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RESPONSE OF BROAD BEAN PLANTS TO BIO-FERTILIZERS FOR GROWTH, N_2- FIXATION AND PHYTOHOMONES PRODUTION UNDER CULTIVATION IN SANDY AND CLAY SOILS

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ABSTRACT: Three efficient indigenous isolates of *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azospirillum* sp. (ASH21) and *Azotobacter* sp. (ZH21) were used as bio-fertilizers either single or mixed under different levels of inorganic and organic N_2 –fertilizers for broad bean (*Vicia faba* L.) plants cultivated in two types of soil. The results concluded that inoculation with tri-mixture inoculants of RZ11, ZH21 and ASH21 isolates together with organic compost gave the best results with regard to nodulation parameters, nitrogenase activity, and healthy plant growth with high N, P and K contents of broad bean plants grown on both sandy and clay soils. Inoculation with tri-mixture inoculants of RZ11, ZH21 and ASH21 plus the application of 1/2 N dose of inorganic N-fertilizer or receiving recommended dose of compost gave the highest value in nodule formation, plant dry weight nitrogenase activity, gibberellins (GA_3) and indole acetic acid (IAA) production as well as the grain yield of plants grown in both sandy and clay soils. These results referred to the importance of inoculation of broad bean plants with bio-fertilizers to obtain the best performance in growth, nodulation and N_2-fixation.

Key words: Broad bean, bio-fertilizers, nitrogenase activity, gibberellins and indole acetic acid

INTRODUCTION

Nitrogen fixation by the legume-*Rhizobium* symbiotic partnership represents an inexpensive alternative to the use of chemical nitrogen fertilizers in the production of food protein and oil. The process requires that the host crop be adequately nodulated by *Rhizobium* bacteria effective in nitrogen fixation. Inoculation of legumes with suitable rhizobial strains is carrying out in many countries to ensure nodulation (Brock *et al.,* 2003).

A wide range of bacteria in rhizosphere can promote plant growth, orchestrated by rhizosphere bacteria that communicate with the plant using complex chemical signals. A critical process that occurs on the surface of the plant and particularly in the root zone is associative nitrogen fixation in which the nitrogen fixing microorganisms is on the surface of the plant root, the rhizoplane, as well as in the rhizosphere. This process is carried out by representatives of the genera *Azotobacter* and *Azospirillium.* Recent evidence suggests that their major contribution may not be in nitrogen fixation but in production of growth-promoting hormones that increase root hair development and thus greater ability of the plant to take up nutrients (Okon 1984, Prescott *et al.,* 2005).

Also, application of organic fertilizers was shown to enhance the incidence and activities of promoting plant rhizobacteria and stimulating plant growth (Khamis and Metwally, 1998 and Esitken *et al.,* 2003). Al-Kahal *et al.* (2001) found that application of olive oil waste water (OOWW) and olive cake (OC) in combination with *R. leguminosarum* inoculation significantly increased nodulation, shoot dry weight, N and P contents in *Vicia faba* plants. Also, Abdelhamid *et al.* (2004) found that application of composts at a rate of 20 g pot^{-1} significantly increased growth, yield components and total crude protein of broad bean plants.

On the other hand, application of *Rhizobium* inoculants and mineral nitrogen showed remarkable increase on plant growth and production of broad bean (Ayneabeba *et al.,* 2001 and El-Ghandour *et al.,* 2001).

So, there is a widespread interest in the use of combination of bio-fertilizers with organic or inorganic N-fertilizers as an healthy alternative and cheap source for chemical fertilizers (this is the aim of the present study).

MATERIALS AND METHODS

Three experiments were conducted in the Laboratory and Greenhouse of Agric. Microbiology Dept. at the Faculty of Agriculture, Zagazig University, Egypt, to examine the effect of indigenous selected biofertilizers under different levels of inorganic and organic N-fertilizers on the growth and phytohormones production of broad bean (*Vicia faba* L.) plants cultivated in clay and sandy soil of Sharkia Governorate.

Inocula Preparation

Among 64 isolates, three efficient indigenous isolates of *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azotobacter* sp. (ZH21) and *Azospirillum* sp. (ASH21) were chosen as the best indigenous isolates from Sharkia Governorate soils (Salem *et al.,* 2006) to be used in this investigation.

Rhizobium strain was inoculated in yeast extract mannitol (YEM) broth (Allen, 1959) and incubated in the dark at 28 ± 2°C in rotary shaker 120 rpm for 3-5 days. *Azotobacter* and *Azospirillum* isolates were inoculated in modified Ashby's medium (Abd El-Malek and Ishac, 1968) and NFM semisolid malate medium (Day and Döbereiner, 1976), respectively, and incubated in the dark at 28°C±2°C in rotary shaker 120 rpm for 7 days.

Soil Types and Analysis

Two different soil textures were used in these greenhouse experiments, namely clay and sandy soils obtained from Zagazig and Salhia, located in Sharkia Governorate, Egypt. The mechanical and physicochemical anaylsis of both soils were described according to Jackson (1970) and Harrigan and McCance (1966) and listed in Table 1. The soil was collected from top layer (0-30 Cm depth) then distributed in plastic pots (25 Cm diameter and 20 Cm depth) at the rate of 6 kg per pot.

