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INFLUENCE OF BIOFERTILIZATION WITH *Bradyrhizobium* AND PHOSPHATE SOLUBILIZING BACTERIA AND MICRONUTRIENTS APPLICATION ON GROWTH AND YIELD OF SOYBEAN

BY

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ABSTRACT

Two field experiments were conducted at the Experimental Farm, Fac. of Agric. Moshtohor during the two successive seasons of 1999 and 2000 to study the effect of *Bradyrhizobium japonicum* and *Bacillus megaterium* var. *phosphaticum* inoculation in presence of micronutrients application (iron and molybdenum) on soybean nodulation, N_2-ase activity, macro and micro-nutrients content in shoots, growth performance, yield and yield components of soybean plants.

Obtained results show that soybean nodulation and N_2-ase activity were increased in bradyrhizobial inoculated treatments. The highest records of soybean nodulation and N_2-ase activity were observed in the treatment of dual inoculation with *B. japonicum* and *B. megaterium* var. *phosphaticum* combined with foliar application with iron and molybdenum. NH_4-N, NO_3-N, and available-P were higher in the treatments inoculated with phosphate solubilizing bacteria and sprayed with either iron or molybdenum compared with uninoculated ones.

Soybean growth characters were significantly increased with either *B. japonicum* or *B. megaterium* var. *phosphaticum* inoculation compared to uninoculated treatments. The highly significant increase of soybean growth characters was observed in the treatment of dual inoculation combined with foliar application with molybdenum and iron.

Also, obtained results indicated that macro and micro-nutrients content in shoots of soybean plants were the highest in case of dual inoculation and spraying with molybdenum and iron compared to spraying with either molybdenum or iron separately.

Yield and yield components of soybean plants as well as protein and oil yield/ fed were significantly increased with either bradyrhizobial or phosphate solubilizing bacteria inoculation compared to uninoculated treatments. The highest records of yield, yield components, protein and oil yield were observed in the treatment of dual inoculaion in combination with micronutrients foliar application.

INTRODUCTION

The efforts to decrease chemical fertilization by using biofertilizers might reduce financial requirement and environmental pollution. Seed inoculation with effective strains of *B. japonicum* is common agricultural practice as it increases yield of soybean. Several investigators have found increases of soybean nodulation, growth performance and yield due to inoculation (Hegazy *et al* 1993; Ghobrial *et al* 1995; Hegazy *et al* 1997; Mikhaeel *et al* 2000 and Abd El-Fattah, 2001).

It is well known that inoculation of legumes with both rhizobia and phosphate solubilizing microorganisms i.e. VA-mycorrhizae or phosphate solubilizing bacteria increases plant growth and yield greater than that recorded when using either inoculum singularly. Vejsadova *et al* (1989); Ishac *et al* (1994); Kumrawat *et al* (1997); El-Sayed (1998) and Attia & Hassan (2000) generally attributed this increase to the enhanced N uptake due to N_2-fixation. Nevertheless, increase of phosphorus availability resulted in an increase in N_2-fixation has also been suggested to account for the synergistic relationship. Many investigators found that N, P and K concentrations as well as Fe, Mn, Zn and Cu in plant shoots were increased with dual inoculation (N_2-fixers and phosphate solubilizers) compared to the application of each inoculum singularly (El-Ghandour *et al* 1997; Barakah & Heggo, 1998 , Zaghloul, 1999, and Abou Aly & Gomaa, 2002).

The lack of trace elements such as Fe, Zn, Mo, and Mn significantly decrease the nodules system for fixing significant quantities of nitrogen even in the presence of effective strains of rhizobia (Salama & Ghonema, 1990; Hegazy *et al*, 1990; Yanni, 1990; Johal & Chalal, 1994 and Amara & Nasr, 1995). Also, they found that growth characters, seed yield, yield components and the accumulation of total nitrogen and micronutrients in seeds were significantly increased when rhizobial inoculation was accompanied with micronutrients application.

The Egyptian alluvial soils are thought to be deficient in micro-elements as a result of :(a) low percentage of organic matter. (b) Intensive cropping. (c) Reducing added amount of mud to the soil after constructing the High Dam. (d) Addition of NPK fertilization without considering micro-elements needs. (e) The alkaline conditions of soil which decrease the availability of some trace elements such as Fe, Mn, Cu, B and Zn. For these reasons, any information concerning the micronutrients status in both soil and plant in our agriculture practice is sensible agriculture policy.

Therefore, the present investigation was undertaken to study the response of soybean plants to inoculation with phosphate solubilizing bacteria, iron and molybdenum application and their interactions in presence of *Bradyrhizobium* inoculation as well as their effects on growth, yield and yield components of soybean plant.

MATERIAL AND METHODS

Two field experiments were conducted at the Agricultural Research and Experimental Center, Fac. of Agric., Moshtohor during 1999 and 2000 seasons to study the response of soybean (*Glycin max* c.v Giza 82) to inoculation with phosphate solubilizing bacteria and foliar spraying with iron and molybdenum in presence of bradyrhizobial inoculation and their effect on soybean nodulation, growth, yield and yield components.

Mechanical and chemical analyses of the experimental soil are presented in Table (1).

Mechanical and chemical analyses were carried out according to Jackson (1973) and Black *et al* (1982), respectively.

