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EFFECT OF BACTERIAL INOCULATION, MOLYBDENUM AS WELL AS SOME SOIL AMENDMENTS ON SOYBEAN.

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ABSTRACT

Lysimeters experiment was carried out in a wire house study effect of microbial inoculation using three active local strains of *Bradyrhizobium japonicum*, *Azotobacter chroococcum* and *Thiobacillus thioparus*, some soil amendments addition as compost, sulfur, rock phosphate on soybean (Glycine *max L*.) cv. Giza 21. The seeds were dreseed by potassium molybdate. In rhizospheric region total count of bacteria, actinomycetes and fungi at 45, 70 and 95 days from sowing were estimated. Also true nodules formation rate, biological yield as root, shoot length, height and dry matter weight were studied. Economical yield as; number of pods, weights and weight of 100 grains as seed index were studied. In addition, determinations of total nitrogen, phosphorus and potassium in seeds and soil properties before sowing and after harvesting were done. The results obtained cleared that useful effect of combined inoculation by three bacteria under study and interaction with soil amendments addition, which resulted increase in nodulation rate (biological nitrogen fixation process), rhizosphere microbial activity followed availability of phosphorus from rock phosphate, so affect that on plant productivity, seed contain of total nitrogen, phosphorus and potassium. Also maintenance, restoring soil fertility due to these treatments. Generally, organic fertilizers with other treatments gave best results in all parameters from control. The results of this study recommended that application of combined biofertilization (*Bradyrhizobium, Azotobacter and Thiobacillus*) and soil amendments addition (compost, sulfur and rock phosphate) as well as seed dressing by potassium molybdate promoted rhizosphere microorganism's proliferation, plant nutrients availability, nitrogen fixation process gains due these treatments on soybean prameters and productivity, also soil maintenance and restoring fertility.

Keywords: soybean, compost, biofertilizers, *Bradyrhizobium japonicum, Azotobacter chroococcum, Thiobacillus thioparus*, elemental sulfur, molybdenum, rock phosphate.

INTRODUCTION

Symbiosis between legumes and Rhizobia or Bradyrhizobia in atmosphere nitrogen fixation process is a critical to plant production, (Smith and Hume, 1987; Vance, 1997; Pepper, 2000).

Enhancement of legume nitrogen fixation by co-inoculation of *Rhizobium* or *Bradyrhizobium* with some plant growth promoting bacteria (PGPB) as *Azotobacter* is away to improve nitrogen availability in a sustainable agricultural production system (Zhang, *et al.*.,1996; Bullied, *et al.*., 2002). Some rhizobacterial strains promote legume nodulation and nitrogen fixation by producing flavonoid-like compounds and /or stimulating the host legume to producing more flavonoid signal molecules (Parmer and Dadarwal 1999). Organic fertilizer as compost are potentially a major limiting factor in maintaining and restoring soil fertility and in rendering insoluble nutrients available to plants . Soils which received continuous application of farmyard manure showed the highest index of fertility, i.e. total microbial count and specific groups production of CO_2, ammonification, nitrification and cellulose degradation rates as compared with soils receiving continuous application of N, NP and NPK, also increase rhizosphere effecincy of soybean and yields (Ibrahim, 1993; Yaman and Cisoy, 1997, Piqueres *et al..*, 2006). Microbial community structure in rhizosphere soil was strongly influenced by the presence or lack of substrate (organic or inorganic) had an effect on microbial biomass sizing and activity, Starky, *et al..*, 2007. Trace element molybdenum is very essential for Rhizobia or Bradyrhizobia for nodulation and nitrogen fixation process efficiency (; Bhuiyan *et al..*, 1998; Sonboir and sarawgi 1998 Al-Karki 1999, and Campo and Hungria, 2002). Agricultural sulfur fortified *Thiobacillus* sp. application in addition rock phosphate increased available N, P ,K and S uptake due to acidification effect and enhancing nitrogen fixation by Rhizobia, Bradyrhizobia as well as free living bacteria as *Azotobacter* sp. , also increasing rhizospheric microorganisms enumeration and activation , return reflects that on plant productivity (Abdel- Fattah and Hilal, 1985, Shafei 1991; Attia and El-Dosuky 1996 Bayuoumi *et al..*,1997; Elsebaay 2001 and Koreish,*et al..*, 2004).

