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EFFECT OF USING SINGLE AND COMPOSITE INOCULATION WITH *Azospirillum brasilense*, *Bacillus megaterium* var. *phosphaticum* AND *Glomus macrocarpus* FOR IMPROVING GROWTH OF *Zea mays*

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ABSTRACT

The interaction between single and/or composite inoculant of nitrogen-fixing bacteria, phosphate-dissolving bacteria & VA mycorrhiza in association with *Zea mays* was investigated in a pot experiment in clayey soil under green house conditions. The inoculation significantly increased plant dry weight particularly by composite inoculant. The maximum increase in plant growth was recorded in triple inoculant treatments in the presence of the half-dose of N fertilizer. Significant increase in nitrogen, phosphorus and potassium content was observed in inoculated plants, particularly with inoculants of the three microbial form, phosphate-dissolving bacteria and phosphate-dissolving bacteria + VA mycorrhiza. Full dose of N fertilization inhibited nitrogenase activity. On the other hand, the highest acetylene reduction activity was observed among maize plants roots associated with *Azospirillum* either in single or composite biopreparations and in the presence of ¼ N dose after 30 and 60 days. Maize plants inoculation increased numbers of total viable bacteria, phosphate-dissolving bacteria, *Azospirillum* sp as well as the mycorrhizal colonization in the rhizosphere soil. The results obtained from this work demonstrate the potential benefit of inoculating *Zea mays* with a composite inoculum of nitrogen-fixing bacteria, phosphate-dissolving bacteria and VA mycorrhiza.

Key words: Mycorrhiza, Phosphate-dissolving bacteria, Nitrogen-fixing bacteria, *Zea mays*, composite inoculation, clayey soil.

INTRODUCTION

The use of intensive and non-rational rates of mineral fertilizers increases the costs of agricultural production. Most of mineral fertilizer elements are either fixed in the soil or leached to pollute the environment. Therefore, there is a wide interest in the use of combination of mineral- and bio-fertilizer as substituent and cheap source for chemical fertilizers (Hernandez *et al.,* 1995, Hauka *et al.*, 1996 and Farag, 1998).

The promising trend for increasing the efficiency is the use of mixtures microorganisms (Bashan and Levanony, 1990). A significant increases in yield and nitrogen fixation have been observed when wheat and barley plants were inoculated with mixed cultures of bioinoculants (Fayez, 1989). There have been many successful attempts to improve maize supplement by using mixtures of nitrogen-fixing bacteria and mycorrhiza (Pacovsky & Fuller, 1985, Sreeramulua *et al*., 1988 and Hao *et al.*, 1991). The effect of combined inoculation of wheat and rice with both nitrogen-fixing bacteria and phosphate-dissolving bacteria on yield, nitrogen and phosphorus accumulation in plants was more significant than the effect of separate treatments (Kundu and Gaur, 1980 & 1984; Rachewad *et al.*, 1991, Belimov *et al.*, 1995 and Farag, 1998). Maize plants growth and nutrient content were increased by dual inoculation with phosphate-dissolving bacteria and VA mycorrhiza in the presence of rock phosphate (Heggo & Barakah, 1993). Furthermore, the beneficial influence of phosphate-solubilizing bacteria on survival of nitrogen-fixing bacteria in the rhizosphere has been observed (Ocampo *et al*., 1975 and Kundu and Gaur, 1980). Related results have been obtained after inoculation of sorghum with *Azospirillum* and phosphate-dissolving bacteria (Alagawadi and Gaur, 1988).

The aim of this study is to determine the effect of individual and/or combined inoculum of nitrogen-fixing bacteria, phosphate-dissolving bacteria and VA mycorrhiza on *Zea mays* plants grown in clayey soils under different N fertilization levels. The effect of inoculation treatments on nitrogenase activity of plant root system and population of certain bacteria in the rhizosphere region was also investigated in this study.

