Annals Agric. Sci., Ain Shams Univ., Cairo, 47(2), 477 - 500, 2002

EFFECTIVENESS OF DUAL INOCULATION WITH *BRADYRHIZOBIUM* AND ENDOMYCORRHIZAE IN PRESENCE OF DIFFERENT PHOSPHATIC FERTILIZER SOURCES ON GROWTH AND YIELD OF SOYBEAN

[31]

Mehasen[1], S.A.S; R.A. Zaghloul[2] and M.A. El-Ghozoli[3]

ABSTRACT

Two field experiments were carried out during 1999 and 2000 seasons at the Experimental Farm, Fac. of Agric. Moshtohor to study the effect of dual inoculation with *Bradyrhizobium japonicum* and vesicular arbuscular mycorrhizae (VAM) *Glomus mosseae* in presence of either superphosphate or rock phosphate on the nodulation, N_2-fixation, mycorrhizal root infection percentage, macro and micro-nutrients content, growth and yield of soybean plants. Results of this study showed that dual inoculation with *B. japonicum* and VA-mycorrhizae increased the nodulation, N_2-ase activity, mycorrhizal root infection percentage, plant growth and macro (N,P and K) and micro-nutrients (Fe, Zn and Cu) contents of the plants compared to the application of each inoculum singularly. Application of rock phosphate rather than superphosphate increased the abovementioned parameters. Superphosphate application gave lower records of nodulation, N_2-ase activity, mycorrhizal root infection compared to rock phosphate application. Yield and yield components of soybean plants were significantly increased in treatments inoculated with either *Bradyrhizobium* or VAM fungus as well as the combination of them and fertilized with rock phosphate compared to that inoculated and fertilized with superphosphate. The highest records of protein and oil yield were observed in the treatment of dual inoculation and fertilization with rock phosphate. Therefore, rock phosphate using combined with dual inoculation by *Bradyrhizobium and* Endomycorrhizae can be recommended as an alternative for superphosphate to reduce the production costs of soybean.

Key words: Bradyrhizobium, Mycorrhizae, Soybean, Inoculation, Rock phosphate, Superphosphate, Growth and yield

INTRODUCTION

One of the most important leguminous crops is soybean. It was introduced in Egyptian Agriculture about 1960. Its production is rapidly expanded as a result of the high demand for the seeds, that serve as a major and excellent

1- Agronomy Dept., Fac. Agric. Moshtohor, Zagazig Univ., Egypt.
2- Agric. Botany Dept., Fac. Agric. Moshtohor, Zagazig Univ., Egypt.
3- Soils, Water and Environment. Res. Inst., Agric. Res. Center, Giza, Egypt.

(Received May 29, 2002)
(Accepted August 17, 2002)

source of oil and protein for human and livestock consumption.

Generally, leguminous crops are fertilized with mineral nitrogen fertilizers as a starter to benefit the symbiotic nitrogen fixation. Rhizobial inocultation becomes an essential practice for efficient and economical soybean management. Several investigators found increases of soybean yield due to rhizobial inoculation (**Hegazy *et al* 1993; Mehasen, 1994 Ghobrial *et al* 1995** and **Kumrawat *et al* 1997**). It is well known that legumes have higher requirements of phosphours nutrition for growth and effective nodulation and consequently N_2- fixation. Mycorrhizal symbiosis is widespread on legumes root system and legumes species differ in their growth response to mycorrhizal infection. Also, mycorrhizal symbiosis increases phosphorus uptake and subsequently nodulation, plant growth and N_2-fixation (**Barakah *et al* 1998** and **Mikhaeel *et al* 2000**). Regarding the relation between phosphours forms and mycorrhizal infection, **Barea *et al* 1980; Cardoso, 1986; Yassen, 1993** and **Barakah *et al* 1998**) found that mycorrhizal infection was hardly affected by the added rock phosphate, but soluble phosphate significantly depressed the infection by either native or introduced VA-mycorrhizae. Many investigators stated that the dual inoculation of soybean plants with *Bradyrhizobium* and mycorrhizae showed significant increase in their growth characters, nodules dry weight, N_2- ase activity, macro and micro-nutrients content in shoot system as well as sed and protein yield compared to the application of each inoculum alone (**Cardoso, 1986; Vejsadova *et al* 1993; Maksoud *et al* 1995; Soliman *et al* 1996; Shalaby & Hanna, 1998** and **Mikaheel *et al* 2000**).

In this research, the effectiveness of dual inoculation of soybean with *Bradyrhizobium japonicum* and Endomycorrhizae (*Glomus mosseae*) along with using either superphosphate or rock phosphate as P-fertilizers on nodulation, symbiotic N_2-fixation, plant growth, nutrients content and soybean yield has been studied.

MATERIAL AND METHODS

Two field experiments were carried out in the Agriculture Research and Experimentation Center of Fac. Agric. Moshtohor, Zagazig Univ. during 1999 and 2000 seasons to study the response of soybean(*Glycin max* c.v. Giza 21) to dual inoculation with *Bradyrhizobium japonicum* and vesicular arbuscular mycorrhizae (*Glomus mosseae*) and study their effect on soybean nodulation, growth, yield and yield components. Some characteristics of the experimetal soil are presented in Table (1).

Particle size distribution was estimated according to **Jackson (1973)**. While, chemical analysis was determined according to **Black *et al* (1982)**.

Bradyrhizobium japonicum strain ARC 502 was obtained from Biofertilizers Production Unit, Soils, Water and Environment Res. Inst., Agric., Res. Center, Giza, Egypt. While, mycorrhizal fungus *Glomus mosseae* (Soil Goettingen strain) was provided from Tropical Institute, Goettingen University, Fedral R. Germany.

Inocula preparation

For preparation of *Bradyrhizobium* inoculum, yeast mannitol broth medium

Table 1. Some characteristics of the experimental soil

Parameters	Season		Parameters	Season	
	1999	2000		1999	2000
Particle size distribution (%):			**Soluble ions meq/l**		
Coarse sand	18.3	19.2	Ca^{+2}	9.27	8.98
Fine sand	15.1	14.6	Mg^{+2}	6.24	6.71
Silt	15.20	13.4	Na^{+}	2.71	2.92
Clay	51.4	52.8	K^{+}	0.62	0.69
Textural class	Clay	Clay	CO_3^{-2}	-	-
Organic matter (%)	1.72	1.78	HCO_3^-	8.37	8.52
P^H (1:2.5 suspension)	8.21	8.14	Cl^-	4.81	4.95
Total -N (%)	0.23	0.28	SO_4^{-2}	5.32	5.83
			Microelements		
Total-P (%)	0.16	0.21	Available Fe (ppm)	18.7	20.2
Total-K (%)	0.48	0.50	Available Zn (ppm)	5.33	5.62
$CaCO_3$ (%)	0.57	0.51	Available Mn (ppm)	3.60	3.90
E.C (dsm^{-1})	1.85	1.93	Available Cu (ppm)	2.71	2.94

(Vincent, 1970) was inoculated with the effective strain (*Bradyrhizobium japonicum*), then incubated at 32°C for 7 days.

For preparation of *Glomus mosseae* inoculum, pots of 30 cm diameter were filled with autoclaved clay loam soil. The soil of each pot was inoculated with VAM fungus *G.mosseae*. Five onion seedlings were transplanted in each pot as a host plant. After 12 weeks, spores of VAM were collected from the rhizosphere and roots of onion were extracted by wet sieving and decanting technique **(Gerdmann and Nicolson, 1963)**. VAM spores were counted by the method described by **Daniels and Skipper (1982)**.

Except for control treatments, soybean seeds were successively washed with water and air-dried. Then, seeds were soaked in cell suspension of *Bradyrhizobium japonicum* (1ml contains about 8.4 x 10^7 viable cells) for 30 min. Gum arabic (16%) was added as an adhesive agent perior to inoculation. The inoculated seeds were air dried for one hour before sowing.In uninoculated treatments with *Bradyrhizobium*, soybean seeds were treated by using uninoculated N-deficient medium instead of *Bradyrhizobium* culture.

