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EFFECTIVENESS OF DUAL INOCULATION WITH *AZOSPIRILLUM* AND PHOSPHATE SOLUBILIZING MICROORGANISMS ON GROWTH AND YIELD OF *Zea mays* L.

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ABSTRACT: Two field experiments were carried out during 1997 and 1998 seasons to study the effect of inoculation with *Azospirillum lipoferum* combined with either the vesicular arbuscular mycorrhiza (VAM) *Glomus mosseae* or the phosphate solubilizing bacterium (PSB) *B. megatherium* var *phosphaticum* on the growth and yield of maize.

The results of this study showed that inoculation with *A. lipoferum* combined with VAM in the presence of full dose of rock phosphate gave the highest values of CO_2 evolution and N_2-ase activity in rhizosphere soil.

The percentage of mycorrhizal root infection was higher in rock-p than in super-p treatments. Densities of p-dissolving bacteria in the rhizosphere were the highest when maize grains were inoculated with PSB and provided with the full dose of super-p. Ammoniacal and nitrate nitrogen contents in the soil were the highest in VAM inoculated treatment which received full dose of rock-p. The highest values of available-p were recorded in PSB inoculated treatment which received full dose of super-p.

The highest records of growth performance, i.e ear characteristics, straw and grain yields of maize were obtained with VAM combined with *A. lipoferum* inoculation in the presence of the full dose of rock-p. Uninoculated plants grown with inorganic fertilizers gave lower growth performance and lower straw and grain yields compared to inoculated treatments. Dual inoculation of maize with *A. lipoferum* in combination with either VAM or PSB gave higher values of N, P, K and crude protein in grains rather than those inoculated with *Azospirillum lipoferum* alone.

Key words : *Azospirillum lipoferum*, *Glomus mosseae*, *Bacillus megatherium* var. *phosphaticum*, super-p, rock-p, maize.

INTRODUCTION

In Egypt, maize is one of the major field crops. Therefore, efforts are focussed to increase its productivity to full the gap between the local production and human consumption of cereals through growing high producing varieties under the most favourable cultural conditions. Nitrogen and phosphorus fertilization are two of the most important factors in increasing the maize yield .

Several investigators showed that inoculation with *Azospirilla* improved growth and yield of maize crop due to their N_2-fixation activity and production of growth promoting substances (El-Demerdash, 1994 ; Fulchieri and Frioni, 1994 ; Raso, 1996 and Hamdi and El-Komy, 1998) .

Moreover, Ishac and Mostafa (1993) and Medeiros *et al* (1993) found that VAM stimulated growth and nutrients uptake of various host plants. They also found that no appreciable differences were recorded between the effect of super-p and rock-p when conjugated with VAM inoculation.

Mcgonigle and Miller (1996) and Saad and Hammad (1998) reported that mycorrhizal inoculation remarkably increased plant growth , phosphorus uptake , yield and yield components of maize and wheat. This increase was accompanied by a high percentage of mycorrhizal root infection and rhizospheric N_2-ase activity .

Concerning the effect of inoculation with phosphate solubilizing bacteria on plant growth, Heggo and Barakah (1993) found that maize inoculation with phosphate dissolving bacteria increased plant growth , N, P, K content as well as micro-elements content . Shatokhina and Khristenko (1996) also reported that the inoculation of maize with associative N_2-fixing bacteria in combination with phosphate solubilizing bacteria enhanced the biological potential of soil and increased nutrients uptake . Mixed inoculation decreased mineral fertilizer application rate by 50% for maize without any reduction in the yield .

The present study aimed to investigate the effect of dual inoculation with *A. lipoferum* and phosphate solubilizing bacteria or VA mycorrhiza in the presence of two forms of p-fertilizers on the growth and yield of *Zea mays* .

MATERIAL AND METHODS

Two field experiments were carried out in the Agricultural Research and Experimentation Center of Fac. Agric. Moshtohor, Zagazig Univ. , during 1997 and 1998 seasons to study the effect of dual inoculation of maize (*Zea mays* c.v Taba) with *A. lipoferum* in combination with either the phosphate solubilizing bacterium (PSB) *B.megatherium* var. *phosphaticum* or the vesicular arbuscular mycorrhiza (VAM) *Glomus mosseae* on maize growth, yield and yield components .

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

Table 1 .Physical and chemical properties of the experimental soil .

a. Mechanical analysis

Coarse sand %	Fine sand %	Silt %	Clay %	Textural class
2.30	18.45	24.1	55.15	Clay

b- Chemical analysis

Organic matter (%)	PH	T. N. %	T. P. %	E. C. mmhos/cm	$CaCO_3$ %
1.68	8.15	0.30	0.16	0.73	1.36

Cations (meq./L)				Anions (meq./L)			
K^+	Na^+	Ca^{++}	Mg^{++}	Cl^-	HCO_3^-	$CO_3^=$	$SO_4^=$
0.65	0.28	3.8	2.1	0.45	2.9	0.33	3.15

Mechanical analysis was estimated according to Jackson (1973). Whereas , chemical analysis was estimated in saturated soil paste according to Black *et al* (1982) .

Azospirillum lipoferum (MRB 16) and *B. megatherium* var. *phosphaticum* (pure local strain) were provided from Microbiology Dept., Soil , Water & Environment Res. Inst., Agric., Res. Center, Giza, Egypt.

While, mycorrhizal fungus *Glomus mosseae* (Soil Goettinge strain) was provided from Tropical Institute, Goettingen University, Fedral R. Germany.

Inocula preparation

For preparation of bacterial inocula, Dobereiner medium (1978) and Bunt and Rovira medium(1955) modified by Abdel-Hafez (1966) were inoculated with *A. lipoferum* and *B. megatherium* var. *phosphaticum*, respectively, then incubated at 32° C and 30° C, respectively for 7 days.

