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IMPACT OF COMPOSED INOCULATION WITH N_2-FIXING-, PHOSPHATE-SOLUBILIZING BACTERIA AND VESICULAR-ARBUSCULAR MYCORRHIZA ON GROWTH AND NUTRITION OF MAIZE PLANTS IN A CALCAREOUS SOIL

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ABSTRACT

A pot experiment was carried out to study the effect of inoculation with single,dual and triple inoculants of N_2-fixer *A. brasilense*, P-solubilizer *B. megaterium* var.*phosphaticum* and the vesicular-arbuscular (VA) mycorrhizal fungus *Glomus macrocarpus* on growth and nutrition of maize in a calcareous soil amended with rock phosphate (0.5%) & compost (0.5%), and fertilized with low dose of N mineral fertilizer. A significant positive effect on plant growth was obtained after inoculation of maize particularly with composed inoculant, in the presence of ½ dose of N fertilizer. Composed inoculants significantly increased the accumulation of N, P and K in maize plants. N_2-ase activity of maize plant roots was higher when *A. brasilense* and *G. macrocarpus* or *A. brasilense, G. macrocarpus* and *B. megaterium* var. *phosphaticum* were combined particularly in the presence of ¼ dose of N fertilizer. High percentage of VA mycorrhiza colonization in maize roots was formed, indicating good ecological adaptation of the mycorrhizal fungus to the calcareous soil. The introduced biopreparations were able to colonize actively the rhizosphere of maize. The results obtained in this work demonstrate the potential benefit of inoculating maize plants growing in calcareous soil with a composed inoculant of N_2-fixing bacteria (NFB), P- solubilizing bacteria (PSB) and VA mycorrhiza in the presence of low dose of N fertilizer

Keywords: N_2-fixing bacteria, Phosphate-solubilizing bacteria, VA mycorrhiza, Biofertilizers, Maize, Calcareous soil.

INTRODUCTION

In Egypt a considerable attention has been paid in the last years to soil reclamation to increase agricultural production. Among the most promising land for agricultural expansion is the highly calcareous type of soils of the northwestern coastal region. This type of soils is known to be low in their natural fertility due to low N level, P fixation and deficiency of some micronutrients (Balba, 1980). Fertility problems are greatly solved by addition of mineral fertilizers. However, the use of intensive and non-rational rates of mineral fertilizers increases not only the costs of agricultural production in these reclaimed areas but also pollutes the environment and forms a hazardous kinds of food products. Therefore, it is essential to evolve a strategy of integrated nutrient supply by using a judicious combination of mineral fertilizers and biofertilization (Hauka *et al.*, 1996).

A promising trend for increasing the efficiency of biofertilizers is the use of different mixtures of biopreparations as N_2-fixers, and P- & silicate-solubilizers (Okon and Labandera-Gonzalez, 1994 and Hauka *et al.,* 1996). A significant increases in yield and dinitrogen fixation have been observed when wheat and bearly were inoculated with mixed cultures of associative N_2-fixers (Fayez, 1989). There have been successful attempts to improve maize development by using mixtures of N_2-fixers and mycorrhiza (Sreeramulu *et al*., 1988). The effect of combined inoculation of grasses with both NFB and PSB on yield and nutrient accumulation in plants was more significant than the effect of separate treatments (Kundu and Gaur, 1980 & 1984). Furthermore, the beneficial influence of PSB on survival of NFB in the rhizosphere has been observed (Ocampo *et al*., 1975, Kundu and Gaur, 1980 and Alagawadi and Gaur, 1988). In view of these encouraging findings, it is necessary to acquire a through knowledge of interactions between NFB, PSB and VA mycorrhiza.

The present study aims at investigation the effect of combined inoculum of NFB, PSB and VA mycorrhiza in the presence of a low dose of N fertilizer and rock phosphate on maize plants grown in calcareous soils amended with rock phosphate and wheat straw compost. The effect of inoculation treatments on N_2-ase activity of plant root system, VA mycorrhiza colonization and population of certain bacteria was also investigated.

MATERIALS AND METHODS

A pot experiment was conducted during summer 1998 under greenhouse conditions at Agric. Res. Exper. Sta., Fac. of Agric., Mansoura Univ. The experiment was carried out to investigate the effect of maize inoculation with *A. brasilense* as NFB, *B. megaterium* var. *phosphaticum* as PSB and *G. macrocarpus*, separately or mixed, on plant growth, nutrient uptake by maize plants and counts of microorganisms in soil.

