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Influence of rhizobium inoculation combined with azotobacter chrococcum and bacillus megaterium var phosphaticum on growth, nodulation, yield and quality of two snap been (phasealus vulgaris l.) cultivars.

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Abstract

The present investigation was carried out during the two successive summer seasons of 2007 and 2008 at the Experimental Station, Faculty of Agriculture, Cairo University, Giza, to study the effect of inoculation with *Rhizobium leguminoarum bv. phaseoli* (ARC 301) (Rh), *Azotobacter chroococcum* (AZ1) and *Bacillus megaterium var phosphaticium* (BM3) on nodulation , N_2-fixation, population of rhizosphere microorganism (RMO), NPK-Content, yield and pod quality of two snap been (*Phaseolus vulgaris L.*) cultivars, namely Bronco and Paulista under 25% of the recommended dose of NPK chemical fertilizers. Results indicated that inoculation with biofertilizers mixture had a significant effect on snap been growth parameters , nodulation and N_2-fixation. The highest values were recorded with Rh + AZ1 + BM3 in presence of 25% the recommended dose of NPK fertilizers. *Paulista cv.* surpassed cv. Bronco in plant height, plant fresh and dry weights, both number of branches and pods/plant as well as leaf chlorophyll content, whearas the reverse was true concerning the plant yield, early and total green pod yield and dry seed yield per feddan, pod weight and diameter as well as pods dry matter, carbohydrates and fibres. *Rhizobium* (Rh) + *Bacillus megaterium* (BM3) with 25% the recommended dose of NPK significantly increased all traits of vegetative growth, yield and its components and pod characteristics in comparison with the control treatment (uninoculated + 100% NPK). The best interaction treatment regarding plant growth and chlorophyll leaf content was cv. Paulista with Rh + BM3 + 25% NPK. Meanwhile, cv. Bronco with the same treatment was the best regarding yield and its components as well as pod characteristics.

Key words: bacterial inoculation, Snapbean, biofertilizer

Introduction

Snap bean *(Phaseolus vulgaris* L.*)* is one of the most important vegetable crops grown in Egypt for local consumption and exportation. Also, it is widely used as a source of protein and for its high nutritive value for human nutrition.

It is well known that common beans are environmently sensitive and had low levels of nodulation and N_2-fixation because these processes are very sensitive to many factors related to environmental conditions and cultural practices (Semu *et al.,* 1982, Moawad *et al.,* 1998; Ravindar and Chandra, 2008). Thus, the literature describing the effectiveness of *Rhizobium* inoculation in increasing vegetative growth, yield and nitrogen content of common bean plants are contradictory, partially because different bean cultivars (Daba and Haile, 2002) environmental and soil conditions (Hernandez – Armenta *et al.*, 1989; Carvalho *et al.,* 1998) and *Rhizobium* strains (Lalande *et al.,* 1990; Sanoria and Yadav, 1993). For example, inoculation of common bean plants with *Rhizobium* was not effective, when it was conducted in irrigated soil (Carvalho *et al.,* 1998) or when the soil temperature was 38^{o}C, or higher, immediately after inoculation (Hernandez – Armenta *et al.,* 1989) or when the application of nitrogen was increased (Datt, *et al.,* 2006). On the other hand, inoculation of *Phaseolus* vulgaris with *Rhizobium* increased plant hight, pods per plant, fresh weight per plant, seed yield and NPK uptake (Rana *et al.*, 2006).

The major aspect which may increase the yield of legumes is inoculation with free or associated nitrogen fixing bacteria, which can fix nitrogen by themselves (Chripeels and Sadava, 1994), improve plant growth, through producing fungistatic substance (Gupta *et al.,* 1995) or through improving symbiotic parameter of legume *Rhizobium* association (Singh and Subba Rao, 1979).

Moreover, it well be useful to use microelements (such as boron and molybdenum) or vitamin B_{12} as well as arbuscular mycorrhizal fungi (AMF) along with *Rhizobium phaseoli* inoculation to improve growth, yield and nutrient uptake of bean plants (Ismail, 2002; Aryal *et al.,* 2003).

The aim of this investigation was to study the effect of rhizobial inoculation plus (*Azotobacter chroococcum* and *Bacillus megaterium)* on nodulation, plant growth, yield and its components as well as pods quality of shop bean (cvs. Bronco and Paulista) grown in clay-loam soil.

Materials and Methods

This study was carried out during the two successive summer seasons of 2007 and 2008 at the Experimental Station, Faculty of Agriculture, Cairo University, Giza, to study the response of two snap bean (*Phasceolus*

vulgaris, L.) cultivars, namely Bronco and paulista, to inoculation with *Rhizobium leguminosarum* biovar *phaseoli* "ARC301", *Azotobacter chroococum* "AZ1" and *Bacillus megaterium* var. phosphatrcum "BM3".

The physical and chemical properties of the experimental soil (Table 1) were determined according to the method of Jakson (1958).

Table 1: Physicochemical properties of the experimental soil.

