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ENHANCEMENT OF NITROGEN FIXATION, GROWTH, AND PRODUCTIVITY OF *BRADYRHIZOBIUM*-LUPIN SYMBIOSIS VIA CO-INOCULATION WITH RHIZOBACTERIA IN DIFFERENT SOIL TYPES

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ABSTRACT

The current investigation was conducted in two different soil types (sandy and clay loam) at Ismailia and Sids Research stations, Agricultural Research Center, Egypt. The target of this study was to evaluate the promotive effect of various rhizobacterial co-inoculants for enhancing the nodulation, growth and productivity of lupin plants grown in the two different soil types. At preliminary, some plant growth promoting properties could be exerted by the tested rhizobacteria were examined under in vitro conditions. Afterwards, field trails were conducted at Ismailia and Sids sites during the two consecutive seasons of 2005/2006 and 2006/2007.

Results of in vitro examination revealed that all tested rhizobacteria had a positive impression for exhibiting the PGP-properties, with clear superiority of *Pseudomonas* and *Serratia* for producing IAA. In other side, *Bradyrhizobium* and *Bacillus megatherium* failed to excrete cyanid or siderophores.

Results of field trials exerted that sole inoculation of lupin seeds with bradyrhizobia resulted in significant increases in nodulation status, dry matter and total N content of the plants either grown in sandy or clay loam soils after 75 days of planting during the two studied seasons. However, these parameters were significantly enhanced when the lupin plants co-inoculated with bradyrhizobia and any of the tested rhizobacteria in both investigated soils at the two studied seasons. Additionally, lupin yield and it's attributes gave the same tendency as a result of enhancement of nodulation, plant vigour and nitrogen fixation performance. For instance, the highest values of percentage increase attained in seed yield of lupin plants grown in sandy soil were 47.46% due sole inoculation with bradyrhizobia, then these values raised to range from 75.36 to 99.33% as function of co-inoculation with various rhizobacteria, during season of 2005/2006.

The corresponding values attained for clay loam soil were 39.35% and 59.69 to 73.13%, respectively. Moreover, results exhibited that nodulation status and productivity of lupin plants grown in sandy soil (Ismailia site) exerted high responses to sole or/and co-inoculation approaches rather than these grown in the clay loam one (Sids site).

Keywords: *Bradyrhizobium* sp., lupin, co-inoculation, rhizobacteria, soil type.

INDRODUCTION

White lupin (*Lupinus albus* L.) has been cultivated as a grain legume for more than 3000 years in Egypt. It has considerable potential for human and animal nutrition as well as for medical and industrial purposes. Because of its efficient nitrogen fixation system, lupin is an environmental friendly crop and it can improve traditional cereal rotations beside its high value of protein content (35-45%) in low input farming system. Additionally, lupin crop possess a great adaptation to poor soil and dry climates as a legume-*Rhizobium* symbiosis resulting in increasing soil fertility with no additional cost or effort, particularly in reclaimed soils (Julier *et al*., 1994 and El-Sayad and El-Barougy, 2002).

From the main factors which lead to greatly restrict the cultivation of lupin in Egypt (from 21428 feddan in 1957 to 3617 feddans in 2005), low yield, late maturity and susceptibility of common varieties to wilt disease causing by *Fusarium oxysporum*, *F.*sp *lupin* (Christiansen *et al*., 1999).

Biological nitrogen fixation is considered the second important biological process after photosynthesis and it can contribute by about 40-48 million tones of N year^{-1} via agricultural crops. This process accomplishes in nature by few genera of prokaryotic organisms via their ownership genetic information to convert the di-nitrogen molecules to ammonia under mild temperature and normal atmospheric pressure. These nitrogen fixing organisms can fix atmospheric nitrogen through different systems, including free living, associative and symbiotic. Biological nitrogen fixation of *Rhizobium*-legume symbiosis is considered the more efficient and important for agricultural environment, particularly sustainable agricultural systems (Havelko *et al.*, 1982; Jenkinson, 2001 and Jensen and Hauggaard, 2003).

Bacteria are by for the most abundant organisms in soil and they a key role in nutrient cycling and soil fertility. The rhizosphere, the zone of 1-2mm around plant roots, is rich in nutrients and provides niches different from those in bulk soil for bacteria to thrive (Van Loon and Bakker, 2003). Plant associated bacteria that are able to colonize root are called rhizobacteria and can be classified into beneficial, deleterious and natural groups on basis of their effect on plant growth. Beneficial rhizobacteria that stimulate plant growth are referred to as plant growth promoting rhizobacteria or PGPR (Davison, 1988 and Kloepper *et al*., 1989). Several mechanisms have been postulated to explain how PGPR may affect the growth and development of inoculated plants. These mechanisms of growth promotion appear to comprise direct or indirect or both. Direct growth promotion occurs through production of metabolites by PGPR such as phytohormones and improvement of nutrients availability (Kloepper *et al*., 1991). Indirect growth promotion can causes by restricting the activity and populations of plant pathogens or deleterious microorganisms (Van- Loon and Bakker, 2003).

Enhancement of nodulation and biological nitrogen fixation of legumes by co-inoculation with rhizobacteria are becoming a practical way to improve nitrogen availability in sustainable agriculture production system (Bai *et al*., 2002). There is evidence for a number of modes for PGPR stimulation of legume-*Rhizobium* symbiosis, but the most commonly implicated mode is phytohormones induced stimulation of root growth, resulting in providing more sites for rhizobial infection and nodulation (Vessey and Buss, 2002). Additionally, PGPRs can promote legume nodulation and nitrogen fixation by producing flavonoid like compounds and/or stimulating the host legume to produce more flavonoid signal molecules (Parmar and Dadarwal, 1999 and Bai *et al*., 2002). Hence, co-inoculation of legumes with rhizobia and rhizobacteria can improve nodulation, plant growth resulting in increasing the productivity and quality of yield (Dashti *et al*., 1997; Dileep Kumar *et al*., 2001; Garcia *et al*., 2004 and Abdel-Wahab *et al*., 2006).