Inorganic Fertilizers

The recommended doses of the inorganic fertilizers for broad bean are, 100 kg fed^{-1}, 75 kg fed^{-1} ammonium sulphate (20.5 % N), for sandy and clay soil, respectively, 200 kg fed^{-1}, 150 kg fed^{-1} calcium superphosphate (15.5 % P_2O_5) for sandy and clay soil, respectively. While potassium sulphate is 50 kg fed^{-1} for both soils. Calcium superphosphate and potassium sulphate were added just before sowing in both soils for all treatments, while ammonium sulphate was added in two equal doses, before cultivation and after 15 days from planting in sandy soil, while in clay soil, it was added after 10 days from planting.

Table 1. The locations of soil samples selected for isolation of *Rhizobium, Azotobacter* and *Azospirllum*

Type of soil analysis	Properties	Salhia soil	Zagazig soil
Physical analysis	pH	8.12	7.6
	EC mmhos.Cm^{-1}	3.10	2.67
Mechanical analysis	Fine sand %	24.45	27.1
	Coarse sand %	71.15	7.12
	Silt %	2.00	12.97
	Clay %	2.40	52.82
	Type of soil	Sandy	Clay
Chemical analysis	Cationes		
	Ca^{++}	0.19	0.54
	Mg^{++}	0.30	0.66
	Na^{+}	0.79	1.49
	K^{+}	0.06	0.07
	Aniones		
	HCo_3^{-}	0.31	0.48
	Cl^{-}	0.43	1.28
	So_4^{--}	0.61	1.52
	Co_3^{--}	Traces	Traces
Total nitrogen %		0.015	0.14

Organic Fertilizer

Garbage compost was provided from the organic fertilizers company, Cairo, Egypt. It has an organic carbon of 30 %, total nitrogen 1.0 %, moisture, 35 % and pH 7.5 ± 0.2. Garbage compost was thoroughly mixed with soil 20 days before cultivation in both sandy and clay soil [recommended dose is 5 ton fed^{-1} (0.5 %) in sandy soil and 2 ton fed^{-1} (0.2 %) in clay soil].

Experimental Design and Treatments Study on the Selected Biofertilizers on Growth of Broad Bean Plants under Different Levels of N-Application

Pots were filled with non-sterile sandy or clay soils as mentioned before. The sterilized seeds of broad bean (Giza 843, from Agronomy Dept. Agric. Res. Center. Giza, Egypt) were soaked in *Rhizobium*, suspension at the rate of 1×10^9 cfu/ml for two hours. In addition, control seeds were soaked in distilled water. Five seeds were sown and then 5 ml of cell suspension was applied over the seeds in each treated pot and covered with soil.

The first greenhouse experiment was carried out to examine the effect of selected bio-fertilizers on growth of broad bean plants. Pots were amended with the following treatments: control (uninoculated), inoculated with RZ11, inoculated with RZ11 + ZH21, inoculated with RZ11 + ASH21, and inoculated with RZ11 + ZH21 + ASH21. The experimental design was completely randomized with 5 treatments and 3 replicates.

The second greenhouse experiment was carried out to examine the effect of using bio-fertilizers with or without inorganic fertilizers on growth of broad bean plants. Pots were amended with the following treatments: control (uninoculated and without inorganic N-fertilizer), uninoculated + normal field dose of inorganic N-fertilizer, inoculated with RZ11, inoculated with RZ11 + normal field dose of inorganic N-fertilizer, inoculated with RZ11 + 1/4 the normal dose of inorganic N-fertilizer, inoculated with RZ11 +1/2 the normal dose of inorganic N-fertilizer, inoculated with RZ11 + 3/4 the normal dose of inorganic N-fertilizer, and inoculated with RZ11, ZH21 and ASH21 + 1/2 the normal dose of inorganic N-fertilizer. The experimental design was completely randomized with 8 treatments and 3 replicates.

The third greenhouse experiment was carried out to examine the effect of selected biofertilizers with or without organic fertilizers on growth of broad bean plants. Pots were amended with the following treatments: control (uninoculated and without organic N-fertilizer), uninoculated + normal field dose of organic N-fertilizer, inoculated with RZ11, inoculated with RZ11 + normal field dose of organic fertilizer, inoculated with RZ11 + 1/2 normal dose, inoculated with RZ11 + double normal field dose, and inoculated with RZ11, ZH21 and ASH21 + normal dose of organic fertilizer. The experimental design was completely randomized with 7 treatments and 3 replicates.

In all the three experiments, after ten days, plants were thinned to three plants/pot and watered daily with tap water (up to 60% whc). After 55 days from planting, plants were uprooted. Plant tops were dried at 70 °C, weighed and ground. Immediately after removal of the tops, response samples of the roots containing nodules from each plant of the respective treatment were taken, transferred to the reaction jars for determining the nitrogenase activity using the acetylene reduction assays method (Hardy *et al.,* 1973). Nodules were then counted and separated from root, dried and weighed. Nitrogenase activity was estimated on bases of per hours per gram weight of nodules. The nitrogen content in both shoots and roots was then determined colorimetrically using Nessler solution according the method described by Naguib (1969).

Total phosphorus content in root and shoots was determined colorimetrically by the hydroquinone method as described by Snell and Snell (1954). Total potassium content in roots and shoots using a Carlzeiss flame-photometer with acetylene burn described by Brown and Lilleband (1964) was also determined.