MATERIALS AND METHODS

A pot experiment was conducted during summer 1998 under greenhouse conditions at Agricultural Research and Experiments Station, Faculty of Agriculture, Mansoura University. This experiment was carried out to study the effect of maize inoculation with either *Azospirillum brasilense* as nitrogen fixing bacteria (NFB), *Bacillus megaterium* var. *phosphaticum* as phosphate-dissolving bacteria (PDB) and *Glomus macrocarpus* as VA mycorrhiza, separately or mixed, in the presence of different N levels (full, ½ and ¼ dose of N fertilizer) and rock phosphate as a source of non soluble P, on some rhizosphere microorganisms, N_2-ase activity of roots, plant growth and nutrient uptake by maize plants.

Grains surface sterilization:

Maize grains (*Zea mays* L. var. triple hybrid 310) kindly provided by Agron. Dept., Sakha Agric. Station were used in this experiment. Grains were selected to be similar in size and weight. Grains were surface sterilized with ethanol (1 minute) followed by 30% H_2O_2 for 5 minutes and subsequently washed with distilled water.

Grains inoculation:

Bacterial inocula:

NFB was kindly provided and identified by the Agricultural Microbiology Department, Soil, Water and Environ. Res. Inst., ARC. PDB was obtained from Microbiol. Dept., Fac. of Agric., Mansoura Univ. The first organism was grown on modified nitrogen-deficient semi-solid malate medium (Hegazi *et al.*, 1979) at 30°C for 48 h. The second organism was grown on Bunt and Rovira liquid medium (1955) modified by Abdel Hafez (1966) at 30°C for 72 h. The suspensions obtained (1 x 10^8 cells/ml each) were used for grains inoculation at the rate of 10 ml single inocula, 5 ml of each culture in binary inoculants or 3.33 ml of each culture in composite inoculants. The same doses were added to each pot after 10 days of sowing.

VA mycorrhiza inoculum:

Mycorrhiza were obtained from Fac. of Sci. Mansoura Univ. For inoculation with VA mycorrhiza to increase the availability of phosphorus in rock phosphate, a cheaper but sparingly-soluble phosphorus fertilizer, grains planted over the thin layer from segments of highly infected onion roots. Five ml of spores suspensions (about 180 spores/ml) were added on the grains and then covered with some of the same soil.

Mineral fertilizers:

N was added in the form of ammonium sulfate (recommended full dose 80 Kg N/fed, ½ dose 40 Kg N/fed and ¼ dose 20 Kg N/fed). P was added in the form of rock phosphate (RP) (0.5%). K in the form of potassium sulfate (50 Kg K/fed).

Plant growth conditions:

10 kg., portions of clayey soil (2.9% $CaCO_3$, 0.2% total N, 23.8 ppm available P, 1.69 meq/100 g available K and pH 8.2. It contained 42.8% clay, 30.2% silt and 22.3% sand) were collected from 0-20 cm layer, Faculty of Agriculture Farm, Mansoura Univ. The soil was air dried, ground to pass throw 2.0 mm sieve and put in plastic pots (30 x 45 cm). The soil was mixed with rock phosphate (RP); 26.4% P_2O_5, (Abou Zaabal Phosphate Fertilizers Co.) at the rate of 0.5% (w/w) before sowing. Grains were distributed at the rate of five grains / pot, then thinned to one seedlings / pot after three weeks of sowing. 16 experimental treatments were included in this study. Three replicate pots received each treatment. One experimental treatment included no inoculation no fertilization (control). One experimental treatment included a full dose addition of nitrogen (80 kg N/fed). Seven experimental treatments included a half dose nitrogen addition (40 kg N/fed) and microbial inoculation. Seven experimental treatments included a quarter dose nitrogen addition (20 kg N/fed) and microbial inoculation. Irrigation stood at 60% of the 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. After 30 and 60 days, plants were uprooted and both plants and amended soil were analysed.