The objective of this work was to determine the effect of combined inoculation by *Bradyrhizobium japonicum, Azotobacter chroococcum* and *Thiobacillus thioparus*, and some soil amendments as; compost, sulfur, rock phosphate as well as seed dressing by potassium molybdate on nodulation rate , plant parameters, soybean productivity and soil fertility.

MATERIALS AND METHODS

Bacterial isolation:

Bradyrhizobium japonicum was isolated from effective nodules of host soybean plants. After eliminate soil particles by stream tap water washing, nodules were surface sterilized by immersion in mercuric chloride solution conc.1/1000 for three minute, then rinsed by sterilized water much times. Nodules were crushed in 5ml sterilized water in Petri dish under aseptic condition. Cell suspension of Bradyrhizobia were streaked on (YEM) agar medium with add Congo red 0.0025 mg / l^{-1} to distinguish from related soil bacteria as *Agrobacterium sp*. then re-streaked on the same medium, A pure colony was picked and microscopically examined by Gram stain reaction to be purification confirmed according to microbiological protocol Allen 1961,Vincent 1970 and Murray *et al.,* 1984.

Bradyrhizobial soybean group was confirmed belong to this group by Koch's postulates applied by re- infection and re- isolation of soybean seedling of each five isolates.Only one of pure cultures, former isolated from soybean plant by using sand culture method. Nodules formation was checked and chosen best isolate on base true nodules formation number to complete this paper.

The most effective isolate was propagated by inoculation in flasks 250 ml each contain 100ml broth (YEM) and stationary incubation at 30 °C for 74 h cells /ml up to (320 x10^{-6} **) .**

Azotobacteria: *Azotobacter chrococcum* was isolated from Egyptian fertile soil, purified, identified according to Bergy’s 1984, also practically tested on free nitrogen fixating process within four isolates was chosen best it . Biomass produced of the better strain was achieved by inoculation in flasks 250ml each contain 100ml of Base 77 broth medium and stationary incubated at 30 c^0 for 96 h cells /ml. up to (290×10^6).

Thiobacteria: Chemolithotrophic (Sulfur oxidizing bacteria) *Thiobacillus thioparus* was also isolated from Egyptian soil, purified, identified as Starkey 1966 and Murray *et al.*., 1984. Capacity on sulfur oxidation according to Public Health Association 1975 as well as atmosphere nitrogen fixing as oligo-nitrophilic bacteria as Rai and Sreenivasa 1994 practically was done. The inoclum propagated in flasks 250 ml each contain 100 ml of broth a modified thiosulfate medium by add 2 mg / l^{-1} yeast extract and 5 g of sodium thiosulfate / liter for increasing biomass yield, medium composition are (g/ l^{-1}), $Na_2HPO_4 . 7H_2O$ 2.27; KH_2PO_4 1.8; $MgCl_2.7H_2O$ 0.1; $(NH_4)_2 SO_4$ 1.098; Mn $Cl_2 . 2H_2O$ 0.023; $CaCl_2$ 0.003; $FeCl_3 .6H_2O$ 0.033; Na_2CO_3 1.0 and $Na_2S_2O_3.5H_2O$ 5.0* after autoclaving flaskes were inoculating and stationary incubated at 28˚C for 7days cells / ml up to (130×10^6).

Lysimeter soil preparation:

Experimental soil was crushed, air-dried, sieved, soil amendments were added and thoroughly mixed with surface layer as type treatments follows:

-without any inoculation or soil amendments addition as control.

-Inoculation by *Bradyrhizobium japonicum* alone.

-Inoculation by *Bradyrhizobium japonicum* with seed dressing by potassium molybdate 1/2 kg /feddan only.

-Inoculation by *Bradyrhizobium japonicum* with adds compost $10m^3$ / fed only.

-Inoculation by *Bradyrhizobium japonicum* with adds sulfur, rock phosphate 100 kg / fedd of each in addition compost only.