Extraction and Determination of Phytohormones (IAA, IBA and GA_3) from Root Plants

The extraction from fresh root sample was carried out according to method of Shindy and Smith (1975). Standard and extracts of hormonal compound were determined according to method described by Frankenberger and Brunner (1983) and Grolamys and Servando (1997).

At maturity the rest of all plants were harvested and seed index and total nitrogen were determined.

Finally, the data were subjected to statistical analysis as a complete randomized design according to **Snedecor and Cochran (1980)**.

RESULTS AND DISCUSSION

Response of Broad Bean Plants to Bio-fertilizers under Cultivation in Sandy and Clay Soils

A pot experiment was conducted to evaluate the response of broad bean plants (Giza 843) grown in sandy and clay soils to inoculation with either RZ11 or with ZH21 or with ASH21 or with tri-mixture inoculants of RZ11+ ZH21+ASH21. The response of the plants was evaluated by determining the nodule formation, plant dry weight, nitrogenase activity, nitrogen, phosphorus and potassium contents as well as the grain yield of broad bean plants.

Data in Table 2 showed that the number of nodules/plant significantly increased by inoculation of seeds with rhizobia alone or with either of nitrogen fixing isolates of bacteria ZH21 (*Azotobacter*) and ASH21 (*Azospirillum*) compared to the uninoculated broad bean seeds in both sandy and clay soils. Inoculated seeds with tri-mixture inoculants significantly giving the highest values of nodule number (average 20.33 nodules/ plant). While the highest value of nodule dry weight was obtained by the treatment inoculated with RZ11 alone in clay soil (42.33 mg/plant), followed by the treatment inoculated with RZ11 + ZH21 (38.33 mg/plant) in sandy soil.

Concerning nitrogenase activity ,the highest value was obtained by treatment inoculated with RZ11 + ZH21 giving 5.342 μ mole C_2H_4 $h^{-1}g^{-1}$ dry nodules followed by the treatment inoculated with tri-mixture inoculants showing 4.635 μ mole C_2H_4 $h^{-1}g^{-1}$ dry nodules in sandy soil. The nitrogenase activities of the different treatments were in parallel with those obtained by nodule numbers and dry weight of nodules. These results confirmed those reported by Salem *et al.* (1983) and Abdelhamid *et al.* (2004). They demonstrated that *Rhizobium*-inoculation significantly increased the number and dry weight of nodules of broad bean cultivated in newly reclaimed soil of Egypt. In addition, there was a beneficial effect due to application of co-inoculants containing other micro-organisms, which are able to improve

Table 2. Effect of bio-fertilizers on the growth and N_2-fixation of broad bean plants cultivated in sandy and clay soil

Soil textures	Number of nodules/ plant		Nodules dry weight (mg/plant)		Nitrogenase activity µ mole C_2H_4/h/g dry nodule		Shoot dry weight g/plant		Root dry weight g/plant		Total N/shoot (mg/plant)		Total N/ root (mg/plant)		Total P/plant (mg/plant)		Total K/plant (mg/plant)		Weight of 100 seeds (g)		N% in seeds	
Fertilization treatments	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay
Control	4.67	0.00	6.33	0.00	0.128	0.00	1.30	1.13	0.24	0.22	37.71	33.73	3.63	3.47	4.82	3.69	44.70	41.99	37.12	49.15	3.18	3.20
Inoculation by RZ11 *	17.33	16.67	16.00	42.33	4.040	3.905	2.07	1.99	0.55	0.45	83.45	78.70	11.96	10.81	12.53	9.35	110.87	99.60	57.20	74.10	3.57	3.82
RZ11 * + ZH21	23.33	14.67	38.33	16.00	5.342	0.969	2.52	1.91	0.46	0.42	88.24	66.87	9.10	9.62	14.52	8.64	109.58	83.43	61.00	79.00	3.67	3.81
RZ11 * + ASH21	12.67	16.67	16.33	15.67	4.362	1.137	1.98	2.71	0.56	0.63	73.70	98.62	11.10	13.40	14.67	14.79	72.04	137.39	54.60	80.80	3.53	3.87
RZ11+ZH21+ ASH21 **	20.33	20.33	31.33	21.00	4.635	1.850	2.26	2.68	0.42	0.58	88.77	109.61	8.71	12.15	13.67	16.18	102.72	150.14	58.55	79.05	3.75	3.86
Soil texture av.	15.67	13.67	26.40	19.00	-	-	2.02	2.08	0.45	0.46	74.37	77.51	8.90	9.89	12.04	10.53	87.98	102.51	53.69	72.42	3.54	3.71
LSD for	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.
0.05	NS	3.70	NS	NS	-	-	NS	0.38	NS	0.10	NS	15.88	NS	2.07	NS	2.19	9.87	15.60	2.08	3.29	0.05	0.08
0.01	NS	5.45	NS	NS	-	-	NS	0.56	NS	0.15	NS	23.38	NS	3.05	NS	3.22	NS	22.96	3.06	4.83	0.07	0.11

* Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11).

** Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azotobacter* spp. (ZH21) and *Azospirillum* spp. (ASH21).

legume growth and nodulation (Salem *et al.*, 1984, Mishra *et al.*, 1999 and Rodelas *et al.*, 1999).

