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RESPONSE OF FABA BEAN PLANTS TO INOCULATION WITH *Rhizobium leguminosarium* AND OTHER RHIZOBACTERIA UNDER THREE NITROGEN LEVES IN NEWLY RECLAIMED SOIL

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ABSTRACT

A field experiment was conducted twice at the farm of the Environmental Studies and Research Institute, Minufiya University, Sadat City (Minufiya Governorate), during the two successive winter seasons of 2006/2007 and 2007/2008 to study the response of faba bean (*Vicia faba* L. cv. Giza 461) to inoculation with *Rhizobium leguminosarium* individually or mixed with rhizobacteria known to produce plant growth- promoting substances (PGPR), i.e., *Azospirillum brasilense* , *Bacillus megaterium* or *Bacillus polymyxa* as co inoculation under three levels of mineral nitrogen fertilizer (0 , 20 , 40 kg N /fed), in a newly reclaimed desert (sandy soil).

Results showed that nodulation, plant growth parameters and bacterial activities in soil, varied depending on nitrogen dose and introduced bacteria. Co-inoculation with *Rhizobium* and PGPR resulted in significant increases in number and dry weight of root nodules, as well as nitrogenase activity of the root system as compared with the single inoculation with *Rhizobium* alone or control (un-inoculated plants). Mixed inoculation improved bacterial activities in rhizosphere soil, dehydrogenase, and phosphatases (acid and alkaline) enzymes and total bacterial count, compared to the single inoculation. In respect to nitrogen fertilizer , the highest rate applied(40 kg N /fed.) gave the maximum value of bacterial activities, as compared with the other doses, except nitrogenase activity, which was favored with the lower N dose(20 kg).

The parameters of faba bean growth, seed yield and its attributes were generally promoted with the applied treatments, i.e., the biofertilizer significantly enhanced plant growth rate and parameters at 40 D and 85 days age. Mixed inoculation (R.l.+ *B. polymyxa*) produced the highest values of plant height , shoot and root dry weights. The same treatment exhibited the greatest number of branches, number of pods, number of seeds / pod, seed index and seed yield /plant, and consequently highest seed yield /fed ,e.g., average increase in yield /fed. was (67.45% & 63.62%) for the two seasons, respectively. No significant differences could be detected between the two doses of nitrogen fertilizer (20 and 40 kg N/fed.) in most studied traits, except seed yield per plant and per fed. Inoculation with R.l. + *B.megaterium* or *B. polymyxa* combined with 40 kg N/ fed. resulted in the highest values of most studied traits, except the nitrogenase activity, which was higher with 20 kg N/fed.

Keywords: Beneficial rhizobacteria, legumes, *Rhizobium leguminosurm*, nitrogen fertilization, sandy Soil.

INTRODUCTION

Co-inoculation of legumes with rhizobia and rhizobacteria producing plant growth- promoting substances (PGPR) has received increasing attention in recent years. One of the most studied PGPR in relation to its interaction with the bacterial genus *Rhizobium* are those belonging to the genera *Azospirillum* and *Azospirilla*, they are free-living N_2-fixing rhizobacteria which, live in close association with plants and are capable of increasing the yield of important crops grown on various soils and climatic regions. The reviewed data from field inoculation experiments showed statistically significant increases in yield on the order of 5-30% in 60-70% of published reports (Okon and Labandera-Gonzalez, 1994). The benefits observed following inoculation with *Azospirillum* were mainly attributed to improved root development and enhanced water and mineral uptake. Available evidence indicated that secretion of plant-growth promoting substances by bacteria was at least partly responsible for those effects (Okon and Vanderleyden, 1997). Indirect mechanisms used by PGPR include antibiotic protection against pathogenic bacteria, reduction of iron available to phytopathgens in the rhizosphere, synthesis of fungal cell wall- lysing enzymes, and competition with detrimental microorganisms for sites on plant roots. Direct mechanisms of plant growth by PGPR include the provision of bio-available phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plants by siderophores, production of plant hormones like auxins, cytokinins and gibberellins and lowering of plant ethylene levels (Glick, 1995; Glick *et al.*,1999). Co-inoculation of several legumes with *Azospirillum*, *Azotobacter*, *Bacillus* and *Rhizobium*, or inoculation with *Azospirillum* or *Azotobacter*, alone in the case of naturally-nodulated legumes, was shown to benefit plant growth in the greenhouse and in the field (Burdman *et al.*, 1998). The increase in dry-matter production and nitrogen content of dually inoculated plants might be attributed to earlier and enhanced nodulation, higher N_2-fixation rates and a general improvement of root development (Okon and Itzigsohn, 1995). Dual inoculation with *Rhizobium* and *Azospirillum* or *Azotobacter* could either stimulate or inhibit nodule formation and growth in a given symbiotic system, depending on the concentrations and timing of inoculation, besides improving the microbiological activity in the rhizosphere (Kohler *et al.*, 2007). Also, Cassan *et al.* (2008) found that single and combined inoculation with *Azospirillum* and /or *Bradyrhizobium* increased significantly shoot length and shoot and root dry weights of corn and nodulation of soybean.

Faba bean (*Vicia faba*), as the most important food legume has a potential necessity for Egyptian people. The national faba bean cultivated area over the last five years is 131.000 hectare, with an average productivity of 2.86 t/ha. Faba bean production in Egypt is still limited and fails to face the local increasing consumption of the crop. So, increasing crop production is one of the major targets of the agricultural policy and can be achieved by both increasing the cultivated area and its productivity.

The objective of this study is to evaluate the response of Egyptian faba bean cultivar to inoculation with *Rhizobum leguminosurm* alone or in combination with either *Bacillus polymyxa*, or *Azospirillum brasilense* or *Bacillus megaterium* under the effect of varying levels of mineral nitrogen on nodulation, plant growth and bacterial activities in rhizosphere soil and yield of faba bean crop.

MATERIALS AND METHODS

Field experiments were carried out at the farm of the Environmental Studies and Res. Institute, Minufiya Univ. (Sadat City – Minufiya Governorate), Egypt, where the soil is sandy (newly reclaimed desert), in two successive growth seasons, i.e. 2006/2007 and 2007/2008. Some physical and chemical properties of the experimental soil were determined according to **Page *et al.*,(1982)**and their data are presented in Table(1a,b).

Table (1): Analytical data of the experimental soil

(A):Physical properties

$CaCO_3$ (%)	Organic matter (%)	Particle size distr., (%)			Texture class
		Sand	Silt	Clay	
1.9	0. 3	88.6	4.8	6.6	Sandy

(B): Chemical Properties

PH*	EC** dS. m^{-1}	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Ca^{++}	Mg^{++}	K^+	Na^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}
7.63	1.82	0.36	0.32	0.14	0.56	-	0.41	0.36	0.61

*In the 1:2.5 Soil: water suspension. **In the soil paste extract.