Therefore, the main targets of this study were to investigate the effects of different rhizobacterial co-inoculants on the lupin-*Bradyrhizobium* symbiosis, growth and yield of lupin plants grown in two different soil types (sandy and clay). Also, this study was concerned with evaluation of some PGPR properties of selected rhizobacteria in vitro.

MATERIALS AND METHODS

Microorganisms and inoculants preparation:-

a-Bradyrhizobia and rhizobacteria:-

Bradyrhizobium sp. (*Lupinus*), mixture of local isolates, ARC401 and ARC408 were used as a basic inoculant for lupin seeds. Local isolates of rhizobacteria, *Bacillus polymyxa*, *Bacillus megatherium*, *Bacillus subtilis*, *Serratia* sp. and *Pseudomonas fluorescens* were used as separately co-inoculants with bradyrhizobia. All microorganisms used were kindly provided by Biofertilizers Production Unit, Soils, Water and Environment Research Institute, ARC, Giza, Egypt.

b- Preparation of bacterial inocula:

Bradyrhizobia was grown on yeast extract mannitol broth medium (**Vincent, 1970**), incubated at 28 ºC for three days until early log phase (5 x 10^9 CFU/ ml culture). Vermiculite supplemented with 10% Irsh peat was packed in polyethylene bags (300 g carrier per bag), then sealed and sterilized by gamma irradiation (5.0 x 10^6 rads). Bradyrhizobia culture was injected into the carrier to satisfy 60% of water holding capacity. *Pseudomonas* and *Serratia* were grown on King's medium (Atlas, 1995), incubated for 48 hr at 28 ºC to maintain population of 3x10^9 CFU/ml culture. The three *Bacillus* species used were grown on nutrient broth medium (Difico Manual, 1984) and incubated for 48 hr at 28 ºC where they attained populations of 6.5 x 10^9 , 3.2 x 10^9 and 5.5 x10^9 CFU / ml culture for *B. polymyxa*, *B. subtilis* and *B. megatherium*, respectively. Afterwards, each culture of rhizobacteria was injected in the sterilized carrier as mentioned before in preparation of bradyrhizobial inoculents. Inoculation of lupin seeds with bradyrhizobia or / and rhizobacteria was done at rate of 300 g of each inoculants / 40 kg seeds their coating with inoculants using 16% Arabic gum solution as adhesive agent.

Soil used:

Two different soil types were used. The main physical, chemical and microbiological properties of the experimental sites were determined and presented in Table (1).

Assay of rhizobacteria for PGPR-properties in vitro:-

Rhizobacteria were tested for their ability to promote plant growth through determination the main PGPR-properties, namely production of IAA (Gordon and Weber, 1951); hydrogen cyanide (Bakker and Schippers, 1987) and siderophores (Alexander and Zuberer, 1991) as well as their ability to solubilize phosphate on DCP media (Frioni, 1990).

Table (1): The main physical, chemical and microbiological traits of the two experimental sites during the two studied seasons

Soil property	Season 2005/2006		Season 2006/2007	
	Ismailia	Sids	Ismailia	Sids
Particle size distribution %				
Sand	91.0	19.6	90.60	19.50
Silt	3.63	33.8	4.60	34.0
Clay	5.37	46.6	4.80	46.5
Texture grade	Sandy	Clay loam	Sandy	Clay loam
Chemical properties:				
Saturation percent (SP%)	20.20	49.20	21.50	48.77
pH (soil paste)	7.52	7.78	7.6	7.72
EC (dSm^{-1})	0.30	1.10	0.31	1.04
Soluble cations (meq/L)				
Ca^{++}	0.72	3.10	0.71	3.00
Mg^{++}	0.50	1.28	0.60	1.36
Na^{+}	1.60	5.40	1.60	5.12
K^{+}	0.14	0.80	0.30	0.98
Soluble anions (meq/L)				
$CO_3^{=}$	0.00	0.00	0.00	0.00
HCO_3^{-}	1.77	1.62	1.60	1.51
Cl^{-}	0.60	1.50	0.70	1.72
$SO_4^{=}$	0.59	7.46	0.80	7.23
Organic matter (%)	0.36	0.96	0.40	0.91
tal-N (%)	0.020	0.039	0.022	0.041
C/N ratio	10.47	14.31	10.57	12.90
Total soluble-N (ppm)	22.1	84.20	19.90	76.50
Available –P (ppm)	5.90	12.40	5.3	10.67
Available –K (ppm)	82.60	265.2	94.50	311.60
DTPA-extractable Fe (ppm)	3.00	7.67	2.8	8.60
DTPA-extractable Mn (ppm)	1.50	4.22	1.40	4.31
DTPA-extractable Zn (ppm)	1.60	3.12	1.70	4.10
DTPA-extractable Cu (ppm)	0.90	4.00	0.70	3.81
Microbiological properties				
Total bacteria (cfu/g soil)	$4.3x10^4$	$1.3 x10^6$	$8.6 x10^4$	$9.8 x 10^5$
Total fungi (cfu/g soil)	$1.8 x10^3$	$7.3 x10^4$	$5.8 x10^3$	$5.8 x10^4$
Total actinomycetes (cfu/g soil)	$8.6 x 10^3$	$9.5 x10^5$	$1.0 x10^4$	$4.7 x 10^5$
Dehydrogenase activity (µg TPF/100 g soil/24hr)	48.6	648.7	52.8	661.3

Field experiments:

Two consecutive field experiments were excuted during the winter seasons of 2005/2006 and 2006/2007 at two different locations, namely Ismailia Res. Station (Ismailia Governorate) and Sids Res. Station (Bani Sweif Governorate) to study the influence of inoculation of lupin (*Lupinus albus* L., cv. Giza 1) with *Bradyrhizobium* sp. either solely or dually with different rhizobacteria on nodulation, growth, N-content, yield and yield components. The following co-inoculation treatment were conducted in both sites:

1-Control (without inoculation)
2-Inoculation with *Bradyrhizobium* only (Br.)
3-Br + Co-inoculation with *Serratia* sp.
4-Br + Co-inoculation with *Bacillus polymyxa*
5-Br + Co-inoculation with *Bacillus subtilis*
6-Br + Co-inoculation with *Bacillus megatherium*

7-Br + Co-inoculation with *Pseudomonas fluorescens*

Complete randomized block design with four replicates was used using plot area, 10.5 m^2 for experimental units. All treatments received the recommended doses from superphosphate (15.5% P_2O_5) at rates of 200 kg /fed for Ismailia site and 150 kg /fed for Sids one. Potassium sulphate (48% K_2O) was applied at rate of 50 kg /fed for both sites. Ammonium sulphate (20.5% N) was added at rates of 20 kg N/fed for Ismailia site and 15 kg N/fed for Sids one. In addition, Ismailia site was topdressed with 10m^3 from pre-composted farmyard manure.