Data showed also that application of bio-fertilization increased the shoots and roots dry weight of broad bean plants over the control treatment grown in both sandy and clay soils. The treatment inoculated with tri-mixture inoculants gave average of 2.47 g/plant for shoots dry weight. While the highest values of root dry weights were obtained also by plants inoculated with RZ11 mixed with ASH21 in both sandy and clay soils, being 0.56 and 0.63 g/plant, respectively. The statistical differences among bio-fertilization treatments were highly significant ($p \leq 0.01$). The obtained results agreed with those reported by Salem *et al.* (1981); Hassan *et al.* (1990) and Hassan (1997), who found that, the average values of plant fresh and dry weights of snap bean significantly increased by seed inoculation with *Rhizobium* as compared with the control. In this respect also Shumin *et al.* (2004) found that the dual inoculation of broad bean with *R. leguminosarum* and *G. mosseae* increased the plant height, chlorophyll content and number and weight of nodules of broad bean. The biomass of broad bean increased by 21.5 and 20.7 % when inoculated with *G. mosseae* alone and/or combined with *R. leguminosarum*, respectively.

Data in Table 2 also show that application of bio-fertilizers significantly increased the total nitrogen content in shoots and roots comparing with the control treatment. The highest values in shoots were also obtained by the treatment inoculated with tri-mixture inoculants, showing 88.77 and 109.61 mg N/plant in both sandy and clay soils, respectively. While, in roots, the treatment inoculated with RZ11 and ASH21 gave the highest value (13.40 mg N/plant) in clay soil, followed by the treatment inoculated with RZ11(11.96 mg N/plant) in sandy soil. The differences among treatments were highly significant ($p \leq 0.01$). Similar results were also obtained by Salem *et al.* (1981); Hassan *et al.* (1990); Hamaoui *et al.* (2001); Brock *et al.* (2003) and Abdelhamid *et al.* (2004).

Regarding the total phosphorus content in whole plant, the highest value (16.18 mg P/plant) was obtained by the treatment inoculated with tri-mixture inoculants in clay soil. The control treatment had the lowest values (4.82 and 3.69 P/plant) in both sandy and clay soils, respectively. These results confirmed the findings reported by

Rodelas *et al.* (1999) and Abdelhamid *et al.* (2004).

The total potassium content in whole plant shows a great variation in both soils. Inoculation with RZ11 only gave the highest value (110.87 mg K^{-1}) in sandy soil, while inoculation with both RZ11 plus ASH21 gave the highest value (150.14 mg K^{-1}) in clay soil. The statistical differences among fertilization treatments were highly significant ($p \leq 0.01$). These results verify the findings reported by Patel *et al.* (1985); Rodelas *et al.* (1999) and El-Sawah (2000).

The clay soil produced significantly higher seed index, being 72.42 g/100 seeds than sandy soil which was 53.69 g/100 seeds. Application of bio-fertilizers (RZ11, ZH21 and/ or ASH21) increased the nitrogen percent in seeds. The highest values were 3.75 and 3.87 % N % in seeds of broad bean plants grown in both sandy and clay soils, respectively. In this respect, Pathak *et al.* (1997); Dadarwal *et al.* (1997) and Hedge *et al.* (1999) explained the role of bio-fertilizers in leguminous crops. They reported that these bio-fertilizers play an important role in enhancing crop productivity through nitrogen fixation, phosphate solubilization, plant hormone productivity, ammonia excretion and to control various plant diseases.

García *et al.* (2004) reported that the inoculation modes of plant growth promoting rhizobacteria (PGPRs) plus rhizobia play a very important role in the produced effects since the PGPRs interact synergistically with the root nodule bacteria.

Response of Broad Bean Plants to Bio-Fertilizers and Inorganic N-Fertilizers under Cultivation in Sandy and Clay Soils

Data in Table 3 show the effect of bio-fertilization and inorganic N-fertilizers application on nodulation, nitrogenase activity, plant dry weight and total nitrogen, phosphorus, and potassium contents, as well as grain yield of broad bean plants. The results showed that the highest number and dry weight of nodules were obtained by treatment the plants with 1/2 N (10 kg nitrogen / fed) + inoculation with isolate RZ11 in sandy soil giving 24.33 nodules/plant and 53.33 mg/plant, respectively and treatment receiving only 1/4 N (5 kg nitrogen /fed) + inoculation with isolate RZ11

Table 3. Effect of bio-fertilizers and inorganic N-fertilizers on the growth and N_2 - fixation of broad bean plants cultivated in sandy and clay soil

