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INFLUENCE OF INOCULATION BY *rhizobium phasoli* AND *Thiobacillus thioparus* WITH MOLYBDENUM AND MANGANESE ON NODULATION, RHIZOSPHERE ACTIVITY AND GROWTH UNDER ORGANIC-INORGANIC SOIL AMENDMENTS OF COMMON BEAN

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ABSTRACT

Pots experiment was carried out 2005 to study the influence seed inoculation of common bean (phaseolus *vulgaris L*,) cv. Bronco by *Rhizobium phaseoli*. Among eight Rhizobium sp. isolates was collected from host common bean plants. Isolates purified, belonging to common bean Rhizobium group and able to true nodules was tested, and the formation most effective of these isolates was chosen to complete this investigation. The inoculation combined with sulfur oxidizing bacteria (Thiobacillus *thioparus)* isolated from Egyptian fertile soil and practically tested. Experiment treatments contain seed inoculation by Rhizobium with or without Thiobacillus, in addition seed treatments by molybdenum separately or with manganese before sowing immediately. Also soil pots amendment by sulfur , gypsum two levels (1 and 2 ton / faddan) , rock phosphate as well as compost by two levels (5 and 10 m^3/ faddan) . Nodulation rate was determined by enumeration of all plant treatments, as well as fresh and dry weight recorded at 25 days from planting. Rhizosphere activity that were determined of three main microorganisms groups i., e. bacteria , actinomycetes and fungi at three different growth stages (seedling , flowering and pod formation) . After 67days from planting (during pod maturity stage), plant vegetative, fruiting and pods number characteristics were tabulated of all treatments as well as chemical determination of total nitrogen, phosphorus and potassium in whole plant mixture. Data obtained revealed that positive effect of all treatments from control by different degrees between it. Also compost treatment was the best from other absolutely and add 10m3/feddan was better than from 5m3/ feddan. In contrast gypsum add by rate one ton/feddan was better than 2 ton /feddan on plant values. In addition, seed treatments with minute concentration of molybdenum with or without manganese promote nodule formation and nitrogen fixation process.

The results of this investigation recommended that application biofertilizer by Rhizobium common bean group combined with sulfur oxidizing bacteria (Thiobacillus *thioparus)* and organic-inorganic soil amendments addition lead to increase plant productivity of common bean

Keywords *Rhizobium phaseoli, Thiobacillus thioparus*, common bean, nodulation, rhizosphere microorganism's activity, molybdenum, manganese, NPK plant uptake.

INTRODUCTION

Rhizobia group is very significant in nitrogen supply by symbiotic leguminous plant, from these common bean (Phaseolus vulgaris L), which consider cash crop. Agriculture sulfur addition and inoculation by *Rhizobium* as well as *Thiobacillus* enhancement nitrogen fixation also NPK uptake Shafei 1991, Attia and Eldosuky1996, and Koreiche; et al., 2004. Organic fertilizer improved that all soil and plant characteristics, increase rhizosphere activity by microorganisms proliferation, also rendering insoluble nutrients available to plant Ibrahim 1993 ,Elsebaey 2004, Piquese et al., 2006 and Stark, et al., 2007 . Increase in rhizosphere activity that, this can be benefit in a solubilize rock phosphate and stimulate nutrient (N.P. K and Ca) uptake was observed with fungus – bacteria associated with rhizosphere .Toro et al., 1996, Bayoumi et al., 1997. Sonboir and sarawgi 1998 and Al-Karaki 1999. Seed dressing by *Rhizobium* and trace nutrients i.e. molybdenum and manganese have promotion nodule formation also atmosphere nitrogen fixation process (symbiotic or a symbiotic) and plant yield increase Bayoumi et al., 1997, Uzbek et al: 1998, Bhulyan et al., 1998, Al-karaki 1999, Vilimelin et al . 2000 and Campo and Hungria 2002 and Bacem , et al.,(2007).The aim of this investigation known effect inoculation by *Rhizobium* common bean group combined with *Thiobacillus thioparus* . In addition, seed dressing by molybdenum and / or manganese under organic- inorganic soil amendments. That on nodulation rate, rhizosphere microorganisms proliferation and reflect return on common bean productivity especially these natural factors were applied by dependant on native soil nutrients without any chemical fertilizers addition as known bio-organic agriculture system.