-Co-inoculation by *Bradyrhizobium japonicum*, *Azotobacter chroococcum* and *Thiobacillus thioparus* without any soil amendments.

-Co-inoculation by *Bradyrhizobium japonicum*, *Azotobacter chroococcum* and *Thiobacillus thioparus* as well as soil amendments sulfur, rock phosphate, compost and seed dressing by potassium molybdate.

* sterilized by filtration

All treatments were designed in three replicates of each treatment also soil physical and chemical properties were analyzed before sowing as control Table (1).

Table (1): Physical and chemical properties of lysimeter soil before planting

Physical				Chemical													
				Cat ions(meq/ l^1)									Anions(meq/ l^1)				
Clay %	Silt %	Sand %	Soil texture	pH	E.c (ds/m)	O.M %	C %	CEC meq/ 100g	Na+	K+	Ca++	Mg++	So4=	Hco3=	CL-	Co3-
23	12	65	Loamy Sand	79	0.1	1.6	0.94	14.7	0.4	0.01	0.1	0.2	0.29	0.11	0.4	0.00

Inoculation: Soybean seeds *(Glycin max L.*) cultivar Giza 21 was kindly from Agric., Research Center. Seeds were inoculated with the test obtained bacteria isolates as separate or together (1:1:1) vol. as type treatment by soaked in culture broth for one hour with 10g of Arabic gum add. Inoculated seeds drained and air dried in shadow then dressed by molybdate as treatment type, directly sowing and irrigated up to (W.H.C) and follow it as plant condition.

Total microbial count of Rhizosphere: Rhizospheric soil microorganisms were achieved of bacteria, actinomycetes and fungi as mean count Cfu / g^{-1} rhizosphere soil dry weight at 45, 70 and 95 days from planting. A pour plate method was used and suitable medium of each microbial group as, bacteria on (SYEA), actinomycetes on (IOSSA) and fungi on (PDA).

Nodules numbers and weight: nodule numbers were counted after 45 days from planting. Fresh nodules for each plant were weighted.

Plant and soil properties: After 120 days from sowing plant uprooted of each treatment only then plant parameters recorded averages as; root, shoot length and height (cm / plant), dry weight (g / plant) as a biological yield, pods (no./plant), pods weight (g / plant) as economical yield and weight of 100 grains / g as well as gains due to these treatments compared by control. Soil properties (physical and chemical) as well as organic matter (O.M %), carbon (C %), electrical conductivity (EC ds/m), (CEC) meq/100g dry soil and pH according to Jakson (1973) and Page *et al.*., (1982). Also determination of total nitrogen, phosphorus and potassium of seeds as percentage was done (Jakson, 1973)

RESULTS AND DISCUSSION

Results in Table (2) revealed the effect of bacterial inoculation and wher soil amendments on total count of bacteria, actinomyctes and fungi in rhizosphere region of soybean .In general treatments with organic fertilizer (compost) either alone or with other additives gave the best results than other treatments absolutely as rhizosphere microorganisms prolifiration. The organic matter plays an important role in soil improvement, plant and microbial nutrition from long time. It results Co2, organic acids, cellulose decomposers, plant growth promoting as microbial activity, this reflect on plant parameters. Data showed that inoculation with *Bradyrhizobium* either abne or with *Azotobacter* and *Thiobacillus* greatly increased the total microbial count in rhizosphere of soybean. The highest increased percentages over the control in bacteria, actinomycetes and fungi counts treatments were achieved by using the mixed inoculum, sulfur, rock phosphate, compost and molybdenum. The increasing percentages are, (322 , 236 , 159 %) bacteria , (289 ,311 , 300%) actinomysetes and (340 , 238 , 227%) fungi at 45 , 70 and 95 days from planting respectively . By comparing treatment applied with *Bradyrhizobium japonicum* alone was the lowest , which percent are , (24 , 25 , 5 %) , (10 , 7 , 5 %) and (40 , 15, 21 %) respectively.Data reveald that potassium –molybdate had a stimulatory effect on total microbial counts .This may be due to its positive effect on plant growth and its reflect on microbial count in rhizospheric region. Results obtained were in line with those obtained by, Abdel-Fatah and Hilal 1985, Ibrahim 1993, Attia and El-Desuky 1996, El-Sebaay 2001, John *et al.* 2004, Piqueres *et al.* 2006 .