Bradyrhizobium japonicum strain USDA 110 and *B.megaterium* var. *phosphaticum* (pure local strain) were obtained from Biofertilizers Production Unit, Soil, Water and Environment Res. Inst., Agric. Res. Center, Giza, Egypt.

Inocula preparation.

For preparation of bradyrhizobial inoculum, yeast mannitol broth medium (Vincent, 1970) was inoculated with effective strain (*Bradyrhizobium japonicum*), then incubated at 32°C for 7 days. While, phosphate solubilizing bacteria inoculum was prepared by inoculating of Bunt and Rovira medium (1955) modified by Abdel-Hafez (1966) with *B. megaterium* var *phosphaticum*, then incubated at 30°C for 7 days.

Before cultivation, except the control treatment, the experimental soil plots 10.5 m^2 (3x3.5m) were supplied with calcium superphosphate at a rate of 30 kg P_2O_5/fed

Soybean seeds were successfully washed with water and air-dried. Then, seeds were soaked in cell suspension of *Bradyrhizobium japonicum* (1ml contains about $10x10^7$ viable cells) for 30min. Gum Arabic 16% was added as an adhesive agent prior to inoculation. Regarding the treatments without rhizobial inoculation, soybean seeds were treated by using the same manner, but with uninoculated N-deficient medium.

Regarding the phosphate solubilizing bacteria treatments, soybean seeds were soaked in cell suspension of *B. megaterium* var *phosphaticum* (1ml contains about $1.2x10^8$ viable cells) for 30 min. The inoculated seeds were air-dried for one hour before sowing. Except for control treatments, all plots were supplemented with a half dose of inorganic N-fertilizer (20 kg N/fed) as ammonium nitrate (33.5% N) in three equal doses i.e. at sowing, after 30 and 60 days from sowing. A control treatment was carried out where the soil was left without fertilization and seeds were not inoculated. Another control was also carried out where the seeds were sown without inoculation, but the soil was fertilized with the recommended doses of nitrogen and phosphorus i.e. 40 kg N and 30 kg P_2O_5/fed as ammonium nitrate and calcium superphosphate, respectively.

Table (1): Mechanical and chemical analyses of the experimental soil.

Mechanical analysis

Soil particle size distribution	Unit	Seasons	
		1999	2000
Coarse sand	%	16.10	16.88
Fine sand	%	14.21	14.96
Silt	%	13.50	14.60
Clay	%	56.19	53.56
Textural class		Clay	Clay

Chemical analysis

Parameters	Unit	Seasons	
		1999	2000
Organic matter	%	1.82	1.94
Total nitrogen	%	0.27	0.31
Total phosphorus	%	0.13	0.16
Total potassium	%	0.52	0.58
Iron	ppm	24.8	27.0
Zinc	ppm	2.80	3.20
Manganese	ppm	16.70	17.80
Copper	ppm	2.07	2.31
Cations			
Ca^{++}	meq/L	10.9	11.12
Mg^{++}	meq/L	6.80	5.86
Na^{+}	meq/L	5.54	6.80
K^{+}	meq/L	0.76	0.80
Anions			
CO_3^{--}	meq/L	5.05	4.82
HCO_3	meq/L	4.85	6.13
Cl^{-}	meq/L	9.30	7.18
SO_4^{--}	meq/L	4.80	6.45
$CaCO_3$	%	0.46	0.51
pH		8.21	8.16

Micro-elements, iron as sequestrone, 6% (100 ppm) and molybdenum as MoO_3 (10 ppm) were sprayed after 30 and 60 days of planting. Each micro-element was dissolved in 200 L/fed and sprayed onto the plants. All treatments were arranged in a randomized complete block design using four replicates per treatment.

This experiment included the following treatments:

1- Uninoculated and unfertilized control.
2- Uninoculated and fertilized control.

3- Iron (100 ppm)	4- Iron + *B. megaterium*.
5- Molybdenum (10 ppm).	6- Molybdenum + *B. megaterium*.
7- Iron + molybdenum.	8- Iron + molybdenum + *B. megaterium*.

Cultivation process.

Cultivation process was performed by sowing four inoculated or uninoculated seeds in hills at rows with a distance of 15 cm between hills and 70 cm between rows. After sowing, soil was directly irrigated to provide suitable moisture for inocula. The normal culture practices for growing soybean were followed as recommended in the region.

Sampling and determinations.

Rhizosphere soil samples of the developed plants were taken at vegetative (35 days) and flowering (75 days) stages. The samples were analyzed for NH_4-N and NO_3-N according to Bremner and Keeny (1965) and available phosphorus according to (A.P.H.A. 1992).

Data of nodules number, nodules weight/ plant and N_2-ase activity in nodules were estimated at flowering stage (75 days-old). N_2-ase activity was estimated according to Hardy *et al* (1973).

Total nitrogen, phosphorus and potassium content were determined in soybean shoots at the 35 and 75^{th} day after planting by using microkjeldahl according to A.O.A.C., 1980; A.P.H.A, 1992 and Dewis & Freitas (1970), respectively.

Iron, zinc and copper content were estimated in soybean shoots at the 35 and 75^{th} day after planting using atomic absorption spectrophotometer (Perkin Elmeo model 3110), while molybdenum was determined by the method described by Purvis and Peterson (1956).

Crude protein and oil content were estimated in soybean seeds. Crude protein was calculated according to the following equation:

% Crude protein = % total nitrogen x 6.25 (A.O.A.C., 1980).