Soil textures	Number of nodules/ plant		Nodules dry weight (mg/plant)		Nitrogenase activity μ mole C_2H_4/h/g dry nodule		Shoot dry weight g/plant		Root dry weight g/plant		Total N/shoot (mg/plant)		Total N/ root (mg/plant)		Total P/plant (mg/plant)		Total K/plant (mg/plant)		Weight of 100 seeds (g)		N% in seeds	
Fertilization treatments	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay
Control	4.67	0.00	6.33	0.00	0.128	0.000	1.30	1.13	0.24	0.22	37.71	33.73	3.63	3.47	4.82	3.69	44.70	41.99	37.25	49.15	3.18	3.20
Inoculation by RZ11 *	17.33	16.67	16.00	42.33	4.040	3.905	2.07	1.99	0.55	0.42	83.48	78.70	11.96	9.75	12.53	6.61	110.87	100.75	57.20	74.10	3.57	3.82
Nitrogen dose (N)	5.00	0.00	6.67	0.00	2.137	0.000	2.23	1.38	0.43	0.35	80.08	52.35	7.69	7.07	12.71	6.53	93.21	75.61	55.25	58.65	3.59	3.47
N + RZ11*	16.67	0.00	23.33	0.00	4.817	0.000	2.16	1.52	0.48	0.33	79.79	66.03	9.41	7.19	13.48	8.09	105.14	72.44	59.20	65.45	3.65	3.65
1/4 N + RZ11*	16.33	14.67	18.00	70.67	3.489	2.377	1.67	1.64	0.31	0.32	63.96	56.98	5.83	6.82	11.45	7.22	71.45	70.88	49.10	66.40	3.55	3.57
1/2 N + RZ11*	24.33	8.33	53.33	36.33	5.446	1.264	2.25	1.74	0.43	0.47	88.85	67.53	12.63	10.62	12.73	7.67	97.20	94.98	58.00	75.70	3.80	3.73
3/4 N + RZ11*	16.67	14.33	15.33	20.33	3.362	0.082	1.80	1.91	0.45	0.45	68.47	70.20	9.01	9.86	10.33	9.26	81.16	95.53	54.00	73.15	3.71	3.76
1/2 N+ RZ11+ZH21+ASH 21**	19.67	8.00	35.00	16.67	7.423	1.427	2.15	2.00	0.48	0.50	95.64	76.25	10.19	9.21	15.52	9.77	99.91	94.78	59.20	74.05	3.81	3.81
Soil texture av.	15.08	7.75	21.75	23.29	-	-	1.95	1.66	0.42	0.38	74.75	62.72	8.79	8.00	11.70	7.35	87.95	80.87	53.65	67.08	3.61	3.63
LSD for	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertiliz ation	Soil texture	Fertili zation
0.05	2.14	4.27	NS	23.07	-	-	0.23	NS	NS	0.09	9.60	19.19	NS	2.15	1.19	2.38	NS	20.48	0.93	1.85	NS	0.08
0.01	3.09	6.18	NS	NS	-	-	NS	NS	NS	0.13	NS	27.79	NS	3.11	1.72	3.44	NS	29.65	1.34	2.68	NS	0.11

* Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11).

** Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azotobacter* spp. (ZH21) and *Azospirillum* spp. (ASH21).

N: Recommended dose of nitrogen (20 kg N in sandy soil and 15 kg N in clay soil).

in clay soil showing 14.67 nodules/ plant and 70.67 mg/plant, respectively. The differences between soil textures were not significant.

The highest value of nitrogenase activity was obtained by treatment of the plants with 1/2 N dose + inoculation with tri-mixture inoculants (RZ11, ZH21 and ASH21) in sandy soil, showing 7.423 μ mole C_2H_4/h/g nodules dry weight. In clay soil, however the plants did not form nodules and hence no nitrogenase activity was detected. This could be attributed to the high amount of nitrogen in clay soil as seen in chemical analysis (Table, 1) which could be deleteriously affected the nodule formation in such soil.

These results are in agreement with those obtained by Hassan *et al.* (1990). Who found that the increase of dose of nitrogen fertilizer led to marked decrease in nodulation of broad bean and amount of N_2-fixed. Also clay soil showed relatively lower percentage of nitrogen derived from atmosphere as compared with both calcareous and sandy soils. In this respect, Narendra *et al.* (1996) and Hussein *et al.* (1997). Recently, Hamaoui *et al.* (2001) demonstrated that inoculation with *Azospirillum brasilense* significantly enhanced nodulation by native *Rhizobium* and improved root and shoot development of chickpea and broad bean plants when compared to the uninoculated control.

Our results concluded that inoculation with tri-mixture inoculants of RZ11, ZH21 and ASH21 isolates plus the application of 1/2 dose inorganic N gave the best results with regard to number of nodules, nodules dry weight and nitrogenase activity in broad bean plant cultivated in sandy and clay soils.

The results showed that the highest value of shoot dry weight was obtained by treatment receiving 1/2 N dose + RZ11 in sandy soil and treatment receiving 1/2 N dose + inoculated with tri-mixture inoculants of RZ11, ZH21 and ASH21 in clay soil, being 2.25 and 2.00 g/plant, respectively. The differences between soil textures were significant ($P \leq 0.05$).

In addition, the highest average root dry weight (0.49 g/plant) was recorded for plants treated with 1/2 N dose + inoculated with tri-mixture inoculants of RZ11 + RZ21 and ASH21 as compared with the averages of all other treatments of both sandy and clay soils.

The obtained data are in agreement with those reported by Hassan *et al.* (1990), El-Ghandour and Galal (1997), Namdeo and Gupta (1999) and Ayneabeba *et al.* (2001). The latter revealed that the inoculation of broad bean plants with bio-fertilizers and applied inorganic N-fertilizer gave the best results with regard to shoots, roots and whole plant dry weights.

Dong *et al.* (2005) reported that, while N application can supply sufficient nutrients to improve plant production, it also leads to a worldwide concern about environmental contamination resulting from excessive nitrate leaching. N_2-fixing bacteria may be important for plant nutrition by increasing N uptake by the plants, and playing a significant role as plant growth promoting rhizobacteria (PGPR) in the biofertilization of crops.