Bacterial strains:

Strains of *Rhizobum leguminosurm*, *Bacillus polymyxa*, *Bacillus megaterium* and *Azospirillum brasilense* were obtained from Soils, Water and Environment Res. Inst., ARC, Giza,. *Azotobacter* was pre-cultured in a modified Ashby's medium (Hegazi and Niemela ,1976).The bacterial strains were grown in a nutrient broth liquid medium for 2 days at 30 ^{0}C. Cultures were then centrifuged at 1000 rpm. for 30 min at 10 ^{0}C . The sediment was re-suspended in 5 ml sterilized 0.8 %KCl (w/v). Bacterial suspension was again shacked for 5 min. This material was considered as an inoculum

Agricultural Practices:

Prior to cultivation the manured area was divided into plots, each of 13.5 m^2 including 6 rows 3 m in length and 0.75 m in width, Faba bean (*Vicia faba*) variety 461 seeds were planted on November 1 and 5 in 2006 and 2007, two respectively, at per hill and 20 cm apart on rows after inoculation with the assigned bacterial agents. Drip irrigation took place immediately. Amounts of nitrogen fertilizer were added as ammonium sulfate (20.6 %N) at three levels, i.e., 0, 20 & 40 kg N/fed. Each dose was applied at three equal additions 18 days after sowing and ten days intervals. Potassium in the form of potassium sulfate (48 % K_2 O) and phosphorus as calcium supper phosphate (15.5 % P_2O_5) were added as recommended by the Egyptian Ministry of Agriculture at soil preparation.

Split plot design with four replications was used, where the levels of nitrogen were allocated in the main plots and the biofertilizer treatments were distributed in the sub plots. The experimental area had been under ordinary cultivation practices along both seasons.

Plant growth criteria, namely, plant height, shoot dry weight and root dry weight, were determined at 40 and 85 days, as well as number of root nodules, dry weight of nodules, nitrogenase activity, dehydrogenase, acid phosphatase and alkaline phosphatase were determined in rhizosphere soil at 40 and 75 days after sowing. Crop growth rate was calculated based on plant height, shoot and root dry weights as averages of the two seasons according to the following equation : -

$GGR = (W2 - W1) \times (1/Ga) / (T2-T1)$ g / m^2/ day

Were calculated for dry weight / plant according to the above mentioned equation where W1 and W2 = total dry weight / plant (g) at T1 and T2 date of sampling, respectively. Whereas, Ga = ground area / plant.

Assay of Enzyme Activities and Bacterial counts in Soil:

Activities of the enzymes under study were determined according to the following methods:

- Dehydrogenase: colourimetrically, for the 2,3,5- triphenyl formazan (TPF) produced from the reduction of 2,3,5- triphenyl tetrazolium chloride (TTC), using acetone for extraction.....(Thalmann.,1967).
- Nitrogenase: by means of gas liquid chromatograph for ethylene produced from the reduction of acetylene....(Hardy *et al.*, 1973).
- Phosphatases: colorimetrically, using a modified universal buffer (MUB) at pH 6.5 for acid phosphatase and at pH 11 for alkaline phosphatase ... (Tabatabai,1982).
- Total bacterial numbers were determined using agar plate technique (Difco Manual, 1985).

At maturity, a sample of five guarded plants were uprooted to measure number of branches, number of pods , number of seeds /pod , seed index (100 seed weight) and seed yield /plant, then the four inner rows were harvested to calculate seed yield /fed . Analyses of variance were computed according to Gomez and Gomez (1984) using the least significant difference at 0.05 level, to compare the differences among means.

RESULTS AND DISCUSSION

Nodulation Status of Faba Bean and Nitrogenase Activity:

Data in Table (2 a & b) showed that nodules number, dry weight of nodules and nitrogenase activity in both successive seasons, increased significantly due to the co-inoculation with *Rhizobium leguminosarum* plus *Azospirillum brasilense, Bacillus megaterium* or *Bacillus polymyxa*, as compared with the plants inoculated with *Rhizobium leguminosarum* alone or the un-inoculated plants on both 45th and 70th day after sowing (DAS).

Nitrogen fertilizer improved nodulation status. Increases in nodules number with *Rhizobium leguminosarum* reached (74.77 and 32.71 %) above the un-inoculated plants at45th and 70th DAS, respectively. These increases were more significant by using the mixed inoculation. Increases with *R. leguminosarum* plus *Azospirillum brasilense* reached (97.56 and 112.81 %) and with *R. leguminosarum* plus *Bacillus megaterium* were (115.27 and 124.62 %) and with *R. leguminosarum* plus *Bacillus polymyxa* reached (101.36 and 117.31 %) at 45th and 70th DAS , respectively. Likewise, dry weight of nodules was increased with such inoculations. *Rhizobium leguminosarum* gave increases reached (26.77 and 18.58 %) above the un-inoculated plants on 45th and 70th DAS, respectively. Co-inoculation with *Azospirillum brasilense* gave (29.5 and 23.78%) and *Bacillus megaterium* (33.28 and 21.01%) and *Bacillus polymyxa* (31.82 and 21.67%) above the control at 45th and 70th DAS, respectively. Nitrogenase enzyme activity had positively responded to the co-inoculation through its higher values, compared to those obtained by *R. leguminosarum* alone (Table2 a & b). The highest N_2-ase activity levels were recorded at the 70th DAS compared to 45th DAS. Increases in such enzyme activity above the control reached (154.8 and 410.48 %) with *R. leguminosarum* at both 45th and 70th DAS, respectively. Co-inoculation were superior in enhancing of N_2-ase activity, when gave (447.89 and 707.58 %) above the control by using *R. leguminosarum* plus *A. brasilense* at 45th and 70th DAS, respectively. The increases recorded due to the use of the mixed inoculum of *R. leguminosarum* plus *B. megaterium* were 311.71 and 537.16%) above the control at 45th and 70th DAS, where as they were (439.78 and 656.86 %) with *R. leguminosarum* plus *B. polymyxa*. Increases occurred upon using the co-inoculation was (115.03, 61.57 and 11.84%) above *R. leguminosarum* alone at 45th DAS and (58.19, 24.81 and 48.26%) at 70th DAS with mixed *Azospirillum brasilense, B. megaterium* or *B. polymyxa*, respectively. Results in Table (2 a & b) revealed that the highest values of number and weight of root nodules and N_2-ase activity were attained with the co-inoculation, as compared with the single inoculation. Enhancement of nodulation status is actually due to root proliferation and thus boosting the N_2-ase activity. Plazinski and Rolfe (1985) suggested that, rhizobacteria could stimulate additional infection sites that were later occupied by rhizobia. Rodelas *et al.* (1999) suggested that the increase resulted in a higher accumulation of N per plant. While concentration of such nutrient (N %) in plant tissues remained similar to that found in the control plants, suggesting that the entire additional N absorbed was used to aid plant growth and development. The distribution of N between plant parts was slightly modified by some combined inoculation treatments. Such results indicate that several PGPR such as *A. brasilense, B. megaterium* and *B. polymyxa* used in this study exerted significant effects on *R. leguminosarum* symbiosis, which were only observed when this combination of microbial strains was used. Such promotion of nodulation and N_2-ase activity in many legume species has been confirmed by many investigators (Rodelas *et al.*, 1996 and 1999 , Abdel-Wahab and Said, 2004 and El-Howeity, 2004 and 2008).