Microbiological analysis:

Samples from rhizosphere soil of maize plants were taken after 30 and 60 days from sowing to determine total bacterial number using Allen (1969) medium, nonsymbiotic nitrogen fixer (*Azospirillum brasilense*) using semi-solid malate medium of Döbereiner (Barber *et al.*, 1976) and most probable number (MPN) technique (Cochran, 1950), the prepared tubes were incubated at 30°C for 2 weeks and phosphate-dissolving bacteria using Bunt and Rovira (1955) modified by Abdel Hafez (1966). The prepared dishes were incubated at 30°C for 4 days for total bacterial count and 48 h for PDB. Mycorrhizal infection was estimated microscopically on a sample of fresh root as described by Trouvelot *et al.* (1986), after clearing and staining (Phillip and Hayman, 1970).

Acetylene reduction activity:

The nitrogenase activity of the roots was determined by the acetylene reduction technique at 30 and 60 days after sowing. Plants were carefully removed from pots and freed of excess soil by gentle shaking, placed in bottles stoppered with suitable silicon rubber suba seals. Following the removal of 10% of the gaseous atmosphere, acetylene was injected in bottels to give 10% of acetylene : air mixture. Gas samples of 0.1 ml were assayed for ethylene content by gas chromatography (Perkin Elemer Sigma 4 gas chromatography) after 2 and 24 hr. The N_2-ase activity was calculated and expressed as nanomoles C_2H_4 oven dry roots/hr. Control samples (inoculated without acetylene) and acetylene standards were tested for ethylene contamination (Hardy *et al*., 1973).

Chemical analysis:

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

The effect of using different inoculation treatments and different N fertilization levels on growth of *Zea mays* plants is presented in Table (1). In general, inoculation with NFB, PDB and VAM, in the presence of ½ dose of N fertilizer increased dry weights of maize to a degree approximately similar to those in full dose of N. The maximum increase in dry weight of maize over control (without inoculation or microbial fertilization) was 212.67% in soil inoculated with triple inoculant and N fertilized with half dose after 60 days, under such conditions the total dry weight reched 22.82 g/plant. Triple inoculant, however, increased dry weight to a greater extent than either singular or dual inoculants particularly after 60 days. Several authors found that the used microorganisms may benefit the plant growth by nitrogen fixation (Hegazi, 1983) by producing growth hormones (Hauka *et al.*, 1990) and by cycling the nutrients (Hauka *et al*., 1996).

Table (1): Effect of microbial inoculation and N fertilization on dry weights of *Zea mays* (g/plant).

Treatments	30 (day)			60 (day)		
	Root Dry Wt	Shoot Dry Wt	Total Dry Wt	Root Dry Wt	Shoot Dry Wt	Total Dry Wt
Non-inoculated Treatments:						
Non N-fertilized control	0.31	0.97	1.28	3.83	6.90	10.73
Full dose of N	0.57	1.07	1.64	4.98	10.88	15.86
Half dose of N:						
NFB	0.53	1.95	2.48	5.06	8.00	13.06
PDB	0.37	1.27	1.64	6.31	11.59	17.90
VAM	0.44	1.13	1.57	4.93	10.48	15.41
NFB + PDB	0.54	1.22	1.76	6.40	8.26	14.66
NFB + VAM	0.61	1.75	2.36	3.85	10.78	14.63
PDB + VAM	0.65	2.00	2.65	6.58	9.26	15.84
NFB +PDB + VAM	0.55	1.53	2.08	9.64	13.18	22.82
¼ dose of N:						
NFB	0.46	1.79	2.25	3.94	9.02	12.96
PDB	0.39	1.37	1.76	4.78	9.92	14.70
VAM	0.54	1.62	2.16	3.22	7.75	10.97
NFB + PDB	0.33	1.05	1.38	4.12	7.49	11.61
NFB + VAM	0.34	1.32	1.66	4.39	10.50	14.89
PDB + VAM	0.33	1.11	1.44	3.10	9.93	13.03
NFB +PDB + VAM	0.34	1.17	1.51	5.54	10.14	15.68
F-test	**	**	**	**	**	**
N-LSD 5%	0.121	0.26	0.25	0.40	0.64	0.80
N-LSD 1%	0.152	0.34	0.32	0.52	0.84	1.05

NFB, nitrogen-fixing bacteria (*A. brasilense*), PDB, phosphate-dissolving bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza, N-LSD, new least significant difference.