MATERIALS AND METHODS

Bacterial isolation:

Rhizobia : common bean Rhizobia group (*Rhizobium phaseoli)* was isolated from host plants by selection numbers of true nodules , then washed and surface sterilized by immersion fore three minutes in mercuric chloride . After that rinsed by sterilized water, under aseptic condition in sterilized Petri dish contain 5ml sterilized water, nodules crushed to make cell suspension of Rhizobia, cells inoculated on (YEM) agar medium and incubated at 30°C.

A pure colony was picked up and re-streaked on the same medium with add 0.002g/l ^{1} Congo red to avoid from soil related bacteria as *Agrobacterium sp.* Growth colonies obtained were examined microscopically by Gram's staining to confirming purification . Kokh's postulats was applied that to insurance these isolate belong to Rhizobia common bean group by re-infection of the same host plant by using sand culture method , from this technique can be choice most effective isolate have able to plant infection and nodule formation:-

Rhizobia inoculum : a pure mother culture was inoculated in 250ml jars contain 100ml (YEM) broth medium and stationary incubate at 30c^{o} for 72 huores cells /ml^{1}(375x 10^{6}) nearly .Allen 1961 ;Vincent 1970 and Bergy.s Manual 1984 .

Thiobacteria; sulfur oxidizing bacteria (Thiobacillus *thioparus)* was former isolated from fertile Egyptian soil. purified and practical tested by the same author in further studies as microbiological protocol.

Thiobacteria inoculum; the purified culture of *Thiobacillus thioparus* was inoculated in flasks 250ml , each contain 100ml of thiosulfate broth medium a modified by add 2mg /l^{1} yeast extract and sodium thiosulfate by rate 5g/l^{1} only to increase biomass bacteria. After inoculate flasks stationary at 28c^{o} for 8 days cells /ml^{1} 138x10^{5} nearly (Patricio; et al., 2003).

Rhizosphere microorganisms activity; rhizosphere soil microorganisms were enumerated of three main groups i. e. bacteria , actinomysetes and fungi at three plant growth stages (seedling , flowering and pod formation) . A pour plate method was used with suitable medium of each microorganisms as follow; (SYE) bacteria; (IOSS) actinomysetes and (PDA) for fungi. That rhizosphere microbial was estimated as mean CFU/g rhizosphere soil dry weight of all treatments.

Soil experiment preparation: experiment soil was crushed, sieved, physical and chemical analyzed before planting data presented in table (1).

Table(1):Physical and chemical analysis of experiment soil before sowing.

Physical										Chemical							
										Cations(meq/l)				Anaions(meq/l)			
Sand %	Clay %	Silt %	Texture	OM %	OC %	CEC meq/ 100g	pH	EC ds/ m	Na+	K^+	Mg^{++}	Ca^{++}	So4=	Hco3=	Cl^-	Co3-
64	22	14	Loamy sand	1.2	0.69	13.4	7.8	0.11	0.4	0.11	0.1	0.2	0.29	0.4	0.11	0.0

Soil filled in earthen pots 25cm diameter by rate 7 kg/ pot and three pots per treatment as follow:

- sowing without any bacterial inoculation or soil amendments as control.
- inoculation by *Rhizobium phaseoli* alone.
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* only.
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as soil amendment sulfur and rock phosphate 100kg /feddan of each .
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as soil amendment sulfur , rock phosphate and seed dressing by potassium molybdate 1/2kg/feddan .
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as soil amendment sulfur, rock phosphate and seed dressing by manganese sulfate 1/2kg/feddan .
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as soil amendment sulfur, rock phosphate also seed dressing by potassium molybdate and manganese sulfate together 1/2kg/fedd of each.
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as sulfur and rock phosphate also seed dressing by potassium molybdate and manganese sulfate together 1/2kg/fedd of each and compost addition 10m3/fedd .
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as soil amendment by sulfur, rock phosphate also seed dressing by potassium molybdate and manganese sulfate together 1/2kg/fedd of each and compost addition $5m^3$/fedd .
- inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as soil amendment by gypsum 1 ton/fedd , rock phosphate also seed dressing by potassium molybdate and manganese sulfate together 1/2kg/fedd of each and compost addition 5m3/fedd .