Table (2a): Effect of rhizobacteria and nitrogen fertilization on root nodulation and nitrogenase activity of faba bean plants at 45 and 70 days after sowing (first season)

Treatment		Number of nodules/ plant				Dry weight of nodules (mg/ plant)				Nitrogenase activity*			
		0 kgN/fed.	20 kgN/ fed.	40 kgN/ fed.	mean	0 kgN/ fed.	20 kgN/ fed.	40 kgN/ fed.	mean	0 kgN/ fed.	20 kgN/ fed.	40 kgN/ fed.	mean
45 days after sowing													
Control		11	13	14	12.67	200.00	254.00	254.00	236.00	5..54	7.29	6.06	6.675
R.legminoserium		20	23	26	23.00	291.00	306.00	305.00	300.67	11.61	14.81	11.73	13.27
R.l.+Azosprillum		22	27	29	26.00	291.00	280.00	342.00	304.33	36.95	42.08	30.93	36.505
R.l.+B.megateriu		23	29	30	27.33	298.00	319.00	335.00	317.33	25.05	30.29	24.4	27.345
R.l.+B.polymyxa		23	27	29	26.33	300.00	312.00	324.00	312.00	28.49	30.86	33.23	32.045
Bio mean		19.80	23.80	25.60	23.07	276.00	294.20	312.00	294.07	25.53	29.51	25.07	27.29
LSD, at 0.05	N	2.3				18.41				3.51			
	Bio	3.16				7.62				5.23			
	Nx Bio	4.36				21.56				5.11			
70days after sowing													
Control		16	17	19	17.33	332	348	359	346.33	12.25	15.67	14.22	14.05
Rh.legminoserium		26	32	33	30.33	393	422	428	414.44	51.08	61.47	54.12	55.56
R.l.+Azosprillum		31	36	40.3	35.77	411	438	455	434.67	86.88	103.58	93.31	94.59
R.l.+B.megaterium		35	39	40	38.00	386	440	461	429.00	65.17	76.05	72.73	71.32
R.l.+B.polymyxa		33	38	39	36.67	418	434	443	431.67	71.37	79.05	73.45	74.62
		28.20	32.40	34.26	31.62	388.00	416.40	429.27	411.22	57.35	67.16	61.57	62.03
LSD, at 0.05	N	1.75				17.65				3.41			
	Bio	3.16				9.43				8.16			
	Nx Bio	4.23				12.33				12.78			

* μ mol C_2H_4 / hr / g dry weight of plant.

Table (2 b): Effect of rhizobacteria and nitrogen fertilization on root nodulation and nitrogenase activity of faba bean plants at 45 and 70 days after sowing (second season)

Treatment	Number of nodules/ plant				Dry weight of nodules (mg/ plant)				Nitrogenase activity *			
	0 kgN/fed.	20 kgN/ fed.	40 kgN/ fed.	mean	0 kgN/ fed.	20 kgN/ fed.	40 kgN/fed.	mean	0 kgN/ fed.	20 kgN/fed.	40 kgN/fed.	mean
45 days after sowing												
Control	12	14	15	13.67	206	253	260	239.67	5.96	8.12	7.01	7.03
R.legminoserium	19	22	28	23.00	285	305	317	302.33	20.94	23.73	19.02	21.23
R.l.+Azosprillum	20	26	32	26.00	290	317	328	311.67	34.58	41.09	33.31	36.33
R.l.+B.megaterium	25	32	31	29.33	297	322	331	316.67	25.68	31.07	28.02	28.26
R.l.+B.polymyxa	22	26	32	26.67	296	320	329	315.00	35.89	45.32	41.93	41.05
	19.60	24.00	27.60	23.73	274.80	303.40	313.00	297.07	24.61	29.87	25.86	26.78
LSD, at 0.05 — N	3.14				9.06				3.26			
LSD, at 0.05 — Bio	4.32				8.09				5.84			
LSD, at 0.05 — N x Bi	5.18				11.53				13.07			
70days after sowing												
Control	16	18	18.67	17.56	341	357	390	362.67	12.96	15.51	15.49	14.65
R.legminoserium	29	36	36	33.67	405	440	434	426.33	58.3	68.95	65.38	64.21
R.l.+Azosprillum	34	40	40	38.00	411	447	471	443.00	87.8	108.65	88.18	94.88
R.l.+B.megaterium	36	42	41	39.67	414	456	468	446.00	69	87.34	78.17	78.17
R.l.+B.polymyxa	35	41	40	38.67	402	440	451	431.00	85.03	120	107.46	104.16
	30.00	35.40	35.13	33.51	394.60	428.00	442.80	421.80	62.62	80.09	70.94	71.21
LSD, at 0.05 — N	4.16				23.87				7.61			
LSD, at 0.05 — Bio	7.14				15.88				11.93			
LSD, at 0.05 — N x Bi	9.79				15.92				19.55			

* µ mol C_2H_4 / hr / g dry weight of plant.

Enzyme Activities in Rhizosphere Soil

A) Dehydrogenase activity (DA):

Dehydrogenase activity (DA) is frequently used as a measurement of the overall microbial activity in soil. Changes in DA during both seasons of the different treatments are presented in Table (3a & b). Results showed that DA increased by time, application N-fertilization and inoculation with ***R. leguminosarum*** individually or mixed with *A. brasilense* or *B. megaterium* or *B. polymyxa* as co inoculation. Data showed that inoculation with *R. leguminosarum* alone increased DA up to (88.27 and 53.18 %) over the control at 45th and 70th DAS, respectively. However, these increases were more pronounced with the co-inoculation. Increases with *A. brasilense* reached (103.25 and 79.83%) and with *B. megaterium* up to (101.79 and 69.32 %) and with *B. polymyxa* were (115.31 and 95.31%) at 45th and 70th DAS, respectively. Dehydrogenases catalyze the electron transfer yielding energy required for the anabolic processes of various microorganisms inhabiting the soil. Through such reactions, the energy source, or electron donor, is converted to a simpler form that could be utilized by other organisms (plants and microbes). Hence, such enzymes are not only responsible for oxidation-reduction processes, but are also involved in the recycling of elements in soil for the benefit of both macro- and micro flora (Burns, 1982).

