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EFFECT OF BIO-INOCULATION, COMPOST AND FARMYARD MANURE ADDITION ON COMMON BEAN PLANT

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ABSTRACT

Field trial was conducted during season 2010 at Itay El-Baroud Center Bohera Governorate, to study the effect of bio-inoculation of common bean (*phaseolus vulgaris L,*) cv. Montano (a one of export variety) with *Rhizobium phaseoli, Thiobacillus thioparus and Bacillus megatherium v. phosphaticum* with or without organic manure as comparison between (Farmyard manure and compost by rate 10m^3/fedd. of each). Also soil was amended with agricultural sulfur and milled rock phosphate 50kg/fedd. as separately or combined treatments. Plant characteristics were measured as means of true nodule formation numbers, plant heights, no. of pods, fresh and dry weights of each character as well as NPK content of whole plant treatment. The obtained results revealed the positive effect of combined treatments then separately. Organic manure combined with three bacterial inoculants as well as soil amendments by sulfur and milled rock phosphate gave the best results compared with the other treatments. However, compost treatments as combined were better than farmyard manure. Also NPK of whole plant contents was higher amount due to combined treatments than separately. From these results could be of value as for productivity and reduction of pollution recommended that application of a bio-organo-fertilizer for common bean production.

Keywords: *Rhizobium phaseoli, Thiobacillus thioparus, Bacillus megatherium* v. *phosphaticum* common bean, nodulation, farmyard manure, compost, NPK plant uptake

INTRODUCTION

Rhizobium phaseoli has a special consideration in atmosphere nitrogen fixation by symbionate with common bean and servese NPK uptake. On the other hand *Thiobacillus thioparus* work on sulfur oxidation to release sulfate to plant and increase soil fertility. However phosphate dissolving bacteria *(Bacillus megatherium v. phosphaticum*) have important role in phosphorus availability and plant uptake. (Elkotkat 1993, Shafei 1991, Attia and Eldosuky1996, Koreiche; et.al.2004, Elsebaay and Elkotkat 2007). Organic fertilizers improve soil fertility and supply common bean plants by nutrients (Ibrahim 1993, Elsebaay 2004, Piquese *et al.*; 2006, Stark, *et al.*; 2007 and Elsebaay and Elkotkat 2007). Also it enhance of rhizosphere microorganisms proliferation and activation, which effect on rock phosphate solubilization and agricultural sulfur oxidation as well as availability of NPK and Ca uptake by plant were observed with fungus, bacteria and actinomycetes associated with rhizospheric zone (Toro *et al* 1996, Bayoumi *et al.* 1997, Sonboir and Sarawgi 1998, Al-Karaki 1999 , Elkotkat 1993 and Madhaiyan *et al.*, 2009). In this object some bacterial group have positive were more effective on atmospheric nitrogen fixation symbiotic or non-symbiotic on plant yield (Bayoumi *et al.* 1997, Uzbek and kalataevsky 1998,Buhlyan *et al.*; 1998, Al-karaki 1999, Vilimiene *et al.*; 2000 and Campo and Hungria 2002).

The aim of this investigation is to study the effect of common bean inoculation with (*Rhizobium phaseoli, Thiobacillus thioparus* and *Bacillus megatherium v. phosphaticum*) addition soil amendments by farmyard manure and / or compost as well as addition of agricultural sulfur and rock phosphate milled as separately or combined, on common characteristics without any mineral fertilizers addition. However depend on microbial unlimited activation with organic manure only to enough plant nutrition requirements as well known a bio-organo-agriculture farming system.

MATERIALS AND METHODS

Bacterial isolation and characterization:

Rhizobium phaseoli; common bean Rhizobial group was isolated from host common been plants by selection numbers of true nodules. After washing and surface sterilization by immersion in mercuric chloride solution 1/1000 it. Then rinsed by sterilized water, under aseptic condition crushed in Petri dish contain 5ml sterilized water to make cell suspension of Rhizobia and streak on (YEM) medium, incubated at 30 c^0. A pure colony was picked and re-streaked on the same medium more time with add 0.002g/l Congo red to avoid from related soil bacterial groups as *Agrobacterium sp.* .The result colonies obtained were examined by light microscope, Gram stain to confirm purification , after that Koch's postulates applied to insure that isolates (5 isolates) belong to common bean Rhizobial group (*Rhizobium phaseoli)* and (choice the effective it on nodule formation) with host to complete study. Inoculum of Rhizobia ; a pure mother culture was inoculated in jars 250ml contain 100ml/jar of (YEM) broth medium and stationary incubated at 30c° for 72 hours mean cells /ml up to ($375x\ 10^6$) nearly.