-inoculation by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* as well as gypsum 2 ton/fedd and rock phosphate also seed dressing by potassium molybdate and manganese sulfate together 1/2kg/fedd of each and compost addition 5m3/fedd .

Soil amendments were add to soil and thoroughly mixed by surface layer of each treatment alone. Common bean seed cultivar (Bronco) was kindly from Agriculture Research center, seeds was inoculated just before sowing by soaked in *Rhizobium phaseoli* and *Thiobacillus thioparus* broth cultures each alone or together by 1:1 (vol. /vol.) for one hour with add 10g Arabic gum. After that seeds were air dried in shadow, also seeds were treated with molybdenum and / or manganese by moistened amount and thoroughly mixed. Experiment was planting by rate 5 seeds /pot and irrigated up to water holding capacity and follow irrigation as plant condition.

Plant characteristics; plants of all treatments at 25 days old samples was taken to determine nodulation by count in addition fresh and dry weight . After that, all experiment plants at 67days old were uprooted and separate of parts (roots, shoots and pods) of each treatment alone. Plant parameters vegetative measurements were recorded as means of; length (cm/plant), fresh and dry weight (g/plant) of root and shoot, plant fruiting as pods numbers, fresh and dry weight /plant.

Plant chemical analysis; plant of different treatments were dried, sample10 g of whole plant of each treatment was taken to determine of total nitrogen, phosphorus and potassium according to, Onken and Sunderman 1977, Olsen 1965 and Jackson 1973.

Results and Discussion

Soil microorganisms playing very significant role in preparation of plant nutrition, in the peak of these rhizosphere soil microorganism's activity. In this research different treatments of common bean were showed positive effect on microbial sizing and percent increasing presented data in table (2) results revealed that rhizosphere soil microorganisms was icreased compared with control which noticed increase percent of bacteria, actinomysetes and fungi proliferation in rhizosphere soil. However the highest percentage was found in combined treatment by *Rhizobium*, *Thiobacillus*, molybdenum, manganese, sulfur, rock phosphate and compost addition by rate $10m^3$/feddan reached, up to (311,274,360%),(517, 495,418%) and (333,475,400%) of bacteria, actinomycetes and fungi at three different growth stages (25, 45and 55 days from planting in respectively). The same treatment found that but compost add by rate 5m3/feddan was littlie percent from previous but any way was better from rest treatments .

Than Also, treatment by the same factors but substitute befor treatment sulfur by gypsum by rate 1 ton /feddan and compost by rate $5m^3$/feddan was better than from gypsum by rate 2 ton/feddan, also compost add by rate$10m^3$/fedd increasing percent up to (288, 223, 344 %), (453, 424, 355%) and (367,325,360%) of the same microorganisms and interval plant growth. In the same field treatment by *Rhizobium phaseoli* alone was the lowest from other treatments reached up to (11, 002, 37%), (33, 33, 29%) and (006, 25, 008 %) compared by control respectively. From long time organic matter known great benefit of soil microorganisms and fertility, this fact confirmed that by Ibrahim 1993, Elsebaay 2004, Piquese et al., 2006 and Stark et al., 2007.

Table(2):Interaction between *Rhizobium phaseoli, Thiobacillus thioparus*, molybdenum, manganese on rhizosphere soil microorganisms under soil amendments of common bean (mean count cfu /g rhizosphere soil dry weight).

