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Field Response of Snap Bean (*Phaseolus* vulgaris L.) To N_2-fixers *Bacillus Circulans* and Arbuscular Mycorrhizal Fungi Inoculation Through Accelerating Rock Phosphate and Feldspar Weathering

[1]O.N. Massoud, [1]Ebtsam M. Morsy and [2]Nadia H. El-Batanony

[1]Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.
[2]Environmental Studies and Research Institute (ESRI), Sadat City, Menoufiya University, Egypt.

Abstract: This study was conducted in two successive seasons of 2006-2007 in a private farm at Kafr Dawood, Menoufiya, Egypt. It aimed to investigate the effect of inoculation of snap bean plants with biofertilizers (AM- mycorrhizal fungi, symbiotic N_2-fixers (*Rhizobium* sp.) and asymbiotic N_2-fixers (*Azospirillum* sp. and *Azotobacter* sp.) in addition to *B. circulans* on soil amended with natural alternatives of fertilizers like rock phosphate and feldspar and their effect on growth parameters, physiological and microbiolgical parameters. In addition, snap bean (*Phaseolus vulgaris*, L.) fresh yield and its components. The obtained results indicated that the treatment No. 9 (mixture of AM- fungi, symbiotic and asymbiotic N_2-fixers and *Bacillus circulans* + rock phosphate + feldspar) was superior in plant height, number of branches, number of nodules per plant and fresh yield (ton/fed) when compared with control and the uninoculated plants. Inoculation with produced also the highest values of N_2-fixers and *B. circulans* populations in the rhizosphere of snap bean plants cultivated in soil amended with rock phosphate and feldspar and produced the highest infection percentage of AM- fungi. The interaction among the studied factors had significant effects on most studied characters. So, our study supports the new trend of using biofertilizers and natural alternatives as beneficial cheap sources of fertilization for sustainable agriculture.

Key words: Nitrogen fixers, *B. circulans,* AM-fungi, rock phosphate, feldspar, snap bean plant.

INTRODUCTION

The most important goal of agriculture is the production of high quality, safe and inexpensive food for an ever-increasing worldwide population. With the increasing problems associated with the use of synthetic chemicals in agriculture (impacts on health and the environment, resistance development in plant pathogens and pests, etc.) there has been an ever-increasing interest in the use of beneficial microorganisms to improve plant health and productivity while ensuring safety for human consumption, and protection of the environment, particularly for the developing countries (Avis *et al*., 2008).

Phaseolus vulgaris L. is one of the most important economic vegetable crops. It is grown for the production of green pods (Snap beans) in addition to dry beans. The recent major problem facing the farmers is the high coast of chemical fertilizers. Therefore, the utilization of biofertilizers is considered as a promising alternative in particular for the developing countries.

Phosphorus and potassium are essential elements for plant nutrition. However, in natural conditions, most of soil P and K contents are found in both mineral (rock phosphate and K-feldspar) and organic forms which are poorly soluble (Badr, 2006; Drever and Vance, 1994).

Although the circulating water is certainly a major weathering agent, the possible role of the biochemical agents in the weathering of rocks and minerals have received great interest in recent years, especially in soil environments (Hinsinger *et al*., 2001). Many researchers showed that microbes can accelerate weathering of minerals and rocks by producing organic acids, phenolic compounds, siderophores and possibly other metabolites which influence pH and redox conditions. In addition direct contact between bacteria and minerals may be important in mineral alteration reaction, as microbial surfaces can complex with metal ions. Some recent reports

Corresponding Author: O.N. Massoud, Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.

showed that silicate dissolving bacteria played a promotion role in the release of Si, Fe and K from feldspar (Badr, 2006; Hinsinger *et al*., 2001; Stryiakova *et al*., 2003; Duponnois *et al*., 2005).

Numerous studies have identified microbial groups which could solublize mineral phosphate and improved plant phosphorus nutrition (Gadd, 1999; El-Tarabily *et al*., 2008). One of these microorganisms are arbuscular mycorrhizal fungi (AM) which have been found to be essential components of sustainable soil-plant system (Schriener *et al.*, 2003), they increased plant uptake of phosphorus and nitrogen. Furthermore, Plants inoculated with AM fungi utilized more soluble phosphorus from rock phosphate than uninoculated plants (Antunes and Cordoso, 1991). The main explanation is that mycorrhiza developed an extended mycelium which increased the root P absorbing sites. This mycorrhizal effect has been frequently observed in rock phosphate amended soils and induced good stimulations of the plant growth and phosphorus foliar content (Duponnois *et al*., 2005).