For preparing VAM inoculum, pots of 30 cm in diameter were filled with autoclaved sandy loam soil. The soil in each pot was inoculated with VAM fungus *Glomus mosseae*. Five onion seedlings were transplanted in each pot as a host plant .

After 8 weeks, spores of VAM were collected from the rhizosphere and roots of onion and extracted by wet sieving and decanting technique (Gerdmann and Nicolson, 1963) . VAM spores were counted by the method described by Musandu and Giller (1994) .

Before cultivation, the experimental soil plots $14m^2$ (4 × 3.5m) were divided into four parts, the first and the second parts were supplied with calcium superphosphate at a rate of 30 kg P_2O_5 /fed. to represent the full dose of phosphorus or half this amount , respectively .Whereas, the third and the fourth parts were supplied with rock phosphate at the same rates mentioned above.

Except for control treatments, maize grains were successfully washed with water and air-dried. Grains were than soaked in cell suspension of *A. lipoferum* (1 ml contains 10^8 viable cell) for 30 min. Gum arabic (16%) was added as an adhesive agent prior to inoculation and then divided into two groups. The first group was inoculated with *Bacillus megatherium* var. *phosphaticum* (1 ml contains 10^8 viable cell) with the same manner mentioned above, whereas the other was left without inoculation for cultivation of mycorrhizal treatments. Regarding the mycorrhizal treatments, plots which have been prepared for inoculation with VA mycorrhiza, were provided with a mycorrhizal spore suspension and infected root segments of onion. The extracted mycorrhizal spore suspension containing abut 100–120 spores ml^{-1} was used as standard inoculum (10 ml m^{-1}) for mycorrhizal treatments. The seeding rate was 80g/plot. The inoculated grains were air dried for one hour before sowing.

Except for control treatments, all plots were supplemented with a half dose of inorganic nitrogen fertilizer (45 kg N/ feddan) as ammonium sulphate in three equal doses, i.e at cultivation, after 30 and 60 days of cultivation.

A control was carried out where the soil was left without fertilization and grains were treated by using N-deficient medium instead of *Azospirillum* inoculum. Another control was also prepared where the grains were sown without inoculation, but the soil was fertilized with the recommended doses of nitrogen and phosphate, i.e 90 kg N and 30 kg P_2O_5 /fed. using ammonium sulphate. and calcium superphosphate, respectively. Also, they were added in three equal doses as mentioned above.

Experimental design

The treatments were arranged in a randomized complete block design with four replicates as follows:

1- Control.
2- Fertilized control (90 kg N + 30 kg P_2O_5 /fed.).
3- Superphosphate(30 kg P_2O_5 /fed.).
4-Rock phosphate(30 kg P_2O_5 /fed.).
5-Vesicular arbuscular mycorrhiza (VAM)+super-p(30 kg P_2O_5 /fed.).
6- VAM + rock-p (30 kg P_2O_5 /fed.).
7- VAM + super-p(15 kg P_2O_5 /fed.).
8- VAM + rock -p (15 kg P_2O_5 /fed.).
9- Phosphate solubilizing bacteria (PSB) + super-p (30 kg P_2O_5 /fed.).
10- PSB + rock-p (30 kg P_2O_5 /fed.).
11- PSB + super-p (15 kg P_2O_5 /fed.).
12- PSB + rock-p (15 kg P_2O_5 /fed.).

Cultivation

Cultivation was performed by sowing four inoculated or uninoculated grains in hills at rows with a distance of 25 cm between hills and 70 cm between rows. After sowing, soil was directly irrigated to provide a suitable moisture for inocula. The normal culture practices for growing maize were followed as recommended in the region.

Sampling and determinations

Rhizosphere soil samples of the developed plants were taken at vegetative (35 days) and heading (70 days) stages. The samples were microbiologically analyzed for N_2-ase activity, CO_2 evolution, densities of inorganic phosphate dissolving bacteria and mycorrhizal root infection. Also, rhizosphere soil samples were chemically analyzed for ammoniacal and nitrate nitrogen and available phosphorus as follows :

A. Microbiological analyses

A. 1. Nitrogenase activity in rhizorphere soil according to Hardy *et al* (1973).

A.2. Carbon dioxide (CO_2) evoluted by soil microorganisms using the method described by Page *et al* (1982)

A.3. Densities of phosphate dissolving bacteria by the standard plate method described by Bunt and Rovira medium (1955) modified by Abdel-Hafez (1966).

A.4. The percentage of mycorrhizal root infection of maize plants assessed microscopically by the slide method as described by Mosse and Giovanetti (1980).

B. Chemical analyses

B.1. Ammoniacal and nitrate nitrogen were determined according to Bremner and keeny (1965).

B.2. Available phosphorus extracted from soil according to Olsen *et al* (1954) and colourimetrically determined according to American public Health Association (APHA, 1989).

C. Growth parameters

After 70 days from sowing, plant height (cm), stem diameter (mm), number of leaves/plant and leaf area of topmost ear (cm^2) were estimated.

D. Yield and its components

At harvest; ear length (cm), ear diameter (cm), weight of grains/ear (gm), weight of cob ear (gm) and weight of 100- grain (gm) were recorded. Also, straw yield, ears yield, biological yield [straw + ears] (ton/fed.) and grain yield (ardab/fed.) were recorded.

Total nitrogen, phosphorus, potassium and crude protein were estimated in grains of maize. Total nitrogen was determined by using microkjeldahl method (A. O. A. C. 1980). Total Phosphorus content was colourimetrically determined according to APHA (1989). Total Potassium content was determined according to Dewis and Freitas (1970).