Thiobacillus thioparus ; sulfur oxidizing bacteria as chemolithotrophic was isolated , identified from Egyptian soil also purified and efficiency tested in former studies by the authors as microbiological protocol (Kshiragar *et al* 2004). For thiobacterial inoculum production was done by inoculation pure culture of *Thiobacillus thioparus* in flasks 250ml of thiosulfate broth medium a modified by add yeast extract 2 mg /l and sodium thiosulfate by rate 5g/l instead of 10g/l to increase biomass bacterial inoculum production . After inoculate flasks were stationary incubated at 28c° for 6 days in this period inoculum cells concentration reached $135x10^6$ /ml nearly .

Bacillus megatherium v. phosphaticum; was isolated from fertile soil by soil dilution then pore plate method was applied by using Bonett & Rovera medium (Elkotkat 1993) The high colony clear zone a rounded was selected then tested microscopically; stained and identified it is belong to *Bacillus megatherium v. phosphaticum* according to(Kshiragar *et al.*, 2004).

Table (1): physico- chemical properties of the experiment soil.

Soil property	value
Physical :	
sand %	18.71
silt %	33.40
clay %	47.89
Texture class	Clayey
chemical :	
Available N ppm	
Available P ppm	
Total carbonate %	2.36
Organic matter %	1.56
Organic carbon %	0.90
CEC meq/100g	49.67
pH 1:2.5	7.95
Ec ds/m	2.14
$Hco_3^{=}$ meq/100g	3.55
Co_3^{-} meq/100g	0.0
Cl^{-} meq/100g	4.21
$SO_4^{=}$ meq/100g	14.50
Mg^{++} meq/100g	3.5
Ca^{++} meq/100g	5.9
Na^{+} meq/100g	11.65
K^{+} meq/100g	0.66

The inoculum of phosphate dissolving bacteria was prepared by a loop from mother culture and inoculated in sterilized Bonett & Rovera liquied medium modified by add 100ml/lof soil fertile extract(sterilized by filtration) autocleave and cooling. The inoculated medium was incubated at 30c° for four inoculum bacterial cells reached $350x10^6$/ml.

Experiment soil site was designed in Itay El-Baroud Center Bohera Governorate and physeco-chemecal analyzed before sowing (data presented in Table 1). The soil was prepared and divided into plots $2x2m^2$ / plot then planned by rate 12 rows/ two rods also amendments by compost, farmyard manure, rock phosphate milled, agric. sulfure as well as plant seeds coated by different bacterial inoculants separately and/or combination as following treatments:

- Inoculated with *R. phaseoli* alone as base treatment.
- Inoculated with *R. phaseoli*, *T. thioparus* and 50kg agricultural sulfur/fed. only.
- Inoculated with *R. phaseoli*, *B. megatherium v. phosphaticum* and 50kg rock phosphate milled /fed.
- Inoculated with *R. phaseoli*, *T. thioparus*, *B. megatherium v phosphaticum*, and 50 kg sulfur and rockphosphate milled /fed.
- Plants amended with farmyard manure by rate10 m^3/ fed. alone.
- Inoculated with *R. phaseoli* and soil amended by farmyard manure 10 m^3/fed.
- Inoculated with *R. phaseoli* , *T. thioparus*, add 50 kg sulfur/ fed.and farmyard manure by rate 10m3/fed.
- Combined inoculum of *R. phaseoli* , *T. thioparus*, *B. megatherium* + 50 kg sulfur + rock phosphate milled and $10m^3$ farmyard manure /fed.

- Soil amended with compost alone.
- Inoculum of *R. phaseoli* and compost by rate 10m³/ fed. alone.
- Combined inoculum of *R. phaseoli*, *T. thioparus* + 50kg agricultural sulfur and compost10m³ /fed.
- Inoculation combined by *R. phaseoli* , *T. thioparus* , *B. megatherium* + 50kg sulfur + 50kg rock phosphate milled and 10m³ compost/fed.
- Sowing without any bacterial inoculation or soil amendments as control.

Common bean cultivar seeds (Montano) was cultured as previous treatments at March 2010, also farmyard manure was supplied from some farmers and compost was supplied from some station achieved in the same aerie and others to recycling rice wastes. This two types organic manure were used in this experiment and analyzed before application (Table 2).