Mechanical analysis			**Textural class**	**$CaCO_3$ %**	**EC dS/m**	**PH 1: 2.5**
Clay%	Silt%	Sand%				
51.20	38.58	10.22	Clay-loam	3.5	4.1	7.6
Macroelements (ppm)			**Microelements (ppm)**			
N	P	K	Fe	Zn	Mn	Cu
95	34	718	4.5	3	15	2.8
Soluble anions (meg/L)			**Soluble cations (meg/L)**			
HCO_3^-	Cl^-	So_4^{-2}	Ca^{+2}	Mg^{+2}	Na^+	K^+
1.5	6	29.1	18.9	6.1	9.9	1.5

Bean seeds were sown on 5th March in the two seasons. Before sowing, seeds were coated with a thin film of *Rhizobium leguminosarum* biovar *phaseoli* "ARC301" and the other two bacterial stains separately or in combination using gums Arabic 40%. The experiment was arranged in a split plot design with three replications, where the two cultivars were put in the main plots, and the six biofertilizers treatments were randomly distributed in the sub main plots. The experiment included 12 treatments representing the various combinations of bean cultivars, bacterial strains and NPK recommended fertilizer level.

The bacterial strains *Rhizobium leguminosarum* bvr. *phaseoeli* (ARC 301), *Azotobacter chroococcum* (AZ1) and *Bacillus megaterium* var. *phosphatecium* (BM3) were provided by central Lab. of organic agriculture, ARC, Giza Egypt.. Three different broth media were used: yeast extract mannitol for *Rhizobium* (Vincent, 1970), modified ashby for *Azotobacter* (Hegazy and Neimela, 1976) and Bunt and Rovira (1955) for *Bacillus megaterium* (Bunt and Rovira, 1955). Each bacterium was grown on the appropriate medium and incubated at 28˚C for 3 days until early log phase. Vermiculite supplemented with 10% Irish peat was packed on polyethylene bags as a carrier (300 g per /bag), then sealed and sterilized by gamma irradiation ($5.0 X10^8$ rads). Bacterial culture was injected into steilized vermiculite to satisfy 60% of the maximal water holding capacity, then, the inculation rate were used as 300gm inocula / feddan for each microorganism (50% for seed inoculation and 50% at 15 day after planting).

Microbial inoculation was done twice, the first was before sowing as seed coating and the other was add after 15 day from sowing. Also, total counts of bacteria, actinomycetes and fungi were estimated in rhizosphere soil samples of the two seasons according to Wollum (1982). Nitrogenase activity was measured as acetylene reduction assay (ARA) according to the method described by Hardy et al. (1973).

The six combination treatments were as follows:

1. Untreated plants.
2. Plants received the recommended NPK levels without biofertilizer.
3. Plants inoculated with *Rhizobium leguminosarum* biovar *phaseoli* "ARC301" (Rh) + 25 %(NRL).
4. Plants inoculated with *Azotobacter chroococum* "AZ1"+ 25 % (NPKRL).
5. Plants inoculated with Rh + *Bacillus megaterium* "BM3" + 25 % (NPKRL).
6. Plants inoculated with Rh + AZ1 + BM3+ 25 % (NPKRL).

The last four treatments received only 25% of the recommended NPK dose while the second treatments received 100 % of recommended NPK dose and the first treatment did not received any bio or normal fertilizers considered as controls. The plot area was $13m^2$, consisting of 5 lines (4m length and 65 cm apart). One line was devoted for vegetative growth parameters samples, and the other two lines were used for green pod yield, while the remainder two lines were used for dry seed yield determination. One line was left between every two plots as a guard line. The inoculated or un inoculated seeds were sown in hills at 5cm apart. Three to five seeds were sown in each hill. Ten days after sowing, plants were thinned to two plants per hill.

Five plants from each experimental plot were taken randomly after 60 days from sowing to determine plant length, fresh and dry weights as well as number of branches/plant, number of nodules and their dry weights. The chlorophyll reading in leaves was recorded (at the beginning of flowering) by A Minolta SPDAD chlorophyll-meter, model SPAD 502 (Yadava, 1986).

Pods were harvested at green maturity stage every. 7 day, then counted and weighted. The following characters were recorded.:

1. Weight and number of green pods per plant were measured on ten plants taken randomly from each plot during all harvesting times.
2. Weight of green pods for the first and second harvests taken from each plot was recorded, then

the average yield of green pods/fed. was calculated and considered as early yield per feddan.

3. Weight of green pods taken during all harvestings of green pod yield/plot. Was recorded then calculated as total yield per feddan.
4. At pod maturity stage, dry pods of the two lines that devoted for dry yield from each plot were collected after about 100 days from sowing, then dry bean seeds were separated and weighed to determine the dry yield per feddan.

5. Ten green pods were taken randomly (from the third harvest) from each experimental plot for measuring the average pod weight (g), pod length (cm) and pod diameter (mm).

Hundred gram of fresh leaves and pods (obtained from three plants) taken randomly from each experimental plot at the thrid harvest, were oven-dried at 70°C till constant weight, the dried samples were taken to measure N,P and K in leaves and pods as well as pod dry matter, protein, total carbohydrates and crude fibers according to (Huphries 1956; Taussky and Shorr 1952; Brown and Lilliland 1964; Stewart, 1989 and A.O.A.C (1980).

Statistical analysis of the obtained data was conducted through the analysis of variance according to Snedecor and Cochran (1980). For comparison between means, L.S.D. at 0.05 was calculated.