B) Phosphatase activity (PA)" acid and alkaline phosphatases":

Phosphatases are the biocatalysts that involved in the liberation of mineral phosphorus from its organic sources for the use of growing plants. Table (3a & b) declared that the mixed inoculation gave higher increases reached with *A. brasilense* up to (173.8 and 124.53 %) and with *B. megaterium* (235.81 and 186.66 %) and with *B. polymyxa* (180.51 and 130.77 %) at 45th and 70th DAS, respectively. The same trend was found for PA with N- fertilization and inoculation with *R. leguminosarum* alone or mixed with PGPR improved the activity of such enzyme at both detection times. Increases with *R. leguminosarum* alone were (56.39 and 63.04%) over the control at 45th and 70th DAS, respectively. However, the co-inoculation raised the activity of PA (both acid and alkaline), with *A. brasilense* to (167.83 and 109.62 %) and with *B. megaterium* (236.25 and 128.81 %) and with *B. polymyxa* (97.39 and 112.89 %) at 45th and 70th DAS, respectively. Inoculation of faba bean with *R. leguminosarum* plus PGPR simulated the PA in rhizosphere soil. This may be due to the ability of PGPR in utilizing some organic compounds as carbon and energy sources and thus produce organic acids, which solubilizing insoluble inorganic phosphates. Richardson (2001) found that, solubilization of P in the rhizosphere is the most common mode of action implicated by PGPR that increase nutrient availability to host plants. Dobbelaera *et al.* (2003) suggested that, PGPR stimulate plant growth by facilitating the uptake of minerals N, P and K (NO_3^-, $H_2PO_4^-$, K+) and microelements by plants, however , there was some controversy regarding to the mechanisms that PGPR employ in the uptake of minerals, due to a general increase in the volume and root system, as reflected by increased root number , thickness and length.

Table(3 a): Effect of rhizobacteria and nitrogen fertilization on enzyme activities in rhizosphere of faba bean plants at 45 and 70 days after sowing (first season)

Treatments		DHA µg TPF/ g dry soil / day				Acid Phosphatse (µgP/gdry soil)				Alkalin Phosphatse (µgP/gdry soil)			
		0 kg N/fed.	20kgN/fed.	40kgN/fed.	mean	0 kgN/fed.	20kgN/fed.	40kgN/fed.	mean	0 kgN/fed.	20kgN/fed.	40kgN/fed.	mean
45 days after sowing													
Control		4.75	5.53	6.86	5.71	19.45	30.88	37.59	29.31	10.98	25.64	39.3	25.31
R.legminoserium		6.41	12.95	14.87	11.41	29.28	49.9	75.28	51.49	41.25	51.2	41.03	44.49
R.l.+Azosprilium		7.73	13.6	16.26	12.53	42.97	83.91	120.91	82.60	37.97	76.o3	108.84	73.41
R.l.+B.megaterium		7.11	13.45	16.63	12.40	51.08	101.9	151.56	101.51	43.56	78.2	146.48	89.41
R.l.+B.polymyxa		7.31	14.32	18.45	13.36	44.9	86.64	128.5	86.68	43.28	70.15	97.89	70.44
Mean		6.66	11.97	14.61	11.08	37.54	70.65	102.77	70.32	35.408	56.2975	86.708	59.47
LSD, at 0.05	N	3.20				10.72				9.56			
	Bio	2.31				12.17				8.46			
	N x Bio	5.43				21.18				9.6			
70 days after sowing													
Control		8.25	10.05	12.49	10.26	54.54	70.3	72.24	65.69	52.68	60.62	60.62	57.97
R.legminoserium		18.85	23.7	25.46	22.67	88.42	106.82	120.33	105.19	76.63	87.31	107.75	90.56
R.l.+Azosprilium		19.82	26.52	27.4	24.58	120.25	148.05	165.48	144.59	104.6	142.77	146.62	131.33
R.l.+B.megaterium		19.49	24.83	27	23.77	181.25	197.68	215.9	198.28	119.38	128.81	165.24	137.81
R.l.+B.polymyxa		20.92	26.17	27.97	25.02	124.3	150.18	171.11	148.53	112.81	127.46	136.32	125.53
Mean		17.47	22.25	24.06	21.26	113.75	134.61	149.01	132.46	93.22	109.394	123.31	108.64
LSD, at 0.05	N	3.62				12.91				11.27			
	Bio	3.07				17.05				16.43			
	N x Bio	8.21				23.16				22.17			

Table (3 b): Effect of rhizobacteria and nitrogen fertilization on enzyme activities in rhizosphere of faba bean plants at 45 and 70 days after sowing (second season)

Treatments		DHA µg TPF/ g dry soil / day				Acid Phosphatse(µgP/g dry soil)				Alkalin Phosphatse(µgP/g dry soil)			
		0 kgN/fed.	20kgN/fed.	40kgN/fed.	mean	0 kgN/fed.	20kgN/fed.	40kgN/fed.	mean	0 kgN/fed.	20kgN/fed.	40kgN/fed.	mean
		45days after sowing											
Control		5.32	6.84	7.57	6.58	19.25	29.47	42.46	30.39	12.24	25.35	40.72	26.10
R.legminoserium		7.9	12.08	15.17	11.72	36.07	72.69	77.37	62.04	27.6	34.07	46.07	35.91
R.l.+Azosprillum		8.11	13.12	16.06	12.43	36.56	77.28	128.77	80.87	35.79	66.03	88.5	63.44
R.l.+B.megaterium		7.83	13.1	16.23	12.39	44.42	101.7	150.82	98.98	43.89	76.61	130.7	83.73
R.l.+B.polymyxa		8.25	14.06	16.94	13.08	40.19	83.7	118.48	80.79	37.19	64.86	88.15	63.40
Mean		7.48	11.84	14.39	11.24	35.30	72.97	103.58	70.62	31.34	53.38	78.83	54.52
LSD, at 0.05	N	2.86				18.31				12.98			
	Bio	2.73				17.12				5.43			
	N x Bio	4.18				11.53				14.03			
		70days after sowing											
Control		9	11.31	12.91	11.07	54.54	68.26	75.47	66.09	43.51	53.89	65.05	54.15
R.legminoserium		7..90	8.98	13.1	11.04	88.42	138.57	150.12	125.70	56.9	102.27	117.54	92.24
R.l.+Azosprillum		11.91	13.33	16.04	13.76	120.25	153.64	161.8	145.23	79.04	111.75	120.23	103.67
R.l.+B.megaterium		10.2	12.72	14.07	12.33	181.25	180.94	196.45	186.21	83.54	127.55	145.1	118.73
R.l.+B.polymyxa		14.12	16.83	18.95	16.63	124.3	158.68	166.41	149.80	81.45	121.95	136.15	113.18
Mean		12.08	14.29	16.35	14.24	113.75	140.02	150.05	134.61	68.89	103.48	116.81	96.39
LSD, at 0.05	N	2.53				8.64				10.36			
	Bio	1.26				9.73				8.74			
	N x Bio	8.24				29.57				12.05			

The enhanced phosphatase activity may help the plant to mobilize P and thereby increases the biomass production (Tarafdar and Gharu, 2006). Increases observed for the activities of dehydrogenase, acid and alkaline phosphatases and nitrogenase may be related mainly due to increase in rhizosphere microbial population as a consequence of the inoculation treatments (Aseri and Tarafdar, 2006).