Five lupin plants were uprooted from each plot after 75 days of sowing to evaluate the nodulation status plant dry matter, N-content of shoot. At harvest, seed yield, hundred seed weight, and crude protein of seeds were assayed.

Methods of analysis:

- Soil properties of the two used sites were determined according to Piper (1950) and Page *et al*. (1982).
- Total-N content of lupin shoots was measured as described by Jackson (1973).
- Crude protein percent was determined by multiplying the nitrogen percentage by 6.25.
- Data of plant parameters were subjected to analysis of variance according to the procedures outlined by Sendecor and Cochran (1980).

RESULTS AND DISCUSSION

I-Characterization of applied rhizobacteria for their ability in producing growth promoting substances under in vitro condition:

Some PGP-properties of used rhizobacteria are presented in Table (2). As general, all tested rhizobacteria were apparently able to trigger PGP-properties under in vitro conditions. Results showed that P-solubilization are the common features of all the tested microorganisms grown on synthetic media as expressed by halo clarification zone formed around their colonies (zone diameters ranged from 2.7 to 3.2 cm). It may deduced from these results that inoculation with rhizobacteria efficient in P-solubilization may act to enhance of P-availability against the adverse conditions, which causing the fixation and precipitation of phosphates either the native soil-P or the applied fertilizers, mainly superphosphate. In this concern, Rhichardson (2001) considered the P- solubilization in rhizosphere is the most common mode of action implicated in PGPR that increase nutrient availability to host plants.

Production of IAA was also exhibited by all studied rhizobacteria. *Pseudomonas* and *Serratia* were the best as they produce 19.77 and 18.6 µg/ml, respectively, while other rhizobacteria ranged from 11.5 to 13.63 µg/ml. Indeed, a high proportion of rhizobacteria are capable to produce the plant growth hormone, indol acetic acid (IAA), which act to stimulate root growth and provide it with more branching and surface area (Patten and Glick, 1996 and Glickman *et al*., 1998).

In addition, tested PGPRs were able to excrete cyanide and siderophores, except *Bradyrhizobium* and *B. megatherium*. Cyanide and siderophores has a key role in the biocontrol activity against soil borne phytopathogens, beside the essential function of siderophores in improvement of iron nutrition.

Table (2): Evaluation of the ability of used microorganisms to exhibite some PGP-properties under in vitro conditions

Microorganism	P-solubilization		IAA-production		Siderphores[d] production color Intensity	Cyanogens (HCN) Color Intensity
	Zone[a] clarification	Zone diameter (cm)	Color[b] intensity	µg/ml [c]		
radyrhizobium	+++	2.9	++	11.50	-	-
erratia sp.	+++	2.8	+++	18.60	++	+
.polymyxa	+++	3.1	++	12.75	+	+
.subtilis	+++	2.7	++	13.63	+	+
. megatherium	+++	2.8	++	12.49	-	-
s.fluorescens	+++	3.2	+++	19.77	++	++

a: diameter of the clarification zone around colonies on DCP media plates
b,c: Intensity of the pink to red color and the quantity of IAA produced (µg/ml) in liquid culture
d: Intensity of the orange halo around colonies on the chrome azurol S agar plates
- Negative result + low ++ Moderate +++ High

The ability of such rhizobacteria to exhibit PGP-properties was emphasized by many investigator (Chapot *et al*., 1996; Antoun *et al*., 1998; Bertrand *et al*., 2000; Patten and Glick, 2002; Compont *et al*., 2005 and El-Sayed, 2007). Indeed, such promotion trails are very important as primer indicative practice to evaluate the potential of rhizobacteria utility as PGPR.

II-Influence of co-inoculation with bradyrhizobia and various rhizobacteria on the lupin plants grown in two different soil types:

a-Nodulation status:

Nodulation established on the lupin roots grown in sandy and clay loamy soils after 75 days of planting as affected by co-inoculation with various rhizobacteria are presented in Table (3). It is clear that sole inoculation of lupin with bradyrhizobia resulted in significant increase in number and dry weight of nodules formed on the roots grown either in sandy or clay loamy soil after 75 days of sowing during the two studied seasons. This could be observed from the striking differences between inoculated and uninoculated treatments and emphasized the vital importance to continue inoculation of lupin seeds successively with effective strains that can tolerate the drought conditions prevailed in those areas in addition the commonly poor in their biological activity including the native rhizobia (Table 1).

Additionally, these results extremely indicating that employing of efficient bradyrhizobial strains for inoculating lupin in old clay loamy soil may improving the efficiency of nodulation process against the biotic and abiotic soil factors, which facing the indigenous brayrhizobia resulting in reducing their efficiency (Palaniappan *et al*., 1997 and Vlassak and Vanderleyden, 1997). In this concern, Minamisowa *et al*. (1992) mentioned that problem of inoculated soybean crop in most countries is the occurrence in their soils of highly competitive ineligenous populations of *Bradyrhizobium* strains, which in many cases are less efficient N_2-fixers than the inoculated strains.

Moreover, co-inoculation of lupine with bradyrhizobia and any of tested rhizobacteria tended to remarkably improve the nodulation status of the plants grown in both studied soils during the two investigated seasons as compared to bradyrhizobial inoculations.