Results and Discussion

Vegetative growth:

As shown in Table (2) Paulista cv. exceeded cv. Bronco in plant height, fresh and dry weight, number of branches per plant as well as chlorophyll leaf reading in both seasons. There were significant differences among the bacterial inocula on all vegetative growth traits and chlorophyll reading especially in the two seasons except the number of branches per plant in the second one. Inoculation with *Rhizobium leguminoarum* bv. *phasaoli* (Rh) + *B. megaterium* (BM3)in presence of 25% recommended NPK had the highest values for all triats of vegetative growth followed by Rh+AZ1+BM3 + 25% NPK. The interaction between microbial inoculation and cultivars was significant on plant height, plant fresh weight as well as leaf chlorophyll content. On the other hand, the interaction was not significant on plant dry weight and number of branches per plant especially in the second season. Meanwhile, treated with Rh + BM3 + 25% NPK exhibited the highest values of all studied traits for both cultivars, while uninoculated plants showed the lowest ones. The obtained results are in agreement with those reported by Singer *et al.,* (1996) who concluded that applying 50 or 75% of recommended NPK doses and inoculation with *Rhizobium*. Sp. and *Azosperillum* spp. or *Rhizobium* spp. with soil yeast (Cand. sp) to snap bean plants resulted in vigorous plant growth. Similarly, Abd El-Fattah and Arisha (2000) indicated that, the stimulative effect of *Rhizobium* inoculation on morphological characters of bean plants might be due to that the treated plants with *Rhizobium* fixed high amounts of nitrogen which in turn increased plant growth parameters.

Nodulation and N_2-fixaction:

Data presented in Table (3) indicated that *Rhizobium* inoculum significantly increased numbers and dry weight of nodules and nitrogen fixation activity as compared to uninoculated treatments. Irrespective of cultivar, co- inoculation with *Rhizobium* and *Azotobacter* or *Bacillus megaterium* var *phosphaticum* or together, did enhance the nodulation and N_2 – fixation of snap bean plants. In general, data recorded in the second season was higher than those obtained in the first one. The same trend was obtained for cvs. Paulista and. Bronco. These results are in harmony with those obtained by Abdel fattah and Arisha (2000) and Ravindar and Chandra (2008), who reported that, mixed inoculation with *Rhizobium* and N_2- fixing bacteria or phosphate dissolving bacteria increased nodulation and N_2 fixation of some leguminous plants.

Microbial status:

Data in Table (4) showed that fertilizing snapbean plants with recommended dose of NPK had a negative effect on rhizosphere microorganism (RMO). This treatment scored the lowest number of total bacteria, fungi and actinomycetes compared to the inoculated treatments. Also, in both seasons, inoculation with *Rhizobium* alone or mixed with *Azotobacter* or *Bacillis megaterium* var *phosphaticum* gave the higher number of total bacteria, fungi and actinomycetes compared to uninoculated one. Irrespective of cultivar the triple inocula treatment gave the higher number of RMO compared to others. Similar results were obtained in both seasons for both cultivars. These results are in agreement with those obtaind by Ragab, Mona *et al* (2006) and Ashrafuzzaman *et al* (2009). They reported that inoculation with the plant growth promoting rhizobacteria (*Azotobcter, Bacillus megaterium, Rhizobium*) had simulative effect on the population of rhizosphere microorganism (RMO) and increased their numbers by more than 50% at the end of the experiment .

Table 2: Effect of some bacterial strains on vegetative growth and chlorophyll content of snap bean cultivars during 2007 and 2008 seasons.

Parameters / Cultivars		Plant height (cm).		Plant fresh weight (g.)		Plant dry weight (g.)		No. of branches per plant		Leaf chlorophyll content (SPAD unit)	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Bronco		36.43	35.64	38.26	39.26	6.77	6.90	3.22	3.33	31.21	34.06
Paulista		38.02	38.12	56.99	52.23	9.87	9.67	4.39	4.00	33.47	35.32
L.S.D. at 0.05		1.58	1.01	1.87	1.38	0.46	0.38	0.51	0.45	2.18	1.07
Treatments											
Control (uninoculated)		34.97	32.83	39.74	36.00	7.41	7.63	3.50	3.17	27.89	27.67
Recommended (NPK)		38.28	36.73	48.17	46.33	8.49	8.42	3.84	3.50	33.94	36.40
Rhizobial inoculation ARC 301 (Rh)+ 25% NPK		35.97	35.12	44.50	34.15	8.10	8.11	3.67	3.67	29.87	32.74
Rh+Azotoacter (AZ1) + 25% NPK		37.45	37.17	48.14	44.59	8.22	8.30	3.50	3.83	31.79	35.02
Rh+ *B.megaterium* (BM3) + 25% NPK		39.22	41.55	54.02	53.89	9.25	8.79	4.34	4.00	36.00	38.55
Rh + AZ1 + BM3+ 25% NPK		37.45	37.74	51.20	52.17	8.45	8.49	4.00	3.83	34.57	37.75
L.S.D. at 0.05+ 25% NPK		2.84	1.75	3.25	3.39	0.80	0.65	0.82	N.S	4.78	1.85
Interaction											
Bronco	**Control (uninoculated)**	33.43	30.43	33.07	36.83	5.79	6.05	3.33	3.00	26.27	24.57
	Recommended (NPK)	36.13	35.13	37.60	38.52	6.87	7.03	3.00	3.33	32.70	36.97
	Rhizobial inoculation ARC 301 (Rh) + 25% NPK	35.97	33.60	35.90	36.63	6.61	6.69	3.33	3.00	28.10	33.90
	Rh+Azotoacter (AZ1) + 25% NPK	36.83	35.33	38.37	38.77	6.38	6.87	2.67	3.33	29.60	34.37
	Rh+ *B.megaterium* (BM3) + 25% NPK	40.53	39.87	43.67	44.10	8.28	7.66	3.67	4.00	35.67	37.33
	Rh + AZ1 + BM3+ 25% NPK	35.67	36.10	40.93	42.87	6.69	7.10	3.33	3.33	33.90	37.20
Paulista	**Control (uninoculated)**	36.50	35.23	46.40	35.17	9.03	9.21	3.67	3.33	29.50	30.77
	Recommended (NPK)	40.43	38.33	58.73	54.13	10.13	9.80	4.67	3.67	35.17	35.83
	Rhizobial inoculation ARC 301 (Rh) + 25% NPK	36.97	36.63	53.10	49.67	9.58	9.52	4.00	4.33	31.63	31.57
	Rh+Azotoacter (AZ1) + 25% NPK	38.07	39.00	57.90	50.40	10.05	9.72	4.33	4.33	33.97	35.67
	Rh+ *B.megaterium* (BM3) + 25% NPK	41.90	42.83	64.37	63.67	10.22	9.92	5.00	4.33	36.33	39.77
	Rh + AZ1 + BM3+ 25% NPK	39.23	39.37	61.47	60.37	10.21	9.87	4.67	4.00	35.23	38.30
L.S.D. at 0.05 and N.S= non sig		4.02	2.48	4.59	3.38	1.13	N.S	1.25	N.S	5.34	2.61