Total Bacterial Numbers:

Total bacterial numbers in the rhizosphere soil, of faba bean plants, as affected by N-fertilization and inoculation with *R. leguminosarum* individually or with *Azospirillum* spp. or *Bacillus* spp. are recorded in Table (4). Data revealed that, addition of N-fertilizer and bacterial inoculation led to significant increases in total bacterial counts in the rhizosphere soil of bean plants. Increases occurred with *R. leguminosarum* were (20.61 and 22.33%) at 45 and 70 DAS, respectively.

Table (4): Effect of rhizobacteria and nitrogen fertilization on total bacterial count in rhizosphere soil of faba bean plants in both seasons

Treatment		Total count at 45 DAS				Total count at 70 DAS*			
		0 kgN/ fed.	20 kgN/fed.	40 kgN/ fed.	mean	0 kgN/ fed.	20 kgN/ fed.	40 kgN/ fed.	mean
First Season									
Control		5.54	5.71	5.8	5.68	5.74	5.93	5.94	5.87
R.legminoserium		6.83	7.1	7.37	7.10	6.81	7.14	7.47	7.14
R.l.+Azosprillum		7.03	7.55	7.78	7.45	6.92	7.32	7.92	7.39
Rl.+B.megaterium		6.76	7.1	7.91	7.26	6.77	7.29	7.94	7.33
R.l.+B.Polymyxa		6.89	7.23	7.92	7.35	7.07	7.48	7.94	7.50
Mean		6.61	6.94	7.36	6.97	6.66	7.03	7.44	7.04
LSD, at 0.05	N	0.42				0.38			
	Bio	1.03				1.16			
	N x Bio	1.12				1.32			
Second season									
Control		5.05	5.7	5.83	5.53	5.63	5.73	5.97	5.78
R.legminoserium		6.33	6.5	6.47	6.43	7.04	7.15	7.13	7.11
R.l.+Azosprillum		6.4	6.96	7.43	6.93	7.3	7.9	7.89	7.70
R.l.+B.megaterium		7.3	7.5	7.94	7.58	7.6	8	8.18	7.93
Rl..+B.Polymyxa		7.55	7.66	7.99	7.73	7.74	8.04	8.23	8.00
Mean		6.53	6.86	7.13	6.84	7.06	7.36	7.48	7.30
LSD, at 0.05	N	0.61				NS			
	Bio	1.23				1.06			
	N x Bio	1.16				1.17			

*** days after sowing**

Likewise, the co-inoculation gave increases reached with *Azqspirillum* spp. (28.27 and 29.55%) and with *B. megaterium* (32.38 and 3.11 %) and with *B. polymyxa* (34.52 and 33.16 %) at 45 and 70 DAS, respectively. Results also, declared that the total bacterial counts increased with increasing N-fertilizer rates, particularly in presence of the high rate, compared to the half rate. These data are in harmony with those obtained by El-Howeity (2004),

Plant Growth Criteria

Data presented in Table (5 a & b)shows the effect of the mixed inoculation with PGPR and nitrogen fertilization at two tested periods of faba bean plant growth on plant height, shoot and root dry weights. Significant variations in plant height shoot and root dry weights due to the application of nitrogen fertilizer were recorded during both seasons of the study, at 40 and 75 days after sowing every season, compared to the control .But insignificant differences were observed between the 20 and 40 kg/fed. of nitrogen fertilizer added on most studied traits.

Application of the biofertilizers resulted in the tallest plants and surpassed the control at 40th DAS by (14.15, 17.19, 14.15 and 17.92%) for *R. leguminosarum* , *R. leguminosarum* plus *A. brasilense*, *R. leguminosarum* plus *B. megaterium* and *R. leguminosarum* plus *B. polymyxa*, respectively. The same increases at 70th DAS were (11.84 , 15.56, 13.38 & 17.48 %) with the above mentioned inocula . Likewise, addition of *R. leguminosarum* and co- inoculations resulted in significant increases in shoot dry weights , at 40th DAS by (13.22, 14.02, 11.37 and 26.45 %) and by (8.89 , 19.46 , 12.78 and 22.83 %) at 75th DAS with the same inoculants, *R. leguminosarum* , *R. leguminosarum* plus *A. brasilense*, *R. leguminosarum* plus *B. megaterium* and *R.* *leguminosarum* plus *B. polymyxa*, respectively. Also, all of the experimental treatments led to increases in root dry matter. Increases of the root dry weight above the control were (65.24, 77.43, 69.51 and 90.24 %) at 45th DAS and (19.72 , 71.46 ,53.36 and 81.67 %) at 70th DAS with *R. leguminosarum*, *R. leguminosarum* plus *A. brasilense*, *R. leguminosarum* plus *B. megaterium* and *R leguminosarum* plus *B. polymyxa*, respectively. Interaction between nitrogen fertilization and bioinoculations was found to be insignificant for most traits in both seasons, except at 75 days in the second one. Top response of faba bean plants to inoculation was recorded for the plants supplemented with the highest rate of nitrogen fertilizer together with the co inoculation. This enhancement of plant growth could be due to a possible N_2-fixation and production of plant growth promoting substances by PGPR.

Crop Growth Rate (CGR):

It was obvious that considerable increases were occurred in plant crop growth rate based on each of plant height , shoot dry weight and root dry weight with increasing the nitrogen level up to 40 kg N/fed. (figs. 2, 3, 4, 5 and 6). The same effect was found using the biofertilizer treatments specially *R.leguminosarum* + *B. polymyxa* which was the most effective inoculum. These results are in harmony with those obtained by Mekhemar (2001), Dobbelaere *et al.*(2003) and El-Howeity (2008). Likewise, Rodelas *et al.* (1999) found that mixed inoculation of *Vicia faba* with four different N_2-fixers namely , *Rhizobium / Azospirillum* and *R. / Azotobacter* increased dry weight of plants as compared with inoculation with Rhizobium alone. Such increase might be attributed to changes in total N content, concentration and / or distribution of mineral macro- and micronutrients (K, P, Ca, Mg, Fe, B, Mn, Zn, and Cu).

Table (5 a): Effect of rhizobacteria and nitrogen fertilization on growth criteria of faba bean plants at 45 and 70 days after sowing (frist season)

Treatment		Plant height(Cm)				Shoot d.wt.(g/ plant)				Root d.wt.(g/plant)			
		0 kgN/ fed.	20kgN/ fed.	40kgN/ fed.	N mean	0 kgN/ fed.	20kgN /fed.	40kgN/ fed.	N mean	0 kgN/ fed.	20kgN/ fed.	40kgN/ fed.	N mean
45 days after sowing													
Control		27	30	33.6	30.20	3.12	3.88	4.22	3.74	1.17	1.46	2.02	1.55
R.legminoserium		30.3	35.3	38	34.53	3.81	4.11	4.9	4.27	2.08	2.9	3	2.66
R.l.+Azosprillum		32.6	37	44.6	38.07	3.43	4.29	5.07	4.26	2.31	3.02	3.17	2.83
R. l.+B.megaterium		31.3	35.6	40	35.63	3.4	4.37	4.76	4.18	2.13	2.95	3	2.69
R.l.+B.polymyxa		35	37	41.3	37.77	4	4.86	5.51	4.79	2.48	3.1	3.23	2.94
Bio mean		31.24	34.98	39.5	35.24	3.55	4.30	4.89	4.25	2.03	2.69	2.89	2.53
LSD. at 0.05	N	2.09				1.03				NS			
	Bio	3.18				NS				0.43			
	N x Bi	NS				Ns				0.65			