Oil percentage was determined according to A.O.A.C. (1980) by Soxheelt apparatus and using petroleum ether 40-60 as a solvent.

Growth characters.

After 75 days of sowing, plant height, number of branches and dry weight of whole plant were estimated.

Yield and yield components.

At harvesting, number of pods/ plant, pods weight/ plant, 100-seed weight, seed yield /plant, seed yield /fed, biological yield /fed, oil percentage in seed, oil yield /fed, protein percentage in seed and protein yield /fed were estimated.

Statistical analysis.

Statistical analysis was carried out for growth and yield characters according to Snedecor and Cochran (1989). The differences between the means value of various treatments were compared by Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Effect of phosphate solubilizing bacteria, *Bradyrhizobium japonicum* inoculation and micronutrients application on soybean nodulation and nitrogenase activity.

Data in Table (2) show that nodules number, dry weight of nodules and N_2-ase activity were highly increased in bradyrhizobial inoculated treatments compared to uninoculated ones. Soybean plants which sprayed with either iron or molybdenum gave higher records of soybean nodulation and N_2-ase activity than nonsprayed plants. These results are in harmony with Hegazi *et al* (1995), Mikhaeel *et al* (2000) and Abd El-Fattah (2001) who recorded considerable increases of soybean nodulation and N_2-ase activity due to bradyrhizobial inoculation. Also, Hegazi *et al* (1995) and Amara & Nasr (1995) found that micro-nutrients foliar application significantly increased soybean nodulation and N_2-ase activity.

Data given in Table (2) also indicate that soybean nodulation and N_2-ase activity were higher in the treatments inoculated with phosphate solubilizing bacteria and sprayed with either iron or molybdenum compared to uninoculated ones. Inoculation of soybean plants with phosphate solubilizing bacteria combined with bradyrhizobial inoculation and spraying with molybdenum gave higher records of soybean nodulation and N_2-ase activity compared to spraying with iron. The highest records of nodules number, nodules weight and N_2-ase activity were observed in the treatment of dual inoculation with *B. megaterium* var.*phosphaticum* and *Bradyrhizobium japonicum* combined with foliar application with molybdenum and iron. This was true in the two growing seasons. The higher records of soybean nodulation and N_2-ase activity with iron and molybdenum application can be attributed to that these elements play an essential role in the biochemical reactions of N_2-fixation process, as nitrogenase system is composed of two dissociating protein components, Fe-protein and Mo. Fe-protein.

Effect of phosphate solubilizing bacteria, *Bradyrhizobium japonicum* inoculation and micronutrients application on soil nitrogen forms, and available phosphorus.

Data recorded in Table (3) show that the changes of nitrogen forms and available-P in rhizosphere of soybean plants. Obtained data show that ammoniacal and nitrate nitrogen content and available phosphorus were increased in the treatments inoculated with *Bradyrhizobium japonicum* compared to uninoculated ones. Foliar application with either iron or molybdenum gave higher values of NH_4-N, NO_3-N and available–P in rhizosphere of soybean plants compared to the treatments which had no foliar application.

Table (2): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* inoculation on nodulation and N_2-ase activity of soybean plants.

Treatments	*Bradyrhizobium* + hdn	No. of nodules / plant		Dry weight of nodules mg/ plant		N_2-ase activity (n moles C_2H_4/g dry soil/hr)	
		1999	2000	1999	2000	1999	2000
P0 + PSB0 + Mo0 + Fe0 (control)	–	11.0	13.0	100.8	120.6	52.0	54.3
P1 + PSB0 + Mo0 + Fe0 (Fert. control)	–	16.0	18.0	148.5	156.2	71.6	78.4
P1 + PSB0 + Mo0 + Fe1	+	24.0	27.0	251.0	263.0	121.1	131.2
P1 + PSB1 + Mo0 + Fe1	+	29.0	31.0	296.0	312.0	126.3	138.9
P1 + PSB0 + Mo1 + Fe0	+	30.0	32.0	306.0	323.0	125.1	133.4
P1 + PSB1 + Mo1 + Fe0	+	34.0	38.0	320.0	338.0	151.6	195.2
P1 + PSB0 + Mo1 + Fe1	+	43.0	46.0	486.0	493.0	126.9	145.3
P1 + PSB1 + Mo1 + Fe1	+	48.0	52.0	515.0	530.0	171.2	201.0

P0: Without phosphatic fertilization.
P1: Phosphatic fertilization with superphosphate.
PSB0: Non inoculated with phosphate solubilizing bacteria.
PSB1: Inoculated with phosphate solubilizing bacteria.
hdn: half dose of nitrogen.
Mo0: Without molybdenum spraying.
Mo1: Molybdenum spraying with 10 ppm solution.
Fe0: Without iron spraying.
Fe1: Iron spraying with 100 ppm solution.
Fert: Chemical fertilization (40kg N+ 30 kg P_2O_5/ fed).

Table (3): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* inoculation on nitrogen forms, available phosphorus in rhizosphere of soybean plants.

