Also there were no significant differences between this treatment and treatments of 0, A, B, C, A+B, A+C, B+C with 100% NP and treatments of A+B, B+C, A+B+C with 75% NP.

Average of harvest index (HI) was significantly increased by 4.54% in application of bio-fertilizers A+B+C bacteria, while application of NP at 100% significantly increased HI by 20.03% (Table 6). Interaction between bio-fertilizers and NP had significantly impact on HI. Maximum HI (30.79) was recorded in treatment of zero bacteria with 100% NP. However, there were no significant differences between this treatment and the treatments of A, B, C, A+B, A+C, B+C, A+B+C with 100% NP and treatments of 0, A, B, C, A+B, A+C, B+C, A+B+C with 75 and 50%NP also treatments of 0, B, C, A+B, A+C, B+C, A+B+C with 50%NP and the treatment of A+B+C with 0%NP. In addition control treatment recorded the minimum value of HI (22.66).

The results of field experiment showed that the highest value for agronomic traits, yield and yield components were the treatment of three bacterial genus +100% NP. While, there were no significant differences between this treatment and treatment of three bacterial genera +50% NP for all agronomic traits, spike length, 1000 grains weight. Besides there was no significant differences between the treatment of zero bacteria+100% NP (recommended dose) and the treatment of three bacterial genera+50% NP for number of spike/m^2, biological yield and grain yield.

This increasing in agronomic traits and yield in field experiments referred to the action of bio-fertilizers. It is well known that considerable number of bacterial species associated with plant rhizo-sphere are able to exert a beneficial effect upon plant growth and yield such an improvement might be attributed to N-fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances. Therefore their use as bio-fertilizers for agriculture improvement has been a focus of numerous researchers for a number of years. These results were in agreement with several workers,[28-32].

Results showed that application of mineral fertilizers at all levels increased agronomic and yield, especially the full dose (100% mineral fertilizer). These results were in agreement with those obtained by [32-36]. The agronomic traits and yield was increased when plants inoculated with bio-fertilizers combined with mineral fertilizers, and that because the application of bio-fertilizers which may be attributed to their role by enhancing plant growth due to the availability of different nutrients including N, P and K in addition to several micronutrients. There were several ways in which *A. chroococcum* and *A. brasilense* promoted plant growth. They were associated with their host plant to fix atmospheric nitrogen [37, 38], synthesize siderophores that sequester iron from the soil and provide it to plants, synthesize several different phyto-hormones that can act to enhance various stages of plant growth such as IAA, gibberellin and cytokinin [39-41].

A. brasilense was able to improve root development by stimulation of lateral root differentiation, in addition to promoting the elongation of primary root which enhanced mineral uptake and plant water relationship [42]. On the other hand *P. fluorescence* was very effective for increasing growth and yield of crops as well as plant available P in soil by production of organic acids and phosphatase enzymes, it was able to release low molecular weight organic acids mainly gluconic and keto gluconic acids which through their hydroxyl and carboxyl groups chelate the cations (Al, Fe, Ca) bound to phosphate [43,44]. Plant supply root borne carbon compounds (mainly sugars) which can be metabolized for bacterial growth (symbiotic relationship) [45]. Furthermore, it could be increasing Fe solubility and hence uptake by plant [46].

Finally, by using bio fertilizer with half dose of NP has resulted in the increased plant growth and yield. Besides, using bio fertilizers that contain different microbial strains have led to decrease in the use of chemical fertilizers and have provided high quality products free of harmful agrochemicals for human safety.

Table 6-Effect of Bio-fertilizers and NP levels on yield and yield components

Biological yield (ton.h^{-1})						
NP fertilizer (% of recommended dose)						
Treatment	0	25	50	75	100	Mean
0	14.967**	16.133	16.500	18.300	19.700	17.120
A	15.500	16.600	17.700	18.433	19.800	17.607
B	16.633	17.733	19.133	19.700	20.967*	18.833
C	15.767	17.167	18.300	19.100	19.967	18.060
A+B	18.800	19.000	19.967	20.200	21.133*	19.820
A+C	17.167	18.133	18.567	19.400	20.500*	18.753
B+C	17.600	18.400	19.700	19.967	20.633*	19.260
A+B+C	19.133	19.400	20.000	20.433*	20.967*	19.987
Mean	16.946	17.821	18.733	19.442	20.458	
LSD ≤ 0.05	Bacteria=0.3248		NP=0.2568		Interaction=0.7263	
Grain yield (ton.h^{-1})						
0	3.387**	4.317	4.837	4.930	6.070*	4.708
A	3.953	4.383	4.920	5.274	6.090*	4.919
B	4.163	4.790	5.120	5.520	6.133*	5.145
C	4.117	4.627	5.000	5.320	6.110*	5.035
A+B	4.640	5.320	5.493	5.730*	6.313*	5.499
A+C	4.337	5.077	5.320	5.603	6.220*	5.311
B+C	4.450	5.227	5.477	5.713*	6.263*	5.426
A+B+C	5.253	5.360	5.510	6.000*	6.403*	5.705

Mean	4.288	4.888	5.210	5.508	6.200	
LSD ≤ 0.05	Bacteria=0.3246		NP=0.2566		Interaction=0.7258	
Harvest index (HI)						
0	22.66**	26.74*	29.25*	26.90*	30.79*	27.27
A	25.50	26.50	27.80*	28.49*	30.76*	27.81
B	25.04	27.01*	26.76*	28.01*	29.24*	27.21
C	26.17	26.97*	27.33*	27.85*	30.60*	27.79
A+B	24.66	28.02*	27.52*	28.37*	29.89*	27.69
A+C	25.32	27.98*	28.61*	28.92*	30.35*	28.24
B+C	25.29	28.46*	27.81*	28.64*	30.41*	28.12
A+B+C	27.46*	27.63*	27.55*	29.39*	30.54*	28.51
Mean	25.26	27.41	27.83	28.32	30.32	
LSD ≤ 0.05	Bacteria=1.877		NP=1.484		Interaction=4.197	

0= 0 bacteria A= *A. brasilense* B= *A. chroococcum* C= *P. fluorescens*
*= no significant differences **= control treatment

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