Table (2): some physic- chemical properties of compost and farmyard manure before application.

Compost		Farmyard manure	
property	value	property	value
		Water content %	3.00
Water content %	46.20	Ec(1:5) mmhos/cm	21.72
Ec (1:5)mmhos/cm	7.21	pH (1:5)	7.12
pH(1:5)	7.10	Total N%	2.06
Total N%	2.40	**Available ions:**	
Organic matter%	52.23	Ca meq/100g	36.8
Organic carbon %	29..32	Mg meq/100g	18.65
C/N ratio	13:0.1	Na meq/100g	17.21
		K meq/100g	89.62
		P (ppm)/100g	105.10
		S (ppm)/100g	195.00

Plant measurements were recorded after 62 days from sowing; plants were uprooted separately for nodule formation and numbers, fresh and dry weights. Plant vegetation measurements were length (cm/plant), fresh and dry weight (g/plant) of root and shoot, fruiting measurements as pods number, fresh and dry weight /plant. Plant chemical determination were done by collection of each plant treatment separately, dried, milled and random sample of each treatment. Plants were digested for determination of NPK according to (Chapman and Pratt, 1967).

RESULTS AND DISCUSION

Rhizosphere microorganisms:

Soil microorganisms in rhizosphere zone have a significant role in the availability of plant nutrients, plant growth promoter's secretion and other substance. Soil organic or inorganic amendments as well as bio-inoculants as N2 fixing, sulfur oxidizing and phosphate dissolving bacteria separately or combined with or without soil improvements. They are affective on rhizosphere microorganisms proliferation and activation these are reflect on healthy and productivity of the plant (Elsebaay 2000, Elsebaay and Elkotkat 2007 and Ibrahim 1993). Also addition of organic matter supports microbial growth for longer periods resulting in greater stimulation of sulfur oxidation (Peper and Miller 1978).

Plant characteristics:

Data in Table (3) revealed the effect of bacterial inoculation, compost and farmyard manure addition separately and /or combined on common bean plant characteristics. From these results the highest value was recorded in plants treated with compost + *R. phaseoli* +*T. thioparus* + *B. megaterium v. phosphaticum* + 50kg rock phosphate milled + agric. Sulfur 50kg/fed. Which increased dry weight of nodules, roots, shoots and pods were (0.099, 3.220, 48.00and 25.1g/plant respectively). But the second order of positive effect on plant characteristics was obtained in combined treatment by farmyard manure + *R. phaseoli* +*T. thioparus* + *B. megaterium v. phosphaticum* + 50kg rock phosphate milled + agric. Sulfur 50kg/fed. The values of dry weight of nodules, roots; shoots and pods were (0.025, 1.63, 44.9 and 16.8 g/plant respectively). On the other hand the lowest effect was noticed in plants inoculated with *R. phaseoli* only the dry weight was (0.009, 0.165, 9.6 and 9.2 g/plant of nodules, roots, shoots and pods respectively) compared with control and other treatments. These results as main effects are expected because either the added organic manure and/or agric. Sulfur and rock phosphate milled has an important function in improving soil environment for plant growth. Results were in agreement with many authors from those (Elkotkat 1993, Shafei 1991, Attia and Eldosuky1996, and Koreiche; *et al.*, 2004, Elsebaay and Elkotkat 2007. Madhaiyan *et al* 2009). Also some facts proved by earlier investigation where detected that higher amounts of GA and IAA in culture filtrates in test microorganisms under study this results confirmed that previously by (Graham 1981, Buttery *et al* 1987, Ramos and Boddey 1987, Hardarson1993, Ibrahim 1993, Buhlyan *et al* 1998 and Marta-nez-Romero 2003. Madhaiyan *et al* 2009).

Table (3): Effect of bacterial inoculation, compost and farmyard manure addition separately and/or combination on .common bean plant characteristics (mean character per treatment).

