 Egypt. J. Soil. Sci. **46,** No. 2, pp.219-235 (2006)

Organic Manuring and Biofertilization Approaches as Potential Economic and Safe Substitutes for Mineral Nitrogenous Fertilization

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THE PRESENT work aimed at throwing some light on the possibility of substituting, partially, the amount required of mineral nitrogen for fertilization of a certain crop by organic manuring and biofertilization. Such an investigation would provide knowledge about to what extent these substitutes can replace mineral nitrogen fertilizer and how far they can affect the percentage of fertilizer N recovery (% FNR) by maize plant (*Zea meys*) grown on an alluvial soil from Moshtohor and a calcareous one from El- Noubaria. An additional desired environmental goal can be achieved through minimizing pollution of soil with mineral nitrogen and hence reducing its release to groundwater or surface water. Approaching the aimed goals was executed through performing a factorial pot experiment in a randomized complete block design with three replicates. Rates of N ranging from 0 to 90 mg N kg^{-1} were tried through different combinations between ammonium sulfate labeled with N^{15} (AS) and chicken manure (CM). Soil pots (5 kg each), received basic supplements of P (as rock phosphate) at 100 mg P kg^{-1} and K (as K_2SO_4) at 50 mg K kg^{-1} and micronutrients in a Hogland solution. The pots were sown with either uninoculated maize grains or maize grains inoculated with either *Azotobacter chroeoccum* (Azt), *Azospirillum brasilense* (Azs) or both bacteria together immediately before seeding and left to grow for 60 days. Application of nitrogen in a mineral form or an organic one or in a combination of both the forms increased dry weight of plant and its uptake of N. The inoculated treatments showed higher values than the uninoculated ones, combined application of (Azt + Azs) was more effective than inoculation with either alone. % FNR of the treatments that received combined inoculation were higher than the corresponding ones that received single inoculation with either Azt or Azs, inoculation with Azt seemed more effective than that with Azs, activity of N_2- fixing bacteria was more obvious at rates of applied N less than 30 mg N kg^{-1} beyond which activity of N_2- fixing bacteria decreased. The alluvial soil gave higher dry weight and N uptake than the calcareous one. Biofertilization through inoculation with Azt or Azs as well as applying organic manure could minimize the dose of mineral N required to be applied which is a profitable from the economic point of view and effective in reducing pollution of soil with N.

Nitrogen is a major nutrient element and it is needed in large amounts to increase growth and yield of plant. Its shortage leads to chlorosis (yellow of leaves) and stoppage of growth. It is a part of protein, important constituent of protoplasm, enzymes, the biological catalytic agents which speed up life processes. Nitrogen is also present as a part of nucleoprotein, amino acids, amines, amino sugar, polypeptides and other organic compounds in plants. Nitrogen would often be a limiting factor for growth and biomass production in all environments where there is suitable climate and availability of water to support life (Black, 1954).

Nitrogen fertilizers are economically an expensive input. In many instances less than 60% of the added N is recovered in the (crop + soil) with the remainder being lost by processes such as volatilization, leaching, immobilization and denitrification (Smith *et al.,* 1989 and Yusron & Philips, 1997). Thus, it is necessary to develop fertilizer management practices that can reduce losses and increase the nitrogen use efficiency (Yusron & Philips, 1997). Hegazi *et al.* (1993) stated that addition of N in form of ammonium sulfate at rates of 200 and 400 mg kg^{-1} soil significantly increased nitrogen content and uptake by maize plants. Hussaini *et al.* (2002) reported that the influence of N was significant on shelling percentage and grain yield of maize plants. Wahba (2003) mentioned that increasing N-levels from 80 to 100 kg fed^{-1}. caused significant increase in the dry weight and grain yield of maize plants and its components. Application of organic manures (OM) is a common practice followed by the farmer for obtaining a maximum yield. Such application causes a flush of microbial activity, which adds complexing agents to the soil. Adding organic manures is considered as an improving treatment for soil fertility and hence its contents of N, P, K beside micronutrients, which is consequently reflected on plant growth and yield (Narvaez *et al.*, 2000; Romero *et al.,* 2000;Yuyama & Mesquita, 2000; Enejil *et al.,* 2001; El-Emam, 2002; Ismaiel, 2002 and Mahdy, 2003). Because of the transitory nature of N in soil, its tendency for loss from the soil, and its potential for becoming a pollutant of air and water, fertilizer N should receive more care in its overall management than any other of the primary and secondary plant nutrients.