Crude protein was calculated according to the following equation :

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

Statistical analysis

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

RESULTS AND DISCUSSION

Effect of maize inoculation with *A. lipoferum* combined with either VAM or PSB on CO_2 evolution and N_2- ase activity .

It is clear from the data presented in Table (2) that the rates of CO_2 evoluted from the activity of soil microorganisms and nitrogenase activity in rhizosphere were remarkably increased in inoculated treatments with *A. lipoferum* in combination with either VAM or PSB as compared to uninoculated ones . VAM inoculated treatments gave higher values of CO_2 evolution and N_2- ase activity than PSB inoculated ones .

This result could be attributed to the incidence of maize root infection with VAM . Moreover, growth promoting substances produced by the mycorrhiza fungi and its increasing the availability of most nutrient elements especially phosphorus (Ishac and Mostafa, 1993; Mcgonigle and Miller, 1996 and Saad and Hammad, 1998) .

The highest values of evoluted CO_2 and N_2- ase activity were obtained with VAM combined with *Azospirillum* inoculation and provided with the full dose of rock phosphate. The same trend of results was observed in both 1997 and 1998 growing seasons as well as at vegetative and heading stage of maize growing.

Data also show that, the rates of CO_2 evolution and N_2-ase activity were higher in the treatments inoculated with either VAM or PSB and fertilized by rock-p either full or half dose than those fertilized by super-p. These results confirmed those obtained by Heggo and Barakah (1993) and Barakah *et al* (1998) . They reported that the application of rock phosphate rather than superphosphate increased N_2-ase activity in the rhizosphere. Generally, CO_2 evolution rates and N_2-ase activity were higher in the 2^{nd} season than in the 1^{st} one and these differences between the two seasons are likely to be due to the differences in the climatic conditions .

Effect of maize inoculation with *A. lipoferum* combined with either VAM or PSB on densities of p-dissolving bacteria and mycorrhizal root infection .

Data in Table (3) show clear differences in the root colonization of maize plants grown in VAM inoculated and uninoculated treatments which depended on the indigenous VAM in the soil. Low percentage of mycorrhizal infection in the uninoculated plants indicated that the native VAM fungi are present in the soil but in low density . Rock phosphate fertilized plants showed higher percentages of VAM infection than superphosphate fertilized ones. It

Table 2. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on CO_2 evolution and N_2-ase activity under two sources of P. fertilization .

Treatments	Growth stage / Azos. Inoculum + 45 kg N/fed. of N. fertilizer	Vegetative stage				Heading stage			
		CO_2 evoluted (µg/g dry soil/hr)		N_2-ase activity (n moles C_2H_4 / hr/g dry soil)		CO_2 evoluted (µg /g dry soil/hr)		N_2-ase activity (n moles C_2H_4 / hr/g dry soil)	
		1997	1998	1997	1998	1997	1998	1997	1998
Control	-	36.20	41.6	24.50	28.00	57.20	61.80	30.51	32.00
Fert. Control	-	62.18	66.40	38.30	41.20	81.50	84.00	51.05	54.60
fSP	+	73.80	75.00	46.12	48.00	112.32	119.30	64.50	70.20
fRP	+	76.20	82.40	46.50	48.80	116.00	122.40	66.80	72.60
VAM+fSP	+	119.40	124.10	77.20	85.00	195.30	221.15	96.20	98.60
VAM+fRP	+	172.40	180.60	95.00	99.20	228.24	247.10	124.10	128.60
VAM+hSP	+	108.10	112.10	66.00	72.30	176.60	192.50	79.10	82.30
VAM+hRP	+	116.80	118.82	73.60	80.40	188.10	201.60	96.50	99.33
PSB+ fSP	+	103.62	108.36	72.30	83.10	187.39	215.21	87.20	93.30
PSB+ fRP	+	165.50	176.00	88.40	91.60	212.20	231.50	112.00	116.20
PSB+ hSP	+	98.80	106.20	60.12	66.30	173.18	179.51	76.80	81.50
PSB+ hRP	+	112.30	116.30	68.10	75.60	182.33	198.00	84.20	87.30

Fert., fertilized (90 kg N + 30 kg P_2O_5/fed.) fSP, full dose of Super- P (30 kg P_2O_5/fed.) hSP,half dose of Super- P(15 kg P_2O_5/fed.)

Azos., Azospirillum fRP, full dose of Rock- P (30 kg P_2O_5/fed.) hRP,half dose of Rock- P(15 kg P_2O_5/fed.)

N, nitrogen fertilizer PSB, Phosphate Solubilizing Bacteria VAM, Vesicular Arbuscular Mycorrhiza .

is known that soluble phosphate inhibited the root colonization by either introduced or indigenous VAM while, rock phosphate enhanced the mycorrhizal root infection (Saad and Hammad ,1998 and Barakah *et al* ,1998). They reported that application of soluble phosphatic fertilizers greatly reduced the plant benefit from mycorrhizal infection .

Data also show that, the highest percentage of mycorrhizal root infection was observed in the treatment inoculated with VAM and provided with full dose of rock-p. Moreover, mycorrhizal root infection was higher at heading stage than vegetative stage and this was observed in both growing seasons. This result is in accordance with Fares (1997) who found that the mycorrhizal root infection and number of spores were increased at late growth stage in wheat plants.

Data presented in Table (3) also indicate that , densities of P-dissolving bacteria in the rhizosphere of maize plants tended to increase progressively in all treatments . At heading stage, densities of P-dissolvers were higher than vegetative stage. Such differences may be due to the changes in multiplication rate of P- dissolving bacteria as a result of qualitative changes in nature of the root exudates of the plant during the different growth stages (Abdel-Ati *et al* 1996 and Saad and Hammad, 1998).

The rhizosphere soil of plants inoculated with P- solubilizing bacteria contained higher densities of P- dissolving bacteria than the uninoculated ones. Such results may indicate that the introduced inoculum has the ability to survive and colonize the root zone. Similar results were obtained by Saad and Hammad (1998).