Before cultivation, the experimental soil plots $10.5m^2$ (3 x 3.5 m) were supplied with either calcium superphosphate or rock phosphate at a rate of 30 kg P_2O_5/fed.

Regarding the mycorrhizal treatments, plots which have been prepared for inoculation with VA-mycorrhizae were provided with a mycorrhizal spore suspension. The extracted mycorrhizal spore suspension containing about 120-150 spores/ml was used as a standard inoculum (20 ml/m^2) for mycorrhizal treatments. Nitrogen fertilizer was applied in the form of ammonium nitrate (33.5% N) at a rate of 20 kg N/fed to all treatments in two equal doses before the first and second irrigation.

Experimental design

A split plot design with four replicates was used in this study. The main plots were assigned to the phosphatic fertilizer sources (zero, rock-p and super-p). While, four dual inoculation with *Bradyrhizobium and* mycorrhizal treatments (Br0M0, Br0M1, Br1M0 and Br1M1) were randomly distributed in the sub plots.

Cultivation process

Cultivation process was performed by sowing four inoculated or uninoculated seeds pre hill at ridges with a distance of 10 cm between hills and 60 cm between ridges. Sowing took place on May 25th in 1999 and May 27th in 2000. After sowing, soil was directly irrigated to provide a suitabel moisture for inocula. Before the 1st irrigation, plants were thinned to two plants per hill. The preceding crop was clover in the two seasons. Agronomic practices were followed according to the standard recommendation for soybean.

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 CO_2 evolution according to **Page *et al* (1982)**, 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 dry weight/plant, N_2-ase activity of nodules and mycorrhizal root infection were estimated at flowering stage at the75th day after cultivation. N_2-ase activity was estimated according to **Hardy *et al* (1973)**. Mycorrhizal root infection of soybean plants was assessed microscopically according to **Mosse and Giovanetti (1980)**.

Total nitrogen, phosphorus and potassium content were determined in soybean shoots at 35 and 75 days after planting according to **A.O.A.C (1980)**, **(A.P.H.A, 1992)** and **Dewis & Freitas (1970)**, respectively. Also, iron, zinc and copper were determined in soybean shoots at 35 and 75 days after planting by the atomic absorption, Perkin Elmer model 3110.

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)**. Also, oil content was determined according to **(A.O.A.C,**

1980) by soxholt apparatus using petroleum ether 40-60 as a solvent.

Grwoth characteristics

After 75 days from sowing, ten guarded plants were chosen at random then plant height, number of branches/plant, dry weights of stem, leaves and pods were estimated.

Yield and its components

At harvesting, ten guarded plants were used to estimate number of pods/plant, pods weight/plant, 100-seed weight. Seed yield/plant seed yield/fed and biological yield/fed were recorded from three inner ridges from each experimental plot, then oil yield/fed and protein yield/fed were calculated.

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 multiple range test (**Duncan, 1955**).

RESULTS AND DISCUSSION

Effect of phosphatic fertilizer sources, dual inoculation and their interaction on nodulation, N_2-ase activity and mycorrhizal root infection of soybean plants.

It is clear from data presented in Table (2) that the nodules number and dry weight were remarkably increased in bradyrhizobial inoculated treatments compared to uninoculated ones. Number and dry weight of nodules in bradyrhizobial inoculation treatments were greater than mycorrhizal inoculation. The highest number and dry weight of nodules were observed with dual inoculation and this was true in the two growing seasons.

Regarding the effect of phosphatic fertilization, obtained data show that soybean plants fertilized with either superphosphate or rock phosphate gave higher nodules number and dry weight than nonfertilized plants. Also, soybean plants fertilized with rock phosphate gave higher nodules number and dry weight compared to the plants fertilized with superphosphate. The same trend of results was observed in the two growing seasons. Generally, obtained data show that the nodules number and dry weight were higher in the 2nd season than in the 1st one.

It is not a surprising result that, N_2-ase activity was higher in case of bradyrhizobial inoculated treatments than mycorrhizal inoculated ones. This result is in harmony with those obtained by **Ghobrial *et al* (1995), Kumrawat** (1997) **Mikhaeel *et al*** (2000) and **Abd El-Fattah (2001)** who found increases of soybean nodulation and N_2-ase activity due to rhizobial inoculation. Whereas, the mycorrhizal infection perecentage was higher in case of mycorrhizal inoculated treatments compared to bradyrhizobial inoculated ones. Obtained data also clearly show differences in root colonization of soybean plants grown in VAM inoculated and uninoculated treatments which depended on the

Table 2. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on nodulation, N_2-ase activity and mycorrhizal root infection of soybean plants after 75 day of cultivation (flowering stage).

P-fertilizer sources	Dual in-oculation	No. of nodules/plant		Dry weight of nodules (mg/plant)		N_2-ase activity (n moles C_2H_4/hr/g dry nodules)		Mycorrhizal root infection (%)	
		1999	2000	1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	12	14	136	148	21.3	23.1	4.0	6.0
	Bro+M1	15	18	161	172	35.3	44.1	34.0	39.0
	Br1+Mo	24	23	230	241	69.1	72.4	11.0	14.0
	Br1+ M1	30	33	293	301	78.2	83.5	48.0	51.0
Mean		20	22	205	216	51.0	55.8	24.3	27.5
Rock-P	Bro+Mo	17	16	196	180	24.2	26.6	10.0	14.0
	Bro+M1	20	25	240	263	63.4	64.5	63.0	66.0
	Br1+Mo	41	42	481	493	91.5	92.9	19.0	21.0
	Br1+ M1	43	45	520	536	121.6	130.7	78.0	80.0
Mean		30	32	359	368	75.2	78.7	42.5	45.3
Super-P	Bro+Mo	16	18	173	184	25.0	29.2	8.0	9.0
	Bro+M1	18	22	201	228	47.2	51.3	57.0	59.0
	Br1+Mo	32	38	318	326	86.9	89.8	16.0	18.0
	Br1+ M1	37	41	390	406	101.5	105.4	73.0	76.0
Mean		26	30	271	286	65.2	68.9	38.5	40.5
Over all means	Bro+Mo	15	16	168	171	23.5	26.3	7.3	9.7
	Bro+M1	18	22	201	221	48.6	53.3	51.3	54.7
	Br1+Mo	32	34	343	353	82.5	85.0	15.3	17.7
	Br1+ M1	37	40	401	414	100.4	106.5	66.3	69

Bro, Non bradyrhizobial inoculation. Br1, Bradyrhizobial inoculation.
Mo, Non Mycorrhizal inoculation. M1, Mycorrhizal inoculation.

Table 2. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on nodulation, N_2-ase activity and mycorrhizal root infection of soybean plants after 75 day of cultivation (flowering stage).

P-fertilizer sources	Dual in-oculation	No. of nodules/plant		Dry weight of nodules (mg/plant)		N_2-ase activity (n moles C_2H_4/hr/g dry nodules)		Mycorrhizal root infection (%)	
		1999	2000	1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	12	14	136	148	21.3	23.1	4.0	6.0
	Bro+M1	15	18	161	172	35.3	44.1	34.0	39.0
	Br1+Mo	24	23	230	241	69.1	72.4	11.0	14.0
	Br1+ M1	30	33	293	301	78.2	83.5	48.0	51.0
Mean		20	22	205	216	51.0	55.8	24.3	27.5
Rock-P	Bro+Mo	17	16	196	180	24.2	26.6	10.0	14.0
	Bro+M1	20	25	240	263	63.4	64.5	63.0	66.0
	Br1+Mo	41	42	481	493	91.5	92.9	19.0	21.0
	Br1+ M1	43	45	520	536	121.6	130.7	78.0	80.0
Mean		30	32	359	368	75.2	78.7	42.5	45.3
Super-P	Bro+Mo	16	18	173	184	25.0	29.2	8.0	9.0
	Bro+M1	18	22	201	228	47.2	51.3	57.0	59.0
	Br1+Mo	32	38	318	326	86.9	89.8	16.0	18.0
	Br1+ M1	37	41	390	406	101.5	105.4	73.0	76.0
Mean		26	30	271	286	65.2	68.9	38.5	40.5
Over all means	Bro+Mo	15	16	168	171	23.5	26.3	7.3	9.7
	Bro+M1	18	22	201	221	48.6	53.3	51.3	54.7
	Br1+Mo	32	34	343	353	82.5	85.0	15.3	17.7
	Br1+ M1	37	40	401	414	100.4	106.5	66.3	69

Bro, Non bradyrhizobial inoculation. Br1, Bradyrhizobial inoculation.
Mo, Non Mycorrhizal inoculation. M1, Mycorrhizal inoculation.