Azospirillum spp. is a well documented member of plant growth promoting rhizobacteria (PGPR), which has a beneficial effect on the symbiosis between *Rhizobium* bacteria and legumes. Many studies demonstrated that *Azospirillum*, when coinoculated with *Rhizobium*, has the ability to enhance nodulation and nitrogen fixation of several legumes, including common bean. Under field conditions co-inoculation has shown potential to increase grain yield of various legumes (Remans *et al*., 2008).

Therefore, the main objective of this study is to evaluate the role of biofertilizers (symbiotic and asymbiotic N_2-fixers, *Bacillus circulans* and AM fungi) and the alternative fertilizers (rock phosphate and feldspar), as well as their interaction, on growth and yield of Snap bean cultivar in a field trials.

MATERIALS AND METHODS

Soil:

The experiments were carried out in a private farm located in Kafer Dawood, Menoufiya governorate, Egypt. The soil texture of the experimental area was sandy clay loam, the organic matter content was 1.12%, soil pH reached 7.85 and soil salinity (EC) was 1.87 dS m^{-1}.

Seeds:

Phaseolus vulgaris L. seeds Snap bean cultivar were provided by Unit, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt.

Inocula Preparation:

Asymbiotic N_2 Fixers:

Azospirilum lipoferum and *Azotobacter chroccocum* were obtained from the microbiology department, Agriculture Research Center, Giza, Egypt. The two strains were grown on semisolid malate (Dobereiner *et al*., 1976) and modified Ashby's (Abdel Malak and Ishac, 1968), respectively. The cultures were then mixed together in equal volumes for field inoculation.

Symbiotic N_2 fixers:

Rhizobium sp. was obtained from the microbiology department, Agriculture Research Center, Giza, Egypt. It was grown on yeast extract mannitol medium (YEM) for 5 days at 28°C (Vincent, 1970).

B. circulans:

was obtained from the microbiology department, Agriculture Research Center, Giza, Egypt. It was inoculated It 250 ml conical flasks that possessed 100 ml Aleksandrov's medium (Zahra, 1969) for 4 week at 28°C and then, enriched on nutrient broth medium (Difco, 1985) for 48 hours at 28°C. Inocula of symbiotic, asymbiotic and *B. circulans* (1x 10^8 cell/ml) were added by spraying on hills. One added directly after sowing and the other at 15 days after sowing according to each treatment.

AM Mycorrhiza:

The AM–fungal inoculum was prepared as described by Massoud (1999). Mixed surface sterilized spores of AM – genera via, *Glomus, Gigaspora* and *Acaulospora* were prepared after propagation and mixed with sterilized peat as a carrier (200 spore/ g).Then adhesion using sticker such as Arabic gum and uniformly coated on the seeds (70 spores/ seed) and air dried for 2 hours before planting.

Fertilization:

Nitrogen was applied in the form of urea (46.5%N), phosphorus was applied in the form of super phosphate (15%) while potassium was added in the form of potassium sulphate (48%). Application of NPK was carried at the rate of one third of the recommended dose (40 kg N, 30 kg P_2O_5 and 17 kg K_2SO_4).

Feldspar (orthoclase) was obtained from location represents the sediments of potash feldspar in eastern desert of Egypt between Ras Benas and Quseir. Rock phosphate was obtained from the new valley project. All samples were ground and sieved and 250 µm size fractions were collected. The percent of potassium oxide (K_2O_5) in feldspar was 11.25% and the percent of P_2O_5 in rock phosphate was 30%. Rock phosphate and feldspar were mixed with soil before planting, whereas NPK were divided into three doses periodically.

Field trials:

This study was conducted over two successive seasons during 2006-2007 and 2007-2008. The experimental treatments were arranged in complete randomized plots design with three replicates as the following:

1. Control (full NPK).
2. Rock phosphate (Rp) + feldspar (Fs).
3. Rp + Fs+ AM-mycorrhiza (AM).
4. Rp + Fs+ Rhizobium (R) symbiotic N_2 fixers.
5. Rp + Fs+ Asymbiotic N_2 fixers (*Azospirilum lipoferum* and *Azotobacter chroccocum*).
6. Rp + Fs+ *B. circulans* (B.c).
7. Rp + Fs+ B.c +AM +R (symbiotic N_2 fixers).
8. Rp + Fs+ B.c +AM + Asymbiotic N_2 fixers.
9. Mixture of all (rock phosphate + feldspar + AM-mycorrhiza + *B.circulans* + symbiotic (*Rhizobium*) and asymbiotic N_2 –fixers).