Concerning the total nitrogen content, the highest value in shoots was recorded by the treatment received 1/2 N dose + inoculation with tri-mixture inoculants of RZ11 + ZH21 + ASH21 under growing in sandy soil, giving 95.64 mgN/plant as compared with the control which showed 37.71 mgN/plant. While the highest value in clay soil was recorded by the treatment inoculated with RZ11 only being 78.70 mgN/plant as compared with the control which showed 33.73 mg N/plant.

The total nitrogen content in roots of broad bean plants showed nearly the general trend like shoots. The statistical differences among fertilization treatments were highly significant ($P \leq 0.01$).

These results are in harmony with those obtained by Hussein *et. al.* (1997); El-Ghandour *et al.* (2001) and Brock *et al.* (2003). In addation, Rodelas *et al.* (1999), pointed out that co-inoculants of broad bean with *Rhizobium leguminosarum bv. vicieae* + *Azotobacter* and *Azospirillum* led to changes in total N content concentration and/or distribution of macro and micronutrients, K, P, Ca, Fe, Mg, Mn, B, Zn, and Cu when compared with plants inoculated with *Rhizobium* only.

Data presented in Table 3 exhibit the total phosphorus content in whole broad bean plant as affected by biofertilizers application. Application of 1/2N dose and inoculation with tri-mixture inoculants of RZ11, ZH21 and ASH21 gave the highest values in both sandy and clay soil, being 15.52 and 9.77 mg P/plant, respectively. The lowest values were obtained by the uninoculated treatment (control), being 4.82 and 3.69 mg P/plant in both soils, respectively. The statistical differences among fertilization treatments and between soil textures were highly significant ($p \leq 0.01$). These results confirmed those obtained by Rodelas *et al.* (1999).

Regarding the total potassium content in whole plant, the results showed that the treatment inoculated with RZ11 gave the highest value as compared to the other treatments. The statistical differences among treatments were highly significant ($P \leq 0.01$), but the differences between soil textures were not significant.

In general, the results referred to the importance of inoculation of broad bean plants grown either in sandy or clay soils with *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azotobacter* spp. (ZH21) and *Azospirillum* spp. (ASH21) to realize the highest plant growth containing high values of N,P and K in the whole plant.

In biomass yield, results indicated that there were highly significant ($P \leq 0.01$) differences among fertilization treatments, and between soil textures. The highest value was obtained by the treatment receiving 1/2 N dose + inoculation with isolate RZ11 (75.70 g/100 seeds) in clay soil, as compared with the control.

In addition, data also show that inoculation of broad bean with tri-mixture inoculants of RZ11, ZH21 and ASH21 in both sandy and clay soils resulted in the highest nitrogen (%) in seeds (3.81N%).

Dual inoculation of legumes with *Azospirillum* and *Rhizobium* has been found to increase several plant-growth variable when compared with single inoculation. *Azospirillum* is considered a *Rhizobium* helper by stimulating nodulation, nodule function and possibly plant metabolism. phytohormones produced by *Azospirillum* promoted epidermal-cell differentiation in root hairs that icreased the number of potential sites for rhizobial-infectoion (Andreeva *et al.*, 1993).

These results also emphasize the role of bio-fertilizers application as inoculants for increasing the seeds index of broad bean plants grown in both sandy and clay soils (Hassan *et al.*, 1990; Hussien *et al.*, 1997 and EL-Ghandour *et al.*, 2001).

Response of Broad Bean Plants to Bio and Organic Fertilizers under Cultivation in Sandy and Clay Soil

The response of the plants was evaluated by determining the nodule formation on the root system, the plant dry weight, nitrogenase activity, total nitrogen, phosphorus and potassium contents as well as the grain yield of broad bean plants (Table, 4).

Data presented in Table 4 show that the highest number of nodules (20 nodules^{-1}) was obtained by the treatment receiving recommended dose of compost and inoculated with RZ11 in sandy soil. While the control treatment gave the lowest numbers of nodules in both soils. The differences among fertilization treatments were highly significant ($P \leq 0.01$).

Regarding the dry weight of nodules, the results showed that inoculation of broad bean plants grown in both sandy and clay soils with isolate RZ11 or with tri-mixture inoculants of RZ11, ZH21 and ASH21 and combined with organic fertilizers increased the nodules dry weight in both soils with relatively high values for the last as compared to control treatment. The last treatment gave the highest values of nodules dry weight in clay soil, being 36.67 mg^{-1}. The differences among fertilization treatments were highly significant ($P \leq 0.01$). Concerning the nitrogenase activity, the highest value was recorded by the same treatment receiving compost (5 ton/fed.) and inoculated with tri-mixture inoculants in sandy soil 8.096 μ mole C_2H_4 $h^{-1}g^{-1}$ dry nodules.

The results showed also that the highest shoot dry weight values was obtained by treatment receiving compost and inoculated with tri-mixture inoculants in sandy soil, giving 2.54 g/plant, compared to control treatment (1.30 g/plant). Only the statistical differences among fertilization treatments were significant ($P \leq 0.05$). The root dry weights, showed the same trend. The statistical differences among fertilization treatments were highly significant ($P \leq 0.01$).

