

GROWTH AND YIELD OF MAIZE PLANTS AS AFFECTED BY AZOSPIRILLUM INOCULATION IN PRESENCE OF DIFFERENT NITROGEN SOURCES.

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ABSTRACT

Two field experiments were carried out in the Agricultural Research and Experimentation Center of Fac. Agric., Moshtohor during 1999 and 2000 seasons to study the effect of organic manures (biogas manure or town refuse compost) amendment either alone or in combination with *Azospirillum* inoculum or ammonium sulphate on the growth and yield of maize plants.

Results of this study showed that, highest densities of azotobacters, azospirilla in maize rhizosphere were observed in the treatment of inoculation with *A. lipoferum* and supplementation with the half dose of inorganic N-fertilizer (45 kg N /fed). While, the highest densities of phosphate dissolvers were observed in the treatment of inoculation with *A. lipoferum* in combination with biogas manure. The highest records of CO₂ evolution were observed in the treatment of soil amendment with full dose of biogas manure, 5.555 ton/fed (90 kg N /fed).

Maize grains inoculated with *A. lipoferum* and provided with the half dose of inorganic nitrogen fertilizer showed the highest records of N₂-ase activity, ammoniacal and nitrate nitrogen as well as available phosphorus. Application of full dose of biogas manure gave higher values of NH₄-N and NO₃-N as well as available phosphorus than the application of full dose of town refuse compost (6.338 ton/fed). The highest records of growth performance i.e growth characters, ear characteristics, straw and grain yields of maize were obtained in the treatment of maize grains inoculated with *A. lipoferum* and supplemented with half dose of inorganic nitrogen fertilizer. Generally, organic manure application either solely (full dose) or in combination with *Azospirillum* inoculation or inorganic N-fertilizer (half dose) gave higher records of growth performance of maize plants than the application of full dose of inorganic N-fertilizer alone. Therefore, maize grains inoculation with asymbiotic N₂-fixing bacteria at sowing and application of half the dose of either inorganic or organic N-fertilizers may be recommended as an alternative for inorganic N-fertilizers application alone to minimize the environmental pollution resulted from the excessive use of chemical fertilizers.

Key words: Maize, *Azospirillum lipoferum*, biogas manure, town refuse compost, inorganic N-fertilizer.

INTRODUCTION

One of the major problems of Egyptian soils is their deficiency in organic matter content, not exceeding from 1-2% in all cultivated soils (Abdel-Malek *et al*, 1961). Therefore, the application of organic manures seems to be of a great value for improving biological, chemical and physical properties of soils, thus improving their productivity (Tester, 1990; More, 1994 and Peoples *et al*, 1995). Nevertheless, organic matter application to different soils is essential for successful management through the following:

- 1- increasing both soil fertility and productivity as a result of improving soil properties.
- 2- Minimizing chemical fertilizer application.
- 3- Reducing environmental pollution.

Manuring the soil with either sewage sludge or town refuse compost increased the dry matter yield, grain yield and uptake of NPK by sorghum plants. (Soltan *et al*, 1996). A significant interaction on barley growth was obtained with the combination of compost and inorganic N-fertilizers. This combination was much better in increasing growth characters and barley yield compared with either compost or inorganic N-fertilizer applied alone (Hountin *et al*, 1995). Gagnon *et al* (1997) found that application of compost in combination with inorganic N-fertilizer significantly increased growth characters, grain yield and N uptake of wheat plants. Grain and straw yields of sorghum were increased with the application of town refuse and sewage sludge compost (Mahmoud, 2000). Many earlier investigators reported that high levels of N-fertilizers may be a limiting factor for N₂-fixation process and exhibited a negative effect on N₂-ase activity in various ecosystems (Neyra and Dobreiner, 1977 and Vlassak and Reynders, 1980). Biofertilizers either solely or in combination with certain N-inorganic additives proved to be an efficient tool in increasing available nutrients in soil as well as crop yield. Several investigators showed that inoculation with azospirilla improved growth and yield of maize crop due to their N₂-fixation activity and production of growth promoting substances (Fulchieri and Frioni, 1994; Raso, 1996 and Hamdi and El- Komy, 1998). Moreover, El-Demerdash (1994) reported that maize grains inoculation with associative N₂-fixers resulted in improving the growth of maize plants and consequently gave high grain and straw yields. He also reported that such application can save 50% of the cost of inorganic nitrogen fertilizers.

So, the objective of the present study is to evaluate the effect of organic fertilization (biogas manure or town refuse compost) either alone or in combination with *Azospirillum* inoculation or inorganic N-fertilizer on growth performance and yield of maize plants.

MATERIAL AND METHODS

Two field experiments were carried out in the Agricultural Research and Experimentation Center of Fac. Agric. Moshtohor, Zagazig Univ., during 1999 and 2000 seasons to study the effect of biogas manure and town refuse compost

application either alone or in combination with *Azospirillum lipoferum* inoculation or inorganic N-fertilizer (ammonium sulphate 20.5%N) on growth performance, yield and yield components of maize (*Zea mays* c.v. Taba).

Mechanical and chemical analyses of the experimental soil are presented in Table (1). Also, the chemical analysis of the used organic manures are shown in Table (2).