Table 3. Effect of rhizobial inoculation combined with *Azotobacter chroococcum* and *Bacillus megaterium* var *phosphaticum* on nodulation and N_2-fixation of snap bean cultivars during **2007**and 2008seasons.

Treatments	No. of nodules plant $^{-1}$				Dry weight of nodules mg plant $^{-1}$				*ARA µ mal C_2H_4 h^{-1} $plant^{-1}$			
	Paulista		Bronco		Paulista		Bronco		Paulista		Bronco	
	Season 2007	Season 2008	Season 2007	Season 2008	Season 2007	Season 2008	Season 2007	Season 2008	Season 2007	Season 2008	Season 2007	Season 2008
Control (uninoculated)	**2**	**3**	**0**	**4**	**0**	**1**	**0**	**2**	**0**	**0**	**0**	**0**
Recommended (NPK)	**0**	**1**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Rhizobial inoculation ARC 301 (Rh) + 25% NPK	**43**	**50**	**40**	**56**	**174**	**190**	**164**	**170**	**181.340**	**140.45**	**93.925**	**103.250**
Rh+Azotoacter (AZ1) + 25% NPK	**59**	**49**	**57**	**48**	**189**	**160**	**180**	**197**	**132.791**	**135.240**	**133.420**	**124.426**
Rh+ B.megaterium (BM3) + 25% NPK	**45**	**68**	**42**	**62**	**150**	**199**	**143**	**169**	**120.153**	**143.820**	**177.325**	**140.372**
Rh + AZ1 + BM3+ 25% NPK	**77**	**80**	**64**	**78**	**247**	**250**	**205**	**223**	**140.253**	**150.342**	**111.472**	**120.375**
L.S.D. at 0.05	**5.0**	**6.0**	**5.0**	**6.0**	**22**	**25**	**20**	**20**	**10.15**	**10.45**	**9.62**	**11.47**

*Acetylene Reduction Assay.

Table 4. Effect of rhizobial inoculation combined with *Azotobacter chroococcum* and *Bacillus megaterium* var *phosphaticum* on numbers of total bacteria, fungi and actinomycetes of snap bean rhizosphers during 2007 and 2008 seasons.

Treatments	Fungi (log number)		Actenomycetes (log number)		Total bacteria (log number)	
	Bronco	Paulista	Bronco	Paulista	Bronco	Paulista
		Season 2007				
Control (uninoculated)	4.62	4.70	3.72	3.84	4.56	4.95
Recommended (NPK)	3.25	3.92	3.22	3.32	5.32	5.47
Rhizobial inoculation ARC 301 (Rh) + 25% NPK	4.70	4.79	3.85	3.88	5.92	5.94
Rh+Azotoacter (AZ1) + 25% NPK	4.75	4.80	3.92	3.97	6.54	6.99
Rh+ B.megaterium (BM3) + 25% NPK	4.88	4.91	4.32	4.56	7.25	7.50
Rh + AZ1 + BM3+ 25% NPK	4.90	4.99	4.57	4.59	7.82	7.90
			Season 2008			
Control (uninoculated)	4.73	4.80	3.84	3.86	4.60	5.02
Recommended (NPK)	3.53	4.02	3.34	3.35	4.99	5.52
Rhizobial inoculation ARC 301 (Rh) + 25% NPK	4.62	4.82	3.90	3.90	5.99	6.01
Rh+Azotoacter (AZ1) + 25% NPK	4.77	4.81	3.92	3.92	6.72	6.98
Rh+ B.megaterium (BM3) + 25% NPK	4.85	4.89	4.45	3.99	7.32	7.62
Rh + AZ1 + BM3+ 25% NPK	4.93	4.99	4.60	4.42	7.92	7.49

Yield and its components:

Data presented in Table (5) indicated that pod number per plant of cv. Paulista was higher than that of cv. Bronco, while the reverse was true for pod yield per plant. On the other hand, Bronco cv. exceeded Paulista cv. in early and total green yield per feddan. Dry seed yield per feddan showed the same trend of green pod yield in both studied cultivars.