Soil:

A calcareous soil (34.8% $CaCO_3$ and pH of about 8.0) was obtained from a surface layer (0-20 cm) in Bourg El-Arab region, Egypt. The soil was air dried, ground to pass through a 2.0 mm sieve. The main physical and chemical properties of the soil are recorded in Table (1).

Table (1): Analysis of the used soil.

Physical properties		Chemical properties	
Particle size distribution		$CaCO_3$ %	34.9
Sand %	53.9	pH	7.92
Silt %	2.9	Total N %	0.6
Clay %	7.7	Available P (ppm)	2.1
Texture	Sandy	Available K meq/100 g	0.45
		E. C. m mhos/m	3.1

Cultivar:

Maize grains (*Zea mays* L. cv. triple hybrid 310) were used in this study. They were provided from Agron. Dept., Sakha Agric. Station. Grains were selected to be similar in size and weight.

Organisms:

A. brasilense was obtained from the Agric. Microbiol. Dept., Soils and Water Res. Inst., Egypt. *B. megaterium* var. *phosphaticum* was obtained from Microbiol. Dept., Fac. of Agric., Mansoura Univ., Egypt. Local strain of *G. macrocarpus* was provided from Fac. of Sci., Mansoura Univ., Egypt.

Compost preparation:

2 kg finely powdered wheat straw and 1 kg shredded small pieces of wheat straw were mixed in a pile. Wheat straw pile was amended with 120 g $(NH_4)_2$ SO_4 dissolved in 7.5 L H_2O. The pile was kept at 22°C. Two doses of $CaCO_3$, 20 g each, were added after 2 and 3 days. Moisture was kept constant a round at 40% for 5 weeks. The pile was remixed every two days. The composted wheat straw was mixed with soil at the rate of 0.5% (w/w).

Grains inoculation:

Bacterial inocula:

A. brasilense was grown on modified nitrogen-deficient semi-solid malate medium (Döbereiner, 1978) at 30°C for 48 h. *B. megaterium* var. *phosphaticum* was grown on Bunt and Rovira liquid medium (1955) modified by Abdel Hafez (1966) at 30°C for 72 h. The suspensions obtained (1×10^8 cells/ml each) were used for grain inoculation at the rate of 10 ml single inoculant, 5 ml of each culture in binary inoculant or 3.3 ml of each culture in triple inoculants. The same doses were added to each pot after 10 days of sowing.

VA Mycorrhiza inoculant:

Grains planted over the thin layer from segments of highly infected onion roots and 5 ml of spores suspension (~ 180 spores/ml) were added on the grains and then covered with some of the same soil.

Plant growth conditions:

The soil was mixed with the composted wheat straw and rock phosphate (26.4% P_2O_5, Abou Zaabal Phosphate Fertilizers Co.) at the rate of 0.5% (w/w) of each, before sowing. Each plastic pot (30 x 45 cm) was filled with 10 kg of soil. Grains were distributed at the rate of 5 grains/pot, then thinned to 1 seedling/pot after two weeks of sowing. Fertilization rates were 60 kg N/fed (NH_4NO_3 33.5%) (recommended full dose), 30 kg N/fed (1/2 N dose) or 15 kg N/fed (1/4 N dose) and 48 kg K_2O/fed (K_2SO_4 48%). Irrigation stood at 60% of the water holding capacity (WHC) with tap water. The pots were incubated under greenhouse conditions. All pots were irrigated with equal volume of tap water whenever, the available water content decreased till 40% of WHC. 16 experimental treatments were included. Three replicate pots received each treatment. One experimental treatment didn't receive bio- or N mineral fertilization (control). Another experimental treatment included a full dose addition of N (60 kg N/fed) without biofertilization. Seven experimental treatments received a ½ dose of N addition (30 kg N/fed) and microbial inoculation. Seven experimental treatments received ¼ dose of N addition (15 kg N/fed) microbial inoculation.

The following inoculants were included:

1- *A. brasilense*.
2- *B. megaterium* var. *phosphaticum.*
3- *G. macrocarpus*.
4- *A. brasilense* + *B. megaterium* var. *phosphaticum*.
5- *A. brasilense* + *G. macrocarpus*.
6- *B. megaterium* var. *phosphaticum* + *G. macrocarpus*.