Mechanical analysis was estimated according to Jackson (1973). Whereas, chemical analysis of soil and organic manures was estimated according to Black *et al*, (1982).

Biogas manure and town refuse compost were added before sowing at a rate of 90 kg N / fed., also, the inorganic nitrogen fertilizer (NH₄)₂SO₄ was added at a rate of 90 kg N / fed. to represent the full dose of nitrogen in three equal doses i.e. at sowing, after 30 and 60 days of sowing. All plots were supplemented with calcium superphosphate (15.5 % P₂O₅) at a rate of 30 kg P₂O₅ / fed.

Inoculum preparation and inoculation process

Azospirillum lipoferum Mn3 strain was provided from the Unit of Biofertilizers, Fac. of Agric., Ain Shams Univ., Cairo, Egypt.

Table (1): Physical and chemical properties of experimental soil before cultivation in 1999 and 2000 seasons.

Seasons	1999	2000
A. Particle size distribution		
Coarse sand (%)	2.83	2.48
Fine sand (%)	17.62	16.58
Silt (%)	23.10	23.50
Clay (%)	56.45	57.44
Textural class	Clayey	Clayey
B. Chemical analysis		
Organic matter (%)	1.78	1.86
PH	8.21	8.05
Total nitrogen (%)	0.36	0.48
Total phosphorus (%)	0.19	0.22
CaCO ₃ (%)	0.42	0.56
Cations (meq/l.)		
Na ⁺	0.26	0.30
K ⁺	0.62	0.64
Ca ⁺⁺	3.2	3.41
Mg ⁺⁺	2.4	2.45
Anions (meq/l.)		
Cl ⁻	0.31	0.33
SO ₄ ⁼	3.21	3.41
CO ₃ ⁼	0.35	0.40
HCO ₃ ⁻	2.61	2.66

Table (2): Chemical analysis of organic manures in 1999 and 2000 seasons.

Parameters	Unit	Type of organic manure			
		Biogas manure		Town refuse compost	
		1999	2000	1999	2000
Organic matter	%	56.82	57.12	24.86	26.01
Organic carbon	%	32.95	33.13	14.41	15.08
Total nitrogen	%	1.62	1.69	1.42	1.51
Total phosphorus	%	0.76	0.70	0.68	0.56
Total potassium	%	1.12	1.20	1.31	1.23
C: N Ratio	%	20.33	19.60	10.14	9.99
Iron	ppm	32.0	36.5	23.5	26.1
Zinc	ppm	26.1	30.0	18.8	16.8
Copper	ppm	12.4	18.2	13.3	11.3
Manganese	ppm	9.3	11.6	6.8	7.2

For preparation of *A. lipoferum* inoculum, semi-solid malate medium (Dobereiner, 1978) was inoculated with *A. lipoferum*, then incubated at 32°C for 7 days. Maize grains were successfully washed with water and air-dried. Thereafter, grains were soaked in cell suspension of *A. lipoferum* (1ml contains about 8×10^7 viable cells) for 30 min. Gum arabic (16%) was added as an adhesive agent prior to inoculation. The inoculated grains were air dried for one hour before sowing.

A control was carried out, where the soil was left without fertilization and grains were treated by using uninoculated 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 phosphorus i.e. 90 kg N and 30 kg P₂O₅ / fed as ammonium sulphate and calcium superphosphate, respectively.

Experimental design

A randomized complete block design with four replicates was used. This experiment included the following treatments:

- 1- Control.
- 2- Fertilized control (90 kg N of ammonium sulphate + 30 kg P₂O₅ of superphosphate / fed.).
- 3- *Azospirillum* inoculum + a half dose of ammonium sulphate (45 kg N / fed.).
- 4- Full dose of biogas manure, 5.555ton/fed. (90 kg N / fed.).
- 5- Biogas manure (45 kg N / fed.) + *Azospirillum* inoculum.
- 6- Biogas manure (45 kg N / fed.) + ammonium sulphate (45 kg N / fed.).
- 7- Full dose of town refuse compost, 6.338 ton/fed. (90 kg N / fed.).
- 8- Town refuse compost (45 kg N / fed.) + *Azospirillum* inoculum.
- 9- Town refuse compost (45 kg N / fed.) + ammonium sulphate.

Cultivation process

Cultivation process 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. The area of each plot was 14 m² (4m x 3.5 m). After sowing,

soil was directly irrigated to provide suitable moisture for inoculum. The normal cultivation practices for growing maize were followed as recommended in the region.

Sampling and determinations

After 35 and 70 days of sowing, representative rhizosphere soil samples of the developed plants were taken. These periods are representing the vegetative and heading stages. The samples were microbiologically analyzed for CO₂ evolution, nitrogenase activity, densities of azotobacters, azospirilla and inorganic phosphate dissolvers. Also, rhizosphere soil samples were chemically analyzed for NH₄-N, NO₃-N and available phosphorus as follows:

1. Microbiological analysis

- 1.1. CO₂ evolved by soil microorganisms was estimated using the method described by Page *et al.* (1982).
- 1.2. Nitrogenase activity was estimated according to Hardy *et al.* (1973).
- 1.3. Densities of azotobacters and azospirilla were determined on modified Ashby's medium (Abdel-Malek and Ishac, 1968) and semi- solid malate medium (Dobereiner, 1978), respectively using the most probable number technique (Cochran, 1950). Whereas, the densities of inorganic phosphate dissolvers were determined on Bunt and Rovira medium (1955) modified by Abdel-Hafez (1966) using the plate count method.