70 days after sowing

		Plant height(Cm)				Shoot d.wt.(g/ plant)				Root d.wt.(g/plant)			
Control		99.6	120.3	131.6	117.17	15.35	17.04	18.02	16.80	3.39	4.21	5.06	4.22
R.legminoserium		112.3	122.3	138.3	124.30	16.66	18.56	20.53	18.58	5.39	6.21	6.44	6.01
R.l.+Azosprillum		122	132	141.6	131.87	18.15	19.93	23.19	20.42	6.1	7.7	8.21	7.34
R.l.+B.megaterim		121	132.3	140.6	131.30	16.8	18.91	22.02	19.24	5.59	6.38	7.43	6.47
R.l.+B.polymyxa		126.3	134.6	142	134.30	18.74	20.96	23.51	21.07	6.35	7.7	9.14	7.73
		116.24	128.3	138.82	127.79	17.14	19.08	21.45	19.22	5.36	6.44	7.26	6.35
LSD. at 0.05	N	5.91				1.64				1.06			
	Bio	4.06				1.34				1.14			
	N x Bio	NS				NS				NS			

Table (5 b): Effect of rhizobacteria and nitrogen fertilization on growth criteria of faba bean plants at 45 and 70 days after sowing (second season)

Treatment	Plant height(Cm)				Shoot dwt.(g/ plant)				Root dwt. (g/plant)			
	0 kgN/fed.	20kgN/ ed.	40kgN/ fed.	mean	0 kgN/fed.	20kgN/f ed.	40kgN/ fed.	mean	0 kgN/fed.	20kgN/fed.	40kgN/ fed.	mean
40 days after sowing												
Control	27	26.6	36.6	37	3.34	3.19	3.95	3.49	3.83	1.47	1.57	2.16
R.legminoserium	30.3	34.3	39.6	40.3	3.81	3.61	4.35	3.92	4.29	2.17	3.01	3.12
R.l.+Azosprillum	32.6	34.6	39	40.3	3.38	3.51	4.5	3.80	4.35	2.42	3.29	3.25
R.l.+B.megaterim	31.3	34	38	39.3	3.71	3.49	4.32	3.84	4.25	2.38	3.12	3.13
R.l.+B.polymyxa	35	34	38	40	3.73	4.0	5.05	4.26	4.81	2.82	3.4	3.68
	31.24	32.70	38.24	39.38	3.59	3.56	4.43	3.86	4.31	2.25	2.88	3.07
LSD. at 0.05 N	1.08				0.62				1.04			
Bio	2.45				NS				NS			
N x Bi	NS				NS				Ns			

85 day after sowing

	Plant height(Cm)				Shoot dwt. (g/ plant)				Root dwt. (g/plant)			
Control	103.6	121	129.3	117.97	11.8	15.85	17.47	15.04	3.51	4.65	5.04	4.40
Rh.legminoserium	133	135.3	147.6	138.63	13.86	17.02	18.8	16.56	5.48	6.42	6.44	6.11
R.l.+Azosprillum	135.3	136.3	148	139.87	13.99	18.56	20.14	17.56	6.1	7.93	8.33	7.45
R.l.+B.megaterim	131.3	133	141.6	135.30	13.53	17.21	19.18	16.64	5.95	6.58	7.7	6.74
R.l.+B.polymyxa	134.6	140.6	150.6	141.93	14.19	18.8	21.21	18.07	6.63	7.9	9.28	7.94
	127.56	133.24	143.42	134.74	13.47	17.49	19.36	16.77	5.53	6.70	7.36	6.53
LSD. at 0.05 N	5.03				3.02				1.13			
Bio	3.42				1.05				1.66			
N x Bio	9.17				3.82				3.11			

Table(6):Effect of rhizobacteria and nitrogen fertilization on faba bean yield and its attributes (two seasons)

Treatments	N level	No. Branches/plant		No. Pods/plant		No. seeds/pod		seed indexS2		yield /plant(g)		yield /fed (kg)	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
Control	0	1.00	1.33	20.67	16.33	1.77	1.80	61.18	67.03	22.37	19.76	704.74	622.29
Control	20	1.67	1.33	23.00	16.67	1.87	1.97	71.00	71.57	30.46	23.48	959.60	739.59
Control	40	2.00	1.67	24.00	17.67	1.97	2.00	73.90	74.20	34.91	26.16	1099.66	824.05
		1.56	**1.44**	**22.56**	**16.89**	**1.87**	**1.92**	**68.69**	**70.93**	**29.25**	**23.13**	**921.33**	**728.64**
R.legminoserium	0	1.33	1.67	23.00	17.67	1.83	1.93	73.20	73.87	30.88	25.34	972.64	798.32
R.legminoserium	20	2.33	2.00	25.00	20.33	2.07	2.10	75.77	76.00	39.16	32.55	1233.57	1025.26
R.legminoserium	40	2.67	2.67	27.67	22.33	2.10	2.17	75.87	76.77	44.00	37.07	1386.10	1167.86
		2.11	**2.11**	**25.22**	**20.11**	**2.00**	**2.07**	**74.94**	**75.54**	**38.01**	**31.66**	**1197.43**	**997.15**
R.l.+Azosprillum	0	1.33	2.00	23.67	18.67	2.00	1.97	72.53	72.17	34.43	26.59	1084.40	837.53
R.l.+Azosprillum	20	2.33	2.67	24.33	22.33	2.00	2.07	73.70	73.23	35.79	33.81	1127.53	1065.03
R.l.+Azosprillum	40	3.00	3.33	29.33	24.33	2.13	2.23	75.47	74.53	47.32	40.46	1490.54	1274.33
		2.22	**2.67**	**25.78**	**21.78**	**2.04**	**2.09**	**73.90**	**73.31**	**39.18**	**33.62**	**1234.16**	**1058.96**
R.l.+B.megaterim	0	1.33	1.33	23.00	18.00	1.93	1.93	73.30	74.13	32.53	25.80	1024.83	812.74
R.l.+B.megaterim	20	2.00	2.33	23.67	22.67	2.20	2.20	75.67	75.50	39.25	37.59	1236.47	1184.09
R.l.+B.megaterim	40	2.67	2.33	30.33	23.67	2.27	2.27	76.50	76.73	52.55	41.13	1655.24	1295.54
		2.00	**2.00**	**25.67**	**21.44**	**2.13**	**2.13**	**75.16**	**75.46**	**41.44**	**34.84**	**1305.51**	**1097.45**
R.l.+B.polymyxa	0	1.67	2.00	24.33	18.67	2.17	2.00	73.20	73.97	38.62	27.59	1216.68	868.93
R.l.+B.polymyxa	20	2.67	2.33	26.33	23.00	2.23	2.20	76.40	77.30	44.90	39.15	1414.34	1233.28
R.l.+B.polymyxa	40	3.33	3.33	32.00	24.67	2.53	2.43	78.30	77.97	63.41	46.81	1997.54	1474.51
		2.56	**2.56**	**27.56**	**22.11**	**2.31**	**2.21**	**75.97**	**76.41**	**48.98**	**37.85**	**1542.85**	**1192.24**
Mean of N	0	**1.33**	**1.67**	**22.93**	**17.87**	**1.94**	**1.93**	**70.68**	**72.23**	**31.77**	**25.01**	**1000.66**	**787.96**
	20	**2.20**	**2.13**	**24.47**	**21.00**	**2.07**	**2.11**	**74.51**	**74.72**	**37.91**	**33.32**	**1194.30**	**1049.45**
	40	**2.73**	**2.67**	**28.67**	**22.53**	**2.20**	**2.22**	**76.01**	**76.04**	**48.44**	**38.33**	**1525.82**	**1207.26**
LSD, at 0.05	N	**0.63**	**NS**	**2.05**	**2.14**	**0.12**	**0.15**	**2.53**	**2.64**	**5.32**	**6.19**	**93.52**	**87.66**
	Bio	**0.38**	**0.36**	**1.32**	**1.43**	**NS**	**NS**	**2.27**	**2.19**	**4.87**	**4.92**	**103.24**	**142.16**
	Nx Bio	0.78	0.65	1.o7	2.16	0.13	0.08	5.42	3.91	6.22	2.17	132.27	88.14