7- *A. brasilense* + *B. megaterium* var. *phosphaticum* + *G. macrocarpus*.

Microbiological analysis:

After 30 and 60 days from sowing, rhizospheric soil was microbiologically analyzed for determining the densities of total bacteria, azospirilla and phosphate-solubilizing bacteria on Allen (1969) medium, semi-solid malate medium (Döbereiner, 1978) and Bunt and Rovira (1955) medium as modified by Abdel Hafez (1966), respectively. Most propable numbers were calculated according to Cochran (1950). Mycorrhizal infection was estimated microscopically on a sample of fresh root as described by Trouvelot *et al.* (1986), after clearing and staining (Phillips and Hayman, 1970).

Nitrogenase activity:

The N_2-ase activity of roots at 30 and 60 days after sowing was measured using the acetylene reduction assay (Hardy *et al.*, 1973). Values of N_2-ase activity were recorded as n moles C_2H_4/g plant/h.

Chemical analysis:

After 30 and 60 days from planting, inoculated and uninoculated representative plants (3 plants/replicate) were carefully uprooted. The root was dipped in water to remove soil particles, washed with distilled water. Total dry weight of plant, root dry weight and shoot dry weight were determined after drying at 70°C for 48 hr. The oven dried plants were powdered and mineralized by sulfuric-perchloric acids (Piper, 1950). Content of total nitrogen, P and K was determined by the method of Jackson (1973).

Statistical analysis:

Differences between treatments were determined using the statistical procedures for agricultural research (Gomez and Gomez, 1984) and the significance of differences among treatments was tested at the 5% and 1% probability level. The data were tabulated to reveal the effect of the main studied factors and significances of the different ways of interaction.

RESULTS AND DISCUSSION

Dry-weight yields:

Data presented in Table (2) show that, in general, inoculation with used biopreparations, in the presence of ½ dose of N mineral fertilizer, increased dry weights of maize plants to a degree approximately similar to dry weights in full dose (N) treatments. However, the highest dry weights were recorded in most treatments of composed inoculation. The maximum increase in dry weight of maize over control, after 60 days, was 304.68% in soil inoculated with triple inoculant and fertilized with ½ dose of N fertilizer. Triple inoculant, however, increased dry weight to a greater extent than either singular or dual inoculants. The beneficial effect of used organisms on plant growth can be attributed not only to N_2-fixation (Fayez, 1989) and nutrient availability (Hauka *et al.*, 1990 & 1996), but also to the production of growth substances (Sundaro-Rao, 1988 and Hauka *et al.*, 1990). These results are in agreement with those reported by Kothari *et al.* (1990) and Heggo &

Barakah (1993) who reported that in calcareous soil composed inoculation improved the dry matter of maize plants.

Table (2): Dry weights (g/plant) of maize plants grown in calcareous soil amended with rock phosphate (0.5%) and composted wheat straw (0.5%).

Treatments	30 (day)			60 (day)		
	Root Dry Wt	Shoot Dry Wt	Total Dry Wt	Root Dry Wt	Shoot Dry Wt	Total Dry Wt
A: Uninoculated treatments:						
Mineral N-free control	0.22	0.43	0.65	1.48	3.43	4.91
Full dose of N	0.31	1.00	1.31	5.01	8.77	13.78
B: Inoculated treatments:						
NFB + ½ N dose	0.25	1.02	1.27	2.96	6.63	9.59
PSB + ½ N dose	0.33	0.84	1.17	2.75	7.91	10.66
VAM + ½ N dose	0.44	0.71	1.15	3.70	8.55	12.25
NFB + PSB + ½ N dose	0.22	0.55	0.77	5.14	9.20	14.34
NFB + VAM + ½ N dose	0.52	1.13	1.65	3.07	11.54	14.61
PSB + VAM + ½ N dose	0.32	1.20	1.52	2.36	7.49	9.85
NFB +PSB + VAM + ½ N dose	0.30	1.17	1.47	4.76	10.20	14.96
NFB + ¼ N dose	0.42	0.97	1.39	2.97	6.37	9.34
PSB¼ N dose	0.31	0.94	1.25	1.94	5.81	7.75
VAM¼ N dose	0.37	1.00	1.37	2.00	7.67	9.67
NFB + PSB¼ N dose	0.32	1.00	1.32	2.66	5.67	8.33
NFB + VAM¼ N dose	0.30	1.18	1.48	3.74	9.11	12.85
PSB + VAM¼ N dose	0.27	0.95	1.22	2.42	8.37	10.79
NFB +PSB + VAM¼ N dose	0.34	1.09	1.43	3.01	11.55	14.56
F-test	**	**	**	**	**	**
N-LSD 5%	0.05	0.18	0.19	0.33	0.51	0.48
N-LSD 1%	0.06	0.22	0.24	0.43	0.67	0.63