Moreover , under either of the two P-fertilization treatment, VAM inoculation led to an increase in densities of P-dissolving bacteria in the rhizosphere of maize compared to the control treatments. This finding indicates that presence of VAM fungi may stimulate multiplication of the P-dissolving bacteria . The highest densities of P-dissolving bacteria were observed in the treatment inoculated with P-solubilizing bacteria and provided with the full dose of superphosphate .

Data also show that the densities of P-dissolving bacteria in the rhizosphere of superphosphate fertilized plants were slightly higher than in that of rock phosphate fertilized ones. These results confirmed those obtained by Abdel-Ati *et al* (1996) and Saad and Hammad (1998). Taking the phosphorus level into account, mycorrhizal root infection and densities of P-dissolving bacteria slightly differed according to phosphorus fertilizer level and this trend was occurred in inoculated treatments with either VAM or PSB.

Table 3. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on the percentage of mycorrhizal root infection and densities of inorganic phosphate dissolving bacteria ($\times 10^4$/g dry soil) under two sources of P. fertilization .

Treatments	Growth stage / Azos. Inoculum+ 45 kg N/fed. of N. fertilizer	Vegetative stage: Mycorrhizal root infection (%) 1997	1998	Vegetative stage: Densities of phosphate dissolving bacteria 1997	1998	Heading stage: Mycorrhizal root infection (%) 1997	1998	Heading stage: Densities of phosphate dissolving bacteria 1997	1998
Control	-	11.2	12.5	21.6	23.3	14.1	14.9	43.2	47.6
Fert. Control	-	15.3	15.6	32.3	36.6	17.0	16.8	50.1	52.6
fSP	+	19.6	19.3	40.1	46.3	20.8	21.0	60.3	64.4
fRP	+	22.1	22.8	41.5	48.2	24.2	24.8	63.6	66.8
VAM+fSP	+	56.6	61.3	75.3	81.3	72.8	76.3	88.4	96.3
VAM+fRP	+	65.6	67.0	78.4	86.7	74.5	78.6	84.2	89.6
VAM+hSP	+	57.0	59.1	51.8	55.4	63.5	65.3	72.2	76.8
VAM+hRP	+	62.2	64.0	54.5	58.6	66.0	68.2	68.8	73.2
PSB+ fSP	+	30.1	31.6	101.6	106.5	34.2	35.2	139.0	143.0
PSB+fRP	+	32.0	33.3	99.2	102.8	37.0	38.8	136.0	141.0
PSB+hSP	+	28.2	29.2	70.2	76.3	32.0	33.6	105.3	112.6
PSB+hRP	+	30.3	31.0	68.6	72.3	34.0	35.6	102.4	108.6

Abbreviations : as those stated for Table (2) .

Effect of maize inoculation with *A. lipoferum* combined with either VAM or PSB on nitrogen forms and available phosphorus in soil.

Data presented in Table (4) indicate that ammoniacal and nitrate nitrogen as well as available phosphorus were remarkably increased in inoculated treatments with *A. lipoferum* in combination with either VAM or PSB as compared to uninoculated treatments.

Ammoniacal and nitrate nitrogen as well as available phosphorus concentrations were higher at heading stage than vegetative stage of maize growth. This may be due to the effect of root exudates which increased during heading or flowering stage in various cultivated plants. These results are in harmony with those obtained by Zaghloul *et al* (1996) who reported that biofertilization with associative N_2-fixing bacteria in the presence of phosphate solubilizing microorganisms increased nutrients content in the soil especially at heading stage.

Data also show that the highest values of ammoniacal and nitrate nitrogen were obtained with VAM combined with *A. lipoferum* inoculation in the presence of the full dose of rock phosphate. This finding was reported in both vegetative and heading stages in both growing seasons. On the other hand, the highest values of available phosphorus were recorded with PSB combined with *A. lipoferum* inoculation plus full dose of superphosphate amendment.

Irrespective of control treatments, the lowest values of NH_4-N, NO_3-N and available phosphorus were obtained when superphosphate or rock phosphate were applied without inoculation. These results may reflect the high efficiency of VAM and P-solubilizing bacteria in enhancing the availability of nutrients in soil. Similar results were obtained by Musandu and Giller (1994) and Barakah *et al* (1998).

From data presented in Table (4) it could be generally concluded that NH_4-N , NO_3-N and available phosphorus concentrations were higher in the 2^{nd} season than in the 1^{st} one. This difference between the two seasons may be due to the change in the meterological factors.

Effect of maize inoculation with *A. lipoferum* combined with either VAM or PSB on :-

A. Growth parameters of maize

It is obvious from data in Table (5) that growth characteristics i.e plant height, stem diameter, number of leaves/plant and leaf area of topmost ear were significantly increased with dual inoculation of maize with *A. lipoferum* combined with either VAM or PSB treatments compared to uninoculated ones. This finding was observed in both growing seasons.

The records of growth parameters were higher in the VAM

Table 4. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on nitrogen forms and available phosphorus contents of soil under two sources of P- fertilization.