2. Chemical analysis

- 2.1. Ammoniacal and nitrate nitrogen were determined according to Bremner and Keeny (1965).
- 2.2. Available phosphorus was extracted from soil according to Olsen *et al.* (1954) and colourimetrically determined according to American Public Health Association (APHA, 1992).

3. Growth parameters

After 70 days of sowing, plant height (cm), stem diameter (mm), number of leaves / plant and leaf area of topmost ear (cm²) were estimated.

4. Yield and its components

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

5. Chemical analysis of maize grains

Total nitrogen, phosphorus and potassium were estimated in grains according to mikrokjeldahl method (A.O.A.C., 1980), APHA (1992) and Dewis and Freitas (1970), respectively.

Crude protein was calculated according to the following equation.

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

Statistical analysis

Analysis of variance (ANOVA) of data obtained from growth parameters, ear characteristics, yield and yield components were 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, 1955).

RESULTS AND DISCUSSION

Effect of different soil applications on microbial densities in rhizosphere of maize plants.

Data in Table (3) show that the densities of azotobacters, azospirilla and phosphate dissolvers in rhizosphere of maize plants increased in all soil application treatments compared to control one. At vegetative stage, highest densities of azotobacters and azospirilla were observed in the treatment of maize grains inoculated with *A. lipoferum* and received half the dose of inorganic N-fertilizer. Whereas, the highest densities of P-dissolvers were observed in the treatment of maize grains inoculated with *A. lipoferum* that received half dose of nitrogen (hdn) of biogas manure. At heading stage, the highest densities of azotobacters, azospirilla and P-dissolvers were recorded in the treatments of *Azospirillum* inoculation + half dose of inorganic nitrogen, *Azospirillum* inoculation + biogas manure (hdn) and full dose of biogas manure, respectively. These results are in agreement with those obtained by Zaghloul *et al.* (1996) and Neweigy *et al.* (1997).

Irrespective of control, the lowest densities of azotobacters, azospirilla and P-dissolvers were observed in the treatment of full dose of inorganic N-fertilizer application. The same trend of results was observed in both growing seasons and growth stages.

Data recorded in Table (3) also show that the densities of azotobacters, azospirilla and P-dissolvers in rhizosphere of maize plants tended to increase progressively in all treatments. At heading stage, densities of abovementioned microbial groups were higher than those recorded in vegetative stage. Such differences may be due to the changes in multiplication rate of different soil microorganisms as a result of qualitative changes in nature of the root exudates during the different growth stages (Abdel-Ati *et al.*, 1996).

Also, increase of different microbial groups densities during heading stage is likely to be due to the beneficial effect of root exudates and debris during heading stage in most cultivated plants.

Generally, from data presented in Table (3) it can be concluded that the densities of azotobacters, azospirilla and P-dissolvers were higher in case of *Azospirillum* inoculation in combination with organic manures than its combination with inorganic N-fertilizer. Similar results were observed by several investigators (De-Freitas and Germida, 1990; Shatokhina and Khristenko, 1996

and Moharram *et al.*, 1998) who emphasized that, in addition to the beneficial effect of N₂-fixing bacteria associated with roots of cereal crops in providing them with nitrogen, these bacteria are also reported to produce growth promoting substances which help in increasing the proliferation of different soil microorganisms (Abdel-Jawad, 1998).

Effect of different soil applications on CO₂ evolution and N₂-ase activity.

It is obvious from data recorded in Table (4) that the evolved CO₂ (as an indication for activity of soil microorganisms) was remarkably increased in the treatments of organic manures application either alone or in combination with *A. lipoferum*. The highest values of evolved CO₂ were observed in the treatment of soil amendment with full dose of biogas manure. The same trend of results was observed in both 1999 and 2000 growing seasons as well as at vegetative and heading stages of maize growth. Rates of CO₂ evolution (activity of soil microorganisms) considerably increased with the increasing of growth period reaching their maximal values at heading stage. The same trend of results was obtained in all treatments. These results confirmed those obtained by Neweigy *et al.* (1997) and Moharram *et al.* (1998) who reported that soil amendment with organic manures increased CO₂ evolution rates in rhizosphere soil rather than inorganic N-fertilizers application.

As regard to N₂-ase activity, data presented in Table (4) show that maize grains inoculated with *A. lipoferum* and provided with a half dose of inorganic N-fertilizer gave the highest values of N₂-ase activity. This result was true in vegetative and heading stages of maize growth.