NFB, N_2-fixing bacteria (*A brasilense*), PSB, P-solubilizing bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

N, P and K content in maize:

Data presented in Table (3) show that N, P and K in maize plants were found to be affected positively by inoculation treatments with NFB, PSB and VAM. The increase in nutrient content of maize plants was much higher in inoculated plants that received ½ dose of N mineral fertilizer. Both the content and uptake of nutrients increased with age of maize plants. The increases may be due to more dry matter accumulation with plant age progress and its metabolic processes. The highest accumulation in nutrients was scored by plants inoculated with dual or triple inoculants. The highest accumulation of N, P and K in maize plants corresponded with inoculants of the triple inoculants, NFB + VAM and NFB +PSB. It could be concluded that N, P and K content in maize plants were increased significantly by the synergistic effect mainly between both N2-fixers and P-solubilizers. The present study also revealed that *A. brasilense* is capable of enhancing the absorption of N and P fertilizers by the maize plants. *B. megaterium* also had beneficial effect on P and N nutrition of maize. The intensive assimilation of N and P as well as the enhancement of root uptake capacity may be responsible for the enhancement of K accumulation by the maize plants after inoculation. This may be attributed to the functional link between N, P and K within the plant (Kumar *et al.*, 1979).

Table (3): Nutrient contents (%) in plants and nutrients-uptake (mg/plant) by maize plants grown in calcareous soil amended with rock phosphate (0.5%) and composted wheat straw (0.5%).

Treatments	30 days		60 days		30 days		60 days		30 days		60 days	
	N				P				K			
	%	Uptake	%	Uptake	%	Uptake	%	uptake	%	Uptake	%	uptake
A: Uninoculated treatments												
Mineral N-free control	1.13	7.35	1.68	82.49	0.23	1.50	0.27	13.26	1.18	7.67	2.50	122.7
Full dose of N	1.45	19.00	2.95	406.5	0.28	3.67	0.34	46.85	1.23	19.00	3.55	489.2
B: Inoculated treatments:												
NFB + ½ N dose	1.50	19.05	2.98	237.8	0.32	4.06	0.36	34.52	1.45	18.42	3.60	345.2
PSB + ½ N dose	1.50	17.55	3.32	354.2	0.32	3.74	0.37	39.44	1.45	17.32	3.51	374.2
VAM + ½ N dose	1.53	17.60	2.91	356.5	0.33	3.80	0.38	46.55	1.48	17.02	3.55	434.9
NFB + PSB + ½ N dose	1.52	11.70	2.99	428.8	0.33	2.54	0.35	50.19	1.48	11.47	3.55	509.1
NFB + VAM + ½ N dose	1.54	25.41	2.96	432.5	0.32	5.28	0.38	55.52	1.49	25.58	3.75	547.4
PSB + VAM + ½ N dose	1.48	22.50	2.98	253.5	0.36	5.48	0.38	37.43	1.55	23.71	3.64	358.5
NFB +PSB + VAM + ½ N dose	1.48	21.76	3.01	450.3	0.36	5.15	0.38	56.85	1.56	23.52	3.66	546.0
NFB + ¼ N dose	1.21	16.82	1.75	163.5	0.25	3.48	0.30	28.02	1.60	16.96	2.55	238.2
PSB + ¼ N dose	1.25	15.63	1.75	135.6	0.26	3.25	0.30	23.25	1.22	15.25	2.56	198.2
VAM + ¼ N dose	1.25	17.13	1.75	169.2	0.26	3.56	0.30	29.01	1.22	16.85	2.60	251.4
NFB + PSB + ¼ N dose	1.24	16.37	1.76	146.6	0.25	3.30	0.30	24.99	1.23	16.10	2.61	243.5
NFB + VAM + ¼ N dose	1.26	18.65	1.78	228.7	0.25	3.70	0.30	38.55	1.22	18.20	2.59	332.8
PSB + VAM + ¼ N dose	1.26	15.37	1.76	189.9	0.25	3.05	0.31	33.45	1.23	14.88	2.58	278.4
NFB +PSB + VAM + ¼ N dose	1.26	18.02	1.78	259.2	0.27	3.86	0.33	48.05	1.22	17.59	2.61	380.0
F-test	**	**	**	**	**	**	**	**	**	**	**	**
N-LSD 5%	0.16	2.57	0.19	10.29	0.029	0.23	0.025	4.71	0.32	2.64	0.24	8.98
N-LSD 1%	1.00	3.36	0.25	13.45	0.039	0.301	0.033	6.16	0.39	3.34	0.31	11.74