Each experimental plot consisted of 6 ridges, each of 5 m long and 50 cm wide (15 m^2). The treated seeds were sown when field soil reached appropriate moisture content. After three weeks growing plants were thinned to one plant / hill.

At 30 and 60 days the bacterial population dynamics in the rhizosphere of the plants, the root nodules (number / plant) were recorded. Furthermore, the nitrogenase enzyme activity in plant root nodules was measured as acetylene reduction activity (ARA) by GC analysis according to (Somasegaran and Hoben, 1994), the dehydrogenase activity in the rhizosphere of snap bean plants was determined according to the method described by (Skujins, 1976) and the infection percentage of AM (%) fungi in plant root tissues were also determined at 30 and 60 days. However, the spore numbers of AM mycorrhizal in soil, the analysis of percentage of total nitrogen, phosphorous and potassium in the shoot dry matter of snap bean plants as well as in pods were recorded according to (Jackson, 1958) at harvest. In addition, the plant height (cm/plant), the number of branches / plant, the pods fresh weight (g/plant) and the pods yield (ton/fed) were recorded also at harvest. The data were analyzed for statistical significance using analysis of variance (ANOVA) test according to (Duncan, 1955).

RESULTS AND DISCUSSION

Microbiological Parameters:

The obtained data in Table (1) clearly indicate that, in both experiments during two successive seasons 2006 and 2007, the effect of rock phosphate + feldspar + AM-mycorrhiza + *B.circulans* + symbiotic (*Rhizobium*) and asymbiotic N_2 –fixers (Mixture of all) gave marked increase in values of N_2-fixers populations where the count of *Azospirillum* sp. recorded 6.4×10^6 and 13.6×10^6 CFU /g soil at 30 and 60 days of planting during season 2006. Whereas in 2007 the count of *Azospirillum* sp. increased to reach 7.1×10^6 CFU /g soil at 30 days and 14.50×10^6 CFU at 60 days of planting. All other treatments exhibited less counts and the least numbers of *Azospirillum* recorded with NPK treatment that was $(0.1, 0.15) \times 10^6$ CFU and $(0.11, 0.19) \times 10^6$ at 30 and 60 days respectively. The same trend obtained with the count of *Azotobacter* that recorded slightly counts less than *Azospirillum* sp. However, the mixture of all treatments recorded highly count members of *B. circulans* at 60 days during the two seasons,respectively (15.9×10^6 CFU/g and 17.80×10^6 CFU /g soil) more than asymbiotic N_2-fixers.

Nitrogenase Enzyme Activity:

Data in Table (2) show the nitrogenase activity (µ mole C_2H_4 h^{-1} g^{-1}dry nodule). The highest activity was significantly recorded with the treatment (mixture of all) that included the inoculation of snap bean plants with rock phosphate + feldspar + AM-mycorrhiza + *B.circulans* + symbiotic (*Rhizobium*) and asymbiotic N_2 –fixers. It recorded 5.613 and 5.851 µ mole C_2H_4 h^{-1} g^{-1}dry nodule after 30 days. While they were 51.0 and 50.310 µ mole C_2H_4 h^{-1} g^{-1}dry nodule after 60 days in the two seasons respectively. The least values of N_2- ase activity exhibited with control (full NPK) and other treatments recorded less N_2-ase activity values. Comparing the treatment mixture of all which recorded the best N_2- ase activity to control, data showed that it increased by ~16 and 4.5 fold at 30 and 60 days during season 2006. While N_2- ase activity increased by 14.3 and 4.5 fold at 30 and 60 days during season 2007.

Concerning the number of nodules $plant^{-1}$, data in Table (2) showed that the significant highest values of nodules number were recorded with the treatment mixture of all. It gave the values 85.0 and 121.0 nodules number at 30 and 60 days in the first season where at the second season they gave 87.0 and 125.0 nodules number at 30 and 60 days, respectively. Moreover, the other treatments gave less value. It was markedly noticed that NPK (control) treatment recorded the least numbers of nodules at all.