There are many sources to supply soil with nitrogen, namely organic and mineral N fertilizers and recently microbial inoculation of crop grains by certain free living N_2 – fixing bacteria had a great importance as a new technology. Existing evidences indicate that maize plants respond well to nitrogen fertilization. However, increasing cost of chemical fertilizers has reduced their use considerably. Under such a situation it is imperative to use natural resources to meet partial requirement of the crop needs for N. The technique of microbial inoculation aims at minimizing the amount of applied chemical fertilizers, preventing the pollution which can occur by excessive use of chemical fertilizers and reduces the cost of production. *Azotobacters and Azospirilla* are among the most important and well known heterotrophic bacteria which increase the yield of several crops by fixing the atmospheric nitrogen in soil, (Ram *et al.,* 1985). Inoculation of seeds with these bacteria registered a significant increase in yield at lower levels of nitrogen (Fayez *et al.,* 1985). This work aims at studying the effect of inoculation of maize grains with some free living N_2-fixing bacteria namely, *Azotobacter chroococcum, Azospirillum brasilense* and a mixture of them in the absence or presence of different levels of a mineral N fertilizer $(NH_4)_2SO_4$ (AS) or an organic N one in the form of chicken manure (CM). Such a study can introduce a great device to the farmers through applying the fertilization practices for growing maize in soils of Egypt.

Material and Methods

Used material

Two soils namely, an alluvial clay soil and a calcareous sandy clay loam soil were collected from Moshtohor and El-Noubaria, respectively (0-15 cm soil surface) to be used in the current investigation. According to the American Soil Taxonomy of the Soil Survey Staff (1999) these soils are classified as Typic Torriorthents and Typic Haplocalcids, respectively. The soils were thoroughly mixed, air dried, crushed, sieved to pass through a 2 mm sieve and kept for analysis and the experimental work. The main physical and chemical characteristics of the tested soils are presented in Table 1.

Maize grains (*Zea mays cv.Swc 10*) were supplied by the Maize Research Station, Field Crop Research Institute, Giza, Egypt. Efficient strains of N_2- fixing bacteria namely, *Azotobacter chroeoccum* (Azt) and *Azospirillum brasilense* (Azs) were supplied by the Microbiology Department, Soil Water and Environment Institut, Giza, Egypt. Two nitrogenous sources were used in the current study, *i.e.*, organic nitrogen supplied as a chicken manure (CM), the chemical composition of which is given in Table 2 and mineral nitrogen applied as ammonium sulfate (AS) labeled with N^{15}. The factors under study were (A) six nitrogenous treatments and (B) four biofertilization treatments, *i.e.,* there were 24 treatments each in three replicates. The experimental design was a randomized complete block, factorial. The grains were either grown without inoculation or inoculated with biofertilizers immediately before cultivation where the adhesive glue material was added to 500 mL warm water, splashed on grains and then biofertilizers were added, well mixed with grains and air dried for adhesion. The 6 nitrogen treatments were as follows:

Treatment code	Source of nitrogen	N rate mg kg^{-1}
A	none	0.0
B	CM	15
C	AS (N^{15})	15
D*	CM + AS (N^{15})	30
E*	CM + AS (N^{15})	60
F*	CM + AS (N^{15})	90

* Half rate is in the form of (AS) and the other half is in the form of (CM).

Experimental work

Poly venyle chloride (PVC) pots of 20 cm height and 20 cm diameter were used. Each pot was packed with 4 kg of soil. The packed soil pots received nitrogen at a rate of 0 mg N pot^{-1}, 15 mg N kg^{-1} in the form of chicken manure (CM), 15 mg N kg^{-1} in the form of ammonium sulfate (AS), 30 mg N kg^{-1} half of it in the form of chicken manure (CM) and the other half in the form of (AS), 60 mg N kg^{-1} half of it in the form of chicken manure (CM) and the other half in the form of (AS) and 90 mg N kg^{-1} half of it in the form of chicken manure (CM) and the other half in the form of (AS).

Chicken manure (CM) was thoroughly mixed with soils just before sowing whereas ammonium sulfate (AS) was applied as solution in one dose two weeks after sowing. The pots were seeded with grains of maize (*Zea mays cv.Swc 10*) at a rate of 10 grains pot^{-1}. After two weeks from sowing, the seedlings were thinned to five seedlings pot^{-1}. Phosphorus was added at a rate of 100 mg P kg^{-1} soil to all pots as rock phosphate just before sowing. Potassium, was applied at a rate of 50 mg K kg^{-1} soil as potassium sulfate. Also, micronutrients (as nutrient solution) were applied using Hogland solution after two weeks from sowing. The moisture content was maintained at water holding capacity through daily application of distilled water. The plants were cut just above the soil surface after 60 days from sowing, rinsed thoroughly with distilled water, cut to small pieces, oven- dried at 60-70° for 48 hr, prior to determination of dry weight. Samples of dried plants were ground to pass through a 0.5 mm stainless stell screen.