Table 2. Effect of bacterial inoculation, sulfur, compost, rock phosphate and molybdenum application on total microbial count in soybean rhizosphere soil at different growth stages (Mean Count cfu/g rhizosphere soil dry weight).

Treatments *	Rhizosphere total microbial count								
	Bacteria (Cfu x10^6)			Actinomycetes (Cfux10^5)			Fungi (Cfux10^4)		
	45	70	95	45	70	95	45	70	95
Control	85	125	185	41	56	105	25	65	81
%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bradyrhizobium alone	105	156	195	45	60	110	35	75	98
%	24	25	5	10	7	5	40	15	21
Bradyrhizobium + MO	115	169	205	56	79	125	31	71	110
%	35	35	11	37	41	19	24	9	36
Bradyrhizobium +compost	226	330	385	85	130	256	70	156	220
%	166	164	108	107	132	144	180	140	172
Brady.+sulf.+rock.+compost	320	385	410	115	195	365	90	180	255
%	276	208	122	180	248	248	260	177	215
Brady.+Azot.+Thiobacillus	189	210	240	95	120	180	45	80	130
%	122	68	30	132	114	71	80	23	60
Brady. +Azot. +Thiob. +sulf. + Rock.+compost+MO	359	420	480	159	230	420	110	220	265
%	322	236	159	289	311	300	340	238	227

*** Mo = molybdenum, sulf. = sulfur, rock. = rock phosphate, Azoto. = Azotobacter, Brady. = Bradyrhizobium, Thio. = Thiobacillus.**

Data presented in Table (3) showed increasing in nodule numbers formation and plant parameters due to microbial inoculation and soil amendments addition .The highest increases percentage in nodule numbers achieved by using the mixed bacterial inoculum and soil amemdments (414%) followed by using the triple inoculum only (343%). Potassuim molybdate addition eahanced nodules formation, the increase perccntage was 16% when it was used with *Bradyrhizobium japonicum* while it was 11% with using *Bradyrhizobium japonicum* alone. Also data clearly showed that compost addition either alone or with microbial inculants increased nodule numbers and weights per plant as well as the other plant parameters. The highest values of nodule numbers and weights 36.0 and 3.2 were recorded in plants received the mixed inoculum and other soil amendments .The same trend was observed in nodules weight. These results are ine line with Yaman and Cisoy (1997), Vance (1997) Bhuiyan *et al.* (1998), Parmar and Dodarwal, (1999), Bullied *et al.* (2002), and Campo and Hungria (2002).

Table 3. Effect of bacterial inoculation, sulfur, compost, rock phosphate and molybdenum application on soybean nodulation and plant parameters.

Plant parameters / Treatments*	Nodule No. plant/	Nodule Weight g/ plant	Root Length cm/plant	Root Weight g/plant	Shoot Height cm/ plant	Shoot Weight g/plant	Pods No./ plant	Pods Weight g/plant	Weight 100seeds Per g.
Control	7	0.9	5	0.091	41	1.775	14.5	5.6	16.1
Gain due to%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bradyrhizobium alone	11	1.3	6	0.103	49	1.950	16.5	7.4	17.3
Gain due to%	57	44	20	32	20	10	14	32	7
Bradyrhizobium+MO	16	1.6	7	0.120	65	2.150	18.5	7.9	18.6
Gain due to%	129	78	40	43	59	21	21	41	16
Bradyrhizobium+compost	20	2.1	8.5	0.250	71	2.940	20.1	9.5	20.1
Gain due to %	186	133	70	175	73	66	46	70	25
Brady.+sulf.+rock.+ compost	26	2.6	9	0.260	75	3.020	22.2	10.4	22.1
Gain due to %	271	189	80	186	83	70	53	88	37
Brady.+Azotobac.+Thiobac.	31	2.9	11	0.280	76	3.015	22.1	11	22.6
Gain due to %	343	222	120	208	86	70	52	96	40
Brady.+Azot+Thiob+sulf. + Rock.+compost+MO	36	3.2	12.5	0.310	81	3.350	27.5	13.5	24.2
Gain due to%	414	256	150	241	98	89	90	141	50