NFB, N_2-fixing bacteria (*A. brasilense*), PSB, P-solubilizing bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

The ability of associative N_2-fixers, P-solubilizing bacteria and VA mycorrhiza to increase the mineral N- and P-uptake by plants has been demonstrated previously (Hauka *et al.*, 1990 and Belimov *et al.*, 1995). The effect of increasing nutrient content in plant can be explained by an enhancement of the biological N_2-fixation or by activities of NFB and PSB on N & P cycling in soil as well as the possibility of interaction between the mycorrhizal fungus and PSB in helping the plant to use sparingly soluble phosphate (Azcon *et al.*, 1978 and Azcon-aguilar *et al.*, 1986). Ba and

Guissou (1996) suggested that VAM plants take up more P from soil and rock phosphate than non-VAM plants. On the other hand, VAM are able to increase N-concentration in plants by an indirect P-supply, a direct uptake of N-compounds from soil by hyphae and an indirect effect on nitrate reductase activity in plants (Barea *et al.*, 1986).

N_2-ase activity:

Inoculated maize plants showed low C_2H_2 reduction rates (6.29-39.48 nmol C_2H_4/h. plant) (Table 4). The high level of N_2-ase activity after 60 days was observed in combined inoculation treatments. However, the highest N_2-ase activity was observed among maize plants associated with *A. brasilense* particularly in composed inoculant, *i.e.*, *A. brasilense* + *B. megaterium* var. *phosphaticum* + *G. macrocarpus.* Generally, maximum values of N_2-ase activity was found when soil was fertilized with ¼ dose of mineral N. In contrast, no or slight increase in N_2-ase activity was observed in the roots of maize plants growing in soil inoculated with single inoculum of either *B. megaterium* + *G. macrocarpus*. These findings are in harmony with data of Hegazi (1983).

Table (4): Nitrogenase activity (nmol C_2H_4/g plant/h) of maize plants grown in calcareous soil amended with rock phosphate (0.5%) and composted wheat straw (0.5%).

Treatments	N_2-ase activity (nmol C_2H_2/g plant/h)	
	30 (day)	60 (day)
A: Uninoculated treatments:		
Mineral N-free control	7.17	8.58
Full dose of N	10.85	14.60
B: Inoculated treatments:		
NFB + ½ N dose	14.46	11.83
PSB + ½ N dose	8.67	9.12
VAM + ½ N dose	6.29	6.49
NFB + PSB + ½ N dose	14.58	16.31
NFB + VAM + ½ N dose	14.82	27.57
PSB + VAM + ½ N dose	9.39	17.59
NFB +PSB + VAM + ½ N dose	13.86	24.41
NFB + ¼ N dose	15.29	13.27
PSB + ¼ N dose	13.48	8.27
VAM + ¼ N dose	12.98	8.14
NFB + PSB + ¼ N dose	16.06	17.05
NFB + VAM + ¼ N dose	32.42	38.63
PSB + VAM + ¼ N dose	10.60	24.69
NFB +PSB + VAM + ¼ N dose	21.26	39.48
F-test	**	**
N-LSD 5%	1.47	1.71
N-LSD 1%	1.92	2.24

NFB, N_2-fixing bacteria (*A. brasilense*), PSB, P-solubilizing bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza (*G. macrocarpus*), N-LSD, new least significant difference.