Treatments	Growth stage / Azos. Inoculum + 45 kg N/fed. of N. fertilizer	Vegetative Stage						Heading stage					
		NH_4 - N (ppm)		NO_3 – N (ppm)		Available – P (ppm)		NH_4 - N (ppm)		NO_3 – N (ppm)		Available – P (ppm)	
		1997	1998	1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	-	18.50	21.30	15.60	19.30	23.10	26.20	26.10	30.00	24.50	28.00	30.30	38.60
Fert. Control	-	22.30	26.00	20.60	23.40	43.00	47.30	28.20	34.00	33.60	36.00	48.51	54.30
fSP	+	35.50	38.00	25.60	28.20	68.60	71.20	40.60	38.00	41.30	45.60	96.32	108.90
fRP	+	33.30	35.50	36.00	38.60	72.60	75.33	36.30	38.80	43.40	49.30	112.80	115.84
VAM+fSP	+	47.60	51.00	50.30	54.10	95.60	98.70	64.00	68.40	63.70	67.40	182.20	187.20
VAM+fRP	+	61.30	67.00	60.00	63.00	86.80	94.20	77.00	80.20	84.80	89.20	185.00	190.40
VAM+hSP	+	41.24	42.50	40.00	43.00	82.20	86.60	51.00	56.00	56.30	62.00	118.50	123.20
VAM+hRP	+	42.12	45.00	48.00	51.00	78.60	84.60	60.00	63.00	60.10	63.12	120.80	128.20
PSB+fSP	+	45.30	48.00	46.30	51.20	112.20	116.30	56.00	60.60	60.30	65.60	195.60	199.60
PSB+fRP	+	52.00	55.00	56.00	58.30	103.30	108.20	62.00	68.30	68.00	72.00	190.60	193.50
PSB+hSP	+	38.31	41.50	42.00	45.00	90.60	95.50	45.00	48.00	48.60	51.40	140.40	146.60
PSB+hRP	+	42.21	46.00	41.00	47.00	93.20	97.40	54.00	58.00	50.30	54.20	150.60	156.30

Abbreviations : as those stated for Table (2)

Table 5. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on growth characteristics under two sources of P- fertilization .

Treatments	Characteristics / Azos. Inoculum + 45 kg N/fed. of N. fertilizer	Plant height (cm)		Stem diameter (m m)		No. of leaves/plant		Leaf area of topmost ear (cm^2)	
		1997	1998	1997	1998	1997	1998	1997	1998
Control	-	177.3 ^{e}	176.7 ^{f}	21.00 ^{g}	21.67 ^{f}	10.67 ^{b}	10.33 ^{c}	680.8 ^{e}	681.7 ^{e}
Fert. Control	-	186.7 ^{e}	190.0 ^{f}	24.33 ^{f}	24.33 ^{e}	12.00 ^{abc}	12.33 ^{ab}	701.7 ^{de}	723.3 ^{d}
fSP	+	246.7 ^{bc}	240.0 ^{de}	30.67 ^{de}	29.00 ^{de}	12.00 ^{ab}	12.33 ^{ab}	737.5 ^{cd}	730.0 ^{d}
fRP	+	256.7 ^{cd}	258.3 ^{cd}	28.33 ^{e}	28.67 ^{de}	12.33 ^{ab}	13.00 ^{ab}	738.0 ^{cd}	741.7 ^{cd}
VAM+fSP	+	281.7 ^{a}	282.3 ^{a}	32.00 ^{abc}	33.33 ^{abc}	12.67 ^{a}	13..33 ^{a}	799.0 ^{b}	800.0 ^{b}
VAM+fRP	+	286.0 ^{a}	288.3 ^{a}	34.33 ^{a}	35.67 ^{a}	13.33 ^{a}	13.67 ^{a}	970.0 ^{a}	920.0 ^{a}
VAM+hSP	+	250.0 ^{cd}	274.7 ^{ab}	30.67 ^{bcd}	32.67 ^{bcd}	12.33 ^{ab}	13.33 ^{a}	722.5 ^{cde}	726.7 ^{d}
VAM+hRP	+	273.3 ^{ab}	263.3 ^{bc}	30.67 ^{bcd}	32.00 ^{cd}	12.67 ^{a}	12.67 ^{ab}	727.3 ^{cd}	743.3 ^{cd}
PSB+ fSP	+	270.0 ^{ab}	275 ^{ab}	33.33 ^{ab}	35.00 ^{ab}	12.33 ^{ab}	12.00 ^{abc}	739.0 ^{cd}	768.3 ^{c}
PSB+fRP	+	282.0 ^{a}	281.3 ^{a}	33.33 ^{ab}	35.00 ^{ab}	11.67 ^{ab}	11.33 ^{bc}	765.0 ^{bc}	763.3 ^{c}
PSB+hSP	+	235.0 ^{d}	254.3 ^{cd}	26.67 ^{e}	28.67 ^{e}	12.00 ^{ab}	13.00 ^{ab}	721,7 ^{cde}	713.3 ^{d}
PSB+hRP	+	260.0 ^{bc}	261.7 ^{bc}	30.00 ^{cd}	30.67 ^{de}	12.00 ^{ab}	12.67 ^{ab}	720.0 ^{cde}	725.0 ^{d}

Means followed by the same letter(s) within each column , are not significantly different from each other at 1% level .
Abbreviations : as those stated for Table (2) .

inoculated treatments than PSB inoculated ones. The same trend of results was observed with both the two sources or the two levels of P-fertilization.

Data also emphasize that the growth performance of maize was higher in inoculated treatments with either VAM or PSB which received full dose of P- fertilizer than those received the half dose. These results are in agreement with those reported by Zaghloul *et al* (1996) ; Saad and Hammad (1998) who found that when grain crops were inoculated with VAM higher records of growth performance were recorded than those obtained from inoculation with PSB . This may be due to the production of growth regulators such as auxins, gibberillins and cytokinins by VAM – fungi which affect the production of root biomass and nutrients uptake (Fitter and Garbaye, 1994).

The highest records of growth performance were obtained from VAM combined with *A. lipoferum* inoculation treatment plus full dose of rock phosphate amendment . Similar trends of responses were observed in both growing seasons. Similar results were obtained by Heggo and Barakah (1993) and Mcgonigle and Miller (1996).