Mineral content:

The effect of using different inoculation treatments and different N fertilization levels on nitrogen, phosphorus and potassium content (%) and mineral uptake by *Zea mays* plants is presented in Table (2). In general, inoculation with NFB, PDB and VAM increased significantly nutrients content in maize plants in comparison with control. The effect of inoculation on nutrients content of maize plants was much more intensified in the presence of a half dose of N fertilizer. The highest increase in nutrients was found in plants biofertilized with dual or triple inoculants.

However, the highest increase of N and P in maize plants corresponded with inoculants of NFB and PDB or VAM, respectively. The positive effect of inoculation on nutrients N, P and K content in maize plants probably due to the significantly increased growth due to beneficial associations between biofertilizers and partner which improve quality of plant.

However, the highest increase of N, P and K in maize plants corresponded with inoculants of three microbial form, (PDB) and (PDB + VAM). It may be concluded that N, P and K content in maize plant were increased significantly by the synergetic effect mainly between the phosphate-dissolving microorganisms. The beneficial effect of these microorganisms on nutrients uptake may be attributed to the promoting effect of PDB and VAM fungus on plant growth and dry matter accumulation by an increase uptake of soil phosphorus and possible metabolic effects on the plant. Likewise, it is also interesting to note that increases in root density due to VAM inoculation especially in size should directly enhance uptake of P and other nutrients. It was also noticed that both the content and uptake of each N, P and K increased with age of plant. The increase can be due to more dry matter accumulation with plant age progress and its metabolic processes. These results are in agreement with those reported by (Hauka *et al*., 1990 and 1996). They found that the improvement in plant growth and development due was to enhanced uptake of soil nutrients. On the other hand, VAM are able to increase N-concentration in plants by (a) an indirect P-supply; (b) a direct uptake of N-compounds from soil by hyphae; (C) an indirect effect on nitrate reductase activity in plants (Barea *et al*., 1986). The effect of maize inoculation on increasing nutrient content and uptake in plants was reported by Rachewad *et al.* (1991).

Microbial counts:

The effect of different inoculation treatments and different N fertilization levels on certain bacterial counts in the rhizosphere of *Zea mays* plants is presented in Table (3). The results obtained show that inoculation with NFB, PDB and VAM increased significantly the counts of the tested microorganisms/g soil. The total bacterial counts, in general, showed positive response for all used inoculants. This finding could be due to the active rhizosphere of plant (Hauka *et al.*, 1990). The highest numbers of PDB namely 28.50 x 10^5 cells/g soil was found when the soil was inoculated with both NFB and PDB and fertilized with half dose of N fertilizer. It is interesting to notice that the density of each total bacteria, phosphate-dissolving bacteria and *Azospirillum* spp in the rhizosphere increased with the increase in plant development (Table 3). The data also show that the densities of these microorganisms in the rhizosphere were affected by either biofertilizers form used and N fertilizer level applied, the highest counts were record for interaction between inoculation and half dose of N fertilizer. When the densities of these representative microorganisms were evaluated, it was found that different types differed considerably in the efficiency of biofertilizer form used (Table 3). The highest numbers of *A. brasilense* namely 88.81 x 10^4 cells/g soil was found in NFB + VAM treatment in the presence of ½ dose of N fertilizer.

Table (3): Effect of microbial inoculation and N fertilization on microbial numbers* (/g oven dry soil).