Mycorrhizal infection:

Data presented in Table (5) show that the inoculation with *G. macrocarpus* successfully developed a high level of infection in maize plants, which indicated good ecological adaptation of mycorrhizal fungus to the calcareous soil. Very low infection was observed in the uninoculated control plants. The highest percentages of VAM colonization were found when soil inoculated with *G. macrocarpus* or *G. macrocarpus* + *B. megaterium*. The rate of fungal colonization was higher after 60 days as compared to plants with 30 days. Colonization in soils fertilized with half dose of N were less than ¼ dose-fertilized ones. Azcon-aguilar *et al.* (1986) found that the essential synergistic effect of bacterial inoculation increased the degree of mycorrhizal infection. The high colonization of VA mycorrhiza may be also due to compost addition which can stimulate the growth of hyphae, that would achieve further infection (St-Jhon *et al*., 1983).

Table (5): Level of mycorrhizal infection in maize plants grown in calcareous soil amended with rock phosphate (0.5%) and composted wheat straw (0.5%).

Treatments	30 days			60 days		
	Infection parameters (%)					
	F	M	A	F	M	A
Mineral N-free-uninoculated control	2.30	1.00	0.00	7.30	3.40	2.20
VAM + ½ N dose	20.00	18.00	13.00	71.00	60.10	43.50
NFB + VAM + ½ N dose	18.30	15.00	11.00	73.00	52.30	44.00
PSB + VAM + + ½ N dose	22.90	17.30	14.00	90.50	77.00	57.00
NFB +PSB + VAM+ ½ N dose	20.10	16.30	9.00	65.10	55.00	45.00
VAM + ¼ N dose	29.40	25.00	22.00	100.00	81.00	61.00
NFB + VAM + ¼ N dose	28.10	23.30	18.00	100.00	85.00	60.00
PSB + VAM + ¼ N dose	13.50	11.00	6.00	100.00	75.00	60.00
NFB +PSB + VAM + ¼ N dose	22.00	19.00	13.00	98.10	65.00	53.00
F-test	**	**	**	**	**	**
N-LSD 5%	0.90	1.58	1.03	3.96	1.35	2.28
N-LSD 1%	1.21	2.12	1.39	5.32	1.81	3.07

F, frequency of root infection; M, intensity of cortical infection; A, arbuscular frequency in roots; NFB, N_2-fixing bacteria (*A. brasilense*), PSB, P-solubilizing bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus*), N-LSD, new least significant difference.

Populations of total bacteria, NFB and PSB:

Data presented in Table (6) show that the introduced bacteria were able to actively colonize the rhizosphere of maize plants particularly in the presence of half dose of N. The improvement in the viability of tested microorganisms may be due to the inoculation with different biopreparations (Belimov *et al*., 1995) as well as to the compost and mineral fertilizers addition (Eweda and Vlassak, 1988 and El-Sawah *et al.*, 1995). The density of total bacteria, azospirilla and PSB in the rhizosphere of maize plants increased with the increase in plant development. The densities of tested microorganisms in the rhizosphere of maize plants were affected by biopreparations form used and N fertilizer level. The population size of tested microorganisms indicated an active rhizosphere of plant. The highest numbers of total bacteria was found in most combined inoculation treatments. The highest numbers of P-solubilizing bacteria, namely 14.39 x 10^5 cfu/g was found in the soil inoculated with P-solubilizing bacteria. The maximum response of *A. brasilense* was found when the soil was inoculated with the *A. brasilense* and *G. macrocarpus* in the presence of ½ dose of N fertilizer.

Table (6): Population* of total bacteria, azospirilla and PSB in rhizosphere of maize plants grown in calcareous soil amended with rock phosphate (0.5%) and composted wheat straw (0.5%).

Treatments	Counts of Total bacteria (x 10^6 cell/g soil)		Counts of PSB (x 10^5 cell/g soil)		Counts of *Azospirillum* (x 10^4 cell/g soil)	
	30 day	60 day	30 day	60 day	30 day	60 day
A: Uninoculated treatments:						
Mineral N-free control	0.91	1.61	2.34	3.88	3.15	9.52
Full dose of N	2.01	2.79	4.50	13.14	4.01	11.50
B: Inoculated treatments:						
NFB + ½ N dose	2.95	3.84	6.27	6.88	7.63	17.52
PSB + ½ N dose	4.27	5.28	8.88	14.39	4.83	14.02
VAM + ½ N dose	3.64	3.60	5.75	12.52	4.96	11.51
NFB + PSB + ½ N dose	2.94	4.23	6.25	13.15	6.69	21.28
NFB + VAM + ½ N dose	2.95	8.05	4.97	12.35	7.63	30.04
PSB + VAM + ½ N dose	3.24	6.28	8.13	14.39	5.37	14.76
NFB +PSB + VAM + ½ N dose	4.67	5.51	5.00	14.39	6.88	22.18
NFB + ¼ N dose	2.02	3.02	3.38	4.64	3.50	19.73
PSB ¼ N dose	3.22	4.58	4.25	10.25	3.25	11.77
VAM ¼ N dose	2.97	2.96	3.15	4.75	2.73	13.82
NFB + PSB ¼ N dose	1.01	2.79	4.50	6.00	6.06	15.37
NFB + VAM ¼ N dose	1.60	2.79	3.75	7.39	7.66	22.17
PSB + VAM ¼ N dose	2.04	3.66	6.57	12.52	4.67	14.88
NFB +PSB + VAM ¼ N dose	4.34	4.90	4.38	10.01	6.88	17.89
F-test	**	**	**	**	**	**
N-LSD 5%	0.27	0.55	0.38	0.72	0.65	1.58
N-LSD 1%	0.35	0.71	0.50	0.94	0.85	2.07