Also, it is worthy to notice that the application of organic manures (biogas manure or town refuse compost) in combination with *Azospirillum* inoculum showed higher records of N₂-ase activity than the application of organic manures in combination with ammonium sulphate. Irrespective of control, the lowest values of N₂-ase activity were observed in the treatment of full dose of ammonium sulphate application. These results confirmed those obtained by Vlassak and Reynders (1980) and EL- Demerdash (1994), who reported that the high levels of inorganic fertilizer may be a limiting factor for N₂-fixation process and exhibited a negative effect on N₂-ase activity in various ecosystems.

Data in Table (4) also show that N₂-ase activity values were higher at heading stage than those recorded at vegetative stage. This result can be attributed to the high densities of azotobacters and azospirilla which showed an increase in their counts at heading stage of maize growth (Table, 3).

Generally, CO₂ evolution rates and N₂-ase activity were higher in the 2nd season than in the 1st one and these differences between the two seasons are likely to be due to the difference in the climatic conditions.

Table (3): *Azotobacters, azospirilla* and inorganic phosphate dissolving bacteria (PDB) densities ($\times 10^4$ / g dry weight of soil) in *rhizosphere* of maize plants.

Growth stage Treatments	Vegetative stage						Heading stage					
	Azotobacters		Azospirilla		P.D.B.		Azotobacters		Azospirilla		P.D.B.	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Control	22.50	24.30	21.5	26.80	18.6	21.30	31.60	30.50	26.00	28.40	20.50	23.30
Fert. control (full dose of amm. sulphate)	52.40	58.60	38.6	43.60	44.1	52.60	84.00	90.60	56.20	68.30	60.50	72.20
<i>Azos. lipoferum</i> + amm. sulphate (hdn)	147.2	156.2	96.9	104.3	92.2	98.50	204.6	216.1	120.6	126.1	136.0	140.0
Full dose of biogas manure (BM)	90.60	96.30	60.4	68.00	84.1	76.30	162.0	171.2	100.6	110.3	186.0	184.1
BM(hdn) + <i>Azos. lipoferum</i>	117.2	129.6	86.2	92.30	94.0	104.1	186.3	191.0	148.0	146.6	160.3	168.2
BM (hdn) + amm. sulphate (hdn)	108.4	121.5	81.1	88.50	88.6	82.20	180.2	168.1	124.0	136.3	140.2	144.3
Full dose of town refuse compost (TRC)	66.40	73.60	52.6	56.40	80.1	72.00	120.3	144.5	68.80	72.30	142.3	136.3
TRC (hdn) + <i>Azos. lipoferum</i>	120.2	124.6	74.2	79.00	70.3	84.20	176.5	184.4	108.4	112.2	160.6	172.2
TRC (hdn) + amm. sulphate (hdn)	112.3	118.4	58.0	63.60	60.0	72.10	152.5	168.3	102.3	106.5	145.0	150.2

Fert., fertilized.

Full dose, (90 kg N/ fed.).

Half dose, (45 kg N/ fed.).

TRC, Town refuse compost.

hdn, half dose of nitrogen.

Azos, *Azospirillum*.

BM, Biogas manure.

Table (4): Carbon dioxide evolution and nitrogenase activity in rhizosphere of maize plants.

Growth stage Treatments	Vegetative stage				Heading stage			
	CO ₂ evolved (µg/g dry soil/hr.)		N ₂ -ase activity (n moles C ₂ H ₄ /g dry soil/hr.)		CO ₂ evolved (µg/g dry soil/hr.)		N ₂ -ase activity (n moles C ₂ H ₄ /g dry soil/hr.)	
	1999	2000	1999	2000	1999	2000	1999	2000
Control	11.50	16.20	8.810	12.31	20.61	23.30	14.00	18.00
Fert. control (full dose of amm. sulphate)	60.53	68.47	52.60	55.00	82.20	89.30	66.60	63.40
<i>Azospirillum lipoferum</i> + amm. sulphate (hdn)	80.42	86.36	105.4	116.6	115.6	131.4	130.8	138.2
Full dose of biogas manure (BM)	110.2	121.5	67.80	74.20	134.1	145.6	83.20	92.60
BM (hdn) + <i>Azospirillum lipoferum</i>	86.34	95.30	81.36	92.31	128.8	135.6	124.4	136.8
BM (hdn) + amm. sulphate (hdn)	65.60	68.50	42.80	48.00	104.5	110.1	60.80	76.86
Full dose of town refuse compost (TRC)	98.34	102.6	73.00	80.40	113.5	120.0	81.12	89.90
TRC (hdn) + <i>Azospirillum lipoferum</i>	74.46	104.8	86.10	96.60	125.5	143.1	104.0	117.0
TRC (hdn) + amm. sulphate (hdn)	63.62	81.39	45.20	57.10	118.9	131.4	68.80	72.20

Abbreviations: as those stated for Table (3).

Effect of different soil applications on nitrogen forms and available phosphorus in soil.

Data presented in Table (5) show that ammoniacal and nitrate nitrogen as well as available phosphorus were remarkably increased in the treatment of maize grains inoculated with *A. lipoferum* and provided with the half dose of inorganic N-fertilizer. The same trend of results was obtained in both growth stages and growing seasons of maize plants.