Dehydrogenase Enzyme Activity:

The results in Table (2) showed the positive effect of biofertilizers on dehydrogenase activity in the rhizosphere of snap bean plants cultivated in soil treated with rock phosphate and feldspar. The combined treatment (mixture of all) recorded the highest values of dehydrogenase enzyme activity compared to control and other treatments. The significantly increased values recorded were 35.40 and 63.70 µg TPF (tri-phenylformasan) at 30 and 60 days during season 2006. On the other hand, it recorded 46.10 and 100.30 µg TPF at 30 and 60 days, respectively during season 2007.

Am-mycorrhizal Infiction (%) and Number of Spores:

Data showed that the highest infection percentage of AM-mycorrhiza was obtained in the treatment mixture of all followed by the treatment (RP + Fs + B. c + AM + Rh.) at 30 and 60 days of planting during the two seasons 2006 and 2007 respectively Table (3). The results recorded 94 and 100% infection % during 2006 at 30 and 60 days, while during 2007 they recorded 100% in both 30 and 60 day of planting in the treatment mixture of all. The obtained result of treatment (RP + Fs + B. c + AM + Rh.) seemed not markedly different during the two seasons respectively.

Concerning the number of AM mycorrhizal spores number after harvest, data revealed that there was a significant increase in number of spores in the treatment mixture of all compared with control. It recorded 257 and 341 of spore number per gram soil during 2006 and 2007, respectively. It was noticed that, the number of spores in the second season was higher than it in the first one, it increased up to1.31 fold.

Macronutrients:

The NPK analysis of shoot dry matter of snap bean plants showed that as a result of addition of alternatives and the viability of AM. mycorrhizae fungi and *B. circulans,* there was marked increase in phosphorus and potassium solublization. They recorded significant highest values of phosphorus and potassium with the treatment mixture of all (0.4 P% and 3.2 K% in 1^{st} season; 0.41 P% and 3.9 K% in 2^{nd} season. While N% was 4.5 and 4.8% in both two seasons 2006 and 2007, respectively (Table 4).

Regarding the values of N, P and K in pods, data in Table (5) revealed that there was a significant increase in NPK values in pods, especially in the treatment mixture of all that exhibited better results than the control as well as the other treatments in general. They recorded 4.4 and 4.2 % nitrogen, 0.64 and 0.61% phosphorus and 3.7 and 3.8% potassium in both seasons, respectively. Data of NPK analysis revealed that the values of NPK % almost were the same in pods and in plant.

Growth Parameters:

Data presented in Table (6) showed that the plants inoculated with biofertilizers and AM-mycorrhizal fungi in soil amended with natural alternatives of mineral fertilizers like rock phosphate and feldspar significantly increased plant height, and the highest length of snap bean plants recorded with the treatment mixture of all that

included inoculation the plants with symbiotic and asymbiotic N_2 –fixers + AM mycorrhizae + *B. circulans* compared with control. It gave 66.0 cm / plant and 67.5 cm / plant during two successive seasons 2006 and 2007 respectively. While all other treatments including control exhibited less results.

The same trend recorded with number of branches plant $^{-1}$. They were 12 and 13 branch during the two successive seasons. Regarding to the weight of fresh pods data confirmed the success in use of alternatives fertilizers and / or biofertilization to increase the weight values of fresh pods where the highest weight obtained was with the treatment mixture of all through the two successive seasons being 230 and 233 g plant $^{-1}$. Other treatments including control involved less weight. The treatment mixture of all increased the fresh weight of pods per plant by 1.1 and 1.06 fold over control in two seasons, respectively.

Snap Bean Yield:

Data presented in Table (6) showed a significant effect on fresh pod yield in the two experiments in both seasons. The positive impact of biofertilizers and fertilizers alternatives represented in the treatment mixture of all. It increased significantly the fresh pods yield compared with control and other treatments in both two seasons. The values were 7.36 and 7.46 ton fed $^{-1}$ in both two seasons respectively, whereas control recorded 6.72 and 7.04 ton fed $^{-1}$ in the seasons two season, respectively. This treatment increased the productivity by 1.1 fold over control in both seasons.

Table 1: Effect of inoculation with AM- fungi, N_2- fixers, and *B. circulans* on assimilation of rock phosphate and feldspar and their impact on bacterial populations in the rhizosphere of snap bean.