As shown in Table (5) there were significant differences among the bacterial inoculation for all tested traits of yield and its components. Snap bean Plants treated with rhizobial inoculation + *B.megaterium* (BM3) + 25% NPK, produced significantly higher values of plant green pod yield, early and total green pod yield per feddan as well as dry seed yield. On the other hand, Rh + AZ1 + BM3 + 25%NPK significantly increased the plants green pod yield as well as dry seed yield compared with uninoculated control and recorded nearly equal values of the treatment of 100% NPK. Concerning the interaction between cultivars and bacterial inoculation, the treatment of Rh + *B.megaterium* (BM3) + 25%NPK, followed by the treatment of Rh + AZ1 + BM3 + 25% NPK for cv. Bronco significantly increased the plant yield, early and total green pod yields as well as dry seed yield. Also, the treatments of 100% NPK or Rh + Azotobacter (AZ1) + 25% NPK led to significant higher values of plant green yield and dry seed yield. Meanwhile, in cv. Paulista plants received Rh + BM3 + 25% NPK produced the highest values of plant green yield and dry seed yield. Also, the treatment of Rh + AZ1 + BM3 + 25% NPK followed by 100% NPK (without biofertilizers) significantly increased dry seed yield comparing with the uninoculated control.

The increase in total green pod and dry seed yields might be attributed to the favourable effect of rhizobium inoculation. Similar conclusions were previously reported by Aryal *et al.* (2003) who found that common bean can meet the plant nitrogen requirements by symbiotic N_2-fixation. The percentage of N derived from atmospheric of field grown with *Phasealus vulgaris* was between 38% and 68%. On the other hand, Mikanova et al., (1995) and Ismail (2002) revealed that pea yield increased with the use of phosphate solubilizing inoculation in the absence of fertilizer to a level similar to that obtained with 45kg P/ha alone. The obtained results are in harmony with those reported by Abd El-Fattah and Arisha (2000) and Shehata *et al.,* (2007).

Green pod characters:

As shown in Table (6) Bronco cv. overcame cv. Paulista in pod weight and diameter, dry matter, carbohydrate and fibres content in both seasons. There were no noticeable differences between the two snap bean cultivars concerning pod length as well as protein percentage. Also, there were significant differences among the bacterial inocula for all studied characters of the green pods. The treatments Rh + BM3 + 25%NPK, Rh + AZ1 BM3 + 25% NPK or 100%NPK led to the highest values of green pod characters in both seasons.

In the case of cv. Bronco, the treatments of Rh + BM3 + 25% NPK, Rh + AZ1 + BM3 + 25% NPK and 100% NPK significalty increased pod weight and length compared to uninoculated control. Meanwhile, in cv. Paulsita the treatment of Rh + BM3 + 25% led to the highest pod weight and length. There were no remarkable differences between all tested treatments in both cultivars regarding pod diameter. In addition, treatment of Rh rhizobium + 25% NPK significantly increased pods dry matter content, protein, charbohydrats and fibers (exceeded or approximately equal to 100% NPK) in both cultivars compared to uninoculated control. The present results confirmed those of El-Sayed (1990) who found differences among some common bean cultivars regarding, crude fibers and protein content. Similarly, Singer *et al.* (1996) mentioned that a mixture of three biofertilizers, in general, gave the highest physical properties of snap bean pods even with different levels of NPK applications. Also, other investigators indicated positive effects of *Rhizobium* and *Azospirillum* on plant growth, yield and chemical components of snap been pods (Singer *et al.,* 2000; Shehata *et al.,* 2007).

Leaves and pods elemental (N, P and K) concentration:

As shown in Table (7)snap bean plants cv. Paulista was greater than those of cv. Bronco in leaves content of N and K as well as pods content of N, P and K, the reverse was true concerning leaves content of P. Meanwhile, there were no remarkable differences between the two snap been cultivars concerning the leaves and pods content of N, P and K. Concerning rhizobium treatments, the treatment of Rh + BM3 + 25% NPK followed by Rh + AZ1 + BM3 + 25% NPK significantly gave the higher value of leaves content of N and K as well as pods content of N and P comparing with the unioculated control. On the other hand, plants treated with Rh + AZ1 + BM3 + 25% NPK produced the highest values of leaves content of P in both seasons, while the treatment of Rh + BM3 + 25% NPK led to the highest values of pods content of K compared to the uninoculated control or the treatment of 100% NPK.

Regarding the interaction, it was clear that in cv. Bronco there were no significant differences among the treatments concerning leaves content of N or P, meanwhile the treatments Rh + BM3 + 25% NPK or Rh + AZ1 + BM3 + 25% NPK significantly increased pods content of N,P and K as well as leaves content of K. In cv. Paulista, plants treated with Rh + BM3 + 25% NPK or Rh + AZ1 + BM3 + 25% NPK significantly increased the leaves and pods content of N, P and K in both seasons compared to the uninoculated control.

Table 5: Effect of bacterial strains on yield of snap bean cultivars during 2007 and 2008 seasons.