Treatments*	Rhizosphere microorganisms								
	Bacteria Cfu x 10^6			Actinomysetes Cfu x 10^5			Fungi Cfu x 10^4		
	Days after planting			Days after planting			Days after planting		
	25	45	55	25	45	55	25	45	55
control	45	65	62	30	42	55	14	20	25
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R. alone	50	66	85	40	56	71	15	25	27
%	11	002	37	33	33	29	006	25	008
R+T only	62	75	95	45	65	95	22	32	55
%	37	15	53	50	55	73	47	60	120
R+T +s +r	68	85	115	52	75	115	25	41	62
%	51	31	85	73	79	109	67	105	148
R+T +s +r+ mo	69	89	120	55	81	120	32	45	66
%	53	37	94	83	93	118	113	125	167
R+T +s +r+ ma	68	85	118	56	82	115	35	51	71
%	51	31	90	87	95	109	133	155	184
R+T+ s+ r+ mo+ ma	71	91	132	62	91	130	41	62	81
%	58	40	113	107	117	136	173	210	224
R+T+ s+ r+ mo+ ma+com10	185	243	285	185	250	285	65	95	125
%	311	274	360	517	495	418	333	375	400
R+ T +s +r +mo+ ma+com5	135	195	230	140	185	220	42	62	110
%	200	200	271	367	340	300	180	210	340
R+T+gy2+r+mo +ma+com10	145	190	240	135	195	165	35	52	85
%	222	192	287	350	364	200	133	160	240
R+T+gy1 +r +mo ma+com5	175	210	275	166	220	250	70	85	115
%	288	223	344	453	424	355	367	325	360

* R= *Rhiobium* , T= *Thiobcillus* , s = sulfur , r = rock phosphate, mo = molybdenum , m = mngnese , com 5 = compost 5ton/feddan , com10= compost 10 ton/feddan .

Rhizosphere microorganisms was affect on plant parameters data in Table (3) showed that positive effect of these factors on nodulation no., root, shoot and pods no., as well as fresh and dry weight. In this field results indicate that biofertilizer by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* and seed dressing by molybdenum, manganese as well as soil amendments by sulfur, rock phosphate, compost by rate 10m3/feddan , was better than from rest treatments. Which common bean plant parameters increased percentage reached up to (475, 158, 800%) of nodules, (36, 1404, 969%) of roots, (15, 49, 264%) of shoots, (222, 77, 130%) of pods respectively. While the same treatment but compost by rate 5m^3/feddan was second order. In the other hand the same treatment but substitute sulfur by gypsum add by rate one-ton compost 5m^3 /feddan was better than from 2 ton and 10m^{3}compost/feddan on plant parameters previous. Also, treatment by *Rhizobium phaseoli* alone was lowest compared with control and other treatments. These treatments were found that affect on gain due to % of plant parameters.

Naturally, this could be attributed to the stimulation effect of the growth promoting substances released by microorganisms on rooting initiation and formation, rather than to the effect of control. This was proved by earlier investigation where higher amounts of GA and IAA detected in culture filtrates, these results confirmed that previously by Ewada 1967, Graham1981, Buttery et al., 1987, Ramos and Boddey1987, Hardarson 1993, Ibrahim 1993, Buhlyan et al., 1998 and Mart Ãnez 2003. In addition, seed dressing by molybdenum and /or manganese was better effect from control on nodule formation as showed results confirmed that by Buhlyan et al., 1998; Campo, and Hungria 2002, they were found that seed dressing by molybdenum and other micronutrient promotion nodule formation and biological nitrogen fixation process.

Table(3):Interaction between *Rhizobium phaseoli,Thiobacillus thioparus,* molybdenum and manganese on plant parameters of common bean under different soil amendments addition after harvesting at 62 days from planting (mean value per plant) .