Treatments	*Bradyrhizobium* + hup	NH_4-N (ppm)				NO_3-N (ppm)				Available-P (ppm)			
		Veg.		Flow.		Veg.		Flow.		Veg.		Flow.	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
P0 +PSB0 +Mo0 +Fe0 (control)	–	26.8	28.6	42.2	49.1	41.8	44.2	50.2	52.6	57.6	59.8	61.2	67.3
P1 +PSB0 +Mo0 +Fe0 (Fert. control)	–	52.0	56.9	67.3	74.2	58.4	59.6	83.5	98.6	90.1	92.5	93.9	100.1
P1 +PSB0 +Mo0 +Fe1	+	66.0	69.2	69.1	79.4	82.0	91.2	92.9	106.0	100.3	101.2	111.1	113.1
P1 +PSB1 +Mo0 +Fe1	+	71.4	73.1	78.7	84.3	93.8	106.7	109.4	125.2	129.4	135.6	138.2	143.1
P1 +PSB0 +Mo1 +Fe0	+	60.5	63.0	68.2	71.3	81.5	93.7	111.0	113.1	94.6	96.3	99.6	103.0
P1 +PSB1 +Mo1 +Fe0	+	70.6	72.6	74.5	80.8	86.6	101.6	105.2	120.8	107.3	109.0	115.1	119.3
P1 +PSB0 +Mo1 +Fe1	+	72.1	76.9	80.2	87.1	89.7	93.7	101.2	104.4	96.1	99.3	102.1	112.4
P1 +PSB1 +Mo1+Fe1	+	76.5	81.0	86.1	92.8	99.6	106.2	130.1	136.9	140.4	148.0	151.0	155.5

Abbreviations: as those stated for Table (2).
Veg.: vegetative stage.
Flow: flowering stage.

Data in Table (3) also indicate that nitrogen forms and available-P were higher in the treatments inoculated with phosphate solubilizing bacteria and sprayed with either iron or molybdenum compared to uninoculated ones. Similar trend of results was observed in the two growing seasons. Soybean plants inoculated with phosphate solubilizing bacteria combined with *Bradyrhizobium japonicum* and sprayed with iron gave higher values of nitrogen forms and available phosphorus compared to the plants sprayed with molybdenum only.

The highest records of NH_4-N, NO_3-N and available-P were observed in the treatment of dual inoculation with *B. megaterium* var. *phosphaticum* and *B. japonicum* combined with foliar application with both molybdenum and iron. This may be due to the higher nodulation records (nodules number and dry weight of nodules) as well as N_2-ase activity which were observed in case of dual inoculation combined with foliar application with molybdenum and iron (Table, 2). It is worthy to notice that ammoniacal and nitrate nitrogen content was higher at flowering stage than vegetative one. The same trend of results was observed in all application treatments as well as in the two growing seasons. The higher levels of NH_4-N and NO_3-N recorded at flowering stage can be attributed to the high multiplication of ammonifiers and nitrifiers during flowering stage as a result of qualitative and quantitative changes in nature of the root exudates of cultivated plants during different growth stages. This result is in harmony with those obtained by Neweigy *et al* (1997) and Hanafy *et al* (1998) who found that the ammonifying and nitrifying bacterial densities in rhizosphere were higher at heading (flowering) stage of plant growth rather than other plant growth stages.

Also, data in Table (3) indicate that available-P values in rhizosphere were higher at flowering stage than vegetative one. The higher records of available-P at flowering stage can be attributed to the higher multiplication rate of phosphate solubilizing bacteria which tended to increase progressively with plant growth progression. This result is in agreement with Neweigy *et al* (1997), Barakat & Gaber (1998) and El-Sayed (1998) who reported that available phosphorus content was increased at flowering stage when the plants were inoculated with phosphate solubilizing bacteria.

Effect of phosphate solubilizing bacteria ,*Bradyrhizobium japonicum* inoculation and micronutrients application on growth characters of soybean.

Data presented in Table (4) show that growth characters of soybean plants i.e. plant height, number of branches /plant and dry weight of plants significantly increased with the treatments inoculated with *Bradyrhizobium japonicum* compared to uninoculated ones. Improvement of growth characters of soybean plants as a result of bradyrhizobial inoculation had been reported by many investigators (Hegazy *et al*, 1997; Mikhaeel *et al*, 2000 and Abd El-Fattah, 2001).

Data in Table (4) also show that foliar application of soybean plants with either iron or molybdenum gave significant increase of growth performance compared to soybean plants received no foliar application with micronutrients. Similar results were observed by Hegazy *et al* (1990); Yanni (1990); Johal & Chalal (1994) and Amara & Nasr (1995) who found that significant increases of plant growth was mostly accompanied with micronutrients application.

Soybean plants inoculated with phosphate solubilizing bacteria and sprayed with either iron or molybdenum gave higher records of growth characters compared to uninoculated ones. Soybean inoculation with *Bradyrhizobium japonicum* combined with phosphate solubilizing bacteria and sprayed with molybdenum gave significant improvements in soybean growth characters rather than the plants dually inoculated and sprayed with iron. The trend of results was consistent in the two growing seasons.

The highest records of soybean growth characters were observed in the treatment of dual inoculation with *B. megaterium* var. *phosphaticum* and *B. japonicum* combined with foliar application with both molybdenum and iron. These increases can be attributed to the high records of soybean nodulation and N_2-ase activity (Table, 2). This result is also parallel to the high records of soluble nitrogen forms (NH_4-N and NO_3-N) and available phosphorus in rhizosphere soil (Table, 3) which were observed in the treatment of dual inoculation combined with foliar application with molybdenum and iron.