*** see the note below Table (2).**

Data in Table (4) showed the effect of bacterial inoculation and soil amendments addition on N ,P and K content of soybean seed .The results revealed that N .P and K contentes reached their highest values in plants treated with the mixed inoculum ,received soil amendments and molybdenum they are 11.4, 2, 16 and 7,8% respectively.

Table 4. Effect of bacterial inoculation, sulfur, compost, rock phosphate and molybdenum application on N, P and K contents of soybean seeds.

Treatments *	N%	P%	K%
control	8.8	1.68	5.8
Gain due to%	0.0	0.0	0.0
Bradyrhizobium alone	9.8	1.69	5.9
Gain due to%	11	1	2
Bradyrhizobium+MO	11.1	2.10	7.2
Gain due to%	26	25	24
Bradyrhizobium+compost	11.0	2.12	7.5
Gain due to %	25	26	29
Brady.+sulf.+rock.+compost	11.2	2.13	7.6
Gain due to %	27	28	31
Brady.+Azotobac.+Thiobac.	10.0	1.77	6.5
Gain due to %	25	5	12
Brady.+Azot.+Thiob.+sulf.+ Rock.+compost+MO	11.4	2.16	7.8
Gain due to%	30	29	34

*** see the note below table (2).**

Plants inoculated with Bradyrhizobium and treated with sulfur + rok phosphate +compost have N, P and K contents in seeds higher than that of those inoculated with the mixed inoculum only.

These results were confirmed with, Bayoumi *et al.*(1997), Sonboir and Sarawgi (1998) , Al-Karki (1999), Vilimiene *et al.* 2000, Bullied *et al.* (2002) and Koreish *et al.* (2004)

Data in Table (5) showed the effect of bacterial inoculation, soil amendments and molybdate on maintaining and restoring fertility .Results showed that both cations and anions were affected through the treatmients, the values were over the control. Organic matter and organic carbon were clearly affected with various treatments, especially those received compost and bacterial inoculation either with bradyrhizobium or with the mixed inoculum .Also compost has affected E.C.values either with bradyrhizobium alone or with the mixed inoculum in presence or obsence of other amendments. These results are in line with Abdel-fattah, and. Hilal (1985), Ibrahim (1993), El-Sebaay (2001), Koreish *et al..*, (2004), Stark *et al..* (2007) they found that bacterial inculation and soil amendments increase soil restoring and fertility, this one object of general development as sustainable bio- organo-agricultural production system.

Table (5): Effect of bacterial inoculation, sulfur, compost, rock phosphate and molybdenum application on soil maintaining and soil fertility.

Soil properties / Treatments*					Chemical							
					Cat ions (meq /L)				Anions (meq/L)			
	O.M %	O.C %	PH	E.C ds/m	Na^+	K^+	Ca^{++}	Mg^{++}	$So_4^=$	$HCO_3^=$	Cl^-	Co_3^-
control	0.76	0.44	7.75	0.1	0.4	0.01	0.3	0.3	0.42	0.29	0.4	0.0
Bradyrhizobium alone	0.85	0.49	7.67	0.1	0.3	0.05	0.4	0.2	0.27	0.38	0.3	0.0
Bradyrhizobium+MO	0.99	0.57	7.56	0.2	0.21	0.04	0.22	0.19	0.24	0.23	0.19	0.0
Bradyrhizobium+ compost	1.17	0.68	7.36	0.7	0.8	0.22	0.5	0.6	0.93	0.49	0.7	0.0
Brady.+sulf.+rock.+ compost	1.08	0.63	7.25	0.85	0.8	0.82	0.3	0.7	1.01	0.29	0.7	0.0
Brady.+Azotobac.+ Thiobac	1.16	0.67	7.56	0.3	0.7	0.25	0.7	0.6	1.09	0.56	0.6	0.0
Brady.+Azot.+Thiob.+ sulf.+Rock.+compost+ MO	1.18	0.99	7.11	0.9	0.71	0.24	0.8	0.5	1.08	0.49	0.7	0.0