Treatments	Total count of bacteria (CFU x 10^6/g soil)											
	Azospirillum sp.				*Azotobacter sp*				*Bacillus circulans*			
	1st Season		2nd Season		1stSeason		2nd Season		1stSeason		2nd Season	
	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d
Full NPK	0.10	0.15	0.11	0.19	0.02	0.03	0.02	0.05	0.21	0.29	0.21	0.33
Rp + Fs	0.17	1.70	0.17	1.90	0.02	0.06	0.02	0.07	0.24	1.30	0.25	1.58
Rp + Fs +AM. Mycorrhizae	0.21	2.10	0.22	3.20	0.02	0.90	0.02	0.95	0.28	0.20	0.29	2.40
Rp + Fs + Rhizobium	0.33	2.20	0.34	2.50	0.93	0.95	0.92	0.97	0.31	2.30	0.32	2.50
Rp + Fs +Asymbiotic	0.24	2.60	3.25	2.70	0.02	1.10	0.02	0.95	0.24	2.90	0.25	3.50
Rp + Fs +B. circulance	0.31	1.80	0.32	2.00	0.03	0.80	0.04	0.90	0.33	4.90	0.35	5.40
Rp + Fs + B.c +AM + Rh.	0.28	2.10	0.30	2.40	0.24	1.30	0.26	1.10	1.60	5.10	1.70	5.40
Rp + Fs + B.c +AM + Asymbiotic	0.52	12.00	0.53	11.00	0.35	9.20	0.36	12.00	3.50	14.00	3.50	16.00
Mixture of all	6.4	13.60	7.10	14.50	1.34	11.30	2.1	13.10	4.60	15.90	5.10	17.80

d = time in day, CFU: colony forming unit

Table 2: Effect of inoculation with AM- fungi, N_2- fixers, and *B. circulans* on assimilation of rock phosphate and feldspar and their impact on nitrogenase, dehydrogenase activity and number of nodules in the rhizosphere of snap bean plants.

Treatments	No.of nodules/plant				Nitrogenase activity (μ mole C_2H_2/h/nod.)				Dehydrogenase activity (µg TPF/g dry soil/day)			
	1st Season		2nd Season		1st Season		2nd Season		1st Season		2nd Season	
	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d	30 d	60 d
Full NPK	10.0	25.0	15.0	31.0	0.351	11.310	0.410	11.300	23.70	40.90	33.40	75.80
Rp + Fs	35.0	42.0	35.0	53.0	1.031	17.601	1.231	18.000	15.90	29.90	21.10	42.20
Rp + Fs + AM. Mycorrhizae	41.0	47.0	43.0	59.0	1.510	22.315	1.731	27.610	22.70	34.50	32.30	49.40
Rp + Fs + Rhizobium	66.0	97.0	67.0	100.0	2.735	30.310	3.227	30.000	25.90	30.30	26.30	80.60
Rp + Fs + Asymbiotic	53.0	66.0	53.0	73.0	2.780	33.110	3.120	37.601	21.60	37.90	26.80	52.10
Rp + Fs + B. circulance	45.0	58.0	48.0	63.0	1.150	15.610	1.251	14.310	23.40	40.90	28.10	45.20
Rp + Fs + B.c + AM + Rh.	81.0	111.0	85.0	117.0	3.712	41.210	4.201	45.000	33.20	48.10	42.20	59.90
Rp + Fs + B.c + AM + Asymbiotic	70.0	81.0	71.0	91.0	3.431	47.651	3.921	50.120	21.80	58.50	23.30	88.10
Mixture of all	85.0	121.0	87.0	125.0	5.613	51.000	5.851	50.310	35.40	63.70	46.10	100.3
$L.S.D_{0.05}$	8.80	7.75	6.00	6.11	0.44	3.83	1.26	7.06	2.67	5.08	4.01	9.62

d = time in day, TPF: tri phenylformasan

Table 3: Effect of inoculation with AM- fungi, N_2- fixers, and *B. circulans* on assimilation of rock phosphate and feldspar and their impact on AM infection of snap bean roots and number of spores in the soil.

Treatments	AM infection (%)				No. of spore after harvest/g soil	
	1st Season		2nd Season		1st Season	2nd Season
	30 d	60 d	30 d	60 d	30 d	60 d
Full NPK	30	50	40	50	9	13
Rp + Fs	40	50	43	53	37	59
Rp + Fs + AM. Mycorrhizae	80	78	85	83	169	256
Rp + Fs + Rhizobium	50	65	60	65	69	85
Rp + Fs + Asymbiotic	40	64	60	74	67	88
Rp + Fs + B. circulance	55	72	65	75	43	50
Rp + Fs + B.c + AM + Rh.	71	100	100	100	200	311
Rp + Fs + B.c + AM + Asymbiotic	80	90	85	93	227	335
Mixture of all	94	100	100	100	257	341
L.S.D $_{0.05}$	10.56	8.56	6.89	5.94	4.47	9.84

d = time in day

Table 4: Effect of inoculation with AM- fungi, N_2- fixers and *B. circulans* on assimilation of rock phosphate and feldspar and their impact on NPK% in snap bean plants.