Table 3. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on nitrogen forms, available phosphorus content and CO_2 evolution in rhizosphere of soybean plants.

P-fertilizer sources	Dual inoculation	NH_4-N (ppm)				NO_3-N (ppm)			
		Vegetative stage		Flowering stage		Vegetative stage		Flowering stage	
		1999	2000	1999	2000	1999	2000	1999	2000
Zero –P	Bro+Mo	60.6	67.9	62.2	73.5	46.4	50.4	61.3	69.9
	Bro+M1	72.8	94.8	89.8	104.4	68.5	84.2	78.6	100.6
	Br1+Mo	89.4	101.5	103.0	125.2	79.6	100.0	98.2	103.5
	Br1+ M1	96.1	107.0	129.3	146.1	98.6	113.4	121.5	123.4
Mean		79.7	92.8	96.1	112.3	73.3	87.0	89.9	99.4
Rock-P	Bro+Mo	68.3	71.7	71.6	75.4	53.3	57.8	67.9	73.8
	Bro+M1	83.8	107.4	97.1	128.5	72.3	88.0	82.6	113.3
	Br1+Mo	100.1	115.6	113.5	144.8	91.2	126.0	109.3	117.0
	Br1+ M1	112.5	124.4	181.3	187.8	133.4	152.3	176.1	182.4
Mean		91.2	104.8	115.9	134.1	87.6	106.0	109.0	121.5
Super –P	Bro+Mo	68.9	70.2	70.1	74.7	52.2	57.3	68.1	72.4
	Bro+M1	77.2	100.8	92.7	115.9	69.9	86.5	80.5	104.5
	Br1+Mo	92.7	109.3	110.4	142.3	83.1	124.7	100.4	116.2
	Br1+ M1	103.6	121.2	153.4	170.7	114.2	120.2	146.8	180.8
Mean		85.6	100.4	106.7	125.9	79.9	97.2	99.0	118.5
Over all means	Bro+Mo	65.9	69.9	68.0	74.5	50.6	55.2	65.8	72.0
	Bro+M1	77.9	101.0	93.2	116.3	70.2	86.2	80.6	106.1
	Br1+Mo	94.1	108.8	109.0	137.4	84.6	116.9	102.6	122.2
	Br1+ M1	100.7	117.5	154.7	168.2	115.4	128.6	148.1	162.1

Table 3. Cont.

P-fertilizer sources	Dual inoculation	Available-P(ppm)				CO_2 evoluted (µg/g dry soil/hr)			
		Vegetative stage		Flowering stage		Vegetative stage		Flowering stage	
		1999	2000	1999	2000	1999	2000	1999	2000
Zero –P	Bro+Mo	59.1	68.9	60.2	70.1	30.6	32.4	33.2	35.6
	Bro+M1	112.3	116.4	116.6	121.7	49.7	63.1	57.9	71.5
	Br1+Mo	98.9	110.1	100.7	116.5	36.3	44.4	39.8	53.9
	Br1+ M1	128.1	136.2	130.2	138.3	73.2	76.2	81.3	89.0
Mean		99.6	107.9	101.9	111.7	47.5	54.0	53.1	62.5
Rock-P	Bro+Mo	78.9	91.2	83.4	96.0	32.5	36.9	37.1	48.6
	Bro+M1	125.1	126.8	128.0	139.8	64.7	69.8	70.7	74.8
	Br1+Mo	112.5	118.7	119.8	126.4	41.8	51.9	53.3	63.2
	Br1+ M1	142.8	148.3	145.7	151.4	87.6	90.5	104.9	112.0
Mean		114.8	121.3	119.2	128.4	56.7	62.3	66.5	74.7
Super –P	Bro+Mo	70.3	84.8	77.6	87.3	31.9	35.3	36.0	44.7
	Bro+M1	121.5	121.1	126.2	130.5	56.8	66.4	62.4	67.1
	Br1+Mo	103.3	111.9	115.6	123.1	38.6	50.8	52.1	58.4
	Br1+ M1	136.3	145.7	139.1	147.2	80.3	81.6	96.8	99.2
Mean		107.9	117.1	114.6	122.0	51.9	58.5	61.8	67.4
Over all means	Bro+Mo	69.4	81.6	73.7	84.5	31.7	34.9	35.4	43.0
	Bro+M1	119.6	123.1	123.6	130.7	57.1	66.4	63.7	71.1
	Br1+Mo	104.9	113.6	112.0	122.0	38.9	49.0	48.4	58.5
	Br1+ M1	135.7	143.4	138.3	145.6	80.4	82.8	94.3	100.1

Abbreviations: as those stated for Table (2).

result of the positive qualitative and quantitative changes in nature of the plant root exudates during different growth stages. These results are in harmony with those obtained by **Neweigy *et al* (1997)** and **Hanafy *et al* (1998)** who found that the amonifiers and nitrifiers bacterial densities were higher in rhizosphere during heading stage of plant growth rather than other plant growth stages. Data also emphasize that available phosphorus content and CO_2 evolution were increased with either mycorrhizal or bradyrhizobial inoculated treatments compared to uninoculated ones. Mycorrhizal inoculated treatments showed higher available-P and CO_2 evolution than bradyrhizobial inoculated ones. The highest records of available-P and CO_2 evolution were observed with dual inoculation of soybean plants with *Bradyrhizobium* and VAM fungus. Rock-p treatments gave higher values of available phosphorus and CO_2 evolution than super-p ones. This may be due to the higher rates of mycorrhizal root infection which observed with rock-p treatments (Table, 2). The same trend of results was observed in both growth stages and the two growing seasons. Available phosphorus and CO_2 evolution were also higher during flowering stage than vegetative stage. The higher records of available phosphorus at flowering stage may be attributed to the higher multiplication rate of phosphate dissolvers which tended to increase progressively with plant growth.These results are in agreement with those reported by **Saad & H ammad (1998)**; **Zaghloul (1999)** and **Abou-Aly & Gomaa (2002)** who reported that available phosphorus content was increased during flowering stage when the plants were inoculated with phosphate solubilizing microorganisms and amended with rock phosphate.

With regard to the interaction effect, data in Table (3) indicate that dual inoculation with *Bradyrhizobium* + mycorrhizae combined with phosphatic fertilization showed higher rhizospheric NH_4 –N, NO_3 –N, available-P and CO_2 evolution than either inoculation only or phosphatic fertilization separately. Dual inoculation with *Bradyrhizobium* + mycorrhizae and fertilization with rock phosphate gave the highest values of tested parameters.

Effect of phosphatic fertilizer sources, dual inoculation and their interaction on growth characters of soybean plants.

Data in Table (4) indicate that growth characters of soybean plants i.e. plant height, number of branches/plant and dry weight of stem, leaves and pods/plant were significantly increased with either bradyrhizobial or mycorrhizal inoculation compared to uninoculated controls. Generally, significant increases were observed in most plant growth characters with bradyrhizobial inoculation compared to mycorrhizal inoculation. While, dual inoculation of soybean plants with *Bradyrhizobium japonicum* and VAM fungus (*Glomus mosseae*) gave higher records of growth characters than the application of each inoculum singularly.