Table (3): Effect of co-inoculation with different rhizobacteria on the nodulation status of lupin plants grown in two soil types after 75 days of planting.

Treatments	Season I		Season II	
	Ismailia site			
	No.of nodules /plant	D. wt. of nodules (mg/pt)	No.of nodules /plant	D. wt. of nodules (mg/pt)
Control	12	550.0	12	593.3
***Bradyrhiz* (Br)**	40	877.5	45	926.6
Br + *Serr.*	65	983.3	81	1016.6
Br + *B.poly*.	69	940.0	85	1060.0
Br +*B.subt*.	46	990.0	63	996.7
Br + *B.meg*.	54	953.3	52	957.0
Br+*Ps.fluor*.	72	1004.5	70	1023.3
L.S.D.at 0.05	13.96	100.33	16.61	49.82
	Sids site			
Treatments	**Season I**		**Season II**	
	No.of nodules /plant	**D. wt. of nodules (mg/pt)**	**No.of nodules /plant**	**D. wt. of nodules (mg/pt)**
Control	28	695.3	23	797.1
***Bradyrhiz* (Br)**	95	923.3	93	990.4
Br + *Serr.*	168	1035.3	178	1046.3
Br + *B.poly*.	157	1038.7	181	1047.2
Br +*B.subt*.	118	1011.4	142	1077.6
Br + *B.meg*.	126	1034.3	130	1045.3
Br+*Ps.fluor*.	161	1037.2	151	1052.3
L.S.D.at 0.05	19.13	23.27	25.18	42.28

The improvement of nodulation pattern was further strengthed in dual inoculation with bradyrhizobia in combination with any of PGPR strains indicating their distinct promotive effect on the initiation of nodules formation on the root system. This promotive effect may be occurred by providing the lupin-bradyrhizobia system with some synergistic substances such as auxins and flavoniodes-like compounds, which act to increase the infection sites occupied by rhizobia and in synchronism enhancing the survival and activity of microsymbionet in lupin rhizosphere. In parallel with this prospect, the in vitro study proved the ability of the tested rhizobacteria to excrete IAA and siderophores (Table 2). The essential role of this promoting substances triggered by rhizobacteria such as auxin, flavonid compounds and vitamins for improving nodulation status of leguminous plants has been evidenced by many investigators (Parmar and Dadarwal, 1999; Gage and Margolin, 2000; Marek-Kozoczuk and Skorupaska, 2001; Bai *et al.*, 2002 and Vessey and Buss, 2002). Data in Table (3) also displayed that the response of lupin to sole inoculation or/ and co-inoculation was more obviously in sandy soil (Ismailia site) rather than clay loam one (Sids site) during the two successive seasons. In other words, the percentage increase in mass of nodular tissues initiated on the lupin roots grown in sandy soil at Ismailia site reached to 59.55% due to inoculation with bradyrhizobia and it is raised to 77.13% as function of rhizobacterial co-inoculation (average of their treatments) in comparison to uninoculated treatment during the first season (2005/2006). While, in second season the parallel values of increase were on the average 56.18% and increased to 70.36% as compared with control treatment (mean of their treatments) as compared with uninoculated treatment. The corresponding values for clay loam soil at Sids site were 32.79 and 48.34% during the first season, and 24.25 and 32.20% during the second one.

b. Plant dry matter and shoot-N content:

A perusal of data presented in Table (4) exerted that bacterization of lupin seeds with bradyrhizobia or/ and any of the tested rhizobacteria led to significantly increase the plant dry matter and shoot-N content as compared with uninoculated plants either they grown in sandy or clay loam soil. However, the synergy inoculation between bradyrhizobia and any of the tested rhizobacteria exhibited remarkable increase in shoot, root dry matter and shoot-N content of lupin plants grown in both investigated soils, with relatively surpassing of treatments comprised *Serratia*, *Bacillus polymyxa* or *Pseudomonas*. These results was true in the two studied seasons. These findings clearly elucidated the positive impressions of rhizobacteria, which resulted in boosting the plant vigour. The promotive impression on the plant growth and N-content (as indication to symbiotic performance) may be elucidated by the ability of PGPRs to produce plant growth substances such as auxin and by immobilization of insoluble nutrients to become available to uptake by plants in the rhizosphere such as phosphate and iron (**Chapot *et al***., 1996; Dashti *et al*., 1997; Patten and Glick, 2002 and Gutierrez-Manero *et al*., 2003).

The efficiency of nitrogen fixation performance may expressed by the nitrogen accumulated in the plant tissue. Data exhibited that the percentage increase in shoot-N content of lupin plants grown in sandy soil (Ismilia site) reached to 48.36% due to sole bradyrhizobial inoculation, then it is magnified as a result of co-inoculation with various rhizobacteria, where their values were ranged from 64.25 to 117.86% in the first season (2005/2006). For second season (2006/2007), the percentage increase due sole bradyrhizobial inoculation reached to 45.94%, then it is magnified to range between 76.19 to 117.30% as function of co-inoculation with various rhizobacteria over the uninoculated treatment. The corresponding values attained for clay loam soil (Sids site) were 50.07, 83.78 to 120.59%, respectively during the first season and 53.21, 88.19 to 111.58%, respectively during the second one. Hence, these results indicated that remarkable impression of PGPRs co-inoculation emphasized their key role in improvement of biological nitrogen fixation by lupin-bradyrhizobia system either in the newly reclaimed areas or the old clay loam soils. This findings are in accordance with those obtained by El-Tahlawy (2006) and El-Sayed (2007).