Treatments	1st Season			2nd Season		
	N %	P%	K%	N%	P%	K%
Full NPK	3.50	0.31	2.10	3.90	0.35	3.40
Rp + Fs	3.30	0.25	2.20	3.40	0.27	3.50
Rp + Fs + AM. Mycorrhizae	3.40	0.36	2.10	3.80	0.31	3.40
Rp + Fs + Rhizobium	3.60	0.23	2.00	3.80	0.32	3.60
Rp + Fs + Asymbiotic	3.90	0.28	2.60	3.90	0.29	3.90
Rp + Fs + B. circulance	3.90	0.27	2.00	4.20	0.35	3.50
Rp + Fs + B.c + AM + Rh.	4.30	0.38	2.00	4.70	0.38	3.00
Rp + Fs + B.c + AM + Asymbiotic	3.60	0.35	2.90	3.70	0.37	3.10
Mixture of all	4.50	0.40	3.20	4.80	0.41	3.90
L.S.D $_{0.05}$	0.47	0.05	0.51	0.27	0.06	0.46

N: nitrogen, P: phosphrus,K: potassium .

Table 5: Effect of inoculation with AM- fungi, N_2- fixers and *B. circulans* on assimilation of rock phosphate and feldspar and their impact on NPK % in pods of snap bean plants.

Treatments	1st Season			2nd Season		
	N%	P%	K%	N%	P%	K%
Full NPK 3.80	0.64	2.90	4.20	0.54	3.50	
Rp + Fs 3.03	0.32	2.20	2.90	0.44	3.00	
Rp + Fs + AM. Mycorrhizae	3.10	0.52	3.00	3.80	0.59	3.50
Rp + Fs + Rhizobium	3.60	0.59	3.10	3.80	0.57	3.20
Rp + Fs + Asymbiotic	3.40	0.50	2.90	3.50	0.47	3.00
Rp + Fs + B. circulance	3.60	0.44	2.70	4.03	0.56	3.50
Rp + Fs + B.c + AM + Rh.	4.20	0.35	2.90	4.10	0.51	3.60
Rp + Fs + B.c + AM + Asymbiotic	3.50	0.51	3.30	3.60	0.49	2.80
Mixture of all	4.40	0.64	3.70	4.20	0.61	3.80
L.S.D $_{0.05}$ 0.31	0.04	0.24	0.42	0.02	0.47	

N: nitrogen, P: phosphrus,K: potassium .

Table 6: Effect of inoculation with AM- fungi, N$_2$- fixers, and *B. circulans* on assimilation of rock phosphate and feldspar and their impact on growth characters and snap bean yield.

Treatments	Growth characters				Yields					
	Plant height (cm/ plant)		No. of branches/plant		No. of fresh pods/ plant		Fresh Wt. of pods/ plant		yields ton/fed.	
	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season	1st Season	2nd Season
Full NPK	58	59	7	7	43	45	210	220	6.72	7.04
Rp + Fs	42	41	5	6	25	26	141	148	4.5	4.74
Rp + Fs + AM. Mycorrhizae	47	49.5	7	8	33	36	160	167	5.12	5.34
Rp + Fs + Rhizobium	59	61	9	9	39	39	169	174	5.41	5.57
Rp + Fs + Asymbiotic	61	62.5	9	10	37	38	164	170	5.25	5.44
Rp + Fs + B. circulance	45	47	6	6	34	35	140	147	4.48	4.71
Rp + Fs + B.c + AM + Rh.	65	67	11	12	42	44	210	219	6.72	7.01
Rp + Fs + B.c + AM + Asymbiotic	62	64	11	12	41	44	206	212	6.59	6.78
Mixture of all	66	67.5	12	13	45	48	230	233	7.36	7.46
L.S.D $_{0.05}$	3.08	2.54	1.40	1.72	2.43	2.29	4.28	3.71	0.17	0.16

Discussion:

Current trends in agriculture are focused on reduction in the use of chemical pesticides and inorganic fertilizers, inducing the search for alternatives that enhance environmental quality (Hameeda *et al*., 2006).