Data show that application of bio and organic fertilizers increased the toal N content in shoots and roots of broad bean plants comparing with the control treatment. The highest value in shoots was obtained by the treatment inoculated with tri-mixture inoculants combined with

Table 4. Effect of bio and organic fertilizers on the growth and N_2 -fixation of broad bean plants cultivated in sandy and clay soil

Soil textures / Fertilization treatments	Number of nodules/plant		Nodules dry weight (mg/plant)		Nitrogenase activity µ mole C_2H_4/h/g dry nodule		Shoot dry weight g/plant		Root dry weight g/plant		Total N/shoot (mg/plant)		Total N/ root (mg/plant)		Total P/plant (mg/plant)		Total K/plant (mg/plant)		Weight of 100 seeds (g)		N% in seeds	
	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay	Sandy	Clay
Control	4.67	0.00	6.33	0.00	0.128	0.000	1.30	1.13	0.24	0.22	37.71	33.73	3.63	3.47	4.82	3.69	44.70	41.99	37.25	49.15	3.18	3.20
Inoculation by RZ11*	17.33	16.67	16.00	42.33	4.040	3.905	2.07	1.99	0.55	0.42	83.45	78.70	11.96	9.75	12.53	9.31	110.87	100.75	57.20	74.10	3.57	3.82
Compost dose	13.33	0.00	18.67	0.00	2.990	0.000	1.57	1.68	0.32	0.34	52.05	53.07	5.65	6.84	6.71	6.74	64.71	72.73	47.85	64.40	3.37	3.45
Comp. + RZ11*	20.00	19.67	28.33	36.00	6.692	4.475	1.89	2.26	0.51	0.43	68.42	85.67	10.65	8.78	10.10	7.89	100.00	81.65	56.90	78.70	3.84	3.91
2 Comp. + RZ11*	18.67	8.33	17.67	24.33	5.969	0.153	1.69	1.65	0.43	0.35	65.56	65.77	8.01	7.61	9.33	13.01	87.14	112.26	55.85	68.60	3.76	3.81
1/2 Comp.+RZ11*	16.33	14.33	28.67	17.00	3.903	0.240	1.34	1.78	0.31	0.44	46.56	60.97	5.53	9.72	5.31	7.26	59.69	71.10	47.30	69.40	3.57	3.61
Comp.+RZ11+ZH21+ASH21**	15.00	15.33	25.33	36.67	8.096	2.131	2.54	2.03	0.53	0.42	97.88	79.76	12.40	10.91	14.51	14.54	126.09	112.07	64.10	72.00	3.83	3.83
Soil texture av.	15.05	10.62	20.14	22.33	-	-	1.77	1.79	0.41	0.37	64.52	65.38	8.26	8.15	9.04	8.92	84.74	84.65	52.35	68.05	3.59	3.66
LSD for	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertili.	Soil texture	Fertilization	Soil texture	Fertilization
0.05	1.81	3.38	NS	13.72	-	-	NS	0.49	NS	0.11	NS	19.58	NS	0.25	NS	2.50	NS	21.17	1.61	3.02	0.04	0.07
0.01	2.62	4.91	NS	19.94	-	-	NS	NS	NS	0.16	NS	28.46	NS	0.37	NS	3.63	NS	30.76	2.34	4.39	0.05	0.10

* Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11).

** Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azotobacter* spp. (ZH21) and *Azospirillum* spp. (ASH21).

Comp.: Recommended dose of compost (5 ton/fed. in sandy soil and 2 ton/fed. in clay soil).

recommended dose of compost in sandy soil, being 97.88 mg N/plant. Concerning the total N content in roots, data showed the same general trend like shoot parts. The significant differences among treatments were highly significant ($P \leq 0.01$). This confirmed the previous results of nodulation dry weights and N_2ase activity of broad bean plants indicating the clear impact of bio-fertilizers and organic manure on the productivity of the plants especially in sandy soil. Moherram *et al.* (1998), Subba Roa (1999) and Abdelhamid *et al.* (2004) came also to the same general conclusion.

Concerning the total phosphorus content (Table, 4) in whole plant, the treatment received compost and inoculated with tri-mixture inoculants of RZ11, ZH21 and ASH21 gave the highest values in both sandy and clay soils showing14.51 and 14.54 mg P/plant, respectively. Also the total potassium content, gave the same trend as found in total phosphorus content, the values in both sandy and clay soils were 126.09 and 112.07 mg K/plant, respectively. The differences among fertilization treatments were highly significant ($P \leq 0.01$), but the differences between soil textures were not significant.

The effect of bio and organic fertilizers on biomass yield (g/100 seeds), and nitrogen % of plants grown in both sandy and clay soils are given in table, 4. Results showed that the highest value was obtained by the treatment inoculated with RZ11 combined with compost in clay soil (78.70 g/100 seeds). Result of plants grown in clay soil had higher values than sandy soil, being 68.05 and 52.35 g/100 seeds for the first and second soil, respectively. The differences between soil textures were highly significant ($p \leq 0.01$).

In addition, the inoculation with RZ11 combined with compost gave the highest nitrogen (%) in seeds of broad bean plants growing in both soils, the values were 3.84 and 3.91 N % in sandy and clay soils, respectively. The statistical differences in nitrogen (%) among fertilization treatments and between soil textures were highly significant ($p \leq 0.01$). These results are in agreement with those obtained by Abd EL-Ghany *et al.* (1996) and Sarwar *et al.* (1998).

Also, Badawi (2003) and Desoki, (2004) demonestrated that compost addition could supply growing plant with essential nutrients as well as improved the physical, chemical and biological properties of soil that consequently reflected on rhizospheric microbial activity and subsequently root colonization.