TABLE 1. Some physical and chemical properties of the investigated soil samples.

Soil characteristic	The alluvial soil	The calcareous soil
	Chemical analyses	
Calcium carbonate %	1.43	24.80
Organic matter %	1.98	1.24
EC (dS m^{-1})*	1.75	3.22
pH(1:2.5)	8.12	8.29
CEC $cmol_c$ kg^{-1}	35.21	10.78
Available N (mg kg^{-1})#	89.23	51.87
Available P (mg kg^{-1})#	24.33	3.37
Available K (mg kg^{-1})#	825.35	418.86
Available Fe (mg kg^{-1})#	34.74	21.83
Available Mn (mg kg^{-1})#	11.38	8.87
Available Cu (mg kg^{-1})#	5.95	1.45
Available Zn (mg kg^{-1})#	1.18	0.94
	Soluble cations and anions ($mmol_c$ L^{-1})*	
Ca^{2+}	5.15	14.06
Mg^{2+}	3.20	5.03
Na^+	8.46	12.06
K^+	0.69	1.15
Cl^{-1}	7.00	10.00
HCO_3^-	2.04	2.90
CO_3^{2-}	0.00	0.00
SO_4^{2-}	8.46	19.40
	Particles size distribution	
Coarse sand %	2.27	16.22
Fine sand %	24.81	39.76
Silt %	22.27	25.95
Clay %	50.65	18.07
Textural class	**Clay**	**Sandy clay loam**
Saturation percent %	70	45

*Soil paste extract
Extracts being KCl (for N); $NaHCO_3$ (for P); NH_4- acetate, pH 7.0 (for K), DTPA (for others)

TABLE 2 . Some characteristics of the chicken manure used in the current study.

Characteristic	Value
Moisture %	20.60
Total C %	27.35
Total N %	1.31
C/N ratio	20.9 : 1
Organic matter %	47.16
Total P %	0.57
Total K %	0.93
pH (1: 5) *	6.87
EC (1: 5) dSm^{-1}*	8.16

- Water suspension (pH) and water extract (EC).

Analytical procedures

Soil analysis

- Particle size distribution of the soil samples was conducted according to pipette method described by Piper (1950).
- Soil pH, soil calcium carbonate, electrical conductivity, organic matter content by Walkley & Black method, were all done as described by Page *et al.* (1982).
- Cation exchange capacity was determined using the method described by Chapman & Pratt (1961).
- Available N (KCl- extract), P (Na- bicarbonate extract) and K (NH_4- acetate extract, pH 7.0) were determined using the methods outlined by Jackson (1973).
- Available Fe, Mn, Cu and Zn were extracted by 0.005 M DTPA according to Lindsay & Norvell (1978) and determined using atomic absorption spectrophotometry.
- Soil moisture contents at water holding capacity and wilting point were determined according to the methods of Veihmeyer & Hendrickson (1949).

Organic manure analysis

- EC, pH, organic matter and total P and K were determined using the aforementioned methods; total N was done using the Kjeldahl method described by Jackson (1973).

Plant analysis

- Samples of dry plant material were wet digested using a mixture of concentrated (H_2SO_4 + $HClO_4$) acids according to Jackson (1973) and digests were analyzed for total nitrogen using the microkjeldahl method described by Jackson (1973).

N^{15} analysis

- The Dumas day combustion method *(Fiedler & Proksch,* 1975*)* was used to convert the nitrogen compounds in the dry samples into nitrogen gas. In this method, all the organic or the inorganic nitrogen compounds are converted in one step to N_2 gas as follow:

$$\text{Dry sample} = \frac{CuO + CaO}{550°} \longrightarrow \text{N- gas}$$

The reaction was carried out on dry material of 550 C° for 6 hours, in a closed nitrogen free atmosphere (dicharge) pyrex tuber, using copper oxide (CuO) as an oxidizing agent and calcium oxide (CaO) to absorb water and gases like CO_2. When the reaction was completed and the system reached room temperature the N^{15} / N^{14} ratio was determined by emission spectrometry N^{15} analyzer (Model NoI – 6 PC) following the description of IAEA (2001).