Taking the organic manure source into account, biogas manure application as full dose of nitrogen gave higher values of nitrogen forms (NH_4 and NO_3) as well as available phosphorus than the application of full dose of nitrogen from town refuse compost. It seems that the biogas manure is easily decomposable rather than town refuse compost. Nevertheless, the high records of available phosphorus which observed in biogas manure treatment can be explained by its high content of P-dissolvers densities especially at heading stage in comparison with town refuse compost (Table, 3).

Data in Table (5) also show that half dose of organic manures application in combination with *Azospirillum* inoculation gave higher records of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and available phosphorus compared to half dose of organic manures application in combination with ammonium sulphate (hdn). This was true in both maize growth stages and the two growing seasons.

Irrespective of control, the lowest records of $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and available phosphorus were observed in the treatment of full dose of ammonium sulphate. This result can be attributed to the lower densities of azotobacters, azospirilla and P-dissolvers which showed a decrease in their counts in the treatment of full dose of ammonium sulphate application (Table, 3).

Generally, from data recorded in Table (5) it is worthy to mention that $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and available phosphorus were remarkably increased at heading stage in comparison with vegetative one. Such differences is likely be due to the difference in multiplication rate of different soil microorganisms as a result of qualitative and quantitative changes in the nature of root exudates during different growth stages (De-Freitas and Germida, 1990 and Abdel-Ati *et al.*, 1996).

Effect of different soil applications on growth and yield of maize plants.

A. Growth characters of maize.

It is clear from data presented in Table (6) that maize growth characteristics i.e plant height, stem diameter, number of leaves/plant and leaf area of topmost ear significantly increased in the treatment of maize grains inoculated with *A. lipoferum* and supplemented with a half dose of inorganic nitrogen fertilizer. This may be due to the production of growth regulators such as auxins, gibberillins and cytokinins by azospirilla which positively affected the production of root biomass and nutrients uptake (Fulchieri and Frioni, 1994).

Table (6): Effect of organic manures application alone and in combination with either *A. lipoferum* or ammonium sulphate on growth parameters of maize plants.

Growth parameters Treatments	Plant height (cm)		Stem diameter (mm)		No. of leaves / plant		Leaf area of topmost ear (cm ²)	
	1999	2000	1999	2000	1999	2000	1999	2000
Control	166.3 ^d	171.3 ^d	19.67 ^b	20.00 ^c	10.67 ^c	10.33 ^c	436.0 ^b	424.0 ^d
Fert. control (full dose of amm. sulphate)	200.0 ^c	210.0 ^c	28.33 ^a	32.00 ^a	11.33 ^{bc}	12.33 ^{ab}	607.5 ^c	580.0 ^c
<i>Azos.lipoferum</i> + amm. sulphate (hdn)	251.7 ^a	256.7 ^a	33.33 ^a	34.67 ^a	13.33 ^a	13.67 ^a	659.2 ^a	696.3 ^a
Full dose of biogas manure (BM)	196.7 ^c	216.7 ^{bc}	29.30 ^a	27.00 ^b	12.33 ^{ab}	13.00 ^{ab}	620.0 ^b	646.3 ^b
BM (hdn) + <i>Azos.lipoferum</i>	216.0 ^c	236.7 ^{ab}	27.33 ^a	29.00 ^a	11.33 ^{bc}	13.33 ^a	655.0 ^a	684.0 ^{ab}
BM (hdn) + amm. sulphate (hdn)	206.7 ^c	226.7 ^{bc}	27.67 ^a	29.33 ^a	12.67 ^{ab}	12.67 ^{ab}	647.5 ^a	635.0 ^b
Full dose of town refuse compost (TRC)	195.0 ^c	210.0 ^c	30.33 ^a	25.67 ^b	12.67 ^{ab}	11.33 ^{bc}	618.0 ^b	636.7 ^b
TRC (hdn) + <i>Azos.lipoferum</i>	238.3 ^{ab}	255.0 ^a	30.67 ^a	32.67 ^a	13.33 ^a	12.67 ^{ab}	629.0 ^{ab}	683.7 ^{ab}
TRC (hdn) + amm. sulphate (hdn)	216.7 ^{bc}	226.7 ^{bc}	30.67 ^a	32.00 ^a	13.33 ^a	12.33 ^{ab}	630.0 ^{ab}	640.0 ^b

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 (3).

Taking the organic manure source into account, insignificant differences were observed in maize growth characteristics when full dose of nitrogen was amended from either biogas manure or town refuse compost. The same trend was observed in the two growing seasons. Except of stem diameter, the obtained results showed that application of organic manures in combination with either *A. lipoferum* inoculation or ammonium sulphate gave higher records of growth characteristics of maize than the application of full dose of nitrogen from the two organic manures under investigation. This observation was consistent in both growing seasons.

These results confirmed those obtained by Hountin *et al.* (1995) and Gagnon *et al.* (1997) who noticed a significant response of growth of barley and wheat with the combination of compost and inorganic N-fertilizer. Irrespective of control treatment, data in Table (6) clearly emphasize that the lowest records of growth performance were obtained when the full dose of ammonium sulphate was applied as a sole source of N-fertilizer.