*** see the note below table (2).**

Conclusion

It cold be concluded from previons results that inoculation of soybean plants with *Bradyrhizobium japonicum* , *Azotobacter chrococcum*, and *Thiobacillus thioparus* as mixed bacterial inoculum as well as soil amendments addition , sulfur , compost , rock phosphate and dressing the seeds an ammonium molybdate improved plant growth ,plant yield as well as N ,P and K content in plant grains .The treatments have a positive role in soil maintaining and restoring fertility . So, we could recommended by using the previous treatments in order to gain higher and good yield of soybean.

REFERENCES

Abdel-fattah, A. and M. H. Hilal 1985 Effect of sulphur application on some properties of Egyptian soils , proc. 2nd Arab regional conf. on sulphur and it's usage .vol. 1,pp. 39-53 .

Al-Karki-G N (1999) Rhizobium and phosphorus influence on lentil seed protein and lipid. J. of plant nutrition 1999, 22: 2, 351- 358, 21 refs.

Allen, O. N. (1961) Experiments in soil Bacteriology, purges publications, co., Minnisota, USA.

American Public Health Association (1994) Standard methods for the examination of water and waste water. Washington, P. C.

Attia, K., K., and M., M., El-Desuky (1996) Effect of elemental sulfur and inoculation with Thiobacillus, organic manure and nitrogen fertilization on wheat. Assiut J. of agric. Sci., vol. 27, No. 4, 1996.

Booth, C.; (1971) Methods in microbiology. Vol., 4, pp 195, London and New York Academic press.

Bullied, J., T., J. Buss, and J. K. Vessey. (2002) Bacillus cereus vw85 inoculation effects on growth nodulation and N accumulation in grain legumes Field studies .Can .J. plant Sci 82: 291- 298.

Bayoumi- Na; El-Bagouri-IH; Negm-MA; EL-Eweddy-EA (1997) Effect of sulfur application to calcareous soils on soil sulfur forms, on nutrient uptake and on yield of alfalfa. Desert-Institute –Bulletin, Egypt 1997, pupl. 1999, 47:1, 31-45; 31 ref.

Bhuiyan –MAH ;Khanam-D, Khatun-MR, Hassan- MS(1998) Effect of molybdenum ,boron and Rhizobium on nodulation growth and yield of chick pea .Bulletin of the Institute of Trobical Agric., Kyushu-Univ. 1998, 21: 1-7; 16 ref .

Campo-RJ; Hungria-M (2002) Importance of micronutrients in biological nitrogen fixation. Anais do 11 congresso Brasileiro de sojae Mercosoja, 3-6 junho 2002, Foz do Lguacu, PR, Brazil .Documentos –Embapa-Soja 2002, No.180, 355-366, 22 refs.

El-Sebaay, H.; H.; (1995) Microbiological studies on rhizosphere regions of some plants resistant and susceptible by wilt. M. Sc. Thesis Fac. Agric. Al-Azhar Univ. Cairo Egypt.

El-Sebaay, H.; H. ;(2001) Microbiological and chemical studies on sulfur bacteria. Ph.D. Thesis Fac. Agric. Al-Azhar Univ.; Cairo Egypt.

Ibrahim ,A.,N.,(1993) Role of biofertilizers in maintaining and restoring soil fertility as a main aid for sustainable increase in crop production. Agrokemia Es Talajtan Tom.42.(1993) No. 1-2 p 121 -126 .

Jackson, M. L. 1973 Soil chemical analysis .Printtice Hall, Inc., Englewood cliffs .N.J. Library of congress, USA.

John P. Morrissey, J. Maxwell Dow, G. Louise Mark & Fergal O'Gara (2004) Are microbis at the root of a solution to world food production? Rational exploitation of interactions between microbes and plants can help to transformation agriculture. (Viewpoint)

European Molecular Biology Organization (EMBO) reports vol.5, No. 10, 2004.