NFB, N_2-fixing bacteria (*A. brasilense*), PSB, P-solubilizing bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza *(G. macrocarpus)*, N-LSD, new least significant difference. * Number of total bacterial counts, PSB, azospirilla at zero time before cultivation were 0.50 x 10^6, 1.09 x 10^5, 2.24 x 10^4 cells/g dry soil, respectively.

On the light of the obtained results and under the conditions of this investigation, it can be recommended with a strategy of integrated nutrient supply by using a judicious combination of low dose of N fertilizers, rock phosphate, compost and biopreparations (N_2-fixers, P-solubilizers and VA mycorrhiza).

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تأثير التلقيح المركب من البكتيريات المثبتة للنيتروجين والمذيبة للفوسفات والميكوريزا على نمو وتغذية الذرة فى الأراضى الجيرية

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أجريت تجربة أصص تحت ظروف الصوبة بمحطة التجارب والبحوث الزراعية بجامعة المنصورة (صيف 1998) بغرض دراسة تأثير اللقاحات المركبة من البكتيريا المثبتة للنيتروجين والبكتيريا المذيبة للفوسفات والميكورهيزا على نمو ومحتوى نباتات الذرة من العناصر ، ونشاط إنزيم النيتروجينيز ، ونسبة إصابة الجذور بالميكوريزا ، وأعداد الميكروبات ، وذلك تحت ظروف الأراضى الجيرية لمنطقة برج العرب ، وقد إستخدم فى هذه الدراسة نصف وربع الجرعة الموصى بها من السماد النيتروجينى ، كما تم تدعيم التربة بصخر الفوسفات (0.5%) وكومبوست قش القمح (0.50%) ، وقد أوضحت النتائج أن أعلى زيادة فى الأوزان الجافة للنباتات وفى محتواها من عناصر النيتروجين والفوسفور والبوتاسيوم فى معظم المعاملات الملقحة باللقاحات المختلطة فى وجود نصف جرعة من السماد النيتروجينى ، كما وجد نشاط عالى لإنزيم النيتروجنيز فى جذور نباتات الذرة فى المعاملات الملقحة بلقاحات مركبة (سواء لقاح مزدوج من الآزوسبيريللم + الميكوريزا أو لقاح ثلاثى) خاصة فى وجود ربع جرعة من السماد النيتروجينى المعدنى ، كما وجدت معدلات إصابة عالية لجذور نباتات الذرة بالميكوريزا بما يشير إلى حدوث نمو جيد لهذا الفطر فى الأراضى الجيرية موضع الدراسة خاصة المسمدة بربع جرعة من السماد النيتروجينى ، وقد أدت اللقاحات المستخدمة إلى تأثير إيجابى بزيادة كثافة أعداد المجتمع الميكروبى ، وقد سجلت أكبر أعداد فى معاملات اللقاح الثلاثى المسمدة بنصف جرعة من السماد المعدنى ، وتوصى نتائج الدراسة الحالية بإمكانية ترشيد إستخدام الأسمدة الكيماوية فى الأراضى الجيرية بإستخدام نظام تسميد متكامل من جرعة منخفضة من الأسمدة النيتروجينية وصخر الفوسفات والكومبوست إلى جانب اللقاحات الميكروبية (البكتيريا المثبتة للنيتروجين الجوى والبكتيريا المذيبة للفوسفات والميكوريزا) .