Treatments	Total bacteria (x 10^6)		PDB (x 10^5)		*Azpspirillum* sp (x 10^4)	
	30 (day)	60 (day)	30 (day)	60 (day)	30 (day)	60 (day)
Non-inoculated Treatments: Non N fertilized control	2.56	3.94	2.43	4.48	10.67	15.78
Full dose of N	4.58	9.06	5.72	14.67	11.15	24.69
Half dose of N: NFB	13.83	16.70	4.34	12.54	12.47	76.82
PDB	15.45	17.38	6.20	16.73	12.48	49.65
VAM	17.27	21.01	8.05	23.54	13.48	35.04
NFB + PDB	14.71	16.45	5.58	28.50	21.28	63.02
NFB + VAM	21.52	22.70	10.53	16.64	15.02	88.81
PDB + VAM	18.51	21.39	8.58	26.81	15.02	26.28
NFB +PDB + VAM	23.83	29.39	5.87	18.34	17.37	48.76
¼ dose of N: NFB	12.84	14.32	8.67	9.91	11.51	48.81
PDB	13.33	20.20	10.53	16.11	7.56	29.74
VAM	17.32	18.51	8.72	17.97	9.71	30.04
NFB + PDB	15.13	15.63	7.43	18.39	15.22	30.41
NFB + VAM	13.20	27.27	7.20	25.66	11.40	29.74
PDB + VAM	18.95	21.89	15.49	27.88	14.87	21.06
NFB +PDB + VAM	22.08	28.52	10.53	24.87	13.67	48.89
F-test	**	**	**	**	**	**
N-LSD 5%	1.25	1.24	0.78	0.80	1.29	1.52
N-LSD 1%	1.64	1.62	1.02	1.05	1.69	1.99

NFB, nitrogen-fixing bacteria (*A. brasilense*), PDB, phosphate-dissolving bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza, NLSD, new least significant difference.

*** Number of total bacterial counts at zero time before cultivation 1.87 x 10^6 cell/g dry soil.**

*** Number of PDB at zero time " before cultivation" 1.33 x 10^5 cell/g dry soil.**

*** Number of *Azospirillium* at zero time " before cultivation" 5.2 x 10^4 cells/g dry soil.**

Mycorrhizal infection:

The effect of different inoculation treatments and different N fertilization levels on mycorrhizal infection for *Zea mays* plants is presented in Table (4). The results obtained show that the VAM-inoculated *Zea mays* developed a high level of infection after 60 days, which indicated good ecological adaptation of mycorrhizal fungus to the clayey soil. Half dose of N fertilizer application caused little increase in the colonization of mycorrhiza development. The results also show that the mycorrhizal colonization increased in the presence of lowest level of nitrogen. Generally, The high percentage of arbuscular frequency was recorded after 60 days from cultivation in single inoculation treatment (62.30%) and dual inoculation, NFB+VAM, treatment (67.50%). The role and development of VA mycorrhizal infections in alluvial soils were reported by Kothari *et al.* (1990) and Hao *et al.* (1991). They investigated the effect of VA mcorrhiza and other rhizosphere microorganisms on growth and water relations in maize.

Table (4): Level of mycorrhizal infections as affected with microbial inoculation and nitrogen fertilization.

Treatments	30 days			60 days		
	F%	M%	A%	F%	M%	A%
Non fertilized non inoculated control	5.00	2.300	0.000	12.300	6.200	5.300
Half dose of N:						
VAM	40.20	28.10	17.00	70.00	48.00	33.50
NFB + VAM	30.90	19.30	11.80	70.00	42.00	32.00
PDB + VAM	38.00	21.10	16.80	100.0	66.60	50.90
NFB +PDB + VAM	22.50	13.00	8.90	60.80	40.50	31.80
¼ dose of N:						
VAM	42.50	28.00	20.20	100.0	70.10	62.30
NFB + VAM	42.00	26.00	16.90	100.0	83.00	67.50
PDB + VAM	20.80	13.30	7.40	90.00	50.30	45.00
NFB +PDB + VAM	33.03	18.40	11.00	83.00	50.00	42.01
F-test	**	**	**	**	**	**
N-LSD 5%	1.32	0.96	1.00	2.18	3.86	2.50
N-LSD 1%	1.78	1.29	1.29	2.93	5.19	3.35

F, frequency of root infection; M, intensity of cortical infection; A, arbusculer frequency in roots; NFB, nitrogen-fixing bacteria (*A. brasilense*); PDB, phosphate-dissolving bacteria (*B. megaterium* var. *phosphaticum*); VAM, vesicular-arbuscular mycorrhiza, NLSD, new least significant difference.