Treatments	Plant characteristics											
	Nodules			Roots			Shoots			Pods		
	No.	F. (g)	D. (g)	L. (cm)	F. (g)	D. (g)	L. (g)	F. (g)	D. (g)	No.	F. (g)	D. (g)
control	5	0.013	0.003	8	0.132	0.090	42.3	35.5	8.2.	9.7	15	5.8
R.	12	0.021	0.009	9.5	0.285	0.165	49.6	39.3	9.6	15	16.9	9.2
R+T+ s	12	0.025	0.009	9.6	0.312	0.141	52.3	42.3	10.3	16	18.1	8.3
R+ B +p	16	0.029	0.019	9.9	0.688	0.425	49.5	39.8	10.8	17	20.0	7.91
R+T+B+s+p	19	0.043	0.022	11.3	0.990	0.333	57.8	58.5	15.3	18	21.6	9.8
Farmyard manure	16	0.032	0.019	12.1	0.633	0.432	57.3	58.1	14.8	17	26.3	9.5
Farmyard+R	21	0.032	0.019	14.2	0.899	0.621	68.6	56.3	18.6	32	38.9	14.2
Farmyard+R+T+s	41	0.082	0.026	14.6	2.656	1.800	66.4	62.5	35.5	43	39.2	19.8
Farmyard+T+B+ s+p	32	0.043	0.026	14.8	2.855	1.63	66.2	55.9	44.9	51	45.9	16.8
Compost	22	0.035	0.066	13.5	3.440	0.990	66.5	65.3	45.6	49	39	19
Compost+R	35	0.089	0.036	16.5	3.848	1.756	71.5	59.5	45.6	56	45	18
Compost+R+T+s	38	0.113	0.056	19	3.92	2.80	86.6	66	46	62	47	23
Compost+R+T+B+s+p	39	0.210	0.099	23	5.50	3.220	87.5	72	48.00	65	49.5	25.1

***R = *Rhizobium phaseoli* , T= *Thiobacillus thioparus* ,B= *Bacillus megatherium v. phosphaticum* , F= farmyard manure , P= milled rock phosphate , S= agricultural sulfur.**

NPK plant concentration:

Results in Table (4) showed an increase NPK concentration in whole plant as result of treatments with bio-fertilizer of three inoculants *(R. phaseoli +T. thioparus + B. megaterium v. phosphaticum), compost and/or* farmyard manure as well as 50kg/fedd. agric. sulfur and rock phosphate milled of each. The highest increase in NPK was recorded in plant reserved, compost + *R. phaseoli +T. thioparus + B. megaterium v. phosphaticum* +addition 50kg/fedd. Of each agric. Sulfur + rock phosphate milled the elements were (4.523, 3.541 and 6.321% respectively) compared with control and other treatments. On the other hand combined treatment with farmyard manure + *R. phaseoli +T. thioparus + B. megaterium v. phosphaticum* +addition 50kg/fedd. Agric. sulfur and milled rock phosphate was found in the second order the elements they were (3.562, 1.981 and 3.561% respectively). While the lowest treatment was base treatment (sowing inoculated with *R. phaseioli* alone) the recorded values were (2.8, 0.280 and 1.9% respectively) compared with control and other treatments. separately or combined the highest values of NPK percent record were, 1.495+2.091 and 2.061 of compost+ Rhizobium + Thiobacillus+ sulfur addition alone it is followed by compost + Rhizobium + Thiobacillus+ sulfur and Bacillus + rock phosphate (1.062,1.751 and 2.061 of NPK respectively) compared with control .

Table (4): Effect of bacterial inoculation, compost and farmyard manure addition separately and/or combination on NPK common bean plant concentration (mg/100g dry weight).

Treatments	Plant composition					
	N%	Gain due to	P %	Gain due to	K %	Gain due to
control	2.5	0.0	0.230	0.0	1.5	0.0
R.	2.8	0.3	0.280	0.050	1.9	0.4
R+T+ s	2.76	0.26	0.350	0.120	1.982	0.482
R+ B +p	2.78	0.28	0.285	0.055	1.75	0.25
R+T+B+s+p	2.98	0.48	0.884	0.654	2.93	1.43
Farmyard manure	2.85	0.35	0.991	0.761	2.44	0.94
Farmyard manure +R	3.21	0.71	0.991	0.761	3.25	1.75
Farmyard manure +R+T+s	3.995	1.495	2.321	2.091	3.561	2.061
Farmyard manure T+B+s+p	3.562	1.062	1.981	1.751	3.561	2.061
Compost	2.95	0.45	1.925	1.695	2.983	1.483
Compost + R	3.85	1.35	2.530	2.695	3.65	2.15
Compost+R+T+S	4.21	1.71	3.221	2.991	4.321	2.821
Compost+ R+T+B+s+p	4.523	2.02	3.541	4.355	6.321	4.821

*R = *Rhizobium phaseoli* , T= *Thiobacillus thioparus* ,B= *Bacillus megatherium v. phosphaticum* , F=farmyard manor , P= milled rock phosphate , S= agricultural sulfur.