These results are in accordance with those obtained by (**Vejsadova *et al* 1993; Soliman *et al* 1996; Shalaby & Hanna, 1998** and **Mikhaeel *et al* 2000**). They reported that the dual inoculation of

Table 4. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on growth characters of soybean plants

P-fertilizer sources	Dual inocula-tion	Plant height (cm)		No. of branches per plant		Dry weight of stem (g)		Dry weight of leaves (g)		Dry weight of pods (g)	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	62.0f	80.8I	2.08e	1.85a	9.92h	8.00c	10.25g	10.27h	9.07j	7.68h
	Bro+MI	75.8e	87.2gh	2.80c	2.45a	13.27f	11.88b	18.90e	16.70g	13.99g	12.07g
	BrI+Mo	85.8abc	88.6ef	2.40d	2.75a	14.86de	11.85b	19.69d	17.42f	16.86e	13.63c
	BrI+ MI	82.0cde	91.8c	3.28a	2.98a	18.09a	12.65ab	21.08c	19.15c	18.55b	15.25c
Mean		76.4C	87.1C	2.64B	2.51B	14.04A	11.09B	17.48B	15.89C	14.62C	12.16C
Rock-P	Bro+Mo	80.5cde	86.2h	2.88bc	2.28a	9.77h	11.68b	18.75e	16.65g	13.63h	12.25g
	Bro+MI	90.3a	88.4fg	3.13ab	2.55a	12.05g	12.18b	19.75d	17.60ef	15.68f	13.77c
	BrI+Mo	89.5ab	90.3d	3.13ab	2.90a	17.99a	12.23b	19.86d	18.15d	17.65d	14.38d
	BrI+ MI	89.3ab	95.4a	3.13ab	3.15a	16.70bc	13.98a	23.53a	21.70a	20.01a	18.50a
Mean		87.4A	90.1A	3.06A	2.72A	14.13A	12.51A	20.47A	18.52A	16.74A	14.73A
Super-P	Bro+Mo	77.8de	86.4h	2.78c	2.20a	13.93ef	11.57b	18.19f	16.65g	10.64I	12.07g
	Bro+MI	83.3bcd	87.8fg	2.80c	2.58a	8.96h	12.07b	20.86c	17.30f	15.57f	12.90f
	BrI+Mo	82.3cd	89.7de	3.13ab	2.75a	15.90cd	12.05b	19.89d	17.88de	17.99c	14.18de
	BrI+ MI	85.5abc	93.3b	3.08ab	3.05a	17.34ab	13.15ab	22.93b	20.40b	19.85a	16.90b
Mean		82.3B	89.3B	2.94A	2.64AB	14.03A	12.21A	20.47A	18.06B	15.99B	14.01B
Over all means	Bro+Mo	73.4B	84.4D	2.58C	2.11D	11.21C	10.42C	15.73C	14.52D	11.11D	10.67D
	Bro+MI	83.1A	87.8C	2.91B	2.53C	11.43C	12.04B	19.84B	17.20C	15.08C	12.92C
	BrI+Mo	86.0A	89.5B	2.88B	2.80B	16.25B	12.04B	19.81B	17.82B	17.47B	14.06B
	BrI+ MI	85.6A	93.5A	3.16A	3.06A	17.38A	13.26A	22.51A	20.42A	19.47A	16.88A

Abbreviations : as those stated for Table (2).
Means followed by the same letter (s) within each column, are not significantly different from each other at 5% level.

soybean plants with *B. japonicum* and mycorrhizae showed significant increase in their growth characters.

Concerning the effect of phosphatic fertilization, rock phosphate fertilization treatments showed significant increase in growth characters compared to superphosphate fertilization ones. The same trend of results was observed in the two growing seasons.

Respecting the interaction effect, data in Table (4) show that dual inoculation with *Bradyrhizobium* + mycorrhizae combined with phosphatic fertilization gave significant increase in growth characters compared to soybean inoculation or phosphatic fertilization separately.

The highly significant increase in soybean growth characters was observed in the treatment of soybean plants inoculated with *Bradyrhizobium* + mycorrhizae and fertilized with rock phophate. This result could be attributed to the high levels of N_2-ase activity and mycorrhizal colonization (Table, 2) as well as the high levels of NH_4-N, NO_3-N and available phosphorus (Table, 3) which observed in the treatment of soybean plants inoculated with *Bradyrhizobium* + mycorrhizae and fertilized with rock phosphate.

Generally except for plant height, soybean growth characters were higher in the 2nd season than the 1st season. This difference between the two growing seasons may be due to the changes in the climatic conditions.

Effect of phosphatic fertilizer sources, dual inoculation and their interaction on macro-nutrinets content of soybean shoots.

Data in Table (5) show that the total nitrogen, phosphorus and potassium content in shoots of soybean plants were increased in the treatments inoculated with either *Bradyrhizobium* or mycorrhizae compared to uninoculated treatments. Bradyrhizobial inoculated treatments gave higher levels of total nitrogen than mycorrhizal inoculated ones. Whereas, mycorrhizal inoculated treatments gave higher levels of total phosphorus and potassium compared to bradyrhizobial inoculated ones.

Dual inoculation of soybean plants gave higher levels of macro-nutrients content (NPK) than those recorded in treatments inoculated with either of *Bradyrhizobium* or mycorrhizae.

With regard to the phosphatic fertilization effect, obtained data show that macor-nutrients levels (NPK) were higher in the treatments fertilized with rock phosphate than the treatments fertilized with superphosphate. This result can be attributed to the higher N_2-ase activity and mycorrhizal root infection recorded in such case (Table, 2) these parameters were greater in the treatments fertilized with rock phosphate than the treatments fertilized with superphosphate. These results are in harmony with those obtained by **Ishac *et al* (1994), El-Sawy *et al* (1998), Mikhaeel *et al* (2000)** and **Abd El-Fattah(2001)** who found that rhizobial inoculation incereased total nitrogen in plant shoots in comparison with uninoculated plants. While, mycorrhizal inoculated plants contained higher levels of phosphorus compared to uninoculated ones (**El-Sawy *et al* 1998; Shalaby & Hanna, 1998** and **Mikhaeel *et al* 2000**). Moreover, they reported that the dual inoculation with *Bradyrhizobium*

Table 5. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on macro - nutrients content of soybean shoots.

P-fertilizer sources	Dual inoculation	Nitrogen (%)				Phosphorus(%)				Potassium (%)			
		Vegetative stage		Flowering stage		Vegetative stage		Flowering stage		Vegetative stage		Flowering stage	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	2.13	2.31	2.17	2.52	0.14	0.15	0.16	0.18	1.45	1.39	1.50	1.65
	Bro+M1	2.97	3.18	2.68	3.65	0.23	0.26	0.27	0.29	1.68	1.56	1.75	1.83
	Brl+Mo	3.59	3.92	3.62	3.93	0.16	0.19	0.20	0.22	1.50	1.52	1.62	1.75
	Brl+ M1	3.66	3.82	3.64	4.03	0.24	0.28	0.29	0.34	1.95	1.90	2.10	2.08
Mean		3.09	3.31	3.03	3.53	0.19	0.22	0.23	0.26	1.65	1.59	1.74	1.83
Rock-P	Bro+Mo	3.19	3.30	3.17	3.25	0.24	0.26	0.28	0.27	1.72	1.64	1.91	1.95
	Bro+M1	3.22	3.82	3.62	3.75	0.39	0.35	0.39	0.41	1.96	1.94	2.16	2.16
	Brl+Mo	3.81	4.12	3.99	4.30	0.29	0.31	0.36	0.34	1.90	1.79	2.10	2.05
	Brl+ M1	3.94	4.32	4.16	4.53	0.46	0.49	0.52	0.58	2.40	2.25	2.65	2.62
Mean		3.54	3.89	3.74	4.01	0.35	0.35	0.39	0.40	2.00	1.91	2.21	2.20
Super-P	Bro+Mo	2.91	3.12	3.01	3.08	0.18	0.21	0.25	0.24	1.61	1.55	1.83	1.72
	Bro+M1	3.18	3.46	3.09	3.68	0.33	0.36	0.36	0.38	1.95	1.90	2.01	2.13
	Brl+Mo	3.63	4.10	3.57	4.25	0.23	0.29	0.30	0.32	1.83	1.71	1.80	1.91
	Brl+ M1	3.73	4.26	3.68	4.35	0.35	0.38	0.36	0.39	2.06	2.11	2.55	2.36
Mean		3.36	3.74	3.34	3.84	0.27	0.31	0.32	0.33	1.86	1.82	2.05	2.03
Over all means	Bro+Mo	2.74	2.91	2.78	2.95	0.17	0.21	0.23	0.23	1.59	1.52	1.75	1.77
	Bro+M1	3.12	3.49	3.13	3.76	0.32	0.32	0.34	0.36	1.86	1.80	1.97	2.04
	Brl+Mo	3.68	4.05	3.73	4.16	0.23	0.26	0.29	0.29	1.74	1.67	1.84	1.90
	Brl+ M1	3.78	4.13	3.83	4.30	0.35	0.38	0.39	0.44	2.14	2.09	2.43	2.35