Nitrogen derived from fertilizer (Ndff), nitrogen derived from soil (Ndfs) nitrogen derived from air (Ndfa) and percentage of fertilizer N recovery (%FNR) were calculated according to *Hardarson & Danso* (1990) as follows:

$$\%\ Ndff = \frac{\%\ ^{15}N \text{ atom excess (plant)}}{\%\ ^{15}N \text{ atom excess (fertilizers)}} \times 100$$

$$\%\ Ndfs\ (unin) = 100 - \%\ Ndff$$

$$\%\ Ndfs\ (in) = 100 - (\%Ndff + \%\ Ndfa)$$

$$Nydff = \%\ Ndff \times \text{Total N uptake}$$

$$\%\ FNR = \frac{Nydff}{\text{applied N (mg N pot}^{-1})} \times 100$$

$$A\ soil = \frac{100 - \%\ Ndff\ (unin)}{\%\ Ndff\ (unin)} \times \text{rate of N}$$

$$A\ soil + air = \frac{100 - \%\ Ndff\ (in)}{\%\ Ndff\ (in)} \times \text{rate of N}$$

$$A\ air = A\ (soil + air) - A\ soil$$

$$\%\ Ndfa = A\ air \times \frac{\%\ Ndff\ (in)}{\text{Rate of applied N}}$$

where

% Ndff = Nitrogen derived from fertilizer, is the amount of Ndff / total N-uptake expressed as %.

% Ndfs = Nitrogen derived from soil, is the amount of Ndfs / total N-uptake expressed as %.

% Ndfa = Nitrogen derived from air, is the amount of Ndfa / total N-uptake expressed as %.

Nydff = Nitrogen derived from fertilizer expressed as absolute amount (mg pot^{-1}).

% FNR= Fertilizer nitrogen recover, is the amount of Ndff (mg pot^{-1}) / fertilizer N^{15} added to soil (mg pot^{-1}) X 100.

unin = uninoculated treatments in = inoculated treatments.

Statistical analysis

Analysis of variance for the obtained data was carried out and significant differences among the means of various treatment were distinguished by the LSD at 0.05 level according to Snedecor & Cochran (1980).

Results and Discussion

Effect of form and rate of applied N and the inoculation treatments on dry matter yield of maize plant

Data presented in Tables 3 and 4 reveal that on average dry weight of the maize plants grown on both soils and inoculated or uninoculated treatments increased significantly due to application of N and the increase was progressive with the rate whether in its organic form (CM), or the mineral one (AS) or in the mixture of both. Similar results were reported by Heggi *et al.* (1993), Ibrahim (1997) and Gopal *et al.* (2000).

TABLE 3. Dry matter yield of maize plants (g pot^{-1}) grown on the alluvial soil as affected by biofertilization and different rates of applied N.

N- treatment rate mg kg^{-1} and source (A)	Inoculation treatments				*(B)*
	Uninoculated	Azt.	Azs.	*Azt. + Azs.*	*Mean*
0 mg N	6.15	6.51	6.36	6.66	6.42
15 mg N (CM)	8.85	10.23	9.64	10.98	9.93
15 mg N (AS*)	9.57	11.50	10.72	12.15	10.98
30 mg N (CM/AS)	11.97	13.68	13.05	14.38	13.27
60 mg N (CM/AS)	13.10	13.46	13.31	13.62	13.37
90 mg N (CM/AS)	14.98	15.04	14.98	15.20	15.05
Mean	10.77	11.73	11.34	12.16	

LSD	A	B	AB
(0.05)	0.33	0.27	0.66

Azt. = *Azotobacter chroococcum* Azs. = *Azospirillum brasilense.*
*AS = Ammonium sulfate (labelled with N^{15}) CM = Chicken manure.

Inoculation of maize grains enhanced the effect of increasing rate of N on dry weight of maize plants grown on both soils, as shown by the main effect. Inoculation gave positive response, and was higher with *Azotobacter chroeoccum* (Azt) than with *Azospirillum brasilense* (Azs). Moreover, the combined inoculation with both *Azotobacter chroeoccum* (Azt) and *Azospirillum brasilense* (Azs) resulted in the highest response. However, there were interactions between inoculation and N- treatment. Inoculation affected the response to N, and N-treatment affected the response to inoculation. (1) Regarding the response to N, difference between 30 and 60 mg N kg^{-1} particularly in the alluvial soil was significant under no inoculation. With inoculation, the two rates were rather similar. (2) Regarding the response to inoculation, the interaction was as follows; inoculation gave no significant effect whether in absence of applied N, or where N was given at the high rates of 60 or 90 mg N kg^{-1}. On the other hand, inoculation gave positive response only in presence of N at 15 to 30 mg N kg^{-1}, (added as AS or (AS+CM). Where it was applied at the high rates of 60 to 90 mg N kg^{-1}, inoculation, gave no significant response. Such interactions indicate that in order to obtain an economic use of N, its application could be done along with inoculation. Also, in order to obtain a positive effect from inoculation, applied N should not be excessive. Excessive application of N, especially where chicken manure is present seems to have nullified the effect of inoculation. These findings stand in well agreement with those of Yadav *et al.* (1992); Galal (1993); Radwan & El-Nimr (1996); Salem (2000); Surendra *et al.* (2002); Wu *et al.* (2005) and El Zemrany *et al.* (2006).