B. Ear characteristics.

Data in Table (7) show that ear characteristics i.e. ear length, ear diameter, weight of ear grains, weight of ear cob and weight of 100-grains were significantly increased in inoculated treatments with *A. lipoferum* provided with a half dose of inorganic N-fertilizer. The same trend of results was observed in the two growing seasons. With respect to organic manure source, obtained results clearly show that ear characteristics of maize plants were significantly higher with the application of biogas manure (full dose) than the application of full dose of town refuse compost. Except of ear diameter, data in Table (7) indicate that the treatments of *Azospirillum* inoculation in combination with either biogas manure or town refuse compost gave higher records of ear characteristics than the combination of organic manure with ammonium sulphate using half the dose of both of them. The increase of growth performance and ear characteristics of maize plants due to inoculation with associative N_2 -fixer (*A. lipoferum*) may be due to the capability of this organism to fix nitrogen which could be taken by the growing plants (El-Demerdash, 1994; Raso, 1996 and Hamdi and El-Komy, 1998).

C. Yield of straw, ears, biological and grains .

It is obvious from data presented in Table (8) that the straw, ears and biological yield as well as grain yield of maize plants were significantly increased in the treatments inoculated with *A. lipoferum* compared to uninoculated ones.

The highest values of the abovementioned criteria were obtained in the treatment of maize grains inoculated with *A. lipoferum* and provided with half the dose of inorganic nitrogen fertilizer. The results of season 2000 are emphasized as those obtained from 1999. These results are in harmony with Fulchieri and Frioni (1994), Raso (1996), Badawy *et al.* (1997) and Moharram *et al.* (1998) who reported that azospirilla inoculated treatments scored the highest improvement in maize straw and grain yield when moderate inorganic N-fertilization rates were used.

Table (7): Effect of organic manures application alone and in combination with either *A. lipoferum* or ammonium sulphate on some ear characteristics and weight of 100 grains of maize plants

Characteristics Treatments	Ear length (cm)		Ear diameter (mm)		Weight of grains/ ear (g)		Weight of ear cob (g)		Weight of 100 grains (g)	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
	Control	15.13 ^d	15.60 ^d	3.86 ^d	3.76 ^c	152.6 ^d	156.3 ^d	41.63 ^e	43.00 ^f	30.10 ^d
Fert. control (full dose of amm. sulphate)	18.33 ^c	18.67 ^c	5.03 ^c	4.16 ^b	186.1 ^{abc}	175.0 ^{bc}	63.87 ^{bc}	61.67 ^d	37.37 ^{ab}	36.43 ^{bc}
<i>Azos. lipoferum</i> + amm. sulphate (hdn)	24.00 ^a	24.63 ^a	6.40 ^a	5.70 ^a	247.6 ^a	246.6 ^a	76.80 ^a	81.00 ^a	39.40 ^a	40.60 ^a
Full dose of biogas manure (BM)	22.60 ^{abc}	23.66 ^{ab}	5.60 ^{abc}	5.66 ^a	192.1 ^{abc}	187.4 ^{bc}	61.27 ^c	60.67 ^d	38.80 ^{ab}	39.00 ^{ab}
BM (hdn) + <i>Azos. lipoferum</i>	24.66 ^a	24.00 ^a	6.10 ^{ab}	5.13 ^a	193.2 ^{abc}	202.7 ^{abc}	70.43 ^b	74.00 ^b	38.87 ^{ab}	37.83 ^{ab}
BM (hdn) + amm. sulphate (hdn)	21.60 ^b	22.33 ^{abc}	6.10 ^{ab}	5.16 ^a	179.2 ^{bc}	172.6 ^c	52.47 ^d	52.57 ^e	32.40 ^c	33.17 ^d
Full dose of town refuse compost (TRC)	21.33 ^b	21.67 ^b	5.06 ^c	4.36 ^b	161.3 ^c	170.3 ^c	43.67 ^e	58.60 ^d	34.03 ^{bc}	33.97 ^{cd}
TRC (hdn) + <i>Azos. lipoferum</i>	23.16 ^{ab}	23.33 ^{ab}	5.40 ^a	5.66 ^a	236.8 ^{ab}	238.3 ^a	71.93 ^b	70.07 ^{bc}	39.07 ^a	38.43 ^{ab}
TRC (hdn) + amm. sulphate (hdn)	22.60 ^{abc}	23.00 ^{ab}	5.33 ^{bc}	5.53 ^a	224.7 ^{ab}	202.3 ^{abc}	66.50 ^{bc}	68.33 ^c	36.07 ^{abc}	37.27 ^{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 (3).

Table (8): Effect of organic manures application alone and in combination with either *A. lipoferum* or ammonium sulphate on straw, ear, biological and grain yield of maize plants.