Abbreviations : as those stated for Table (2).

and VAM fungi gave the highest levels of macro-nutrients content of plant shoots. Such trends of results support the obtained results in the current study.

With respect to the interaction effect, data presented in Table (5) indicate that dual inoculation with *Bradyrhizobium* + mycorrhizae combined with phosphatic fertilization gave higher levels of macro-nutrients content of soybean shoots than either soybean inoculatin or phosphatic fertilization separately. The highest levels of macro-nutrients content were observed in the treatment of soybean inoculation with *Bradyrhizobium* + mycorrhizae accompanied with rock phosphate.

Data also show that macro-nutrients (NPK) content of soybean shoots was higher during flowering stage than vegetative one. The same trend of results was obtained in all treatments as well as during the two growing seasons. Similar results were recorded by (**Vejsadova *et al* 1993; Maksoud *et al* 1995** and **Mikhaeel *et al* 2000**).

Effect of phosphatic fertilizer sources, dual inoculation and their interaction on micro-nutrients content in shoots of soybean plants.

Data presented in Table (6) show that micro-nutrients (iron and copper) content of soybean shoots were higher in mycorrhizal inoculated treatments than bradyrhizobial inoculated ones. Whereas, zinc content of soybean shoots was higher in rhizobial inoculated treatments than mycorrhizal inoculated ones. The same trend was observed in the two growing seasons as well as in different growth stages of soybean plants.

The highest micro-nutrients content was observed in the case of dual inoculation compared to inoculation with either *Bradyrhizobium* or VAM individually. This result is in harmony with those reported by (**Cardoso, 1986; Soliman *et al* 1996** and **Shalaby & Hanna, 1998**) who reported that the dual inoculation of soybean plants with *Bradyrhizobium* + mycorrhizae showed the highest increase of micro-nutrients content in shoot system.

Data in Table (6) also indicate that soybean plants fertilized with rock phosphate gave higher micro-nutrients content compared to soybean plants fertilized with superphosphate. This result could be attributed to the higher records of N_2-ase activity and mycorrhizal root infection rate in case of rock phosphate treatments which previously discussed in Table (2).

Respecting the interaction effect, data recorded in Table (6) show that dual inoculation with *Bradyrhizobium* + mycorrhizae combined with phosphatic fertilization gave higher micro – nutrients content of soybean shoots than either soybean inoculation or phosphatic fertilization singularly. The highest micro- nutrients content was observed in the treatment of soybean inoculation with *Bradyrhizobium* + mycorrhizae and fertilized with rock phosphate.

Micro-nutrients content of soybean shoots was higher during flowering stage than vegetative one and this was true in all treatments as well as during the two growing seasons.

Generally, obtained data show that the micro-nutrients content was higher in the 2^{nd} season than in the 1^{st} one. The differences between the two growing

Table 6. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on micro-nutrients of soybean shoots

P-fertilizer sources	Dual inoculation	Iron (ppm)				Zinc (ppm)				Copper (ppm)			
		Vegetative stage		Flowering stage		Vegetative stage		Flowering stage		Vegetative stage		Flowering stage	
		1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	783.0	786.1	794.2	799.5	225.3	227.3	228.1	230.0	75.9	80.2	77.6	81.3
	Bro+M1	1100	1138.6	1342.0	1369.1	312.3	327.8	349.7	358.5	149.7	148.3	156.9	158.7
	Brl+Mo	917	987.2	1003.1	995.4	320.7	348.0	358.6	366.0	144.6	146.7	142.6	156.2
	Brl+ M1	1339	1372.5	1373.2	1407.3	415.8	397.2	418.2	414.1	162.5	163.2	164.3	170.0
Mean		1035	1071.0	1128	1143	318.5	325.1	338.7	342.2	133.2	134.6	135.4	144.6
Rock-P	Bro+Mo	798	887.2	886.7	972.8	241.7	283.1	249.1	346.0	78.2	84.3	80.4	89.5
	Bro+M1	1306.0	1363.3	1370.3	1382.4	369.5	389.0	382.0	421.2	168.3	168.9	169.8	173.7
	Brl+Mo	1113.7	1248.5	1230.0	1260.7	380.6	391.6	396.2	428.1	180.5	187.3	189.9	198.8
	Brl+ M1	1368	1399.1	1442.0	1495.6	427.2	482.0	436.5	493.6	191.2	187.1	198.2	200.6
Mean		1147	1225	1232	1278	357.8	386.4	365.9	422.2	152.6	156.9	159.6	165.7
Super-P	Bro+Mo	792.0	863.4	809.5	962.5	233.1	256.6	240.9	308.0	77.3	83.1	78.8	85.6
	Bro+M1	1270	1283.7	1355.6	1371.2	348.9	387.2	368.3	398.0	160.1	165.2	167.1	169.2
	Brl+Mo	1103	1215.1	1216.2	1236.3	355.4	362.1	384.8	381.8	156.2	159.8	163.7	166.1
	Brl+ M1	1341	1390.8	1390.1	1418.2	418.1	462.0	426.4	479.8	170.5	185.9	172.4	189.3
Mean		1127	1188	1193	1247	338.9	367.0	355.1	391.9	141.0	148.5	145.5	152.6
Over all means	Bro+Mo	791	846	830	912	233.4	255.7	239.4	294.7	77.1	82.5	78.9	85.5
	Bro+M1	1225	1262	1356	1374	343.6	368.0	366.7	392.6	159.4	160.8	164.6	167.2
	Brl+Mo	1045	1150	1150	1151	352.2	367.2	379.9	392.0	160.4	164.6	165.4	173.7
	Brl+ M1	1349	1387	1402	1440	420.4	447.1	427.0	462.5	174.7	178.7	178.3	186.6

Abbreviations: as those stated for Table (2) seasons may be due to the changes in the meterological factors.

Effect of phosphatic fertilizer sources, dual inoculation and their interaction on yield and yield components of soybean plants.

It is obvious from data given in Table (7) that pods number and weights/plant, weight of seeds/plant, 100-seed weight, seed yield/fed and biological yield/fed were significantly increased with either bradyrhizobial or mycorrhizal inoculated treatments compared to uninoculated ones and this observation was consistent in the two growing seasons.

Data in Table (7) also show that significant increases in most studied traits were observed with bradyrhizobial inoculation treatment compared to mycorrhizal inoculation. Whereas, dual inoculation of soybean plants with *B. japonicum* and VAM fungus (*Glomus mosseae*) gave higher values of yield and yield components in comparison with the application of each one solely. These results are in harmony with those obtained by **Kumrawat *et al* (1997); Shalaby & Hanna (1998), Mikhaeel *et al* (2000)** and **Abou-Aly and Gomaa (2002).** The higher yield and yield components which was observed in case of dual inoculation could be attributed to the high N_2-ase activity, mycorrhizal colonization intensity (Table, 2). In addition, higher levels of NH_4-N, NO_3-N, available phosphorus and CO_2 evolution (Table, 3). As well, higher records of soybean growth characters (Table, 4) which were observed in case of dual inoculation treatments.