Generally, obtained data show that soybean growth characters were higher in the 2^{nd} season than in the 1^{st} one. Such differences between the two growing seasons may be due to the change in the climatic conditions

Effect of phosphate solubilizing bacteria, *Bradyrhizobium japonicum* inoculation and micronutrients application on macronutrients content in shoots of soybean plants.

Data given in Table (5) clearly show that total nitrogen, phosphorus and potassium content in shoots of soybean plants significantly increased in the treatments inoculated with *Bradyrhizobium japonicum* compared to uninoculated ones. Also, soybean plants sprayed with either iron or molybdenum gave higher records of N, P and K in shoots rather than nonsprayed plants.

Concerning the effect of phosphate solubilizing bacteria inoculation, data in Table (5) show that soybean plants inoculated with *B. megaterium* var. *phosphaticum* combined with foliar application with either iron or molybdenum have higher values of macronutrients compared to uninoculated ones, and this was true in both growing seasons.

It is obvious from data presented in Table (5) that macronutrients content of soybean shoots was higher in case of dual inoculation with phosphate solubilizing bacteria combined with *Bradyrhizobium japonicum* and sprying with molybdenum and iron compared to spraying with either molybdenum or iron separately. These results confirmed those obtained by Hegazy *et al* (1990), Yanni (1990), Amara & Nasr (1995), El-Ghandour *et al* (1997), Barakah & Heggo (1998) and Abou Aly & Gomaa (2002) who found that combined inoculation of

Table (4): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* inoculation on some growth characters of soybean.

Treatments	*Bradyrhzo-bium* + hdn	Plant height (cm)		No. of branches/ plant		Dry weight of plant (g)	
		1999	2000	1999	2000	1999	2000
P0 +PSB0 +Mo0 +Fe0 (control)	–	71.0 d	72.0 d	1.8 d	2.1 d	25.00 d	26.10 c
P1+PSB0+Mo0+Fe0 (Fert. control)	–	72.0 c	73.0 d	1.9 d	2.3 cd	30.50 cd	31.90 bc
P1 +PSB0 +Mo0 +Fe1	+	74.5 c	74.0 cd	2.0 cd	2.5 b	31.00 cd	33.00 bc
P1 +PSB1 +Mo0 +Fe1	+	75.0 c	76.0 c	2.4 bc	2.7 c	32.50 c	36.20 b
P1 +PSB0 +Mo1+Fe0	+	85.0 b	86.0 b	3.5 a	3.0 b	38.20 b	43.00 a
P1 +PSB1 +Mo1 +Fe0	+	87.0 a	88.0 ab	3.6 a	3.2 b	38.70 b	45.00 a
P1 +PSB0 +Mo1 +Fe1	+	86.0 ab	89.0 ab	3.5 a	3.6 a	38.40 b	44.00 a
P1 +PSB1 +Mo1+Fe1	+	86.0 ab	90.0 a	3.6 a	3.8 a	39.70 a	46.00 a

Abbreviations: as those stated for Table (2).

Table (5): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* on NP & K content in shoots of soybean plants.

Treatments	*Bradyrhizobium* + upd	Nitrogen %				Phosphorus %				Potassium %			
		Veg.		Flow.		Veg.		Flow.		Veg.		Flow.	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
P0 + PSB0 + Mo0 + Fe0 (control)	–	2.49	2.54	2.61	2.86	0.15	0.18	0.19	0.21	2.10	2.30	2.23	2.42
P1 + PSB0 + Mo0 + Fe0 (Fert. control)	–	3.01	3.22	3.36	3.47	0.26	0.28	0.30	0.33	2.13	2.38	2.28	2.53
P1 + PSB 0 +Mo0 +Fe1	+	3.11	3.39	3.60	3.75	0.28	0.30	0.33	0.35	2.43	2.52	2.48	2.67
P1 + PSB 1 +Mo0 +Fe1	+	3.45	3.58	3.71	3.84	0.30	0.34	0.36	0.38	2.56	2.71	2.59	2.79
P1 + PSB 0 +Mo1+Fe0	+	3.32	3.40	3.73	3.81	0.29	0.32	0.32	0.37	2.51	2.56	2.63	2.69
P1 + PSB 1 +Mo1 +Fe0	+	3.54	3.69	3.86	3.92	0.33	0.36	0.35	0.39	2.60	2.70	2.71	2.75
P1 + PSB 0 +Mo1 +Fe1	+	3.46	3.78	3.77	4.05	0.33	0.35	0.36	0.39	2.57	2.49	2.68	2.75
P1 + PSB 1 +Mo1 +Fe1	+	3.66	3.91	3.84	4.13	0.36	0.42	0.44	0.46	2.63	2.73	2.80	2.83

Abbreviations: as those stated for Tables (2&3).

symbiotic N_2-fixing bacteria and phosphate solubilizers in the presence of micronutrients application gave the highest macronutrients content of plant shoots.

Data in Table (5) also emphasize that macronutrients content of soybean shoots was higher at flowering stage rather than vegetative stage. This was observed in all application treatments as well as in the two growing seasons. The higher records of macronutrients at flowering stage is likely to be due to the increase of NH_4-N, NO_3-N and available-P content in rhizosphere soil which was observed at flowering stage under different treatments (Table, 3).

Effect of phosphate solubilizing bacteria, *Bradyrhizobium japonicum* inoculation and micronutrients application on micronutrients content in shoots of soybean plants.