Table (4): Effect of co-inoculation with different rhizobacteria on the dry matter and shoot N-content of lupin plants grown in two soil types after 75 days

Treatments	Ismailia site					
	Season I			Season II		
	Shoot d.wt g/plant	Root d.wt g/plant	Shoot N-content mg/plant	Shoot d.wt g/plant	Root d.wt g/plant	Shoot N-content mg/plant
Control	10.29	1.17	314.7	11.32	1.28	318.0
***Bradyrhiz* (Br)**	14.56	2.16	466.9	15.29	2.31	464.1
Br + *Serr.*	22.10	3.49	685.6	21.31	3.29	646.5
Br + *B.poly*.	19.70	3.11	634.7	21.85	3.50	679.0
Br +*B.subt*.	19.95	2.73	618.7	20.30	2.85	634.6
Br + *B.meg*.	16.33	2.61	516.9	17.60	2.80	560.3
Br+*Ps.fluor*.	22.41	3.07	680.3	22.31	3.07	691.0
L.S.D.at 0.05	4.21	0.83	151.01	3.73	0.84	114.49

Treatments	Sids site					
	Season I			Season II		
	Shoot d.wt g/plant	Root d.wt g/plant	Shoot N-content mg/plant	Shoot d.wt g/plant	Root d.wt g/plant	Shoot N-content mg/plant
Control	12.50	3.18	364.3	13.60	3.68	386.0
***Bradyrhiz* (Br)**	18.60	4.26	546.7	17.40	4.74	591.4
Br + *Serr.*	25.20	5.21	779.5	25.60	5.49	792.5
Br + *B.poly*.	24.80	5.34	744.6	24.50	5.40	767.0
Br +*B.subt*.	23.60	4.76	669.5	24.4	4.85	726.4
Br + *B.meg*.	25.20	4.90	799.2	25.0	5.00	816.7
Br+*Ps.fluor*.	26.20	5.29	803.6	24.80	5.76	782.2
L.S.D.at 0.05	1.44	0.93	48.43	0.95	0.53	45.61

c. Lupin yield and some it's attributes:

Seed yield, hundred seed weight and seed protein content of lupin plants grown in two different soils along two consecutive seasons as affected by co-inoculation with bradyrhizobia or / and various rhizobacteria were presented in Table (5).

It is clear that sole inoculation of lupin seeds with *Bradyrhizobium* sp. led to significant increases in seed yield, hundred seed weight and seed protein content of plants grown in both studied sites during the two investigated seasons (2005/2006 and 2006/2007). Additionally, when these seeds co-inoculated with bradyrhizobia and any of the tested rhizobacteria, the lupin yield parameters are significantly magnified in the two investigated soils. Obviously, the highest values of seed yield, hundred seed weight and seed protein content attained as a result of co-inoculation approach were 770.00 kg/fed; 31.73 g and 30.85%, respectively during the first season and they were 789.90 kg/fed; 31.93 g and 30.81%, respectively during the second one for lupin grown in sandy soil (Ismailia site). The corresponding values for clay loam soil (Sids site) reached to 1096.7 kg/fed; 33.41 g and 31.85%, respectively during the first season and they were 1129.80 kg/fed; 33.72 g and 32.50%, respectively during the second one. However, results exhibited that productivity of lupin plant grown in sandy soil exerted high responses to sole or co-inoculation approaches rather than those grown in clay loam soil. In other meaning, sole inoculation of lupin seeds sown in sandy soil at

Ismailia site with bradyrhizobia resulted in increasing seed yield by 47.47% over the control (uninoculated treatment), then this increase surpassed and ranged from 75.36 to 99.33% due to their co-inoculation with various rhizobacteria during the first season (2005/2006). The corresponding values attained in the second season (2006/2007) were 44.84%, which it surpassed to range from 66.34 to 87.22%, respectively. The corresponding values obtained for clay loam soil (Sids site) were 39.35% and 59.69 to 73.13%, respectiviely during the first season and 33.27%; 50.66 to 64.79%, respectively during the second one. These results clearly indicated that the promotive effects of PGPRs on the plant vigour may be reflected on the productivity and quality of lupin plants grown either in sandy or clay loam soils. These dramatic effects of PGPRs on the lupin yield and some it's attributes may be originated as an end result of the boosting the nodulation, growth and nitrogen fixation performance as well as saving the bioprotection against phytopathogens, which are triggered by the valuble mode of actions in lupin rhizosphere (Glick, 1999; Luz, 2001; Vessey, 2003, Abdel-Wahab *et al*., 2006 and El-Sayed, 2007).

Table (5): Response of lupin yield and some it's components to different rhizobacterial co-inoculation in two different soil types

Treatments	Ismailia site					
	Season I			Season II		
	Seed yield kg/fed	Hundred eeds weigh (g)	ßeed crude protein%	Seed yield kg/fed	undred seed weight (g)	Seed crude protein %
Control	386.30	28.19	26.91	421.90	28.60	26.76
***radyrhiz* (Br)**	569.67	30.59	28.76	611.10	30.66	28.89
Br + *Serr.*	770.00	31.57	30.71	789.90	31.93	30.81
Br + *B.poly.*	755.90	31.73	30.85	760.50	31.84	30.87
Br +*B.subt.*	677.40	31.20	30.44	701.80	30.93	30.29
Br + *B.meg*.	708.20	31.40	30.60	739.43	31.11	30.48
Br+*Ps.fluor*.	748.50	31.70	30.70	771.83	31.73	30.62
L.S.D.at 0.05	55.73	0.66	0.39	60.20	0.52	0.35
	Sids site					
Treatments	**Season I**			**Season II**		
	Seed yield kg/fed	**Hundred eeds weigh (g)**	**Seed crude protein%**	**Seed yield kg/fed**	**undred seed weight (g)**	**Seed crude protein%**
Control	633.47	29.50	28.44	685.60	29.44	27.95
***radyrhiz* (Br)**	882.73	30.89	30.14	913.71	31.07	30.80
Br + *Serr.*	1096.70	32.88	31.85	1129.80	33.59	32.50
Br + *B.poly.*	1057.50	33.24	31.63	1118.50	33.72	32.33
Br +*B.subt.*	1011.60	32.51	30.85	1032.90	32.76	31.60
Br + *B.meg*.	1022.40	32.90	30.10	1037.70	33.06	31.70
Br+*Ps.fluor*.	1065.30	33.41	31.75	1119.00	33.38	31.87
L.S.D.at 0.05	82.67	0.37	1.09	115.04	0.39	1.18

From these results, it has been concluded that exploitation of co-inoculants of rhizobacteria is becoming an efficient approach for enhancing the productivity of legumes (Bai *et al*., 2002) such as lupin cultivated in sandy and old clay loam soil as well as the ability of rhizobateria to provide co-inoculated legumes with natural bioprotection against pytopathogen such as

Fusarium (Luz, 2001; Abdel-Wahab *et al.*, 2006 and El-Sayed, 2007). Hence, these co-inoculants may considered one of the most magnitude strategies for overcoming the problems, which entangled the extension of lupin cultivation in Egypt at least in the newly reclaimed areas to magnify the key role of this crop in improvement of soil fertility.