Faba Bean Yield and Some of Its Attributes:

The response of faba bean yield and its attributes to nitrogen fertilization and inoculation with *R. leguminosarum* individually and co-inoculation with *Azospirillum spp.* or *Bacillus* spp. is listed in Table (6). Results showed that faba bean yield and its attributes were significantly affected by nitrogen fertilization. Increasing the nitrogen rates caused significant increases in numbers of branches and pods / plant, number of seeds /pod , seed index and seed yield per plant and per feddan. In the other hand, slight but insignificant increases were detected for number of branches, in both seasons due to doubling the nitrogen levels (from 20 to 40 kg N/fed). In the second season, numbers of pods, and seeds/pod and seed yield /plant exerted increases. Seed yield / fed. of faba beans fertilized with mineral nitrogen exceeded significantly the control, i.e. by 52.5 and 27.8%in the first season and 53.2 and 15 % in the second season, by 20 and 40 kg N/fed., respectively. In this concern, Mahmoud *et al.* (2006) reveled that 40 kg nitrogen/fed as soil treatment has significantly increased plant height, leaf area, total dry weight, number of pods, number of seeds per pod, seed yield. However, seed protein and carbohydrate proper accumulation required higher doses. Ihsanullah *et al.* (2008) stated that seed yield and shoot dry weight indicated significant quadratic relation with the increasing N rates between 0 and 200 kg ha^{-1}.

The co inoculation especially with R. *leguminosarum* plus *B. polymyxa* attained the highest values of numbers of branches, and pods /plant, number of seeds and yield /plant and yield /fed., compared to the control and *R. leguminosarum* alone. The promotive effect of rhizobacteria as co inoculation with rhizobia on crop yield and its attributes has been demonstrated by many investigators (Rodleas *et al.*,1999, Abdel-Wahab and Said, 2004 and El-Howeity, 2004 & 2008).*R.leguminosarum* plus *B. polymyxa* as co-inoculation displayed significant increases in all yield characters compared to the control or *R. leguminosarum* alone or with other bacterial inoculants in both seasons. Average increases in yield/fed. reached (67.45 and 63.62%) for both seasons, respectively. It is worthy to note that the same treatment had higher number and dry weight of nodules, nitrogenase activity and total count of bacteria in rhizosphere soil. Followed by *R.leguminosarum* plus *B.megaterium* (41.65 and 50.62%) without significant differences with (*R+B.* polymyxa), then *R. leguminosarum* plus *A.. brasilense* (33.95 and 45.33%), and finally *R. leguminosarum* alone (29.96 and 36.85%) above the control for both seasons, respectively. Introduction of the chemical nitrogen fertilizer enhanced plant elongation and branching, which led to highest dry weights of shoots and roots of faba bean plants (Table 5 a,b),and higher number of pods and weights of pod and seeds (Table 6).No significant differences between both of the nitrogen doses (20 and 40 kgN/fed.). Regarding the interaction between the bio-agents and chemical nitrogen fertilizer on yield and its attributes,Table (6) showed that yield components displayed significant variations between the applied treatments and the response of faba bean to the biofertilizers and the chemical nitrogen fertilizer. Babiker *et al.*(1995) Nitrogen fertilization alone or N fixation significantly improved both yield and protein content, because plants make use of N added or fixed for growth and development and for seed production and composition.

Fig (1) Crop growth rate of faba bean based on plant height as affected by nitrogen levels

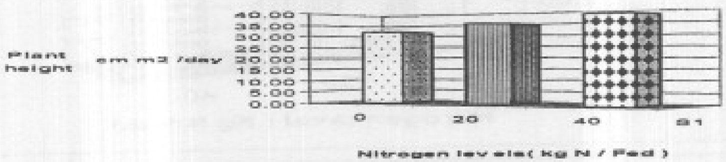


Fig (2) Crop growth rate of faba bean based on plant height

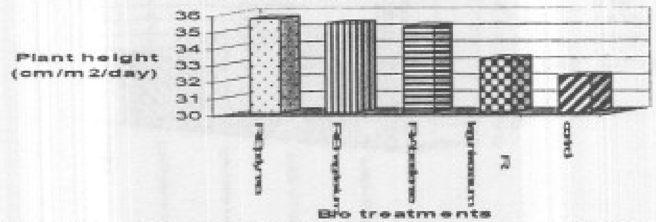


Fig (3) Crop growth rate of faba bean based on shoot dry weight as affected by nitrogen levels

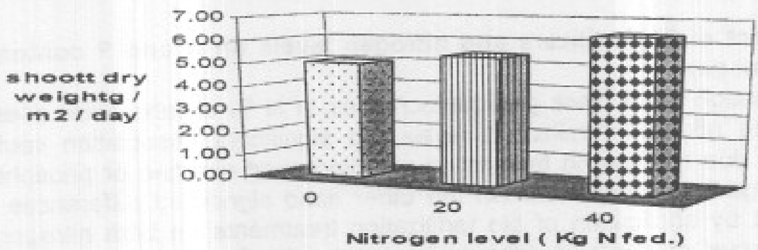


Fig (4) Crop growth rate of faba bean based on shoot dry weight .