Parameters / Cultivars		Green pods yield/plant				Green pods yield (ton/fed.)				Dry seed yield (ton / fed).	
						Early		Total			
		Weight (g.)		No. of pods							
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Bronco		73.13	73.84	18.06	19.00	0.55	0.56	3.58	3.60	0.77	0.79
Paulista		67.74	68.44	20.78	20.06	0.48	0.50	3.21	3.29	0.69	0.69
L.S.D. at 0.05		3.96	4.00	1.22	1.03	0.05	0.06	0.33	0.31	0.07	0.07
Treatments											
Control (uninoculated)		64.12	64.65	18.34	17.84	0.46	0.48	3.03	3.10	0.49	0.54
Recommended (NPK)		75.52	73.79	20.00	20.17	0.53	0.54	3.36	3.55	0.73	0.77
Rhizobial inoculation ARC 301 (Rh) + 25% NPK		65.35	65.35	18.50	18.67	0.50	0.51	3.32	3.36	0.60	0.60
Rh+Azotoacter (AZ1) + 25% NPK		66.68	68.68	19.00	19.84	0.52	0.52	3.44	3.40	0.67	0.68
Rh+ *B.megaterium* (BM3) + 25% NPK		79.02	78.77	21.00	21.00	0.56	0.58	3.71	3.59	0.96	0.97
Rh + AZ1 + BM3+ 25% NPK		71.94	75.69	19.67	19.69	0.54	0.54	3.56	3.58	0.92	0.90
L.S.D. at 0.05		6.86	6.93	2.11	1.84	0.09	0.10	0.59	0.47	0.12	0.12
Interaction											
Bronco	**Control (uninoculated)**	65.87	65.97	16.67	17.00	0.44	0.46	2.92	3.04	0.54	0.57
	Recommended (NPK)	82.40	75.70	19.33	19.33	0.55	0.57	3.59	3.76	0.80	0.83
	Rhizobial inoculation ARC 301 (Rh) + 25% NPK	66.47	66.63	17.00	18.33	0.51	0.52	3.38	3.46	0.70	0.71
	Rh+Azotoacter (AZ1) + 25% NPK	66.53	70.03	17.67	19.67	0.55	0.53	3.60	3.49	0.76	0.80
	Rh+ *B.megaterium* (BM3) + 25% NPK	83.87	83.53	19.33	21.00	0.59	0.64	3.92	3.79	0.10	0.98
	Rh + AZ1 + BM3+ 25% NPK	83.67	81.17	18.33	18.68	0.58	0.57	3.79	3.78	0.88	0.87
Paulista	**Control (uninoculated)**	62.37	63.33	20.00	18.67	0.48	0.50	3.13	3.16	0.44	0.50
	Recommended (NPK)	68.63	71.87	20.67	21.00	0.50	0.51	3.15	3.35	0.66	0.70
	Rhizobial inoculation ARC 301 (Rh) + 25% NPK	64.23	64.07	20.00	19.00	0.48	0.50	3.25	3.27	0.49	0.50
	Rh+Azotoacter (AZ1) + 25% NPK	66.83	67.33	20.33	20.00	0.50	0.51	3.29	3.32	0.58	0.56
	Rh+ *B.megaterium* (BM3) + 25% NPK	74.17	74.00	22.67	21.00	0.53	0.52	3.51	3.39	0.98	0.95
	Rh + AZ1 + BM3+ 25% NPK	70.20	70.03	21.00	20.68	0.49	0.51	3.32	3.38	0.95	0.93
	L.S.D. at 0.05	9.70	9.79	2.58	2.30	0.13	0.13	0.84	0.74	0.17	0.17

Table 6: Effect of bacterial strains on pod characteristics and chemical compounds of snap bean cultivars during 2007 and 2008 seasons.