Nitrogenase activity:

The effect of different inoculation treatments on nitrogenase activity is presented in Table (5). The results show that inoculated *Zea mays* showed highly significant C_2H_2 reduction rate (12.58 – 66.80 nmoles C_2H_4/h./g plant). Relatively high nitrogenase activity was observed in plant roots of ¼ dose treatment. However, the highest nitrogenase activity was observed among maize plant roots associated with *A. brasilense.* These findings are in harmony with data of Hegazi *et al.* (1979) on maize plants.

Table (5): Effect of microbial inoculation and N fertilization on nitrogenase activity (nmoles C_2H_4/g plant/h).

Treatments	30 (day)	60 (day)
Non-N fertilized Non-inoculated control	10.24	17.73
Full dose of N	8.46	9.64
Half dose of N:		
NFB	33.17	21.11
PDB	15.21	19.67
VAM	18.73	25.21
NFB + PDB	28.87	44.21
NFB + VAM	39.98	37.44
PDB + VAM	24.95	17.47
NFB +PDB + VAM	32.90	39.50
¼ dose of N:		
NFB	26.53	35.42
PDB	16.36	12.58
VAM	28.24	38.67
NFB + PDB	59.92	39.17
NFB + VAM	36.69	66.80
PDB + VAM	20.61	15.18
NFB +PDB + VAM	53.21	52.40
F-test	**	**
N-LSD 5%	2.25	2.11
N-LSD 1%	2.95	2.75

NFB, nitrogen-fixing bacteria (*A. brasilense*), PDB, phosphate-dissolving bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza, NLSD, new least significant difference.

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تأثير إستخدام التلقيح المفرد والمركب بالآزوسبيريلم برازيلينس والباسيلس ميجاتيريم وجلومس ماكروكاربس على تحسين نمو الذرة

فتحى إسماعيل على حوقة
قسم الميكروبيولوجى ـ كلية الزراعة ـ جامعة المنصورة ـ المنصورة ـ مصر .

إستهدف البحث دراسة تأثير إستخدام لقاحات مفردة ومركبة من البكتيريا المثبتة للنيتروجين والبكتيريا المذيبة للفوسفات وكذلك الميكوريزا على تحسين نمو الذرة ومحتواها من العناصر وكذلك أعداد الميكروبات فى ريزوسفير هذه النباتات بالإضافة إلى نسبة إصابة الجذور بالميكوريزا ، ونشاط إنزيم النيتروجينيز فى الجذور ، وذلك تحت ظروف الأراضى الطينية فى تجربة أصص تحت ظروف الصوبة بمحطة التجارب والبحوث الزراعية بكلية الزراعة – جامعة المنصورة ، وقد إستخدمت فى هذه الدراسة مستويات مختلفة من كبريتات الأمونيوم كمصدر للنيتروجين المعدنى (الجرعة الموصى بها كاملة بالإضافة إلى نصف وربع هذه الجرعة) ، كما إستخدم صخر الفوسفات كسماد فوسفاتى رخيص الثمن.

وقد أظهرت النتائج مايلى :

وجدت زيادة معنوية فى الأوزان الجافة للنباتات وفى محتواها من عناصر النيتروجين والفوسفور والبوتاسيوم وذلك فى المعاملات الملقحة باللقاحات الميكروبية المركبة فى وجود نصف الجرعة من السماد النيتروجينى مقارنة بالجرعة الكاملة أو ربع الجرعة خاصة بعد 60 يوم من الزراعة .