Effect of Bio-Fertilizers Only or Combined with Inorganic or Organic N-Fertilizers on Ga_3, Iaa and Iba Content in Roots of Broad Bean Plants

The three isolates, RZ11, ZH21 and ASH21, used in this study were able to produce GA_3, IAA and IBA in pure cultures. Data in Table 5 show that the highest values of gibberellins (GA3) and Indole acetic acid (IAA) were obtained by the treatment inoculated with tri-mixture inoculants combined with the recommended compost dose in sandy soil, being (101.05 µg /plant and 1.27µg/plant fresh roots, respectively) followed by the treatment inoculated with RZ11 combined with ½ dose of inorganic N-fertilizers in clay soil (74.88 µg/plant fresh roots) concerning GA_3 and the treatment inoculated with RZ11 only in clay soil (1.35 µg/plant fresh roots) concerning IAA.

Regarding the IBA, the highest values were obtained by the treatment inoculated with RZ11 rely in both sandy and clay soils, being 344.15 and 513.01 µg/plant fresh roots, respectively. Our results demonstrated that RZ11, ZH21 and ASH21, produced the highest amounts of GA_3, IAA and IBA and these isolates significantly increased the different growth parameters of broad bean plants. The obtained results are in agreement with those reported by Mishra *et al.* (1999) and Rodelas *et al.* (1999). They found that the interest in developed Co-inoculants contains other micro-organisms, which are able to improve legume growth, included rhizobacteria which promote nodulation, nitrogen fixation, plant vigor and yield via such mechanisms as phyto hormones, antibiotic or metal binding compound production.

In the same subject Dakora (2003) found that new roles for plant and rhizobial molecules had been detected in soil and mixed plant cultures involving symbiotic legumes *Rhizobium* (species *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, *Allorhizobium*, *Sinorhizobium* and *Mesorhizobium*) produce chemical molecules that can influence plant development.

Our results indicated that selected PGPR are able to promote broad bean plants growth and yield

Table 5. Effect of bio-fertilizers only or combined with inorganic or organic N-fertilizers on GA_3, IAA and IBA prodution in root of broad bean plants

Treatment	GA_3 (µ g/g fresh roots)		IAA (µ g/g fresh roots)		IBA (µ g/g fresh roots)	
	Sandy	Clay	Sandy	Clay	Sandy	Clay
Control	5.71	13.11	0.40	0.62	17.58	4.61
Inoculation by RZ11*	22.05	26.42	1.01	1.35	344.15	513.01
Comp. + RZ11+ZH21+ASH21 **	101.05	52.24	1.27	0.77	72.11	183.78
1/2 N+ RZ11*	16.20	74.88	0.73	0.69	166.68	232.61
1/2 N+ RZ11+ZH21+ASH21 **	73.47	66.96	1.01	0.81	296.80	45.79
RZ11+ZH21+ASH21 **	49.14	23.61	0.71	1.00	222.64	131.89

* Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11).
** Inoculation with *Rhizobium leguminosarum bv. vicieae* (RZ11), *Azotobacter* spp. (ZH21) and *Azospirillum* spp. (ASH21).
N: Recommended dose of nitrogen.
Comp: Recommended compost dose

and reduce the need for chemical fertilizers. In view of environmental pollution due to excessive use of fertilizers and high costs of production of fertilizers, PGPT strains have potential to be used for the sustainable and environmentally benign crop production.

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استجابة نباتات الفول البلدي للقاحات الحيوية و تأثيرها علي النمو وتثبيت النيتروجين وإنتاج الهرمونات النباتية عند زراعتها في أراضي رملية وطينية

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قسم الميكروبيولوجيا الزراعية – كلية الزراعة – جامعة الزقازيق

تم استخدام ثلاث عزلات محلية من الرايزوبيم (RZ11) و الأزوسبيرلم (ASH21) والأزوتوبكتر(ZH21) علي درجة عالية من الكفاءة كسماد حيوي مفردة او خليط منها مع مستويات مختلفة من السماد العضوي والمعدني لنبات الفول البلدي المزروع في نوعين من الأراضي.

وقد اوضحت النتائج ان التلقيح بخليط من الثلاث عزلات معا و هي RZ11,ASH21,ZH21 في وجود الكومبوست (كسماد عضوي) اعطي احسن النتائج بالنسبة لتكوين العقد و نشاط انزيم النيتروجينيز و كذلك زيادة نسبة النيتروجين و الفوسفور والبوتاسيوم في النباتات النامية في كلا من التربة الرملية و الطينية. كذلك التلقيح بخليط العزلات RZ11,ASH21,ZH21 بالإضافة الي نصف الجرعة الموصي بها من السماد النيتروجيني المعدني او الجرعة الموصي بها من السماد العضوي اعطي اعلي القيم بالنسبة لتكوين العقد ووزن النبات الجاف ونشاط انزيم النيتروجينيز و انتاج الهرمونات النباتية مثل الجبريلينات (GA3) و الاندول حمض الخليك (IAA) بالاضافة الي انتاج الحبوب في النباتات النامية في كلا من الاراضي الرملية و الطينية. هذه النتائج تشير الي اهمية تلقيح نباتات الفول البلدي باللقاحات الحيوية للحصول علي افضل النتائج في النمو وتكوين العقد و تثبيت النيتروجين.