TABLE 4. Dry matter yield of maize plants (g pot^{-1}) grown on the calcareous soil as affected by biofertilization and different rates of applied N .

N- treatment rate mg kg^{-1} and source (A)	Inoculation treatments				(B)
	Uninoculated	Azt.	Azs.	Azt.+ Azs.	Mean
0 mg N	4.22	4.46	4.34	4.55	4.39
15 mg N (CM)	6.06	7.64	6.98	8.25	7.23
15 mg N (AS*)	6.34	8.40	7.74	9.10	7.89
30 mg N (CM/AS)	8.24	10.36	9.98	10.72	9.83
60 mg N (CM/AS)	9.19	9.41	9.31	9.53	9.36
90 mg N (CM/AS)	10.42	10.54	10.48	10.59	10.51
Mean	7.41	8.47	8.14	8.79	

LSD	A	B	AB
(0.05)	0.35	0.28	0.70

Data in Tables 3 and 4 show also that dry weight increment percentages attained due to inoculation as compared with the dry weight of the uninoculated treatments seemed highest upon application of *Azospirillum brasilense* (Azs) in soil receiving 15 mg N kg^{-1} and lowest at the highest rate of the applied N-fertilizers (90 mg N kg^{-1}). This occurred in both the alluvial and calcareous soils, meanwhile ammonium sulfate (AS) at a rate of 15 mg N kg^{-1} produced higher increment percentage of maize dry weight than the same rate of applied nitrogen in the form of chicken manure (CM). Maize plants grown on the calcareous soil showed higher response to the increase in rate of the applied nitrogen and the inoculation treatments than the alluvial soil. Such a finding may be a final product of the low natural fertility of the calcareous soil and its lower content of total and available nitrogen than the alluvial one.

Effect of form and rate of applied N and inoculation treatments on nitrogen uptake by maize

Tables 5 and 6 demonstrate that on average (*i.e.*, main effect) applying N and increasing its rate, increased significantly N- uptake by maize plants grown on both soils. The N- uptake values became higher when the fertilization treatments were associated with inoculation. The N- uptake values seemed higher in the alluvial soil than the calcareous one. The main effect shows that inoculation with *Azotobacter chroeoccum* (Azt) was of more pronounced effect on increasing N- uptake in both the alluvial and calcareous soils. Combined inoculation with (Azt + Azs) was of the most positive effect. These results are similar to those obtained by Galal (1993) who stated that combination of ammonium sulfate (AS) with either *Azotobacter chroeoccum* (Azt) or *Azospirillum brasilense* (Azs) produced more values of N- uptake than with chemical fertilizers only. The lower values of N- uptake achieved by the plants grown on the calcareous soil as compared with those taken up by the plants grown on the alluvial soil might be attributed to the lower fertility and consequently the lower nitrogen content of the calcareous soil than the alluvial one. Moreover, loss of nitrogen by volatilization as ammonia NH_3 in the calcareous soil may have occurred. As the case with response of maize yield, there were significant interactions concerning response of N uptake.

(1) Regarding response to N- treatment, it was significant only in the calcareous soil. Increasing the rate of N from 30 to 60 mg N kg^{-1} showed a positive effect only where no inoculation was done. Under conditions of inoculation, the increase from 30 to 60 mg N kg^{-1} caused a decrease which was particularly significant under inoculation with (Azt + Azs). (2) Regarding response to inoculation, in both soils inoculation was of no significant positive response under conditions of no N application; it was significant only where N was applied. Besides, *Azotobacter* surpassed *Azospirillum* only where N was applied at the lowest rate.

TABLE 5. N uptake (mg pot^{-1}) by maize plants grown on the alluvial soil as affected by biofertilization and different rates of applied N.