Koreish, E., Menaria-B-L., and Pushpendra-Singh (2004) Effect of NPK and S combination on yield and NP content of soil after harvest of soybean (*Glycin max L*.) cv. Merpl. Annals of Agric. Research 2004 , 25 (1) 162-163.

Kiss-S (1999) Enzymology of soils inoculated with microorganisms .studia-univ. –babes-bolyai, biologia, 1999, 44: 1-2, 3-44, 67ref.

Murray,R.G.E.,D.G. Bryant., M.R.Krieg., J.W.Malvlder.,N.pfening., P.H.A. sneath and J.T.staly(1984) Bergey,s manual of systematic Bacteriology .(Eds) Williams and wilkinsco., Baltimoae,landonpress.

Page, A., L., Miller, R., H., and Keeny, D., R., (1982) Methods of soil analysis part 2: Chemical and Microbiological properties .second edition.Agron. J. 9:2.

Parmar, N., and K. R., Dodarwal. (1999) Stimulation of nitrogen fixation and induction of flavonoid-like composition rhizobacteria. J. Appl .Microbiol. 86: 36-64

Pepper, I., L., (2000) Beneficial and pathogenic microbes in agriculture .In R. M. Maier *et al.*.,(ed.). Environmen. Microb. Academic press San Diego.

Piqueres, A., P., V., Edel-Hermann, C., Alabouvette and C. , Steinberg (2006) Response of soil microbial communities to compost amendments . Soil Biolog. And Biochem. , March 2006, Pages 460-47 vol. 38, ISSUe 3.

Rai,-P -V, Sreenivasa,-M-N (1994) Nitrogen fixation by chemolithotrophs and non-chemolithotrophs. Biology and application of nitrogen fixing organisms; problems and prospects. 1994; 119-128.

Shafei, A.M.M. (1991) Effect of sulfur addition on some crops yield and soil properties of clay alkaline soil. M. Sc. Thesis, Fac. Agric. Al-Azhar Univ. Cairo Egypt.

Sonboir-HL; Sarawgi-Sk (1998) Effect of phosphorus bacterial cultures and micronutrients growth, yield and balance sheets of N and P in chickpea. Annals of Agricultural Research 1998, 19:4,488-491; 5 ref .

Smith, D. L. and D. j. Hume. (1987) comparison of assay methods for nitrogen fixation utilizing with bean and soybean. Can. J. plant Sc. 67: 11-19.

Starkey, R. L. (1966) Oxidation and reduction of sulfur compounds in soil .soil science, 101 (4): 297-306.

Stark, C., L., M., Condron, A., Stewart, H., J., D.and M. O. Callaghan (2007) Influence of organic and mineral amendments on microbial soil properties and processes. Appl. Soil Ecology, vol. 35, Issue 1, January 2007, pages 79-93.

Szabo, I.; M.; (1974) Microbial communities in a forest rendzina ecosystem. Academia Budapest Hungary.

Vance, C., P., (1997) Enhanced agricultural sustainability through biological nitrogen fixation. In A. legocki *et al.* (ed.) biological fixation of nitrogen for ecology and sustainable of agriculture. NATO AS series Springer-verlag, Heidelberg, Germany.

Vilimiene-R; Sliesaravicius-A; and Pranaitis-P (2000) Effects of nodule bacteria (Bradyrhizobium *lupine*) strains on lupine (*Lupinus angustifolius L., lupinus luteus L*.) Productivity. Sodininkysteir-Darzininkystee. 2000, 19: 3 (3), 265- 272. 13 refs.

Vincent, J., M., (1970) A manual for the practical study of root nodule bacteria. Blackwell scientific publication. Oxfor England .

Yaman-M; and Cisoy-AS (1997) the effect of inoculation with bacteria (Rhizobium *japonicum L*.) and nitrogen application on yield and seed weight in soybean. Anadolu. 1997, 7:1, 21-29; 16 ref.

Yuming Bai, Xiaomin Zhou, and Donald l. smith (2003) Enhanced soybean plant growth resulting from co-inoculation *Bacillus* strains with *Bradyrhizobium japonicum.* Crop Science, 43: 1774-1781.