Treatments*	Plant parameters											
	Nodules			Roots			Shoots			Pods (eco. yield)		
	No.	F. (g)	D. (g)	L (cm)	F. (g)	D. (g)	L. (g)	F. (g)	D. (g)	No.	F. (g)	D. (g)
control	4	0.012	0.002	7.5	0.130	0.080	40.3	30.5	7.0	9.0	15	5.0
Gain due to %	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
R. alone	9	0.019	0.006	8.2	0.252	0.113	44.6	33.2	8.1	12	16.6	7.2
Gain due to %	125	58	200	009	94	41	11	009	16	33	11	44
R+T only	11	0.021	0.008	8.3	0.283	0.115	45.3	35.2	9.5	13	17.0	7.3
Gain due to %	175	75	300	11	118	43	12	16	36	44	13	46
R+T +s +r	13	0.023	0.010	8.9	0.588	0.325	46.5	36.8	10.3	14	18.0	7.5
Gain due to %	225	92	400	19	352	306	13	21	47	55	20	50
R+T +s +r+ mo	15	0.023	0.011	9.3	0.790	0.233	47.8	38.5	11.3	15	18.6	7.8
Gain due to %	275	92	450	24	508	191	19	26	61	66	24	56
R+T +s +r+ ma	14	0.022	0.011	9.1	0.609	0.331	47.3	38.1	10.8	14	18.3	7.5
Gain due to %	250	83	450	20	368	314	17	25	54	55	22	50
R+T+ s+ r+ mo+ ma	17	0.024	0.012	9.4	0.854	0.445	48.6	40.3	12.6	16	18.9	8.2
Gain due to %	325	100	500	25	557	456	21	32	80	77	26	64
R+T+ s+ r+ mo+ ma+com10	23	0.031	0.018	10.2	1.955	0.855	56.5	45.5	25.5	29	26.5	11.5
Gain due to %	475	158	800	36	1404	969	15	49	264	222	77	130
R+ T +s +r +mo+ma+com5	19	0.027	0.016	9.4	1.855	0.766	52.2	42.3	23.6	23	23.6	10.6
Gain due to %	375	125	700	25	1327	858	30	39	237	155	57	112
R+T+gy2+r+mo +ma+com10	18	0.026	0.015	9.5	1.450	0.640	50.5	41.2	22.1	18	20.5	8.9
Gain due to %	350	117	650	27	1015	700	25	35	216	99	37	78
R+T+gy1 +r +moma+com5	19	0.026	0.016	9.6	1.845	0.755	51.3	40.5	20.6	21	22.5	9.6
Gain due to %	375	117	700	28	1319	844	27	33	194	133	50	92

* see the note below table 2.

Table (4):Interaction between *Rhizobium phaseoli, Thiobacillus thioparus*, molybdenum and manganese on NPK uptake in whole plant of common bean at 62 days from planting.

Treatments*	Plant composition		
	N %	P %	K %
control	2.2	0.220	1.3
Gain due to %	0.0	0.0	0.0
R. alone	2.6	0.240	1.5
Gain due to %	18	009	15
R+T only	2.65	0.250	1.551
Gain due to %	20	14	19
R+T +s +r	2.78	0.285	1.75
Gain due to %	26	30	35
R+T +s +r+ mo	2.89	0.684	1.95
Gain due to %	31	211	50
R+T +s +r+ ma	2.74	0.881	1.94
Gain due to %	24	300	49
R+T+ s+ r+ mo+ ma	2.97	0.985	2.25
Gain due to %	35	348	73
R+T+ s+ r+ mo+ ma+ com 10	2.995	1.920	2.95
Gain due to %	36	773	126
R+ T +s +r + mo+ ma+ com5	2.880	1.550	2.65
Gain due to %	31	605	104
R+T+gy2+r+mo + ma + com10	2.750	1.420	2.35
Gain due to %	25	545	81
R+T+gy1 +r + moma + com5	2.850	1.530	2.65
Gain due to %	30	595	104

***See the note below table 2.**

Refer to whole plant content from NPK, results in Table (4) revealed that increasing in gain percentage of these elements as naturally result of biological activity affected by these treatments. In this text biofertilizer and organic-inorganic soil amendments was better than from rest, also sulfur and compost by rate $10m^3$/feddan was first order, followed by compost add by rate $5m^3$/feddan. However treatment with gypsum by rate 1 ton/feddan with $5m^3$compost/feddan gave, results better than from gypsum 2ton and compost 5m3/feddan. In the same time, treatment inoculation by *Rhizobium phaseoli* alone was lowest treatment absolutely compared with control and rest treatments. Which seed dressing by molybdenum and /or manganese lead to increase in phosphorus, potassium and nitrogen up take as showed of presented data this results supported by Hardarson 1993, Sonboir and Sarawgi 1998 and Campo and Hungria 2002.