Cultivars		Pod's characteristics						Chemical compounds of snap bean pods							
		Weight (g.)		Length (cm.)		Width (mm)		Dry matter %		Protein %		Carbohydrate %		Fibers %	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Bronco		3.91	4.05	11.94	11.83	88	87	10.63	10.42	22.36	22.07	60.72	60.56	10.34	10.27
Paulista		3.31	3.63	11.82	11.70	69	66	10.23	10.00	22.63	22.65	59.77	59.86	9.67	9.64
L.S.D. at 0.05		0.16	0.25	0.42	0.37	0.7	0.6	0.35	0.41	N.S.	N.S.	0.56	0.67	0.29	0.40
Treatments															
Control (uninoculated)		3.20	3.37	11.17	11.24	70	70	9.27	9.65	21.33	20.68	56.12	56.29	9.07	9.38
Recommended (NPK)		3.73	3.81	12.19	12.12	82	81	11.19	10.90	23.07	22.52	61.90	61.69	10.37	10.32
Rhizobial inoculation ARC 301 (Rh) + 25% NPK		3.37	3.55	11.33	11.40	74	72	9.80	10.02	21.37	21.29	75.62	57.87	9.50	9.48
Rh+Azotoacter (AZ1) + 25% NPK		3.64	3.63	11.65	11.53	79	75	10.50	10.45	21.69	22.27	61.25	61.40	9.98	10.03
Rh+ *B.megaterium* (BM3) + 25% NPK		3.88	4.07	12.69	12.34	88	82	11.25	10.94	24.55	24.20	63.07	62.43	10.73	10.47
Rh + AZ1 + BM3+ 25% NPK		3.85	3.85	12.27	11.97	80	79	10.58	10.57	22.95	23.22	61.53	61.57	10.37	10.07
L.S.D. at 0.05		0.27	0.43	0.73	0.65	10	11	0.60	0.72	0.89	1.17	0.97	1.15	0.49	0.70
Interaction															
Bronco	**Control (uninoculated)**	3.50	3.73	11.13	11.20	88	80	9.10	9.33	21.13	20.53	57.40	57.77	9.53	9.83
	Recommended (NPK)	4.13	4.07	12.50	12.27	90	93	11.70	11.23	22.83	22.27	62.10	61.67	10.53	10.73
	Rhizobial inoculation ARC 301 (Rh) + 25% NPK	3.60	3.87	11.33	11.50	80	80	10.10	9.60	21.17	20.80	58.47	58.60	10.07	9.97
	Rh+Azotoacter (AZ1) + 25% NPK	4.00	4.03	11.70	11.53	87	83	10.50	10.43	21.77	22.37	61.47	61.63	10.33	10.10
	Rh+ *B.megaterium* (BM3) + 25% NPK	4.13	4.50	12.87	12.57	103	93	11.73	11.27	24.83	24.90	63.27	63.03	11.13	10.87
	Rh + AZ1 + BM3+ 25% NPK	4.10	4.10	12.47	11.90	90	90	10.63	10.63	22.90	23.07	61.63	61.83	10.43	10.13
Paulista	**Control (uninoculated)**	2.90	3.00	11.20	11.27	63	60	9.43	9.97	21.53	20.38	54.83	54.80	8.60	8.93
	Recommended (NPK)	3.33	3.53	11.87	11.97	73	67	10.67	10.57	23.30	22.77	61.70	61.70	10.20	9.90
	Rhizobial inoculation ARC 301 (Rh) + 25% NPK	3.13	3.23	11.33	11.30	67	63	9.50	10.43	21.27	22.17	56.77	57.13	8.93	9.00
	Rh+Azotoacter (AZ1) + 25% NPK	3.27	3.23	11.60	11.53	70	67	10.50	10.47	21.60	22.17	61.03	61.17	9.63	9.97
	Rh+ *B.megaterium* (BM3) + 25% NPK	3.63	3.63	12.50	21.10	73	70	10.77	10.60	24.77	24.00	62.87	61.83	10.33	10.07
	Rh + AZ1 + BM3	3.60	3.50	12.07	12.03	70	67	10.53	10.50	23.00	23.37	61.43	61.30	10.30	10.00
L.S.D. at 0.05		0.38	0.61	1.04	0.82	16	15	0.85	1.01	1.27	1.66	1.37	1.63	0.70	0.98

Table 7: Effect of bacterial strains on N, P and concentrations (%) of snap bean leaves and pods during 2007 and 2008 seasons.

Cultivars		Leaves NPK (%)						Pods NPK (%)					
		Nitrogen		Phosphours		Potassium		Nitrogen		Phosphorus		Potassium	
		2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Bronco		1.96	2.09	0.63	0.67	2.18	2.21	3.58	3.53	0.55	0.51	2.71	2.69
Paulista		2.18	2.23	0.59	0.63	2.32	2.37	3.61	3.62	0.58	0.60	3.01	2.98
L.S.D. at 0.05		N.S.	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S	N.S
Treatments													
Control (uninoculated)		1.88	1.78	0.58	0.60	2.31	2.28	3.41	3.31	0.57	0.54	2.78	2.81
Recommended (NPK)		1.96	2.01	0.52	0.61	2.11	2.06	3.69	3.60	0.58	0.56	2.80	2.72
Rhizobial inoculation ARC 301 (Rh) + 25% NPK		2.01	1.99	0.58	0.52	2.43	2.39	3.42	3.41	0.50	0.51	2.67	2.61
Rh+Azotoacter (AZ1) + 25% NPK		2.19	2.16	0..61	0.58	2.50	2.54	3.47	3.56	0.63	0.60	2.74	2.80
Rh+ *B.megaterium* (BM3) + 25% NPK		2.41	2.33	0.64	0.66	2.99	2.92	3.93	3.87	0.73	0.69	3.11	3.00
Rh + AZ1 + BM3+ 25% NPK		2.18	2.11	0.68	0.72	2.80	2.73	3.67	3.72	0.71	0.68	2.91	2.99
L.S.D. at 0.05		0.26	0.31	0.09	0.11	0.46	2.39	0.19	0.27	0.09	0.12	0.23	0.18
Interaction													
	Control (uninoculated)	1.94	1.93	0.48	0.51	2.50	2.57	3.38	3.28	0.58	0.56	2.69	2.73
	Recommended (NPK)	2.01	2.10	0.55	0.50	2.44	2.53	3.85	3.16	0.63	0.61	2.58	2.61
Bronco	**Rhizobial inoculation ARC 301 (Rh) + 25% NPK**	2.26	2.21	0.57	0.51	2.61	2.63	3.39	3.33	0.60	0.56	2.80	2.75
	Rh+Azotoacter (AZ1) + 25% NPK	2.22	2.17	0.60	0.62	2.71	2.76	3.48	3.58	0.63	0.61	2.90	2.87
	Rh+ *B.megaterium* (BM3) + 25% NPK	2.18	2.29	0.62	0.65	2.81	2.90	3.97	3.98	0.79	0.81	3.16	3.24
	Rh + AZ1 + BM3+ 25% NPK	2.16	2.21	0.70	0.72	2.96	2.91	3.66	3.69	0.77	0.47	3.10	3.06
	Control (uninoculated)	1.87	1.92	0.53	0.56	2.62	2.71	3.44	3.33	0.52	0.58	2.96	2.88
	Recommended (NPK)	2.19	2.24	0.58	0.49	2.71	2.67	3.03	3..24	0.55	0.59	2.80	2.72
Paulista	**Rhizobial inoculation ARC 301 (Rh) + 25% NPK**	2.02	2.29	0.55	0.58	2.70	2.82	3.45	3.48	0.60	0.64	2.91	2.88
	Rh+Azotoacter (AZ1) + 25% NPK	2.17	2.23	0.61	0.65	2.87	2.92	3.46	3.54	0.67	0.66	3.01	2.96
	Rh+ *B.megaterium* (BM3) + 25% NPK	2.46	2.35	0.84	0.79	3.08	2.99	3.96	3.84	0.82	0.77	3.69	3.52
	Rh + AZ1 + BM3+ 25% NPK	2.30	2.34	0.79	0.80	3.11	3.06	3.68	3.74	0.80	0.79	3.30	3.38
L.S.D. at 0.05		0.32	0.40	0.21	0.21	0.38	0.33	0.20	0.22	0.17	0.15	0.41	0.39