It is clear from data presented in Table (6) that micronutrients (iron, zinc, copper and molybdenum) were increased in the treatments inoculated with *Bradyrhizobium japonicum* compared to uninoculated ones. Foliar application of soybean plants with either iron or molybdenum gave higher records of micronutrients in shoots compared to the treatments which have no foliar application. Moreover, micronutrients content in shoots was higher in the treatments inoculated with phosphate solubilizing bacteria and sprayed with either iron or molybdenum compared to uninoculated ones. Soybean plants inoculated with *B. megaterium* var. *phosphaticum* combined with *B. japonicum* and sprayed with iron gave higher values of iron content in soybean shoots compared to the plants which sprayed with molybdenum. While, zinc, copper and molybdenum content in soybean shoots were higher in case of spraying with molybdenum than spraying with iron. Similar trends were observed in both growth stages and the two growing seasons.

The highest records of micronutrients were observed in dual inoculation treatment with phosphate solubilizing bacteria and *Bradyrhizobium japonicum* combined with foliar application with molybdenum and iron. These results are in accordance with Salama & Ghonema (1990), Gohal & Chalal (1994) and Amara & Nasr (1995) who reported that total nitrogen and micronutrients in seeds were significantly increased when rhizobial inoculation was accompanied with micronutrients application. It is worthy to mention that the micronutrients content in soybean shoots was higher at flowering stage than vegetative one and this was observed in all application treatments as well as during the two growing seasons.

Effect of phosphate solubilizing bacteria, *Bradyrhizobium japonicum* inoculation and micronutrients application on yield and yield components of soybean plants.

Data presented in Table (7) show that the pods number, pods weight /plant, seed yield /plant, seed yield /fed, 100-seed weight and biological yield /fed were significantly increased with bradyrhizobial inoculated treatments compared to uninoculated ones. These results are in harmony with Ghobrial *et al*(1995), Hegazy *et al* (1997), Mikhaeel *et al*(2000) and Abd El-Fattah (2001) who found

Table (6): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* inoculation on micro-nutrients content in shoots of soybean plants.

Treatments	*Bradyrhizobium* + hdn	Iron (ppm)				Zinc (ppm)				Copper (ppm)				Molybdenum (ppm)			
		Veg.		Flow.		Veg.		Flow.		Veg.		Flow.		Veg.		Flow.	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
P0 +PSB0 +Mo0 +Fe0 (control)	–	372.5	406.7	400.1	432.3	137.2	139.5	143.1	152.1	108.3	110.3	116.0	121.0	34.2	42.4	45.0	51.4
P1 +PSB0 +Mo0 +Fe0 (Fert. control)	–	483.2	486.0	505.7	508.9	162.1	170.0	181.6	178.7	120.8	129.2	139.5	142.1	53.6	62.2	55.8	77.2
P1 +PSB0 +Mo0 +Fe1	+	636.9	701.2	661.3	719.5	188.2	195.0	209.0	218.0	189.9	193.3	208.1	230.6	81.3	94.2	90.5	97.1
P1 +PSB1 +Mo0 +Fe1	+	800.0	815.2	821.4	840.1	205.7	209.6	221.5	225.2	192.4	199.5	211.2	238.0	90.2	100.6	92.8	103.8
P1 +PSB0 +Mo1 +Fe0	+	583.9	620.1	612.2	639.7	185.2	193.7	219.5	234.1	193.4	201.2	216.0	243.1	111.4	130.6	117.8	188.4
P1 +PSB1 +Mo1 +Fe0	+	632.4	655.2	650.3	678.7	212.3	221.2	260.1	272.1	196.1	206.0	219.1	255.6	119.2	132.8	122.0	197.0
P1 +PSB0 +Mo1 +Fe1	+	920.4	987.0	999.2	1200	278.2	292.4	307.6	380.2	228.6	245.1	278.1	301.2	113.6	123.1	126.4	137.5
P1 +PSB1 +Mo1+Fe1	+	926.0	1002	1177	1298	325.4	338.7	362.1	421.6	287.1	297.2	308.0	324.6	120.0	139.3	142.0	152.8

Abbreviations: as those stated for Tables (2&3).

Table (7): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* on yield and yield components of soybean.