Taking the p-source into account, data in Table (7) show that yield and yield components of soybean plants were significantly increased in the treatments fertilized with rock phosphate compared to the treatments fertilized with superphosphate.

These results indicate the important role of VAM in phosphorus mobilizing from the unavailable sources such as rock phosphate. Therefore, rock phosphate as a cheap source of phosphorus could substitute superphosphate for soybean fertilization in the presence of vesicular arbuscular mycorrhizae (VAM).

With respect to the interaction effect, data presented in Table (7) indicate that dual inoculation with *Bradyrhizobium* + mycorrhizae combined with phosphatic fertilization showed significant increase in yield and yield components of soybean plants compaerd to soybean inoculation or phosphatic fertilization separately. The high significant increase of yield and yield components of soybean plants was observed in the treatment of soybean inoculation with *Bradyrhizobium* + mycorrhizae accompanied with rock phosphate.Similar results were observed by (**Barakah *et al*, 1998; El-Sawy *et al*; 1998** and **Zaghloul, 1999**).

Effect of phosphatic fertilizer sources, dual inoculation and their interaction on protein and oil yield of soybean seeds

Data in Table (8) indicate that protein and oil yield (kg/fed.) were significantly increased in soybean plants inoculated with either *Bradyrhizobium* or mycorrhizae compared to uninoculated ones.

Table 7. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on yield and yield components of soybean plants

P-fertilizer sources	Dual Inoculation	No. of pods /plant		Weight of pods/plant (g)		Weight of seeds/plant (g)	
		1999	2000	1999	2000	1999	2000
Zcro-P	Bro+Mo	40.3g	36.4g	25.77f	22.33j	12.43b	12.55h
	Bro+M1	51.8de	46.4gh	34.90cde	33.30gh	17.90a	16.67efg
	Brl+Mo	53.1c	50.5ef	35.95abcd	36.78de	19.63a	17.35de
	Brl+ M1	54.7b	55.1bc	36.38abc	39.47bc	20.98a	19.42c
Mean		49.9C	47.1C	33.25B	32.97C	17.73B	16.50C
Rock-P	Bro+Mo	51.1e	44.7hi	35.13bcde	31.77hi	18.05a	16.27fg
	Bro+M1	52.1d	50.3ef	34.43de	36.25ef	18.45a	17.48de
	Brl+Mo	54.4b	53.3cd	35.35dcde	38.60cd	20.30a	18.15d
	Brl+ M1	56.5a	57.9a	37.45a	42.97a	22.00a	22.35a
Mean		53.6A	51.5A	35.59A	37.40A	19.70A	18.56A
Super-P	Bro+Mo	50.1f	42.8I	34.00e	30.05i	17.83a	15.77g
	Bro+M1	51.5de	48.2fg	35.38bcde	34.75fg	18.33a	17.22def
	Brl+Mo	54.1b	52.1de	35.53bcde	37.83cde	19.80a	17.63de
	Brl+ M1	55.6a	56.7ab	36.78ab	40.67b	21.38a	20.65b
Mean		52.8B	49.9B	35.39A	35.83B	19.33A	17.82B
Over all means	Bro+Mo	47.1D	41.3D	31.63C	28.05D	16.10C	14.87D
	Bro+M1	51.8C	48.3C	34.87B	34.77C	18.23BC	17.13C
	Brl+Mo	53.9B	52.0B	35.61B	37.73B	19.91AB	17.71B
	Brl+ M1	55.6A	56.6A	36.87A	41.04A	21.45A	20.81A

Table 7. Cont.

P-fertilizer sources	Dual Inoculation	Weight of 100-seed (g)		Seed yield (Kg/fed.)		Biological yield (ton/fed.)	
		1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	15.43h	12.52i	865g	823j	2.83e	3.50h
	Bro+M1	17.10f	16.60fg	1154e	1090gh	3.53abcd	3.97efg
	Br1+Mo	17.77de	17.02f	1280c	1130ef	3.58abcd	4.09cde
	Br1+ M1	18.40c	19.00c	1312bc	1178c	3.78ab	4.25ab
Mean		17.17A	16.29C	1153C	1057C	3.43A	3.76C
Rock-P	Bro+Mo	16.90fg	16.25gh	1089f	1078h	3.45cd	3.88g
	Bro+M1	17.33ef	17.15ef	1220d	1133ef	3.55abcd	3.92fg
	Br1+Mo	18.20cd	18.10d	1312bc	1165cd	3.68abc	4.04cdef
	Br1+ M1	20.02a	20.58a	1389a	1315a	3.81a	4.18bc
Mean		18.11A	18.02A	1253A	1173A	3.62A	4.09A
Super-P	Bro+Mo	16.50g	15.95h	1086f	1020i	3.38d	3.87g
	Bro+M1	17.10f	16.98f	1181e	1109fg	3.49bcd	4.02def
	Br1+Mo	18.02cd	17.75de	1303bc	1145de	3.65abcd	4.16bcd
	Br1+ M1	19.15b	19.65b	1329b	1218b	3.80a	4.33a
Mean		17.69A	17.58B	1225B	1123B	3.58A	4.00B
Over all means	Bro+Mo	16.27D	14.91D	1013D	974D	3.22C	3.50B
	Bro+M1	17.17C	16.91C	1185C	1111C	3.52B	3.97C
	Br1+Mo	18.00B	17.63B	1298B	1147 B	3.64B	4.09B
	Br1+ M1	19.19A	19.74A	1343A	1240A	3.80A	4.25A

Abbreviations : as those stated for Table (2).
Means followed by the same letter (s) within each column, are not significantly different from each other at 5% level.

Table 8. Effect of Phosphatic fertilizer sources, dual inoculation and their interaction on percentage and yield of protein and oil of soybean seeds

P-fertilizer sources	Dual inoculation	Protein percentage in seeds		Protein yield (Kg/fed)		Oil percentage in seeds		Oil yield (Kg/fed)	
		1999	2000	1999	2000	1999	2000	1999	2000
Zero-P	Bro+Mo	33.70a	33.10ef	291.5g	272.4j	19.35a	18.95e	167.4g	155.6i
	Bro+M1	35.13a	37.16a	405.1e	372.2gh	20.15a	20.80bcd	232.4e	226.7fg
	Br1+Mo	34.95a	32.94f	447.3c	390.7ef	21.10a	20.75bcd	271.8c	234.5de
	Br1+ M1	35.42a	32.92f	464.9b	418.6c	21.90a	21.38ab	286.5b	253.7b
Mean		34.80B	34.03A	402.02C	363.5C	20.63B	20.47A	239.5C	217.6C
Rock-P	Bro+Mo	34.75a	34.05cde	378.4f	366.9h	20.13a	20.48cd	219.4f	220.6g
	Bro+M1	35.20a	34.35cd	429.4d	389.0f	20.75a	21.17abc	254.7d	239.8cd
	Br1+Mo	35.28a	35.03bc	462.9b	408.0d	21.67a	20.80bcd	284.7b	242.3c
	Br1+ M1	35.75a	35.70b	496.6a	469.4a	22.60a	21.67a	313.9a	285.2a
Mean		35.24A	34.78A	441.8A	408.3A	21.29A	21.03A	268.2A	247.0A
Super-P	Bro+Mo	34.63a	33.72def	375.2f	344.0i	19.73a	20.17d	211.2f	205.8h
	Bro+M1	35.10a	34.25cd	414.5e	379.9g	20.58a	20.90bcd	248.7d	231.8ef
	Br1+Mo	35.33a	34.78bcd	460.3bc	398.2e	20.75a	21.40ab	271.2c	245.1c
	Br1+ M1	35.70a	35.42b	474.4b	431.3b	21.92a	21.38ab	291.3b	260.3b
Mean		35.19A	34.54A	431.1B	388.4B	20.74B	20.96A	255.6B	235.7B
Over all Means	Bro+Mo	34.36B	33.63C	348.4D	327.7D	19.73D	19.87C	199.3D	194.0D
	Bro+M1	35.14AB	35.25A	416.4C	380.4C	20.49C	20.96B	245.3C	232.8C
	Br1+Mo	35.18AB	34.25B	456.8B	399.0B	21.17B	20.98B	275.9B	240.6B
	Br1+ M1	35.63A	34.68AB	478.6A	439.8A	22.14A	21.48A	297.2A	266.4A

Abbreviations: as those stated for Table (2).
Means followed by the same letter (s) within each column, are not significantly different from each other at 5% level.