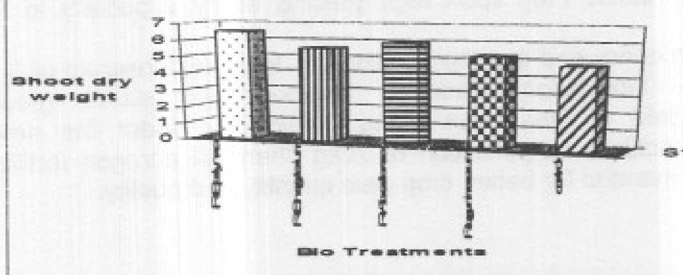


Fig (5) Crop growth rate of faba bean based on root dry weight as affected by nitrogen levels

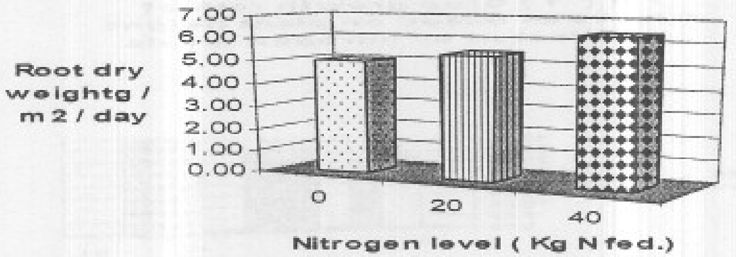
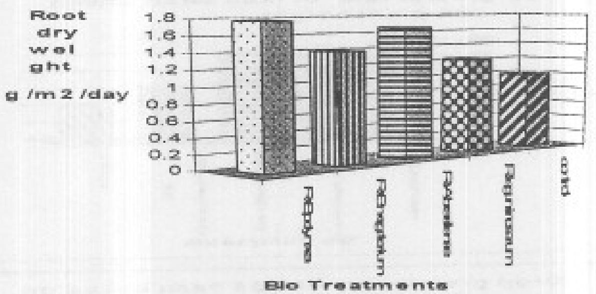


Fig (6) crop growth rateof faba bean based on root dry weight .



The Effect of biofertilizers and nitrogen levels on N and P contents of faba bean seeds:

Although there were gradual increases in N % of faba bean seeds by increasing nitrogen fertilization levels, no significant association could be obtained due to nitrogen fertilization affecting seed nitrogen or phosphorous percentage in both season. On the other hand significant differences were observed by application of bio fertilization treatments on both nitrogen and phosphorous contents in faba bean seeds. *R.+B.polymyxa* exhibited the maximum values of nitrogen percentage , while *R. + B megaterium* recorded the highest values of phosphorous percentage of faba bean seeds in both seasons . These results may sport high yielding ability especially in the second season.

It could be concluded that the addition of *R. leguminosarum* plus *B. polymyxa* as co- inoculation enhanced the nodulation status, growth characters and yield of faba bean plants particularly under the newly reclaimed land conditions. Application of 20kg chemical nitrogen fertilizer /fed. is also recommended for better crop yield quantity and quality.

Table (7): The effect of biofertilizers and nitrogen levels on NP contents of faba bean and seeds

		N %				P %			
N levels		0	20	40	Mean	0	20	40	Mean
Control		3.5	3.8	3.9	3.73	0.256	0.257	0.276	0.263
R.legminoserium		3.6	3.9	4	3.83	0.321	0.348	0.342	0.337
R.+Azosprillum		4.1	4.3	4.2	4.20	0.355	0.361	0.363	0.360
R.+B.megaterium		4.3	3.9	4.9	4.37	0.462	0.372	0.365	0.400
R.+B.polymyxa		4.4	4.6	4.6	4.53	0.395	0.397	0.373	0.388
mean		3.98	4.10	4.32	4.13	0.358	0.347	0.344	0.350
LSD at 0.05	N	NS				NS			
	Bio	0.42				0.094			
	NxBio	0.71				NS			
Second season									
Control		3.6	3.8	3.87	3.76	0.234	0.233	0.237	0.235
Rh.legminoserium		3.8	3.88	3.91	3.86	0.327	0.352	0.367	0.349
Rh.+Azosprillum		3.92	3.94	3.95	3.94	0.307	0.361	0.358	0.342
Rh.+B.megaterium		3.92	3.87	3.91	3.90	0.382	0.387	0.392	0.387
Rh.+B.polymyxa		3.97	4.01	4.11	4.03	0.38	0.385	0.374	0.380
mean		3.84	3.9	3.95	3.90	0.326	0.344	0.346	0.338
LSD at 0.05	N	NS				NS			
	Bio	0.08				0.122			
	NxBio	0.14				NS			

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استجابة الفول البلدي للتلقيح المشترك بالريزوبيا وبكتيريا الريزوسفير تحت ثلاث مستويات من التسميد الازوتى فى الاراضى حديثة الاستصلاح

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تم إجراء تجربة حقلية مرتين فى مزرعة معهد الدراسات والبحوث البيئية -جامعة المنوفية (فرع الـسادات) , خلال موسمين شتويين متتالين٢٠٠٦/٢٠٠٧ و٢٠٠٧/٢٠٠٨, وذلك لدراسة مدى استجابة الفول البلدي للتلقيح بالريزوبيا المتخصصة (ريزوبيوم ليجيومينوزيرم) منفردة أو مختلطه مع البكتيريا المنتجة لمنشطات النمو فى منطقـة الجـذور (الازوسبيريللم برازيلينس- باسلس ميجاتيريم - باسلس بوليمكسا) كتلقيح مشترك تحت ثلاث مـستويات مـن التـسميد النيتروجينى المعدنى (٠ و ٢٠ و ٤٠ كج نيتروجين للفدان).

وأظهرت النتائج أن كلا من التسميد البكتيري والمعدني المستخدم كان له تأثيرا معنويا على عملية تكوين العقد الجذرية وصفات نمو للنبات و النشاط البكتيرى وكذا الزيادة فى عدد ووزن العقد البكتيرية على جذور نباتـات الفـول البلدي و كان للتسميد المختلط تأثيرا معنوياعلى تنشيط إنزيم النيتروجينيز مقارنة بالتلقيح بالريزوبيا منفردة أو الكنترول.

وأدى التلقيح المختلط الى تحسين النشاط البكتيرى فى منطقة الريزوسـفير بـصفة عامـة وخاصـة إنزيمـات الديهيدروجينيز و الفوسفاتيز الحامضى والقلوى وكذلك زيادة أعداد البكتيريا مقارنـة بـالتلقيح بالريزوبيـا منفـردة والكنترول.

وأعطى التسميد المعدني بجرعته العليا (٤٠ كجم نيتروجين /فدان) أعلى القيم مع النشاط البكتيري ماعدا إنـزيم النيتروجينيز حيث أظهرت أفضل قيمة مع الجرعة الأقل (٢٠كجم نيتروجين /فدان) .

كما اظهرت النتائج زيادة المحصول ومكوناته معنويا باستخدام التلقيح المختلط وخاصة مع الباسلس بوليمكسا الذى أعطى أعلى القيم. ولم يظهر التسميد المعدني فروقا معنوية بين المستويين ٢٠ و ٤٠ كج فى المحصول ومكوناته.