The inoculation of snap bean with the biofertilizers (symbiotic and asymbiotic N_2-fixers, *Bacillus circulans* and AM fungi) and the alternative fertilizers (rock phosphate and feldspar), as well as their interaction, improved the plant development and productivity as indicated by increasing all the tested parameters. The improvement may be as a result of the important role of these microorganisms in improving soil fertility and plant development i.e. nitrogen fixation, symbiotic (*Rhizobium* spp.) and asymbiotic (*Azospirillum, Azotobacter* and *Bacillus* spp.) and releasing certain nutrient elements (P, Fe, Zn, Mn and K) in addition to contributing with some plant growth substances (Abd El-Mageed *et.al*., 2004,).

In the meantime, both rock phosphate and feldspar as natural fertilizers alternatives had great effect on growth and yield of snap bean. Also, data indicated that growth responsibility and yield quantity were improved as a result of the interaction between bio and natural fertilizers that had significant effect on most characters and this may be due to the reaction of these microorganisms. It is now well established that AM- fungi modify root function (i.e. root exudation) (Marshner *et al*., 1997). Furthermore, AM- fungi can exude substances that have a selective effect on the microbial community in the rhizosphere and in the soil (Linderman, 1988). AM fungal colonization of plant roots can affect bacterial communities associated with these roots directly and indirectly. Direct interactions include provision of energy-rich carbon compounds derived from host assimilates, which are transported to the mycorrhizosphere via fungal hyphae, changes in pH of the mycorrhizosphere induced by the fungus, competition for nutrients, and fungal exudation of other inhibitory or stimulatory compounds. Indirect interactions can also take place in the form of mycorrhiza mediated effects on host plant growth, root exudation, and soil structure (Johansson *et al.*, 2004).

Rock phosphate and feldspar can be solubilized or weathered under the influence of physical and biological agents (Hinsinger *et al*., 2001; Duponnois *et. al*., 2005). Biological weathering or biochemical weathering is made by microorganisms which produce organic acids, phenolic compounds, protons and siderophores (Duponnois *et. al*., 2005). Soluble organic acids affecting rock phosphate weathering in soils could be of high molecular weight (humic substances) or low molecular weight produced by plant roots and soil microorganisms (Ochs, 1996; Duponnois *et. al*., 2005). These low molecular weight organic acids produces by plant roots and soil microorganisms are very effective in promoting mineral dissolution.

Mycorrhizal fungi can solubilize surrounding weatherable minerals through excretion of organic acids such as a-ketoglutaric acid. This organic compound could exert a selective influence on soil microbial communities though a multiplication of a-ketoglutarate catabolizing microorganisms (Duponnois *et. al*., 2005). Soil microorganisms using organic acids excreted by AM- fungi, could also act as solublizing agents against rock phosphates and feldspar. The results obtained proved that K% was increased in *phaseolus* plant which consequently improved the plant growth and yield. This improvement could be explained on the bases that, the main source of K for plants growing under natural conditions comes from the weathering of K minerals (e.g. feldspar) and organic K-sources such as composts and plant residues. On the other hand the weathering process can be further mediated by organisms and their metabolites through respiration of plant roots and microbial degradation of organic matter which can elevate carbonic acid concentrations in the soils and ground water, leading to an increase in the weathering rates of minerals. In addition to carbonic acid, direct contact between bacteria and minerals may be important in mineral alteration reaction, as microbial surfaces can complex with metal ions. Microorganisms attached to mineral surfaces can also create micro-environments where concentrations of acidity and redox activity can be substantially elevated (Badr, 2006).

The above results clearly showed that AM- fungi inoculation combined to different nitrogen fixers and *B. circulans* is highly beneficial to the growth of snap bean plants. This combination optimizes the P and K-solubilization from the rock phosphate and feldspar and increased microbial activity in the rhizosphere of snap bean plants. So, from our obtained results it was concluded that, the weathering of rock phosphate and feldspar by AM-mycorrhizal fungi and *B. circulans* bacteria led to release of P and K ions that led to encouragement the snap bean growth and consequently the diverse of rhizospheric microflora.

So, our study supports the new trend of using biofertilizers and chemical alternatives as beneficial cheap sources of fertilization for sustainable agriculture.

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