Treatments	*Bradyrhizobium* + hdn	No. of pods/ plant		Weight of pods/plant (g)		Seed yield/ plant (g)		Seed yield (kg /fed)		100-seed weight (g)		Biological yield (kg/fed)	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
P0 +PSB0+Mo0 + Fe0 (control)	–	24.20^{cd}	26.60^{de}	37.0^{d}	41.2^{e}	16.90^{e}	19.80^{e}	710^{e}	750^{e}	17.2^{f}	15.0^{c}	2.70^{d}	2.70^{c}
P1 +PSB0 +Mo0 +Fe0 (Fert. control)	–	26.40^{c}	31.20^{d}	40.9^{c}	42.2^{de}	20.00^{d}	23.00^{d}	872^{d}	860^{d}	18.0^{e}	16.7^{b}	3.15^{b}	3.30^{b}
P1 + PSB 0 +Mo0 +Fe1	+	28.00^{c}	32.20^{cd}	42.0^{c}	44.5^{d}	21.40^{d}	23.90^{d}	931^{c}	920^{c}	17.9^{e}	16.2^{b}	3.10^{b}	3.40^{b}
P1 + PSB 1 +Mo0 +Fe1	+	29.00^{bc}	34.00^{cd}	46.4^{b}	49.6^{c}	21.60^{d}	24.00^{d}	1009^{c}	1025^{c}	18.4^{d}	16.0^{b}	3.20^{b}	3.60^{b}
P1 + PSB 0 +Mo1+Fe0	+	39.00^{a}	39.90^{c}	60.3^{a}	62.0^{b}	26.20^{c}	26.50^{c}	1387^{b}	1452^{ab}	21.4^{c}	20.4^{a}	3.99^{a}	4.23^{a}
P1 + PSB 1 +Mo1 +Fe0	+	40.00^{a}	40.70^{bc}	61.0^{a}	64.0^{ab}	27.90^{b}	28.20^{b}	1395^{b}	1469^{a}	22.0^{ab}	20.2^{a}	4.02^{a}	4.25^{a}
P1 + PSB 0 +Mo1 +Fe1	+	38.00^{a}	41.90^{a}	58.7^{ab}	63.9^{ab}	28.60^{a}	30.30^{a}	1417^{a}	1450^{ab}	22.30^{a}	20.3^{a}	3.90^{a}	4.26^{a}
P1 + PSB 1 +Mo1 +Fe1	+	40.00^{a}	40.90^{b}	60.4^{a}	65.0^{a}	29.10^{a}	30.60^{a}	1424^{a}	1478^{a}	21.90^{b}	20.5^{a}	4.13^{a}	4.29^{a}

Abbreviations: as those stated for Table (2).

that significant increases in yield and yield components of soybean plants due to bradyrhizobial inoculation.

Soybean plants which sprayed with either iron or molybdenum gave significant increase in yield and yield components of soybean plants compared to non sprayed plants. Nearly similar results were obtained by Hegazy *et al* (1990), Johal & Chalal (1994) and Amara & Nasr (1995) who found that seed yield and yield components of leguminous plants were significantly increased when rhizobial inoculation was accompanied with micronutrients application.

Data in Table (7) also indicate that soybean plants inoculated with *Bradyrhizobium* in combination with phosphate solubilizing bacteria and spraying with micronutrients showed significant increase in yield and yield components of soybean plants compared to individual inoculation with *Bradyrhizobium japonicum*. These results are in agreement with Vejsadova *et al* (1989); Ishac *et al* (1994); Barakat & Gaber (1998); El-Sayed (1998) and Attia & Hassan (2001) who reported that inoculation of legumes with both rhizobia and phosphate solubilizing microorganisms increases plant growth and yield. This increase in plant growth and yield can be attributed to the enhancement of nitrogen uptake due to N_2-fixation. Nevertheless, increase of phosphorus availability resulted in an increase in N_2-fixation has also been suggested to account for the synergistic relationship.

It is worthy to notice that inoculation of soybean plants with *Bradyrhizobium japonicum* combined with phosphate solubilizing bacteria and spraying with molybdenum gave higher records of yield and yield components of soybean plants compared to spraying with iron. The higher records of yield and yield components of soybean plants in case of dual inoculation and molybdenum spraying can be attributed to the high records of soybean nodulation and N_2-ase activity (Table, 2); high records of available-P (Table, 3) and improvement of plant growth (Table, 4) which were observed in the treatment of dual inoculation with *Bradyrhizobium japonicum* an *B. megaterium* var. *phosphaticum* combined with molybdenum application. The same trend of results was observed in the two growing seasons.

Generally, obtained data show that yield and yield components were higher in the 2^{nd} season than in the 1^{st} one. Such differences between the two growing seasons are likely to be due to the changes in the meteorological factors.

Effect of phosphate solubilizing bacteria, *Bradyrhizobium* inoculation and micronutrients application on protein and oil yield of soybean plants.

Data in Table (8) show that protein and oil percentage in seed and protein and oil yield /fed were significantly increased in the treatments inoculated with *B. japonicum* compared to uninoculated ones. Foliar application of soybean plants with either iron or molybdenum gave significant increase in protein and oil percentage. Protein and oil yield were significantly increased with foliar application compared to the treatments which had no foliar application. These

Table (8): Effect of phosphate solubilizing bacteria inoculation, iron and molybdenum application in presence of *Bradyrhizobium japonicum* inoculation on some chemical constituents of soybean seeds.

Treatments	*Bradyrhizobium* + hdn	Protein %		Protein yield (kg/fed)		Oil %		Oil yield (kg/fed)	
		1999	2000	1999	2000	1999	2000	1999	2000
P0 +PSB0 +Mo0 +Fe0 (control)	–	30.21 c	30.10 c	214.49 d	225.75 d	18.00 d	18.20 d	127.80 d	136.50 d
P1 + PSB0 + Mo0 + Fe0 (Fert. control)	–	30.50 c	30.50 c	265.96 c	262.30 c	19.10 c	19.00 c	166.55c	163.40 c
P1 + PSB0 + Mo0 + Fe1	+	31.30 c	31.20 b	291.40 c	287.40 c	19.40 c	19.30 c	180.61 b	177.56 c
P1 + PSB1 + Mo0 + Fe1	+	33.60 bc	34.90 a	339.02 b	357.72 b	20.60 b	21.50 ab	207.85 b	220.37 b
P1 + PSB0 + Mo1 + Fe0	+	34.30 b	35.30 a	475.74 a	512.55 a	21.30 b	21.30 ab	295.43 a	309.27 a
P1 + PSB1 +Mo1 +Fe0	+	34.60 b	35.90 a	482.67 a	527.37 a	21.60 ab	21.00 ab	301.32 a	308.49 a
P1 + PSB0 + Mo1 + Fe1	+	35.40 a	35.20 a	501.61 a	510.40 a	22.01 a	20.70 b	311.88 a	300.15 a
P1 + PSB1 + Mo1 + Fe1	+	35.50 a	35.40 a	505.52 a	523.21 a	22.30 a	22.31 a	317.55 a	329.74 a

Abbreviations: as those stated for Table (2).

results are in agreement with those reported by Hegazy *et al* (1990); Johal & Chalal (1994) and Amara & Nasr (1995).