N- treatment rate mg kg^{-1} and source (A)	Inoculation treatments				(B)
	Uninoculated	Azt.	Azs.	Azt. + Azs.	Mean
0 mg N	60.3	76.9	74.3	83.3	73.7
15 mg N (CM)	117.6	164.3	149.7	186.7	154.6
15 mg N (AS*)	136.1	187.1	168.8	215.3	176.8
30 mg N (CM/AS)	208.0	293.6	279.1	326.1	276.7
60 mg N (CM/AS)	273.9	300.5	290.7	309.1	292.1
90 mg N (CM/AS)	349.4	367.3	364.7	375.4	364.1
Mean	190.9	231.6	220.2	249.3	

LSD	A	B	AB
(0.05)	7.34	6.00	14.69

TABLE 6. N uptake (mg pot^{-1}) by maize plants grown on the calcareous soil as affected by biofertilization and different rates of applied N.

N- treatment rate mg kg^{-1} and source (A)	Inoculation treatments				(B)
	Uninoculated	Azt.	Azs.	Azt. + Azs.	Mean
0 mg N	43.0	51.7	48.8	54.6	49.5
15 mg N (CM)	75.5	112.5	101.3	125.7	103.7
15 mg N (AS*)	79.0	125.4	113.1	141.6	117.0
30 mg N (CM/AS)	135.5	216.7	201.2	227.9	195.3
60 mg N (CM/AS)	180.1	203.1	195.7	208.4	196.8
90 mg N (CM/AS)	232.3	244.7	242.0	251.9	242.7
Mean	125.7	159.0	150.4	168.4	

LSD	A	B	AB
(0.05)	7.83	6.39	15.66

It seems that heavy doses of nitrogen fertilizers may have inhibited N_2- fixing activities. Galal *et al.* (2000) and Rout *et al.* (2001) reported that the positive response to biofertilizer decreased by increasing rate of the applied nitrogen. They attributed the effect of the N_2- fixing bacteria to their production of growth promotion substances. They added that free- living bacteria change root morphology, *i.e.*, increase root growth, hence enhance nutrient uptake.

Effect of form and rate of applied N and inoculation on persentage of fertilizer nitrogen recovery (% FNR)

Data presented in Tables 7 and 8 show that values of the percentage of fertilizer nitrogen recovery (%FNR) significantly increased due to increasing rate of the applied nitrogen fertilizers regardless of its source. Values for the alluvial soil seemed higher than in the calcareous one. Values seemed to be higher for the inoculated plants than the uninoculated ones. Moreover, inoculation with *Azotobacter chroeoccum* (Azt) resulted in values higher than with inoculation with *Azospirillum brasilense* (Azs). Inoculation with *Azotobacter chroeoccum* (Azt) + *Azospirillum brasilense* (Azs) gave the most pronounced effect except where N was applied at its highest rate when all inoculated treatments gave rather similar response. These results agree with those of El-Akel (1997) and Galal *et al.* (2000) who found that inoculation of wheat with Azospirillum increased the efficient use of both nitrogen and phosphorus. Galal (2003) reported that the maximum values of total nitrogen recovery were attained with dual inoculation of diazotrophs.

TABLE 7. Percent of fertilizer N recovery (% FNR) by maize plants grown on the alluvial soil as affected by biofertilization and different rates of applied N.

N- treatment rate mg kg^{-1} and source (A)	Inoculation treatments				(B)
	Uninoculated	Azt.	Azs.	Azt. +Azs.	Mean
15 mg N (AS*)	26.7	30.7	28.3	34.5	30.1
30 mg N (CM/AS)	39.6	46.1	45.1	49.3	45.0
60 mg N (CM/AS)	53.0	56.1	55.0	57.0	55.3
90 mg N (CM/AS)	60.8	63.0	62.9	64.2	62.7
Mean	45.1	49.0	47.8	51.3	

LSD	A	B	AB
(0.05)	0.96	0.96	1.66

TABLE 8. Percent of fertilizer N recovery (% FNR) by maize plants grown on the calcareous soil as affected by biofertilization and different rates of applied N.

N- treatment rate mg kg^{-1} and source (A)	Inoculation treatments				(B)
	Uninoculated	Azt.	Azs.	Azt. + Azs.	Mean
15 mg N (AS*)	15.1	19.3	17.9	21.5	18.5
30 mg N (CM/AS)	22.2	30.2	28.5	31.2	28.0
60 mg N (CM/AS)	32.1	34.7	33.8	35.2	34.0
90 mg N (CM/AS)	39.3	40.8	40.6	41.7	40.6
Mean	27.2	31.2	30.2	32.4	

LSD	A	B	AB
(0.05)	1.23	1.23	2.45

Effect of inoculation on contribution percentages of air, soil and fertilizer to nitrogen content of maize plants

Data presented in Tables 9 and 10 show that values of Ndff (where the source of fertilizer N was totally AS at 15 mg N kg^{-1} with no inoculation) were11.7% and 10.35% in the plants grown on the alluvial and calcareous soils, respectively. Little change occurred when the rate was 30 mg N kg^{-1} where the source was partly AS and partly CM at overall rate of 30 mg N kg^{-1}. Increasing rate of applied nitrogen fertilizer was accompanied by increases in Ndff % in both soils. Inoculation with *Azotobacter chroeoccum* (Azt), *Azospirillum brasilense* (Azs) or both together slightly decreased Ndff %.