The conclusion of the obtained results

The aim of this study was to evaluate the net effect of Bradyrhizobial inoculation solely or in combination with different species of rhizobacteria in relation with lupin plants grown in two different soils, sandy and clay loam. The average means of calculated data of the two experimental seasons and the percentage of increase over control treatments are presented in Table (6) from which the following could be evidenced:

1-Rhizobial inoculation led to proportional increase in the accumulation of plant materials of shoots and roots as well as total N content of shoot, seed yield and crud protein of seeds as compared with control treatment (uninoculated). The percentage of increases were on the average 37.74, 82.1, 46.45, 46.10 and 7.41, respectively for plants grown in sandy soils, while that grown in clay loam soil, the values were 37.93, 31.19, 51.6, 36.2 and 8.1%, respectively.

2-Co-inoculation with Bradyrhizobia in association with any of the tested rhizobacteria led to further increases in all above mentioned parameters and strengthened the stimulating effect of inoculation. The corresponding values (average of all the tested rhizobacteria) reached to 80.13, 173.2, 90.5, 77.43 and 13.03, respectively for plants grown in sandy soil. The parallel values in clay loam soil were 82.18, 61.52, 71.85, 57.79 and 11.51 in clay soil.

3-It is worthy to mention that the response of lupin plants to co-inoculation were more pronounced in sandy soil (Ismailia site) than that in clay loam soil (Sids site). Although the later achieved higher seed yield (986.23 kg/fed) as compared with plants grain in sandy soil (672.31.6kg/fed). These could be related to the differences in soil fertility between those two types of soil.

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REFERENCES

Abdel-Wahab, A. F.; G.A.A. Mekhemar; Heba Sh. Shehata and A.A.Hanali (2006). Effect of plant growth bioprotecting and promoting rhizopacteria and compost on the healthy and productivity of peanut crop in sandy soil. Minufia J. Agric. Res., 31:1323-1348.

Alexander, D.B.; and D.A. Zuberer (1991). Use of chrome azurol 5 reagent to evaluated siderophore production by rhizospher bacteria. Boil . Fertil. Soils, 2:39-45.

Antoun, H.; J. Chantal; N.Goussard; R. Chapot and R. Ialanele (1998). Potential *Rhizobium* and *Bradyrhizobium* species on plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Raphanus sativius* L.). Plant Soil, 204:57-67

Atlas, R.M.(1995). Handbook of Media for Environment Microbiology. CRC. Press, Boca Raton, F1.

Bai, Y. ; A. Souleimanov and D.L. Smith (2002). An inducible activator produced by *Serratia proteamaclans* strain and its soybean growth promoting activity under greenhouse conditions.J.Exp.Bot., 53:149-502

Bakker, A.W. and B. Schippers (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pesuomonas sp. Mediated plant growth stimulation. Soil Biol. Biochem.,4:451-457.

Bertrand, H.; C. Plassard; X.Pimochet; B. Touraine; P. Normaned and J. C. Cleyet-Mared (2000). Stimulation of the ionic transport system in *Brassiea napus* by a plant growth *Chromobacteria* sp. Can.J. Microbial., 46:229-236.

Chapot, R.; H.Antoun and M.P. Cescas (1996). Growth promotion of maize and lettuce by phosphate solubilizing *Rhizobium legumino Sarum* biovar. Plant Soil,184:321.

Christiansen, J.L; S. Raza and R. Ortiz (1999). White lupin (L.albus) germplasm collection and preliminary in situ diversity assessment in Egypt. Genetic Resources and Crop Evaluation, 46:169-174.

Compont, S. ; B. Duffy; J. Nowak; C. Clement and E. Ait Barka (2005). Use of plant growth promoting bacteria for biocontrol of plant diseases, principles, mechanisms of action, and fulture prospects. Appl. Environ. Microbiol., 72:4951-4959.

Dashti, N. F. Zhang; R. Hynes and D.L. Smith (1997). Application of plant growth promoting rhizobacteria to soybean (Glycine max L.) increases protein and dry matter yield under short season conditions. Plant Soil, 188:33-41.

Davison, J.(1988). Plant beneficial bacteria. BioTechnology, 6:282-286.

Difico Manual (1984). Dehydrated culture media and Reagents for Microbiology. 10th Edition. Difico Laboratories, Detroit, Michigan, USA.

Dileep-Kumar, B.S.; I.Berggren and A.M.Martensson (2001). Potential for improving pea production by co-inoculation with fluorescens Psedomonas and *Rhizobium*. Plat Soil, 229:25-34

El- Sayad, Z. S. and Ebtehag, El-Barougy (2002). Development iof some white lupin mutant lines high yielding, early maturing and resistant to Fusaruium wilt. Annals Agric. Sci. Ain Shams Univ., Cairo, 47:641-657.

El-Sayed Soaad Y.S. (2007). Utilization of some biological resources in biocontrol and proation of some legume plant growth. M.Sc. Thesis; Fac. of Women for Arts,Science and Education,Ain Shams Univ., Egypt.

El-Tahlawy, Y.A.Gh. (2006). Microbial impact productivity of some medicinal plants. M.Sc. Thesis, Fac. of Agric., Ain Shams Univ., Egypt.

Frioni, L. (1990). Ecologia Microbianade suelo. Depertment de Publicioures YE disciones, Universided de la Republico, Uruguay.