Data in Table (8) also show that significant increases were observed in the percentages and yields of protein and oil when soybean plants inoculated with *Bradyrhizobium* compared to mycorrhizal inoculated one. The highest records of protein and oil yield of soybean were observed in case of dual inoculation with *Bradyrhizobium japonicum* and *Glomus mosseae* and this was observed in the two growing seasons. These results are in accordance with thse reported by **Vejsadova *et al* (1993); Maksoud *et al* (1995)** and **Shalaby & Hanna (1998)**.

Concerning the effect of phosphatic fertilization, data in Table (8) clearly show that rock phosphate fertilization treatments showed significant increase in protein and oil yield of sóybean plants in comparison with superphosphate fertilization treatments. The same trend of results was observed in both seasons.

Regarding the interaction effect, data in Table (8) show that dual inoculation with *Bradyrhizobium japonicum* + mycorrhizae combined with phosphatic fertilization showed significant increase in the percentages and yields of protein and oil of soybean compared to soybean inoculation or phosphatic fertilization separately. The high significant increase of abovementioned parameters was obtained in the treatment of soybean inoculation with *Bradyrhizobium japonicum* + mycorrhizae accompanied with rock phosphate.

CONCLUSION

Generally, it could be concluded that nearly about 50 % of the nitrogen requirements of soybean could be saved by *Bradyrhizobium japonicum* inoculation. That is of great interest especially when public health and environmental pollution were considered.

Also, dual inoculation of soybean with *Bradyrhizobium japonicum* and *G. mosseae* gave vigorous growth and high yield as well as yield components of soybean plants especially with rock phosphate. Thereby, the use of rock phosphate at a rate of 30 kg P_2O_5/fed combined with dual inoculation can be recommended to substitute superphosphate application for reducing the production costs of soybean.

REFERENCES

Abd El-Fattah, F.K. (2001). Stimulatory effect of *Azospirillum and Azotobacter* on symbiotic efficiency between *Bradyrhizobium* and soybean under different rates of nitrogen fertilization. ***J. Agric. Sci., Mansoura Univ., 26 (6): 3961-3973.***

Abou Aly, H.E. and A.O. Gomaa (2002). Influence of combined inoculation with diazotrophs and phosphate solubilizers on growth, yield and volatile oil content of coriander plants (*Coriandrum sativum L*). ***Bull. Fac. Agric., Cairo Univ., 35: 93-114.***

A.O.A.C., Association of Official Agricultural Chemists (1980). Official methods of analysis. 10th Ed. Washington, D. C., U. S.A. P:832.

A.P.H.A, American Public Health Association (1992). Standard methods for the examination of water and waste water. Washington, D. C., U. S. A.

Barakah, G.N.; S.H. Salem and A.M. Heggo (1998). Effect of inoculation with endomycorrhizal fungi (VAM) and *Rhizobium meliloti* on nodulation, nutrition and N_2-fixation of alfalfa grown in calcareous soil. ***Annals Agric. Sci., Ain Shams Univ., Cairo, 43 (1): 49-64.***

Barea, J.M.; J.L. Escudero and C.C. Azcon (1980). Effect of introduced and indigenous VA-mycorrhizal fungi on nodulation, growth and nutrition of *Medicago sativa* in phosphate-fixing soils as affected by p-fertilizers. ***Plant and Soil, 54: 283-296.***

Black, C.A.; D.O. Evans; L.E. Ensminger; J.L. White; F.E.Clark and R.C. Dinauer (1982). ***Methods of Soil Analysis. Part 2, 2nd Ed., pp. 62-68*** Chemical and microbiological properties. Soil Sci. of Am: Inch. Publ., Madison, Wisconsin, U. S. A.

Bremner, J.M. and D.R. Keeny (1965). Steam distillation method for determination of ammonium, nitrate and nitrite. ***Annals Chem. Acta 32: 485-495.***

Cardoso, E.J.B. (1986). Effectiveness of vesicular arbuscular mycorrhizal fungi on soybean inoculated with *Rhizobium japonicum* and receiving rock phosphate as a function of soil type. ***Revista Brasileria de Ciencia do Solo, 10(1): 17-23.***

Daniels, B.A. and H.D. Skipper (1982). Methods for recovery and quantitative estimation of propagules from soil. In Methods and Principles of Mycorrhizal Research. ***Am. Phytopathological Society, pp: 29-35.***

Dewis, G. and F. Freitas (1970). Physical and chemical methods of soil and water analysis. ***F.A.O., Bull., No (10).***

Duncan, D.B. (1955). Multiple range and multiple F. test. ***Biometrics, 11:11-24.***

El-Sawy, M.; E.A. Saleh; M.A. El-Borollosy; T.H. Nokhal; I. Fendrik and M.S. Sharaf (1998). Effectiveness of dual incoulation with diazotrophs and vesicular arbuscular mycorrhizae on the growth and khellin content of *Ammi visnaga*. ***Arab Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 8 (2): 357 – 371.***

Fares, Clair, N. (1997). Effect of indigenous and introduced vesicular arbuscular mycorrhizal fungi on wheat plants. ***Annals Agric. Sci., Ain Shams Univ., Cairo, 42 (1) : 61-71.***

Gerdmann, J.W. and T.H. Nicolson (1963). Spores of mycorrhizal *Endogone* species extracted form soil by wet sieving and decanting. ***Trans. Brit. Mycol. Soc., 46: 235-244.***

Ghobrial, W.N.; Dawlat, N. Abadi; R.Y. Abd El-Kodoos and M.H. Hegazy (1995). Nitrogen fixation potential as affected by *B. japonicum* strains and soybean cultivars for maximizing soybean productivity. ***Annals Agric. Sci., Ain Shams Univ., Cairo, 40(1): 117-128.***

Hanafy, Ehsan, A.; N.A. Newigy; R.A. Zaghloul and El-Sayeda, H. El-Badawy (1998). Inoculation efficiency of rice plants with *Azolla* as a biofertilizer in the presence of different levels of phosphorus. ***Arab. Univ. J. Agric. Sci., Ain Shams Univ., Cairo, 6(1):49-76.***

Hardy, R.W.F.; R.C. Burns and R.D. Holsten (1973). Application of the acetylene ethylene assay for measurement of nitrogen fixation. ***Soil Biol. Biochem., 5: 47-81.***

Hegazy, M.H.; M.S. Abo-Soliman; Dawlat, N. Abadi and Faiza, K. Abd El-Fattah (1993). Effect of *Bradyrhizobium japonicum* inoculation and methods of p-application on soybean. ***Egypt. J. Appl. Sci., 8 (9): 373-387.***

Ishac, Y.Z.; J.S. Angle; M.A. El-Borollosy; M.E. El-Demerdash; M.I. Mostafa and Clair, N. Fares (1994).

Growth of *Vicia faba* as affected by inoculation with vesicular arbuscular mycorrhizae and *R.ieguminosarum* bv. *Vicieae* in two soils. ***Biofertil. Soils, 17: 27-31.***

Jackson, M.L. (1973). ***Soil Chemical Analysis***. pp. 46-52, Prentic-Hall of India, Private, New Delhi.