Inoculation reduced Ndfs. Increasing rate of the applied nitrogen, generally decreased Ndfs under all the inoculation treatments and in both soils. Results indicate that the reduction in Ndfs was more obvious upon inoculation with *Azotobacter chroeoccum* (Azt) than with *Azospirillum brasilense* (Azs) whereas the inoculation with {*Azotobacter chroeoccum* (Azt) + *Azospirillum brasilense* (Azs)} produced the lowest values of Ndfs.

Regarding inoculation treatments on contribution percentages of nitrogen derived from air, data show that inoculation with *Azotobacter chroeoccum* (Azt) was more effective in increasing Ndfa % than inoculation with *Azospirillum brasilense* (Azs). Inoculation with {*Azotobacter chroeoccum* (Azt) + *Azospirillum brasilense* (Azs)} seemed to be of the highest effect on Ndfa at all rates of applied nitrogen in both soils. Values of Ndfa increased slightly by increasing rate of the applied nitrogen from 15 to 30 mg N kg^{-1}. Increasing rate of the applied nitrogen beyond 30 mg N kg^{-1} decreased Ndfa in both soils. The high level of the applied inorganic nitrogen might have inhibited nitrogenase activity and hence reduced both N_2- fixing capacity and Ndfa. These results agree with Roger & Watanabe (1986); Omar (1995); Antoun (1998); El- Komy *et al.* (1993); Galal *et al.* (2000) and Galal (2003) who reported that, inoculation with (*Rhizobium* + *Azospirillum* microorganisms) gave higher values of Ndfa as compared with individual inoculation with *Rhizobium or Azospirillum,* alone.

TABLE 9. **Percentage of nitrogen drived from fertilizer (Ndff)& soil (Ndfs) and air (Ndfa) by maize plant grown on the alluvial soil as affected by biofertilization and different rates of applied N.**

N-treatment rate mg kg^{-1} and source	Inoculation treatments										
	Uninoculated		Azt.			Azs.			Azt. + Azs.		
	Ndff	Ndfs	Ndff	Ndfs	Ndfa	Ndff	Ndfs	Ndfa	Ndff	Ndfs	Ndfa
15 mg N(AS*)	11.78	88.22	9.87	73.91	16.22	10.06	75.46	14.48	9.61	72.10	18.29
30 mg N (CM/AS)	11.45	88.55	9.53	73.09	17.38	9.69	75.15	15.16	9.07	70.31	20.62
60 mg N (CM/AS)	23.23	76.77	22.43	74.15	3.42	22.71	75.10	2.19	22.15	73.19	4.66
90 mg N (CM/AS)	31.34	68.66	30.90	67.72	1.38	31.04	68.02	0.94	30.77	67.43	1.80

TABLE 10. **Percentage of nitrogen drived from fertilizer (Ndff)& soil (Ndfs) and air (Ndfa) by maize plant grown on the calcareous soil as affected by biofertilization and different rates of applied N.**

N-treatment rate mg kg^{-1} and source	Inoculation treatments										
	Uninoculated		Azt.			Azs.			Azt. + Azs.		
	Ndff	Ndfs	Ndff	Ndfs	Ndfa	Ndff	Ndfs	Ndfa	Ndff	Ndfs	Ndfa
15 mg N(AS*)	10.35	89.65	9.25	80.04	10.71	9.46	82.07	8.47	9.11	78.99	11.90
30 mg N (CM/AS)	9.85	90.15	8.36	76.54	15.10	8.50	77.80	13.70	8.12	75.22	16.66
60 mg N (CM/AS)	21.44	78.56	20.49	75.10	4.41	20.75	76.05	3.20	20.25	74.23	5.52
90 mg N (CM/AS)	30.56	69.44	29.99	68.15	1.86	30.21	68.66	1.13	29.82	67.75	2.43

Results reveal that values of Ndfa in maize plants grown on the alluvial soil were, generally, much higher as compared with the corresponding ones of the maize plants grown on the calcareous soil, probably due to higher fertility of the alluvial soil.