CONCLUSION

The application of biofertilizer by *Rhizobium phaseoli* combined with *Thiobacillus thioparus* and seed dressing by molybdenum, manganese as well as organic-inorganic soil amendments, compost, rock phosphate and sulfur or gypsum is strongly recommended of common bean productivily. This practice increase rhizosphere microorganisms activity and will supply the growing plants with their nitrogen , phosphorus , potassium and sulfur as well as micronutrients as result of soil physical , chemical and biological improvment. This in turn will decrease the necessary doses of mineral fertilizers, leading to a significant of crop production coast. In addition, the pollution rates of soil, water, foods and could lowered to a certain degree as a result by this practice.

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أثرالتلقيح بالريزوبيوم فاصولاى والثيوباسيلس ثيوبيرس مع الموليبدنم, المنجنيز على التعقيد وميكروبات الريزوسفيروالنمو تحت ظروف اضـــافات عضـــوية ومعدنيـــة للتربة على الفاصوليا.

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أجريت تجربة أصص لدراسة أثر التلقيح بأكفأ عزلات بكتيريا ريزوبيــوم فاصــولاى ضمن ثمانى عزلات تم عزلها واختبار قدرتها فى تكوين العقـد الجذريـــة وإنتماؤهــا لمجموعــة ريزوبيوم الفاصوليا بالاشتراك مع ببكتيريا أكسدةالكبريت المعدنية (ثيوباسيلس ثيوبيرس) المعزولة من التربة المصرية الخصبة والمختبرة معمليا ومعاملة البذور قبل الزراعة بالموليبدنم والمنجنيــز على معدل التعقيد ونشاط ميكروبات الريزوسفير واستغلال هذا النشاط فى إذابة مطحون صـــخر الفوسفات المضاف للتربة لامداد نبات الفاصوليا بالفوسفوراللازم للنمو والانتــاج فــى ظــروف معاملات التجربة.

وأوضحت نتائج البحث إلى ايجابية تاثير المعاملات مع الاختلاف فيما بينها بالمقارنـــة بالكنترول, وبصفة عامة كانت معاملات التخصــيب الحيوىالمشــترك بالبكتيرياالمــذكورة مــع اضافةالتسميد العضوى(الكومبست)الاعلى فى معدل التعقيد ونشاط ميكروبات الريزوسفير وتحسين قياسات النبات الخضرية والثمرية كذلك محتوى النبات من النتروجين والفوســفور والبوتاســيوم. وكانت أقل المعاملات هى معاملة التلقيح بالريزوبيوم منفردة مقارنة بالكنترول. كما كانت معاملات الكبريت افضل من إضافة الجبس الزراعى, أيضا كان معدل التسميد العضوى ١٠م٣/فدان افضــل من٥م٣ وكان التسميد العضوى افضل على الاطلاق بالمقارنة بالكنترول والمعاملات الأخرى. كما كان تاثير إضافة الجبس الزراعى بمعدل ١طن/للفدان افضل من معدل ٢ طن/فــدان, أيضــا أدى معاملة البذرة بالموليبدنم والمنجنيز قبل الزراعة الى تشجيع تكــوين العقــد وتنشــيط ميكروبــات الريزوسفير فى تيسير الفوسفوروتثبيت الازوت الجوى الازم للنبات.

ويوصى البحث الى تطبيق نظام التخصيب الحيوى بالريزوبيوم والثيوباسيلس و إضافة المواد العضوية والمعدنية المحفزة للنشاط الميكروبى والمحسنة للتربة فى إنتاج الفاصوليا من أجل الاستهلاك والتصنيع والتصدير وخفض معدلات التلوث.