Characteristics Treatments	Straw yield (ton/fed.)		Ear yield (ton/fed.)		Biological yield (ton/fed.)		Grain yield (ardab/fed.)	
	1999	2000	1999	2000	1999	2000	1999	2000
Control	3.16 ^d	3.21 ^d	2.26 ^d	2.63 ^d	5.42 ^d	5.84 ^d	8.610 ^d	8.840 ^f
Fert. control (full dose of amm. sulphate)	4.73 ^{bc}	4.60 ^c	3.16 ^{cd}	3.33 ^{cd}	7.89 ^c	7.93 ^c	16.20 ^c	15.49 ^{de}
<i>Azos. lipoferum</i>+ amm. sulphate (hdn)	7.46 ^a	7.83 ^a	6.53 ^a	5.80 ^a	13.99 ^a	13.63 ^a	22.97 ^a	22.07 ^a
Full dose of biogas manure (BM)	3.66 ^c	3.56 ^{cd}	3.33 ^{cd}	3.53 ^{cd}	6.99 ^c	7.09 ^c	16.44 ^c	15.52 ^{de}
BM (hdn) + <i>Azos. lipoferum</i>	6.53 ^{ab}	6.13 ^b	5.20 ^{ab}	5.26 ^{ab}	11.73 ^b	11.39 ^b	20.15 ^b	18.56 ^b
BM (hdn) + amm. sulphate (hdn)	4.26 ^{bc}	3.93 ^{cd}	3.46 ^c	3.90 ^c	7.72 ^c	7.83 ^c	16.80 ^c	17.42 ^{bc}
Full dose of town refuse compost (TRC)	3.83 ^c	3.86 ^{cd}	3.24 ^{cd}	3.93 ^c	7.07 ^c	7.79 ^c	15.12 ^c	14.41 ^e
TRC (hdn) + <i>Azos. lipoferum</i>	4.00 ^c	4.96 ^{bc}	4.66 ^{bc}	4.86 ^{ab}	8.96 ^{bc}	9.82 ^{bc}	17.64 ^{bc}	17.15 ^{bc}
TRC (hdn) + amm. sulphate (hdn)	4.20 ^c	4.16 ^{cd}	3.73 ^{bcd}	4.30 ^{bc}	7.93 ^c	8.46 ^{bc}	17.59 ^{bc}	16.52 ^{cd}

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 (3).

With regard to organic manures source, data in Table (8) indicate that biogas manure application (full dose) didn't show significant differences on the abovementioned criteria compared to the treatment of town refuse compost. Generally, *Azospirillum* inoculation in combination with either biogas manure or town refuse compost significantly increased maize yield compared to the application of full dose of nitrogen from either organic manures or ammonium sulphate.

In addition, obtained results clearly show that the combination of organic manures with either *Azospirillum* inoculation or ammonium sulphate gave higher straw, ears, biological and grain yields than the application of both organic and inorganic N-fertilizers alone. The same trend of results was observed in the two growing seasons.

Similar results were observed by (Hountin *et al.*, 1995; Gagnon *et al.*, 1997 and Moharram *et al.*, 1998) who found that the application of compost in combination with inorganic N-fertilizer significantly increased grain yield in barley, wheat and maize plants compared to the application of either compost or inorganic N-fertilizer alone. Also, Mahmoud (2000) found that grain and straw yields of sorghum considerably increased with the application of town refuse and sewage sludge compost.

From data presented in Table (8), it could be generally concluded that the straw, ears, biological and grain yields of maize plants were slightly differed in the two growing seasons. This difference between the two growing seasons may be due to the changes in the meteorological factors.

Effect of different soil applications on N, P, K and crude protein contents of maize grains.

Data recorded in Table (9) clearly show that the treatment of maize grains inoculated with *A. lipoferum* and provided with a half dose of inorganic nitrogen gave the highest values of N, P, K and crude protein of grains. These results are in accordance with the findings of Fulchieri and Frioni (1994), Hamdi and El-komy (1998) and Moharram *et al.* (1998) who found that maize grains inoculated with *A. lipoferum* gave higher values of nutrients uptake than uninoculated ones.

It is also clear that the nutrients content (N and P) of maize grains were slightly higher in the treatment amended with biogas manure than the treatment amended with town refuse compost whereas, town refuse compost application gave higher values of potassium content of maize grains compared to biogas manure application.

Maize plants inoculated with *A. lipoferum* and provided with a half dose of nitrogen from either biogas manure or town refuse compost gave higher values of N, P, K as well as crude protein content than those fertilized with either biogas manure or town refuse compost in combination with ammonium sulphate. In this concern, biofertilizers either solely or in combination with certain nitrogen

Table (9): Effect of organic manures application alone and in combination with either *A. lipoferum* or ammonium sulphate on N,P,K, and crude protein content of maize grains.

Parameters Treatments	Total nitrogen %		Crude protein %		Total phosphorus %		Total potassium %	
	1999	2000	1999	2000	1999	2000	1999	2000
Control	1.12	1.16	7.00	7.25	0.320	0.326	0.310	0.318
Fert. control (full dose of amm. sulphate)	1.41	1.44	8.81	9.00	0.424	0.478	0.430	0.450
<i>Azos. lipoferum</i> + amm. sulphate (hdn)	1.87	1.90	11.68	11.87	0.815	0.964	0.640	0.675
Full dose of biogas manure (BM)	1.48	1.56	9.25	9.75	0.459	0.540	0.382	0.400
BM (hdn) + <i>Azos. lipoferum</i>	1.63	1.70	10.18	10.62	0.724	0.890	0.485	0.520
BM (hdn) + amm. sulphate (hdn)	1.47	1.56	9.18	9.75	0.492	0.528	0.440	0.470
Full dose of town refuse compost (TRC)	1.37	1.40	8.56	8.75	0.405	0.448	0.435	0.490
TRC (hdn) + <i>Azos. lipoferum</i>	1.58	1.56	9.87	9.75	0.651	0.675	0.550	0.615
TRC (hdn) + amm. sulphate (hdn)	1.42	1.35	8.87	8.43	0.601	0.568	0.415	0.575

Abbreviations: as those stated for Table (3).

additives proved to be an efficient tool in increasing available nutrients in soil. This is likely to be due to N_2 -fixation and production of growth promoting substances by *Azospirilla* (El-Demerdash, 1994, Raso, 1996 and Hamdi and Elkomy, 1998).