Kumrawat, B.; H.M. Dighe R.V.; Sharma and G.V. Katti (1997). Response of soybean to biofertilizers in black clay soils. ***Crop Research Hisar, 14 (2): 204 – 214.***

Maksoud, H.H.; H. Moawad and R.V. Saad (1995). Performance of soybean as affected by *Bradyrhizobium japonicum* and VA-mycorrhizae under different levels of P and N fertilization. ***Egyptian J. of Microbiology, 30 (3): 401 – 414.***

Mehasen, S.A.S. (1994). ***Effect of Irrigation Treatment, Bacterial Inoculation and Nitrogen Fertilization on Soybean Productivity. pp. 92-104,*** Ph.D. Thesis, Fac.of Agric. Moshtohor, Zagazig Univ. Egypt.

Mikhaeel, F.T.; A.M. Shalaby and Monna, M. Hanna (2000). Dinitrogen fixation and nitrogen assimilation as influenced by dual (VA-mycorrhizal fungi-*Rhizobium*) inoculation in soybean plants. ***Annals Agric. Sci., Ain Shams Univ., Cairo 45 (1): 67-77.***

Mosse, B. and M. Giovanetti (1980). An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. ***New Phytopathology, 84: 489-500.***

Neweigy, N.A.; Ehsan, A. Hanafy; R.A. Zaghloul and El-Sayeda, H. El-Badawy (1997). Response of sorghum to inoculation with *Azospirillum,* organic and inorganic fertilization in the presence of phosphate solubilizing microorganisms. ***Annals. of Agric.Sci., Moshtohor, 35(3):1383-1401.***

Page, A.L.; R.H. Miller and D.R. Keeny (1982). ***Methods of Soil Analysis, Part 2, 2^{nd} Ed. pp. 60-64,*** Am. Soc. Agronomy, Inc. Mad. Wisconsin,U. S. A.

Saad, O.A.O. and A.M.M. Hammad (1998). Fertilizing wheat plants with rock phosphate combined with phosphate dissolving bacteria and VA-mycorrhizae as alternative for Ca-superphosphate. ***Annals Agric. Sci., Ain Shams Univ., Cairo, 43(2) : 445-460.***

Shalaby, A.M. and Monna, M. Hanna (1998). Interactions between VA-mycorrhizal fungus *Glomus mosseae, Bradyrhizobium japonicum* and *Pseudomonas syringae* in soybean plants. ***Acta Microbiol. Polonica, 47(4): 385-391.***

Snedecor, G.W. and W.G. Cochran (1989). ***Statistical Methods. 8^{th} Ed.,*** Iowa State Univ., Press, Iowa, U.S.A.

Soilman, S.; I.A. El-Ghandour and Khadra, A. Abbady (1996). Effect of nitrogen, phosphorus supply and *Bradyrhizobium* and VAM fungus inoculants on dinitrogen fixation in soybean. ***Folia Microbiologica, 41 (2): 197-200.***

Vejsadova, H.; D. Siblikova; M. Gryndler; T. Simon and I. Miksik (1993). Influence of inoculation with *Bradyrhizobium japonicum* and *Glomus claroideum* on seed yield of soybean under greenhouse and field conditions. ***J. of Plant Nutrition, 16 (4): 619-629.***

Vincent, J.M. (1970). ***Manual for the Practical Study of Root-Nodule Bacteria.*** IBP Handbook No. 15, Blackwell Sci. Pub., Oxford,pp. 14-25.

Yassen, A.M. (1993). ***Studies on Endomycorrhizae in Egypt pp. 32-40.***M.

Sc. Thesis, Fac. Agric. Ain Shams Univ., Cairo, Egypt.
Zaghloul, R.A. (1999). Effectiveness of dual inoculation with *Azospirillum* and phosphate solubilizing microorganisms on growth and yield of zea mays. ***Zagazig J. Agric. Res. Vol. (26) No. (4): 1005-1025.***

مجلة حوليات العلوم الزراعية ، كلية الزراعة، جامعة عين شمس، القاهرة، م٤٧، ع(٢)، ٤٧٧ - ٥٠٠، ٢٠٠٢

فعالية التلقيح المزدوج بالبرادي ريزوبيوم والميكوريزا الداخلية في وجود مصادر مختلفة للأسمدة الفوسفاتية على نمو ومحصول فول الصويا

[٣١]

صديق عبد العزيز صديق محيسن[١] - راشد عبد الفتاح زغلول[٢] - محمد عبد المؤمن الغزولى[٣]

١- قسم المحاصيل - كلية الزراعة بمشتهر - جامعة الزقازيق - مصر
٢- قسم النبات الزراعى - كلية الزراعة بمشتهر - جامعة الزقازيق - مصر
٣-معهد بحوث الأراضى والمياه والبيئة - مركز البحوث الزراعية - الجيزة - مصر

أقيمت تجربتان حقليتان خلال موسمى ١٩٩٩، ٢٠٠٠ لدراسة تأثير التلقيح المزدوج ببكتريا العقد الجذرية *Bradyrhizobium japonicum* وفطر الميكوريزا الداخلية *Glomus mosseae* وذلك فى وجود التسميد الفوسفاتى بالسوبر فوسفات أو صخر الفوسفات على تكوين العقد الجذرية و تثبيت النيتروجين و نسبة إصابة الجذور بالميكوريزا، محتوى النبات من العناصر المغذية الكبرى والصغرى وكذلك على نمو وإنتاجية فول الصويا ولقد أوضحت نتائج هذه الدراسة ما يلى:-

أدى التلقيح المزدوج لفول الصويا ببكتريا العقد الجذرية وفطر الميكوريزا الداخلية إلى زيادة تكوين العقد، زيادة معدل تثبيت الأزوت متمثلا فى زيادة نشاط إنزيم النيتروجينيز، زيادة نسبة الإصابة بالميكوريزا وكذلك زيادة محتوى النبات من العناصر المغذية الكبرى (N, P and K) والصغرى (Fe, Zn and Cu) حيث انعكس كل ذلك على زيادة صفات النمو التى درست فى حالة التلقيح المزدوج وذلك بالمقارنة بتلقيح فول الصويا ببكتريا العقد الجذرية أو فطر الميكوريزا الداخلية كل على حده.

أيضا، أوضحت نتائج هذه الدراسة زيادة معنوية فى كل الصفات السابقة وذلك عند التسميد بصخر الفوسفات مقارنا بالسوبر فوسفات حيث لوحظ نقص فى نشاط إنزيم النيتروجينيز ونسبة إصابة الجذور بالميكوريزا عند التسميد بالسوبر فوسفات.

كذلك أدى التلقيح ببكتريا العقد الجذرية وفطر الميكوريزا الداخلية سواء كل على حدة أو مختلطين معا والتسميد بصخر الفوسفات إلى حدوث زيادة معنوية فى محصول فول الصويا ومكوناته وذلك بالمقارنة بالمعاملات الملقحة والمسمدة بالسوبر فوسفات.

كذلك أوضحت النتائج أن أعلى محصول للبروتين والزيت قد تحقق عند إجراء التلقيح المزدوج والتسميد بصخر الفوسفات.

من نتائج هذه الدراسة يتضح أن إجراء تلقيح فول الصويا ببكتريا العقد الجذرية عند الزراعة يقلل من استخدام الأسمدة النيتروجينية المعدنية وهذا يقلل من تلوث البيئة الناتج من استخدام الأسمدة الكيماوية. أيضا عند تلقيح فول الصويا بفطريات الميكوريزا الداخلية يمكن التسميد بصخر الفوسفات الأرخص ثمنا كبديل لسماد السوبر فوسفات مما يقلل من تكاليف إنتاج فول الصويا .

تحكيم: أ.د عبد العظيم احمد عبد الجواد
أ.د إحسان احمد حنفي