The aforementioned results reveal that organic fertilization could substitute the mineral nitrogen fertilizer. Also inoculation with N_2- fixing bacteria especially where available N is in moderate amounts (such as applying a low rate of N) could increase the contribution of the atmospheric air to the nitrogen taken up by the plant. This approach may assist low income farmers in increasing plant production with less fertilizer nitrogen and at the same time reduce the potential hazardous contamination of surface and ground water.

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(*Received* 8/2006;
accepted 10/2006)

التسميد العضوى والحيوى كبدائل إقتصادية وآمنة للتسميد النيتروجينى المعدنى

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يهدف هذا البحث إلى إلقاء بعض الضوء على إمكانية الإحلال الجزئى للتسميد النيتروجينى المعدنى للمحاصيل بالتسميد العضوى والحيوى وإلى أى مدى يمكن أن يحدث هذا الإحلال وتأثيره على كفاءة إستخدام النيتروجين بواسطة نبات الذرة النامى على أرض رسوبية من مشتهر و آخرى جيرية من النوبارية، بالإضافة إلى تقليل التلوث الذى قد يحدث للماء الأرضى بالنترات. لتحقيق هذه الأهداف أجريت تجربة أصص عاملية فى قطاعات عشوائية كاملة فى ثلاث مكررات. تراوحت معدلات النيتروجين لنبات الذرة النامى فى أصص من صفر وحتى ٩٠ كجم نيتروجين للفدان فى توافقات مختلفة بين سماد النيتروجين المعدنى (كبريتات الأمونيوم المرقمة بالـ N15) وسماد مخلفات الدواجن. تم تعبئة الأصص بـ ٥ كجم تربة وتم إضافة معاملات السماد النيتروجينى كما سبق وتم إضافة الفوسفور فى صورة صخر فوسفاتى بمعدل ١٠٠ مللجم / كجم والبوتاسيوم فى صورة كبريتات البوتاسيوم بمعدل ٥٠ مللجم / كجم وأضيفت العناصر الصغرى فى صورة محلول مغذى (محلول هوجلاند). تم زراعة نباتات الذرة الغير ملقحة بالبكتريا والملقحة بالبكتريا المثبتة لنيتروجين الهواء الجوى بإستخدام الأزوتوباكتر *Azotobacter chroococcum* (Azt) والأزوسبيرلم (Azs) *Azospirillum brasilenseense* أو خليط من الميكروبين ونمت النباتات لمدة ٦٠ يوم.

أوضحت النتائج أن إضافة السماد النيتروجينى فى صورة معدنية أو عضوية أو خليط من الصورتين أدى إلى زيادة قيم الوزن الجاف والممتص من النيتروجين مقارنة بمعاملة الكنترول (بدون تسميد)، وإزدادت القيم السابقة نتيجة للتلقيح بواسطة الميكروبات المثبتة لنيتروجين الهواء الجوى بالمقارنة بالغير الملقحة وكان التلقيح المزدوج بخليط من السلالتين أكثر كفاءة من التلقيح في صورة فردية وبالتالى إزدادت القيم المعبرة عن كفاءة استخدام النيتروجين وكانت أعلى القيم نتيجة للتلقيح المزدوج بخليط من السلالتين مقارنة بالتلقيح في صورة فردية، وأوضحت النتائج أيضاً أن التلقيح بالأزوتوباكتر (Azt) كان أكثر فاعلية من التلقيح بالأزوسبيرلم (Azs)، وتوضح الدراسة أن نشاط البكتريا المثبتة لنيتروجين الهواء الجوى كان أكثر كفاءة وتأثيراً عند المعدلات المنخفضة من التسميد النيتروجيني (٣٠ كجم نيتروجين/فدان) وينخفض هذا النشاط بزيادة المعدلات عن ذلك، وأظهرت معاملات الأرض الرسوبية قيما أعلى من الوزن الجاف وكذلك النيتروجين الممتص مقارنة بمعاملات الأرض الجيرية. هذا وتؤكد هذه الدراسة على أهمية استخدام التسميد العضوى والحيوي مع المعدلات المنخفضة من التسميد النيتروجينى والتى قد تصل إلى ٣٠ كجم نيتروجين/فدان لنباتات الذرة النامية على أرض التجربة مما يؤدى إلى زيادة نمو النبات وكذلك امتصاصه للعناصر الغذائية بالمعدلات الملائمة لنموه وهذا ينعكس بدوره على مساعدة نوى الدخول المحدودة من الفلاحين فى زيادة إنتاج هذا المحصول مع أقل كمية من السماد النيتروجينى المعدنى فى وجود السماد العضوى وكذا يساهم فى تقليل التلوث الذى قد يحدث للماء الأرضى من الأسمدة المعدنية مما يساعد على وجود بيئة مأمونة زراعياً.