CONCLUSION

From the above-mentioned results, it can be concluded that maize grains inoculation with associative N_2 -fixers (*azospirilla*) and supplementation with half the dose of inorganic N-fertilizer resulted in improving the growth performance of maize plants and consequently gave higher straw and grain yield than using the full dose of inorganic N-fertilizer without inoculation. Such application can save 50% of the cost of inorganic N-fertilizer.

Also, the growth responses (characteristics) of maize plants and maize yield were higher with the application of organic manures (biogas manure or town refuse compost) in combination with either *A. lipoferum* or inorganic nitrogen fertilizer than the application of full dose of organic manures alone.

Moreover, organic manures application either alone or in combination with biofertilizers gave significantly higher records of growth performance and grain yield of maize than the application of full dose of inorganic nitrogen fertilizer.

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تأثير التلقيح بالأزوسبيريللام في وجود مصادر مختلفة للنيتروجين علي النمو والمحصول في الذرة الشامية.

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أقيمت تجربتان حقليتان بمزرعة مركز البحوث والتجارب الزراعية بكلية الزراعة بمشتهر خلال موسمي ٢٠٠٠، ١٩٩٩ لدراسة تأثير إضافة الأسمدة العضوية (سماد البيوجاز أو كمبوست مخلفات المدن) سواء بمفردها أو مخلوطة مع لقاح الأزوسبيريللام أو مع نصف جرعة من سماد سلفات الأمونيوم علي نمو وإنتاجية الذرة الشامية ولقد أظهرت هذه الدراسة أهم النتائج التالية:-

أعطت معاملة تلقيح تقاوي الذرة عند الزراعة بالأزوسبيريللام والتسميد بنصف جرعة من السماد النيتروجيني المعدني (٥٤ كجم أزوت/فدان) أعلى الأعداد من الميكروبات المثبتة للأزوت (الأروتوباكتر-الأزوسبيريللا). بينما سُجّلت أعلى أعداد من الميكروبات المذيبة للفوسفات في منطقة الريزوسفير عند التلقيح بالأزوسبيريللام مع التسميد بنصف جرعة من سماد البيوجاز.

أدى التسميد بجرعة كاملة من سماد البيوجاز ٥,٥٥٥ طن/فدان (٩٠ كجم أزوت/فدان) إلى زيادة نشاط ميكروبات التربة معبرا عنه بثاني أكسيد الكربون المنطلق في التربة بالمقارنة بالمعاملات الأخرى. لُوِجِظ أعلى نشاط لإنزيم النيتروجينيز في منطقة الريزوسفير عند التلقيح بالأزوسبيريللام والتسميد بنصف جرعة من السماد النيتروجيني المعدني وأيضاً أعطت نفس المعاملة أعلى محتوى من النيتروجين الأمونيومي والنتراتى وكذلك الفوسفور الميسر في التربة. كذلك أوضحت النتائج أن التسميد بجرعة كاملة من سماد البيوجاز أعطى قيماً أعلى من النيتروجين الأمونيومي والنتراتى والفوسفور الميسر في التربة بالمقارنة بالتسميد بجرعة كاملة من سماد قمامة المدن ٦,٣٣٨ طن/فدان (٩٠ كجم أزوت/فدان).

أوضحت هذه الدراسة أيضاً أن هناك فروقاً معنوية في صفات النمو التي دُرِسَتْ وكذلك في المحصول ومكوناته حيث ازدادت هذه القياسات معنويًا عند تلقيح التقاوي بالأزوسبيريللام والتسميد بنصف جرعة من السماد النيتروجيني المعدني.

وعموماً اتضح من نتائج هذه الدراسة أن استخدام الأسمدة العضوية سواء بمفردها (جرعة كاملة) أو مختلطة مع لقاح الأزوسبيريللام أو التسميد المعدني (نصف جرعة) يؤدي إلى زيادة صفات النمو للذرة الشامية والحصول علي محصول أعلى بالمقارنة بالتسميد بجرعة كاملة من السماد المعدني. لذلك ينصح بإجراء تلقيح تقاوي الذرة الشامية عند الزراعة بالبكتيريا المثبتة لأزوت الهواء الجوي اللاكتكافلية مع إضافة نصف جرعة أزوت سواء من الأسمدة الغير عضوية أو العضوية كبديل للأسمدة الكيماوية مما يقلل من التلوث البيئي الناتج من الإفراط في استخدام الأسمدة الكيماوية.