Also refer to role of bio-inoculants and organic matter in availability of micro and macro plant nutrients these agreements with many authers as, (Hardarson 1993, Sonboir and Sarawgi 1998, Elsebaay 2000, Elsebaay and Elkotkat 2007 Ibrahim 1993 and Madhaiyan *et al* 2009).

Results showed that organic fertilizer combined with biofertilizer was better than biofertilizer alone. Moreover soil amendments with compost combined with biofertilizer (three inoculants) was the best and effect from other treatments and control. These results were supported by (Elsebaay

2000, Elsebaay and Elkotkat 2007, Ibrahim 1993, Hardarson 1993, Sonboir and Sarawgi 1998 and Campo and Hungria 2002. Madhaiyan *et al* 2009).

Conclusion

It could be concluded that application of bio-inoculation with *R. phaseoli, T. thioparus* and *B. megatherium var. phosphaticum* as separately and/or combined with organic fertilizer (compost or farmyard manure) add 50kg/fed. of each agricultural sulfur and rock phosphate milled gave the highest values of common bean plant characteristics as a bio-organo-agriculture production system.

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تأثير التسميد الحيوى واضافة الكومبست والسماد البلدى على نبات الفاصوليا

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أجريت تجربة حقلية عام 2010م فى إيتاى البارود بحيرة لدراسة تأثير التلقيح باحسن عزلة بكتيريا ريزوبيوم فاصولاى *Rhizobium phaseioli* من خمس عزلات تم عزلها من عقد صادقة لنبات الفاصوليا واختبار قدرتها فى تكوين العقد الجذرية ذات الكفاءة مرة اخرى وانتماؤها لمجموعة ريزوبيوم الفاصوليا .بالإشتراك مع لقاح بكتيريا أكسدة الكبريت ذاتية التغذية كيماوية الطاقة (ثيوباسيلس ثيوبيرس *Thiobacillus thioparus*) ولقاح البكتيريا المذيبة للفوسفور (باسيلس ميجاتيريوم *Bacillus megatherium v. phosphatecum*) بالإضافة الى التسميد العضوى بى (السماد العضوى الصناعى compost بمعدل 10م^3 للفدان و السماد البلدى بمعدل 10م^3 للفدان مع اضافة 50كجم للفدان مطحون صخر الفوسفات أيضا الكبريت الزراعى بمعدل50كجم للفدان) وذلك فى صورة معاملات فردية أو مشتركة مع معاملة الأساس (التلقيح بعقدين الفاصوليا) للإستفادة من النشاط الحيوى اللامحدود للكائنات الدقيقة فى توفير غذاء النبات بدون تسميد معدنى مع المحافظة على خصوبة التربة .

وأوضحت نتائج البحث الى ايجابية تأثير المعاملات المختلفة (الفردية والمشتركة) بدرجات مختلفة بالمقارنة بالكنترول ومعاملة الأساس. وبصفة عامة كانت معاملات التسميد الحيوى المشترك مع إضافة السماد العضوى الصناعى (الكومبست)الأفضل فى معدلات قياسات النبات تكوين العقد على جذر النبات وكذلك زيادة تركيز النتروجين والفوسفور والبوتاسيوم فى أجزاء النبات . كما كانت أقل المعاملات هى معاملة الأساس (التلقيح بالريزوبيوم منفردة) مقارنة بالكنترول . ايضا تفوق التسميد العضوى الصناعى (الكومبست) على السماد البلدى لكن بصفة عامة كان التسميد العضوى افضل على الاطلاق من التسميد الحيوى بمفردة بالمقارنة بمعاملة الأساس والكنترول.

ويوصى البحث الى تطبيق نظام التسميد الحيوى المشترك بالريزوبيوم والثيوباسيلس وبكتيريا تيسير الفوسفور مع إضافة السماد العضوى الصناعى (الكومبست) أفضل من السماد البلدى من أجل زيادة الإنتاج النباتى بنظام الزراعة الحيوية العضوية بدون تسميد معدنى من أجل المحافظة على خصوبة التربة فى ظل التكثيف الزراعى مع خفض معدلات تلوث التربة والماء والهواء و الحفاظ على الصحة العامة .

قام بتحكيم البحث

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