Gage, D. J. and W. Margolin (2000). Hanging by a thread: invasion of legume plants by rhizobia Curr. Opinion Microbiol. 3:613-617.

Garcia, J.A.L.; A. Probanza; B. Romos; J. Barriuso and F.J.Gutierrez (2004). Effects of inoculation with plant growth promoting rhizobacteria and *Sinorhizobium fredii* on biological nitrogen fixation, nodulation and growth of *Glycine max* cv. Aumi. Plant Soil,267:143-153

Glick, B.RE. (1999). In Biochemical and Genetic Mechanisms Used By Plant Growth Promoting Bacteria (eds.) Gliclc B.R.; C.N. Patten; G.Holguim and D.M. Penrose, Imperid College Press, London, pp.1-13.

Glickman, E.; L. Gardan; S. Jagquet; S. Hussain; M. Elasri; A. Petit and Y.Dessayx (1998). Auxin production is common feature most pathoras of *Pseudomonas syringae*. Mal. Plant Microbe. Interact., 11:156-162

Gordon, S.A. and R.P. Waber (1951). Colorimetric estimation of indoleacetic acid . plant Physial., 26:192-195.

Gutierrez-Manero, F. J.; A. Probanza, B. Ramos; J. J. Colonflores and J. A. Lucas (2003). Effects of cullure filtrates of rhizobacteria isolated from wild lupin on germination, growth and biological nitrogen fixation of lupin seedlings. J. Plant nutr., 26:1101-1115.

Havelko, U.D.; M.G.Bolye and R.W.F.Hardy (1982). Biological nitrogen fixation. Pp:365-422. In F.J.Stevenson; J.M. Bromner, R.D.Hank and D.R.Keeny (eds.) Nitrogen in Agriculture Soils. No.22. the Agron., Plus. Madison Wisconsin, USA.

Jackson, M.I. (1973). Soil chemical Analysis. Prentic-Hall India Private limited, New Delhi.

Jenkinson, D.A. (2001). The impact of humans on the nitrogen cycle with focus on tamperate arable agriculture. Plant Soil, 228:3-15.

Jensen, E.S. and H. Hauggaard-Nielsen (2003). How can increased use of biological N_2 fixation in agriculture benefit the environment?. Plant oil, 252:177-186.

Julier, B.; C. Huyghe and J. Papineau (1994). Dry mattr and nitrogen accumulation in determinate autumn-sown white lupin (L.albus) cv lundsle. Er.J. Agron., 3:153-160.

Kloepper, J. W., R.M. Zablotowicz; E.M.Tipping and K. Lifshitz (1991). Plant growth promotion medilated by bacterial rhizospher colonizers. In: D.L.Keister and P. B. Gregan (eds). The rhizosphere and plant growth. Kluwer, Dordrecht, pp:315-326.

Kloepper, J.W.; R.Lifshitz and R. M. Zablotowicz (1989). Free –living bacteria inocula for enhancing crop productivity. Trends Biotechnology.,7:39-43.

Luz, W. C. (2001). Evaluation of plant growth promoting and bioprotecting rhizobacteria on wheat crop. Fitopatologia Brasileira, 26:597-600.

Marek-Kozoczuk, M. and A. Skorupaska (2001). Production of B-group vitamins by plant growth promoting Pseudomonas fluorescens strain 267 and the importance of vitamins in the colonization and nodulation of rod clover. Biol. Fertil. Soils, 33:146-151.

Minamisowa, K.; T. Seki; S. Onodera; M. Kubolo and T.Asoml (1992). Genetic relotedness of *Bradyrhizobia japonicum* field isolates as revealed by repeated sequences and various other characteristics. App. Environ. Microbiol. 58:2832-2839.

Page, A.L.; R.H.Miller and D.R.Keeney (1982). Methods of Soil Anlysis. II. Chemical and microbiological properties. Soil Sci. Am., Madison Wsconsin, USA.

Palaniappan, S. P.; P.S.Sreedhar; P. Loganathan and J. Thomas (1997). Competitiveness of native *Bradyrhizobium japonicum* strains in two different soil types

Parmar, N. and K. R. Dadarwal (1999). Stimulation of nitrogen fixation and induction of flavonoidlike compounds by rhizobacteria. J. Appl. Microbiol., 86:36-44.

Patten, C.L. and B.R.Glick (1996). Bacterial biosynthesis of indole-acetic acid. Can.J. Microbial., 42:207-220.

Patten, C.L. and B.R.Glick (2002). Role of Pseudomonas putida indol acetic acid in development of the host plant root system. Appl. Environ. Microbial., 68:3795-3801.

Piper, C.S. (1950). Soil and Olant Analysis. 1st Ed. Interscience Puplishers Inc., New York, pp:30-229.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plant s. aust.J. Plant Physiol., 28:897-906.

Snedecor, G.A. and W.G. Cochran (1980). Statistical Methods. 7th Ed., Iowa State Univ. Press, Am., USA, pp.255-269.

Van Loon, L.C. and P. A. H. M. Bakker (2003). Signaling in rhizobacteria-plant interactions. Ecological Studies, 68: 290-330.

Vessey, J. K. and T.J.Buss (2002). *Bacillus cereus* UW85 inoculation effects on growth, nodulation and N-accumulation in grain legumes. Controlled environment studies. Can.J. Plant Sci., 82:282-290

Vessey, J. K. (2003). Plant growth promoting rhizobacteria as biofertilizers. Plant Soil, 255:571-586.

Vincent, J.M. (1970). A manual for the practical study of root nodule bacteria. Black well scientific pubulications, Oxford.

Vlassak, K. M. and J. Vanderleyden (1997). Factors influencing nodule occupancy by inoculent rhizobia. Critical Reviews in Plant Sciences, 16:163-229.