Generally, soybean plants inoculated with *B. megaterium* var. *phosphaticum* combined with *B. japonicum* and sprayed with molybdenum gave higher records of protein and oil yield of soybean plants than plants sprayed with iron only. It is worthy to mention that dual inoculation of soybean plants with symbiotic N_2-fixing and phosphate solubilizing bacteria improved protein and oil yield /fed of soybean plants. The highest records of protein and oil yield of soybean plants were observed in the treatment of dual inoculation with *Bradyrhizobium japonicum* and *Bacillus megaterium* var. *phosphaticum* combined with foliar application with both molybdenum and iron.

CONCLUSION

From the obtained results, it can be concluded that soybean inoculation with specific strains of bradyrhizobia can be reduced nitrogen requirements fertilization to 50%. Such application minimizes the environmental pollution by using fewer amounts of chemical fertilizers. Soybean inoculation with phosphate solubilizing bacteria increases phosphorus availability, macro and micro-nutrients uptake and improves plant growth. Foliar application of micronutrients especially Mo and Fe provides optimal conditions for accumulation of nitrogen by soybean plants since successful nodulation is achieved.

Dual inoculation of soybean with *Bradyrhizobium japonicum* and phosphate solubilizing bacteria in presence of micronutrients (Mo &Fe) foliar application resulted in a considerable improvement of soybean growth, yield and yield components as well as protein and oil yield.

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تأثير التسميد الحيوي بالبرادي ريزوبيوم والبكتريا المذيبة للفوسفات والرش بالعناصر الصغرى على نمو ومحصول فول الصويا

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أجريت تجربتان حقليتان بمزرعة البحوث والتجارب بكلية الزراعة بمشتهر خلال موسمي ١٩٩٩ و ٢٠٠٠ لدارسة تأثير التلقيح بكل من *Bradyrhizobium japonicum* و *Bacillus megaterium var phosphaticum* مع الرش بالعناصر الصغرى (الحديد و الموليبدنم) على تكوين العقد الجذرية ونشاط إنزيم النيتروجينيز ومحتوى المجموع الخضري من العناصر الكبرى والصغرى وكذلك على النمو والمحصول لنباتات فول الصويا.

و لقد أوضحت النتائج أن تكوين العقد الجذرية وكذلك نشاط إنزيم النيتروجينيز قد زاد في المعاملات الملقحة بالبرادى ريزوبيوم وكانت أعلى المعدلات عند التلقيح المزدوج بالبرادىريزوبيوم والبكتريا المذيبة للفوسفات في وجود الرش بالحديد و الموليبدنم. كما وجد أن محتوي التربة من النيتروجين الأمونيومى والنيتروجين النتراتى وكذلك الفوسفات الذائبة كان اعلي في حالة المعاملات الملقحة بالبكتريا المذيبة للفوسفات مع الرش بالحديد أو الموليبدنم و ذلك بالمقارنة بالمعاملات غير الملقحة.

كما أوضحت النتائج أيضا أن التلقيح بالبرادى ريزوبيوم مع البكتريا المذيبة للفوسفات قد أعطى زيادة معنوية في صفات النمو الخضري عن المعاملات الغير ملقحة وكانت أعلى زيادة معنوية في حالة التلقيح المزدوج بكلا النوعين من البكتريا مع الرش بكل من الحديد و الموليبدنم.

أيضا أوضحت النتائج أن محتوى النبات من العناصر الكبرى والعناصر الصغرى كان أعلى في حالة التلقيح البكتيري المزدوج مع الرش بكل من الحديد و الموليبدنم. وقد أدى التلقيح بالبرادى ريزوبيوم أو البكتريا المذيبة للفوسفات إلى الحصول على زيادة معنوية في كل من محصول البذرة و البروتين ومحصول الزيت لنباتات فول الصويا بالمقارنة بالمعاملات غير الملقحة. أظهرت النتائج أن أفضل المعاملات هي تلقيح نباتات فول الصويا بكل من البرادى ريزوبيوم والبكتريا المذيبة للفوسفات مع الرش بالعناصر الصغرى.

مما سبق يتضح أن تلقيح فول الصويا ببكتريا العقد الجذرية عند الزراعة يقلل من استخدام الأسمدة الكيماوية. كذلك يمكن أن يوصي في ضوء نتائج هذه الدراسة بإجراء تلقيح تقاوي فول الصويا بالبكتريا المذيبة للفوسفات لتعظيم الاستفادة من الأسمدة الفوسفاتية تحت ظروف الأراضي المصرية. أيضا يجب الرش بالحديد و الموليبدنم لرفع كفاءة إنزيم النيتروجينيز في تثبيت أزوت الهواء الجوي و هذا ينعكس بالزيادة علي إنتاجية محصول فول الصويا.