The present results confirmed those of Aryal *et al.* (2003) and Shehata *et al.* (2007) who reported that inoculation of snap bean with *Rhizobium* - *Azosirillum* or arbuscular mycorrhizal fungi (AMF) increased leaves content of N and the chemical composition of pods in addition to improve nutrient uptake. The positive effects of inoculation with *Azospirillum brasilense* on plant growth, and consequently on yield and pod characters could be explained by an enhancement of root branching and root growth. These favorable effects on root growth are known to improve the efficiency of mineral and water uptake, and consequently protein production and hormonal activity in inoculated plants (Hamaoui *et al.*, 2001). Additionally, the positive effect of increased phosphorus absorption by bean plants as a result of inoculation with Okadine + Rhizobacterin on vegetative growth may be due to the beneficial effect of P element on the activation of photosynthesis and metabolic processes of organic compounds in plants and hence increasing plant growth (Gardener *et al.*, 1985). Also, the enhancing effect of nitrogen absorption on plant growth may be due to the positive effects of N-element on activating photosynthesis and metabolic processes of organic compounds in plants which in turn, encourage the plant vegetative growth, which exert direct effect on the yield (El-Seifi *et al.*, 2004).

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تأثير التلقيح بالريزوبيا والأزوتوباكتر والباسلس ميجاتيرم علي تكوين العقد الجذرية والمحصول والجودة لصنفين من الفاصوليا الخضراء

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الملخص العربي

أجري هذا البحث خلال موسمي الزراعة للأعوام2007، 2008 في محطة البحوث والتجارب الزراعية التابعة لكلية الزراعة – جامعة القاهرة لدراسة تأثير التلقيح بريزوبيا الفاصوليا وسلالتين من البكتريا (الأزوتوباكتر وباسيلس ميجاتيرم) علي تكوين العقد الجذرية وتثبيت النيتروجين الجوي والنشاط الإنزيمي والمحتوي الميكروبي في منطقة الريزوسفير(منطقة نمو الجذور) وعلي المحصول وجودة ثمار الفاصوليا صنفي برونكو وبوليستا تحت 25% من السماد الكيماوي الموصي به منNPK وقد أظهرت النتائج ما يلي:-

وجد أن معاملات التلقيح بخليط من السلالات أعطت تأثيرا معنويا علي النمو وتكوين العقد الجذريه وتثبيت النيتروجين الجوي وكذلك النشاط الإنزيمي لكلا الصنفين ولقد سجلت أعلي النتائج مع معاملة التلقيح بالخليط بين السلالات (الريزوبيا والأزوتوباكتر والباسلس ميجاتيرم) في وجود 25% من السماد الكيماويNPK الموصي به.

تفوق الصنف بوليستا علي الصنف برونكو بالنسبة لصفات ارتفاع النبات ، الوزن الطازج والجاف للنبات وكلاً من عدد الافرع وعدد القرون للنبات الواحد بالإضافة إلي محتوي الأوراق من الكلوروفيل بينما كان العكس صحيحاً بالنسبة لصفات محصول النبات علي أساس وزن الثمار والمحصول الأخضر المبكر والكلي وكذلك المحصول الجاف للفدان، ووزن وقطر القرن بالإضافة إلي محتوي

من المادة الجافة والكربوهيدرات والألياف. وقد أدي تلقيح بذور الفاصوليا ببكتريا الباسيلس مع استخدام 25% من الكميات الموصي بها من السماد المعدني (NPK) إلي زيادة معنوية في جميع صفات النمو الخضري والمحصول ومكوناته ومواصفات القرون وذلك مقارنة بالكنترول غير الملقح بالبكتريا والمسمد بـ100% من ال NPK الموصي به.

أظهرت معاملات التفاعل بين الأصناف و التلقيح بسلالات البكتريا أن المعاملة بالريزوبيا والباسلس و 25% فقط من الـ NPK كانت أفضل المعاملات مع الصنف بوليستا بالنسبة لصفات النمو الخضري ومحتوي الأوراق من الكلوروفيل، بينما حققت نفس المعاملة مع الصنف برونكو أفضل القيم بالنسبة لصفات المحصول ومكوناته بالإضافة إلي مواصفات جودة الثمار.