Data in Table (5) also show that, maize inoculation with *Azospirillum lipoferum* alone in the presence of either super-p or rock-p induced lower levels of growth performance than the treatments which were inoculated with both the associative N_2-fixer and P-solubilizing microorganisms .

B. Ear characteristics

Data in Table (6) indicate that ear characteristics i.e ear length, ear diameter, weight of ear grains, weight of ear cob and weight of 100-grain were significantly increased in inoculated treatments with *Azospirillum lipoferum* combined with either VAM or PSB compared to uninoculated treatments . Similar trends of results were observed in the two growing seasons.

Except of ear length, the obtained results show that VAM inoculated treatments gave higher records of ear characteristics than the PSB inoculated ones and this observation was consistent in both growing seasons.

The highest records of ear characteristics were obtained with VAM combined with *A. lipoferum* inoculation and provided with the full dose of rock phosphate .

Irrespective of control treatments, the lowest records of ear characteristics were observed when super-p or rock-p were applied without inoculation . This result is in harmony with that obtained by Heggo and Barakah (1993) and Saad and Hammad (1998).

C. Yield of straw, ears, biological and grains

It is obvious from data given in Table (7) that the straw, ears and biological yield as well as grain yield of maize plants were significantly

Table 6. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on ear characteristics and weight of 100-grain under two sources of P- fertilization .

Treatments	characteristics / Azos. Inoculum + 45 kg N/fed. of N. fertilizer	Ear length (cm)		Ear diameter (cm)		Weight of ear grains (gm)		Weight of ear cob (gm)		weight of 100 – grain (gm)	
		1997	1998	1997	1998	1997	1998	1997	1998	1997	1998
Control	-	17.00 ^{e}	19.67 ^{c}	4.43 ^{d}	4.43 ^{e}	146.4 ^{d}	130.4 ^{e}	46.9 ^{e}	48.43 ^{e}	32.33 ^{e}	32.50 ^{e}
Fert. Control	-	19.33 ^{de}	20.00 ^{bc}	4.33 ^{d}	4.76 ^{de}	166.3 ^{cd}	163.6 ^{de}	53.73 ^{de}	56.43 ^{de}	32.33 ^{e}	33.43 ^{de}
fSP	+	20.33 ^{cde}	21.67 ^{abc}	5.13 ^{c}	5.13 ^{bcd}	196.1 ^{abcd}	152.8 ^{de}	56.74 ^{de}	57.27 ^{de}	35.00 ^{de}	33.50 ^{de}
fRP	+	20.5 ^{cde}	21.67 ^{abc}	5.80 ^{bc}	4.90 ^{cd}	196.9 ^{abcd}	174.9 ^{cde}	63.83 ^{cd}	67.43 ^{cd}	36.67 ^{bcd}	35.37 ^{cd}
VAM+fSP	+	24.33 ^{a}	24.00 ^{a}	5.93 ^{ab}	5.50 ^{b}	230.1 ^{ab}	236.2 ^{ab}	82.50 ^{b}	83.33 ^{b}	38.30 ^{abc}	39.40 ^{a}
VAM+fRP	+	23.00 ^{ab}	23.00 ^{ab}	6.53 ^{a}	6.10 ^{a}	239.4 ^{a}	264.8 ^{a}	96.93 ^{a}	99.60 ^{a}	40.67 ^{a}	39.67 ^{a}
VAM+hSP	+	23.33 ^{ab}	22.00 ^{abc}	6.10 ^{ab}	5.26 ^{bc}	200.0 ^{abcd}	205.8 ^{bcd}	75.27 ^{bc}	77.1 ^{bc}	37.27 ^{bcd}	37.53 ^{abc}
VAM+hRP	+	21.33 ^{bcd}	22.33 ^{abc}	6.50 ^{ab}	5.50 ^{b}	212.0 ^{abc}	221.6 ^{acd}	71.00 ^{bc}	85.03 ^{b}	39.00 ^{ab}	38.10 ^{ab}
PSB+fSP	+	24.00 ^{a}	24.33 ^{a}	5.50 ^{bc}	5.20 ^{bcd}	214.7 ^{abc}	226.0 ^{abc}	73.93 ^{bc}	71.87 ^{bc}	38.00 ^{abcd}	38.80 ^{a}
PSB+fRP	+	23.33 ^{ab}	24.33 ^{a}	6.10 ^{ab}	6.03 ^{a}	211.2 ^{abc}	230.0 ^{ab}	77.27 ^{bc}	77.77 ^{bc}	39.33 ^{ab}	39.13 ^{a}
PSB+hSP	+	22.6 ^{abc}	21.67 ^{abc}	5.76 ^{bc}	5.40 ^{b}	199.0 ^{abcd}	205.6 ^{bcd}	65.80 ^{cd}	73.4 ^{bc}	36.83 ^{bcd}	36.17 ^{bc}
PSB+hRP	+	21.00 ^{bcd}	22.67 ^{abc}	5.53 ^{bc}	5.33 ^{bc}	209.0 ^{abc}	189.9 ^{bcd}	65.47 ^{cd}	79.67 ^{bc}	36.67 ^{bcd}	37.53 ^{ab}

Means followed by the same letter(s) within each column , are not significantly different from each other at 1% level .
Abbreviations : as those stated for Table (2) .

Table 7. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on straw, ears, biological and grain yield under two sources of P- fertilization.