وجدت زيادة فى الأعداد الكلية للبكتيريا ومذيبات للفوسفات وكذلك فى أعداد الآزوسبيريللم فى تربة ماحول الجذور حيث وصلت الأعداد الكلية إلى أقصاها فى حالة اللقاح الثلاثى أما مذيبات الفوسفات فقد وصلت أعدادها إلى أقصاها مع اللقاح المزدوج من مثبتات النيتروجين ومذيبات الفوسفات بينما فى حالة الأزوسبيريللم فقد سجلت أكبر عدد فى حالة اللقاح المزدوج من مثبتات النيتروجين والميكوريزا ، وكانت هذه الزيادة جميعها بعد 60 يوم من الزراعة وفى وجود نصف جرعة نيتروجين ، وجدت زيادة فى مستوى الإصابة بالميكوريزا فى المعاملات الملقحة وبخاصة المسمدة بربع جرعة من النيتروجين ، أدى التسميد بالجرعة الموصى بها من النيتروجين إلى تثبيط نشاط إنزيم النيتروجنيز بينما نتج عن التلقيح الميكروبى زيادة فى نشاط هذا الإنزيم خاصة مع اللقاحات المحتوية على الآزوسبيريللم وفى وجود أقل مستوى من السماد النيتروجينى .

Table (2): Effect of microbial inoculation and N fertilization on nutrient content of *Zea mays* (mg/plant).

Treatments	30 (day)		60 (day)		30 (day)		60 (day)		30 (day)		60 (day)	
	N%	N-uptake	N%	N-uptake	P%	P-uptake	P%	P-uptake	K%	K-uptake	K%	K-uptake
Non-inoculated Treatments:												
Non N-fertilized control	1.25	16.00	1.92	206.02	0.25	3.20	0.31	33.26	1.25	16.00	2.65	284.35
Full dose of N	1.55	25.42	3.00	475.80	0.33	5.41	0.38	60.74	1.65	27.06	3.60	570.96
Half dose of N:												
NFB	1.55	38.44	3.05	398.33	0.35	8.68	0.38	49.63	1.66	41.17	3.84	501.50
PDB	1.55	25.42	3.03	542.37	0.34	5.58	0.38	68.02	1.66	27.22	3.85	689.15
VAM	1.61	25.44	3.03	466.93	0.35	5.50	0.39	60.10	1.72	27.00	3.89	599.45
NFB + PDB	1.60	28.16	3.08	451.53	0.34	5.98	0.42	61.57	1.73	30.45	3.90	571.74
NFB + VAM	1.61	38.00	3.20	468.16	0.35	8.26	0.38	55.59	1.73	40.83	3.90	570.57
PDB + VAM	1.65	43.73	3.20	506.88	0.37	9.81	0.47	74.45	1.78	47.17	3.90	617.76
NFB +PDB + VAM	1.65	34.32	3.22	734.80	0.38	7.90	0.45	102.69	1.78	37.02	3.97	905.95
¼ dose of N:												
NFB	1.32	29.70	2.00	259.20	0.28	6.30	0.32	41.47	1.31	29.48	2.70	349.92
PDB	1.32	23.23	2.00	294.00	0.28	4.93	0.32	47.04	1.32	23.23	2.73	401.31
VAM	1.33	28.73	2.00	219.40	0.28	6.05	0.33	36.20	1.33	28.73	2.73	299.48
NFB + PDB	1.28	17.66	2.10	243.81	0.27	3.73	0.31	35.99	1.32	18.22	2.72	315.79
NFB + VAM	1.28	21.25	2.10	312.69	0.27	4.48	0.33	49.37	1.32	21.91	2.75	409.48
PDB + VAM	1.28	18.43	2.11	274.93	0.28	4.03	0.33	43.00	1.35	19.44	2.76	359.63
NFB +PDB + VAM	1.35	20.39	2.14	335.55	0.31	4.73	0.34	53.31	1.35	20.39	2.76	432.77
F-test	**	**	**	**	**	**	**	**	**	**	**	**
N-LSD 5%	0.11	4.09	0.25	19.78	0.064	0.85	0.034	6.13	0.130	3.79	0.24	8.43
N-LSD 1%	0.14	5.71	0.33	25.86	0.071	1.111	0.043	8.01	0.173	4.96	0.33	11.02

NFB, nitrogen-fixing bacteria (*A. brasilense*), PDB, phosphate-dissolving bacteria (*B. megaterium* var. *phosphaticum*), VAM, vesicular-arbuscular mycorrhiza, N-LSD, new least significant difference.