Zhang, F., N. Dashti, H. Hynes, and D. L. Smith (1996) plant growth promoting rhizobacteria and soybean (Glycine *max* L.) Merri nodulation and nitrogen fixation at suboptimal root zone temperature. Ann. Bot. (London) 77: 453-459.

تأثير التلقيح الميكروبي مع الموليبدنم و بعض اضافات التربة على فول الصويا

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أجريت تجربة أحواض ليسمترية لدراسة تأثير التلقيح الميكروبي مع بعض محسنات التربه مثل الكومبوست والكبريت الزراعي وصخر الفوسفات وكذلك معاملة البذور بموليبدات البوتاسيوم علي تكوين العقد ومحصول نبات فول الصويا وكذلك علي صفات التربة الكيماوية والفيزيائية والبيولوجية . استخدمت ثلاث عزلات وتشمل عزلة برادى ريزوبيوم جابونيكم هي اكفأ عزلة ضمن خمس عزلات تم عزلها واختبار قدرتها على عدوى فول الصويا وتكوين عقد فعالة فى تثبيت الازوت الجوى وعزلة الازوتوباكتر كروكوكم المثبتة لازوت الهواء الجوى لاتكافليا.

وأشارت النتائج المتحصل عليها إلى الآتي :

- زيادة معدل تكوين العقد الجذرية فى معاملات التلقيح المشترك مع اضافة بعض محسنات التربة ثم التلقيح المشترك فقط ثم التلقيح بالبرادى ريزوبيوم مع الكومبست فقط ثم البرادى ريزوبيوم مع موليبدات البوتاسيوم وأخيرا التلقيح بالبرادى ريزوبيوم بمفردها على الترتيب بالمقارنة بالكنترول.
- زيادة أعداد (نشاط) ميكروبات الريزوسفير بالمقارنة بالكنترول وكانت أعلاها معاملة التلقيح المشترك مع إضافة الكومبست ثم الكومبست مع البرادى ريزوبيوم ثم باقي المعاملات بالمقارنة بالكنترول .
- ادى زيادة نشاط ميكروبات الريزوسفير الى تاثيرها الايجابى على صفات النبات وتيسير عناصر تغذية النبات ومنها الفوسفور من مطحون صخر الفوسفات
- كما ادى معاملة البذرة بجرعة ضئيلة (1/2كجم/فدان) موليبدات البوتاسيوم الى زيادة تكوين العقد الجدرية ونساط البكتيريا فى تثبيت الازوت وزيادة نسبة النتروجين فى البذور.
- تحسنت صفات النبات (المحصول البيولوجي والاقتصادى) كذلك معدل تمثيل عناصر النتروجين , الفوسفور , البوتاسيوم الكلى بزيادة محتواها فى البذور وكانت معاملة التلقيح المشترك مع اضافة محسنات التربة في المرتبة الأولى بالمقارنة بالمعاملات الأخرى والكنترول .
- كما تحسنت صفات التربة الكيماوية والفيزيقية والبيولوجية (الأثر المتبقي للمحصول) بالمقارنة بالتربة قبل الزراعة مع الكنترول وكانت أفضل المعاملات هي ايضا التلقيح المشترك مع إضافة الكومبست , والكبريت الزراعي ومطحون صخر الفوسفات وموليبدات البوتاسيوم بالمقا رنة بالكونترول و باقي المعاملات.

ويوصى البحث باستخدام التخصيب الحيوي المشترك بالبرادى ريزوبيوم و الأزوتوباكتر والثيوباسيلس مع السماد العضوي (الكومبست) وإضافة محفزات النشاط الميكروبي المعدنية الطبيعية (الكبريت الزراعي- مطحون صخر الفوسفات- وجرعة ضئيلة من موليبدات البوتاسيوم) لكي تنشط الميكروبات خاصة في منطقة الريزوسفيرمما يعمل على تعويض استنزاف التربة من العناصر الغذائية وتحسين صفات النبات المحصولية وتقليل نفقات الإنتاج ومعدل التلوث عامة.