Treatments	parameters / Azos. Inoculum + 45 kg N/fed. of N. fertilizer	Straw yield (ton /fed.) 1997	Straw yield (ton /fed.) 1998	Ear yield (ton /fed.) 1997	Ear yield (ton /fed.) 1998	Biological yield (ton /fed.) 1997	Biological yield (ton /fed.) 1998	Grain yield (ardab /fed.) 1997	Grain yield (ardab /fed.) 1998
Control	-	2.50 ^{c}	3.33 ^{e}	3.10 ^{c}	2.70 ^{f}	5.60 ^{f}	6.03 ^{d}	9.60 ^{e}	9.84 ^{h}
Fert. Control	-	4.20 ^{b}	5.13 ^{d}	4.20 ^{b}	3.90 ^{e}	8.40 ^{e}	9.03 ^{cd}	16.23 ^{d}	16.70 ^{g}
fSP	+	4.53 ^{b}	4.73 ^{d}	4.23 ^{b}	4.76 ^{d}	8.76 ^{de}	9.49 ^{cd}	17.80 ^{cd}	16.96 ^{g}
fRP	+	4.33 ^{b}	5.03 ^{d}	5.46 ^{ab}	5.30 ^{d}	9.79 ^{d}	10.33 ^{c}	17.93 ^{cd}	17.10 ^{g}
VAM+fSP	+	7.56 ^{a}	7.36 ^{bc}	6.00 ^{a}	6.56 ^{ab}	13.56 ^{a}	13.92 ^{b}	21.54 ^{ab}	21.05 ^{ab}
VAM+fRP	+	7.70 ^{a}	9.10 ^{a}	6.32 ^{a}	7.23 ^{a}	14.02 ^{a}	16.38 ^{a}	22.96 ^{a}	21.35 ^{a}
VAM+hSP	+	7.60 ^{a}	6.53 ^{c}	6.20 ^{a}	6.46 ^{ab}	13.80 ^{a}	12.99 ^{bc}	20.89 ^{ab}	19.87 ^{de}
VAM+hRP	+	6.76 ^{ab}	6.93 ^{c}	5.96 ^{a}	6.80 ^{ab}	12.72 ^{bc}	13.73 ^{b}	20.68 ^{ab}	20.14 ^{cd}
PSB+ fSP	+	6.80 ^{ab}	8.16 ^{b}	5.93 ^{a}	6.60 ^{ab}	12.73 ^{bc}	14.76 ^{ab}	19.47 ^{bc}	20.56 ^{bc}
PSB+fRP	+	7.23 ^{a}	7.23 ^{c}	5.16 ^{ab}	6.40 ^{ab}	12.39 ^{c}	13.63 ^{b}	21.47 ^{ab}	21.12 ^{ab}
PSB+hSP	+	6.86 ^{ab}	7.43 ^{bc}	5.46 ^{ab}	5.53 ^{cd}	12.32 ^{c}	12.96 ^{bc}	19.91 ^{bc}	18.53 ^{f}
PSB+hRP	+	7.16 ^{a}	7.16 ^{c}	5.86 ^{a}	6.13 ^{bc}	13.02 ^{ab}	13.29 ^{b}	20.85 ^{ab}	18.86 ^{ef}

Means followed by the same letter(s) within each column , are not significantly different from each other at 1% level .
Abbreviations : as those stated for Table (2) .

increased in treatments inoculated with *A. lipoferum* in combination with either VAM or PSB compared to uninoculated ones.

The highest values of the abovementioned records were obtained with VAM combined with *A. lipoferum* inoculation in the presence of the full dose of rock phosphate . The results of 1998 season emphasized those obtained from 1997.

Taking the p-source into account; straw, ears, biological and grain yields of maize slightly differed according to the source of phosphorus fertilizer and this trend was observed in inoculated treatments with either VAM or PSB. Similar results were obtained by Zaghloul *et al* (1996) and Barakah *et al* (1998) who reported that there was no significant difference in plant growth parameters and yield due to superphosphate or rock phosphate fertilization. These results indicate the important role of VAM as well as PSB in mobilizing phosphorus from the unavailable sources such as rock phosphate.

Therefore,the rock phosphate as a cheap source of phosphorus, could substitute superphosphate for maize fertilization in the presence of phosphate solubilizing microorganisms .

Effect of maize inoculation with *A. lipoferum* combined with either VAM or PSB on N, P and K as well as crude protein contents of maize grains .

Data in Table (8) show that dual inoculation of maize grains with *A. lipoferum* and *B. megatherium* var. *phosphaticum* gave higher values of N, P, K as well as crude protein contents than those inoculated with only associative N_2- fixing bacteria. The same trend of results was observed in the two growing seasons.

Data also show that VAM inoculated treatments gave higher percentages of total nitrogen, crude protein and total phosphorus than PSB inoculated ones . The same trend of results was observed when maize plants were fertilized with either super-p or rock-p as well as when phosphorus fertilizer was added either in full or half dose.

Maize plants inoculated with *A. lipoferum* combined with either VAM or PSB and provided with the full dose of super-p or rock-p gave higher values of total potassium in grains than those provided with the half dose of phosphorus fertilizer .

These results are in harmony with Heggo and Barakah (1993) Zaghloul *et al* (1996) and Saad and Hammad (1998) who reported that the inoculation with phosphate solubilizing microorganisms increased plant growth as wall as plant content of N, P and K.

Table 8. Effect of dual inoculation of maize with *A. lipoferum* in combination with either PSB or VAM on grains content of N , P , K and crude protein under two sources of P- fertilization .