تحسين كفاءة العلاقات التكافلية للترمس والبرادريزوبيا والنمو والانتاجية عن طريق أنواع عديدة من الريزوباكتيريا فى انواع مختلفة من الاراضى

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نفذ هذا البحث فى نوعين مختلفين من الاراضى (رملية وطينية طميية) فى محطتى البحوث بكل من الاسماعيلية وسدس التابعة لمركز البحوث الزراعية. وتهدف هذه الدراسة لتقييم التأثير المشجع للعديد من الريزوباكتيريا من خلال دراسة تأثيرها عل تشجيع عملية تكوين العقد الجذرية ونمو وانتاجية نباتات الترمس فى نوعين مختلفين من التربة. فى البداية تم اختبار الريزوباكتريا المختارة من حيث قدرتها على اظهار الخواص المتعلقة بتشجيع النمو تحت ظروف المعمل. بعد ذلك تم تنفيذ تجربتى الحقل فى موقعى الاسماعيلية و سدس خلال الموسمين المتتالين 2006/2005 و 2007/2006.

أظهرت النتائج المعملية التأثير الايجابى للانواع المختلفة من الريزوباكتريا لاظهار الخواص المتعلقة بتشجيع النمو مع تفوق واضح لكل من السيدوموناس والسراتيا لانتاج اندول حمض الخليك. من ناحية أخرى فشل كل من البرادريزوبيا والبسلس ميجاتريم فى افراز السيانيد والسيدروفورز.

أظهرت نتائج التجارب الحقلية أن التلقيح بالبرادى ريزوبيا بمفردها نتائج التجارب الحقلية أن التلقيح بالبرادى ريزوبيا بمفردها أدى لزيادة معنوية فى حالة التعقيد والمادة الجافة والمحتوى الكلى من الازوت للنباتات النامية فى كلا الارضين الرملية والطينية الطميية بعد 75 يوم من الزراعة بالنسبة للموسمين تحت الدراسة.

بالرغم من ذلك فان هذه القياسات زادت معنويا عندما لقحت بالبرادى ريزوبيا فى كلا الارضين تحت الدراسة خلال موسمى النمو. بالاضافة لذلك فقد أعطى المحصول وبعض مكوناتة نفس الاتجاة المتحصل علية فى المرحلة الخضرية وذلك كمحصلة للتأثير المشجع لعملية التعقيد ونمو النباتات وزيادة كفاءة عملية تثبيت النيتروجين. والمثال على ذلك فان أعلى قيم الزيادة فى محصول البذور للترمس تحصل عليها فى الارض الرملية كانت 47.46% نتيجة التلقيح المفرد بالبرادريزوبيا وعندما حفز التلقيح بالعديد من الريزوباكتريا فلقد تراوحت هذه الزيادة مابين 75.36 و 99.33% خلال موسم النمو 2006/2005. كانت القيم المطابقة المتحصل عليها فى الارض الطينية هى 39.35% ومابين 59.69 و 71.13% على التوالى. علاوة على ذلك أظهرت النتائج أن حالة التعقيد وانتاجية نباتات الترمس النامية فى الارض الرملية (بموقع الاسماعيلية) أبدت استجابة عالية للتلقيح المفرد أو المحفز مقارنة بالنباتات النامية فى الارض الطينية الطميية.

Table (6): The over-all effects of co-inoculation with *Bradyrhizobium* and different species of PGPRs on lupin plants growin in sandy (1) and clay loam soil(2)

(1) Ismailia site (sandy soil)

Treatments / Tested parameters	Average values of the two seasons								Increase % over control (uninoc.)							
	Cont.	Brady. (Br)	Br. + Serr	Br+ B.Poly	Br + B.subt	Br.+ B.meg.	Br.+ Ps.fluor	Average X$^-$	Cont.	Brady. Br	Br. + Serr	Br+ B.Poly	Br + B.subt	Br.+ B.meg.	Br.+ Ps.fluor	Average X$^-$
Shoot D.W.(g/p)	10.81	14.89	21.71	20.78	20.13	16.97	22.36	18.24	-	37.74	100.81	92.2	86.2	56.98	106.85	80.13
Root.D.W.(g/p)	1.23	2.24	3.39	3.31	2.79	2.71	3.07	2.68	-	82.1	175.61	169.1	126.8	120.3	149.5	173.2
Shoot N cont.(mg/p)	317.85	465.85	660.9	656.86	626.65	538.6	685.65	564.62	-	46.45	107.65	106.65	97.2	69.45	115.7	90.5
Seed yield (kg/fed.)	404.1	590.4	779.9	758.2	689.6	723.8	760.2	672.31	-	46.10	92.99	87.63	70.65	79.11	88.12	77.43
Crude protein (%)	26.84	28.83	30.76	30.86	30.37	30.54	30.66	29.84	-	7.41	14.61	14.98	13.15	13.79	14.23	13.03

(2) Sids site (clay loam soil)

Treatments / Tested parameters	Average values of the two seasons								Increase % over control (uninoc.)							
	Cont.	Brady. Br	Br. + Serr	Br+ B.Poly	Br + B.subt	Br.+ B.meg.	Br.+ Ps.fluor	Average X$^-$	Cont.	Brady. Br	Br. + Serr	Br+ B.Poly	Br + B.subt	Br.+ B.meg.	Br.+ Ps.fluor	Average X$^-$
Shoot D.W.(g/p)	13.05	18.0	25.4	24.65	24.00	25.1	25.50	22.24	-	37.93	94.60	88.89	83.91	92.34	95.40	82.18
Root.D.W.(g/p)	3.43	4.5	5.35	5.37	4.81	4.95	5.53	4.85	-	31.19	55.97	56.55	40.20	69.50	115.70	61.52
Shoot N cont.(mg/p)	375.15	568.7	786.0	755.8	697.9	807.9	792.9	683.48	-	51.60	109.50	101.5	86.00	115.3	113.60	71.85
Seed yield (kg/fed.)	659.5	898.2	1113.3	1088.0	1022.3	1030.1	1092.2	986.23	-	36.20	68.81	64.90	55.00	56.19	65.61	57.79
Crude protein (%)	28.19	30.47	32.18	31.98	31.26	30.9	31.81	30.97	-	8.10	14.15	13.44	10.89	9.61	12.84	11.51