Treatments	parameters / Azos. Inoculum + 45 kg N/fed. of N. fertilizer	Total nitrogen %		Crude protein %		Total potassium %		Total phosphorus %	
		1997	1998	1997	1998	1997	1998	1997	1998
Control	-	0.96	0.98	6.00	6.12	0.308	0.326	0.270	0.278
Fert. Control	-	1.03	1.12	6.43	7.00	0.355	0.372	0.378	0.405
fSP	+	1.21	1.26	7.56	7.87	0.386	0.410	0.432	0.464
fRP	+	1.24	1.28	7.75	8.00	0.368	0.385	0.486	0.510
VAM+fSP	+	1.56	1.58	9.75	9.87	0.666	0.674	0.801	0.861
VAM+fRP	+	1.60	1.62	10.00	10.12	0.684	0.692	0.920	0.965
VAM+hSP	+	1.40	1.48	8.75	9.25	0.450	0.470	0.581	0.618
VAM+hRP	+	1.44	1.50	9.00	9.37	0.465	0.520	0.603	0.629
PSB+ fSP	+	1.42	1.46	8.87	9.12	0.586	0.614	0.668	0.714
PSB+fRP	+	1.51	1.55	9.43	9.68	0.648	0.651	0.860	0.900
PSB+hSP	+	1.33	1.36	8.31	8.50	0.516	0.543	0.540	0.556
PSB+hRP	+	1.36	1.40	8.50	8.75	0.455	0.491	0.586	0.600

Abbreviations : as those stated for Table (2) .

Data also show that percentage of crude protein was increased with *Azospirillum* inoculation treatments either singly or in combination with phosphate solubilizing microorganisms. This result is in accordance with Hamdi and El-Komy (1998).

CONCLUSION

From the obtained results, it can be concluded that maize grains inoculation with *A. lipoferum* in the presence of half the recommended dose of inorganic N- fertilizer improved the growth and consequently gave higher straw and grain yields than using the full dose of inorganic N- fertilizer without inoculation. Such application can save 50% of the cost of inorganic nitrogen fertilizer and minimize environmental pollution by using less chemical fertilizer.

Also, the growth characteristics and yield of maize plants inoculated with *A. lipoferum* combined with either VAM or PSB and fertilized with superphosphate were almostly corresponding to of those fertilized with rock phosphate. Therefore, the use of rock phosphate at a rate of 30 kg P_2O_5/ fed. combined with dual inoculation may be recommended as an alternative for superphosphate application to reduce the production costs of maize and phosphate pollution .

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فعالية التلقيح المزدوج بالأزوسبيريللام والميكروبات المذيبة للفوسفات على نمو وإنتاجية الذرة الشامية

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أقيمت تجربتان حقليتان بمزرعة مركز البحوث والتجارب الزراعية بكلية الزراعة بمشتهر خلال موسمى ١٩٩٧، ١٩٩٨ لدراسة تأثير التلقيح بالبكتريا المثبتة لآزوت الهواء الجوى ***A.lipoferum*** فى وجود فطر الميكورهيزا ***Glomus mosseae*** أو البكتريا المذيبة للفوسفات ***B. megatherium*** var ***Phosphaticum*** على نمو وإنتاجية الذرة الشامية (صنف طابا) مع استخدام سماد السوبر فوسفات وصخر الفوسفات حيث أضيفت هذه الأسمدة بمستويين هما ١٥ ، ٣٠ كجم فو٢أ٥ /فدان . ولقد أسفرت نتائج هذه الدراسة عن الآتى :

عند التلقيح بالـ ***A.lipoferum*** فى وجود فطر الميكورهيزا ***Glomus mosseae*** مع إستخدام المستوى الكامل من الفوسفور(٣٠ كجم فو٢أ٥ /فدان) فى صورة صخر الفوسفات ، أعطى ذلك أعلى معدل من تصاعد ك أ٢ فى التربة وكذلك أعلى معدل من نشاط إنزيم النيتروجينيز فى منطقة الريزوسفير . كانت إصابة الجذور بالميكورهيزا أعلى عند التسميد بصخر الفوسفات وذلك مقارنة بالتسميد بالسوبر فوسفات .

أدى التلقيح بالـ ***B. megatherium*** var ***Phosphaticum*** مع استخدام المستوى الكامل من السوبر فوسفات إلى زيادة أعداد البكتريا المذيبة للفوسفات فى منطقة الريزوسفير وكذلك مستوى الفوسفور الميسر فى التربة . عند التلقيح بفطر الميكورهيزا لوحظ زيادة فى محتوى التربة من النيتروجين الآمونيومى والنتراتى بالمقارنة بالبكتريا المذيبة للفوسفات .

لوحظت فروق معنوية فى صفات النمو التى درست وكذلك فى المحصول ومكوناته حيث إزدادت هذه القياسات فى وجود التلقيح بفطر الميكورهيزا والأزوسبيريللام مع إستخدام المستوى الكامل من صخر الفوسفات فى حين أعطت المعاملة الغير ملقحة والمسمدة بالجرعة الآزوتية والفوسفاتية الكاملة (٩٠ كجم ازوت + ٣٠ كجم فو٢أ٥ /فدان) نقص معنوى فى صفات النمو وكذلك فى المحصول ومكوناته بالمقارنة بالمعاملات الملقحة بالآزوسبيريللام وفطر الميكورهيزا أو البكتريا المذيبة للفوسفات .

أدى التلقيح المزدوج بالبكتريا المثبتة لآزوت الهواء الجوى والميكروبات المذيبة للفوسفات إلى زيادة محتوى الحبوب من النيتروجين والفوسفور والبوتاسيوم ونسبة البروتين وذلك بالمقارنة بالتلقيح بالبكتريا المثبتة للآزوت فقط .

من نتائج هذه الدراسة يتضح أن إجراء تلقيح الذرة الشامية بالبكتيريا المثبتة لازوت الهواء الجوى ***A.lipoferum*** يقلل من إستخدام الأسمدة النيتروجينية المعدنية وهذا يقلل من تلوث البيئة الناتج من إستخدام الأسمدة الكيماوية . أيضاً عند تلقيح الذرة الشامية بفطريات الميكورهيزا أو البكتيريا المذيبة للفوسفات يمكن التسميد بالصخر الفوسفاتى الأرخص ثمناً كبديل لسماد السوبر فوسفات وأيضاً تجنب التلوث الفوسفاتى .