INTEGRATION OF DIAZOTROPHS INOCULATION WITH ORGANIC AND INORGANIC FERTILIZATION TO IMPROVE WHEAT AND MAIZE PRODUCTIVITY IN SANDY SOILS

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Abstract

Biomass, nitrogen and grain yields of wheat cvs. Sakha 69 and Giza 163 as well as maize cv. single hybrid 122 were determined to evaluate the response of gramineous crops to diazotroph inoculation, organic and inorganic fertilization in Ismailia sandy soils. Rigorous environmental conditions of the experimental site severely reflected on development of untreated plants which showed N deficiency symptoms at early growth stages. Ammonium sulfate was superior, compared to urea form-N, as the reported increases in biomass yield with the recommended N dose were 100 and 41.9% for wheat cvs. Sakha 69 and Giza 163, respectively. Response of both cultivars to diazotroph inoculation was found to be N-dependent, whereas percentage increases of 165.6 and 164.2 were recorded due to inoculation simultaeously with 50 Kg N fed^{-1} as ammonium sulfate over that in case of the same N level as urea form. Regardless of treatment, cv. Sakha 69 supported higher soil dehydrogenase activity compared to cv. Giza 163 whereas the mean activities were 90.4 and 57.5 µg TPF g^{-1}, respectively. Also, cv. Sakha 69 supported higher nitrogenase activity compared to cv. Giza 163 giving an average increase of 114.9 nmoles $C_2H_4h^{-1}g^{-1}$ root. Plants inoculated and received the rational dose (half the recommended dose) the of N fertilizer contained somewhat lower quantities of protein in their grains than those supplemented with the full N fertilization without inoculation. Incorporation into soil of biogas manure (2.5 ton fed^{-1}) slightly stimulated protein accumulation in grains. Application of 120 kg N fed^{-1} did magnify maize plant vigorousness with percentage increases of up to 213% in biomass production. Rhizobacterin supported ca. 230% higher plant N yield over N fertilization alone. Profuse ear yield of 3.44 ton fed^{-1} was scored by urea form-full N supplied maize. Diazotroph inoculation in combination with 60 kg N fed^{-1} revealed the superior dehydrogease activity in soil (124.1 µg TPF g^{-1} soil). The maximum activity of 385 nmoles $C_2H_4g^{-1}h^{-1}$ recorded in soil inoculated with rhizobacterin together with 60 kg N fed^{-1} as urea form represented a gain of 9.2 kg N fed^{-1}. Raising the N fertilization level decreased the N gain. As expected, the lowest N budget was estimated for untreated soils.

INTRODUCTION

Agriculture of desert soils is faced by the prevalence of a number of rather extreme and detrimental conditions, i.e. very poor sandy soils deficient in nutrients and very low organic matter content, limited water supply and drought conditions. This is in addition to poor quality of irrigation water when using the Nile water mixed with drainage water and future developments of adverse conditions such as sallinity, sun heat and radiation.

To cope with these environmental constraints, various safe agrotechnologies based on biological processes could be of rather significance and substantial input. This includes biological nitrogen fixation (BNF) and plant growth substances producing rhizobacteria (PGPR) compared to agricultural chemicals required to support sustainable agricultural production (Bockman, 1997 and Kennedy *et al.*, 1997). Most production systems utilize both approaches, and take advantage of the almost cost-free input of nitrogen when nitrogen fixing plants are grown.

The present work is aiming at improving the productivity of sandy soils, which represent a major part of the newly reclaimed soils in Egypt.

MATERIALS AND METHODS

Study site:

Sandy soil (organic matter 0.029%, total N 0.007%, pH 7.7 and E.C. 0.42 dSm^{-1}) at Ismailia Experimental Station, Agricultural Research Center (ARC) was selected to conduct two field trials. The experimental site lies at 33^{o}E and 30^{o}S at an altitude of 13 m above sea level. Such area is characterized by the following climate: a) rainfall is scant, irregular and variable, b) temperature fluctuates from 13 to 23^{o}C in winter and 19 to 29^{o}C in summer, c) relative humidity is 50-70% and d) abundance of sunshine.

Host plants:

Seeds of wheat, *Triticum aestivum* L (cvs. Sakha 69 and Giza 163) and maize, *zea maize* (cv. Single hybrid 122) were obtained from Field Crops Research Institute, ARC. Initially, germination rate of each seed type was determined separately, estimates of both were falling in the range 85-90%. Seeds were thoroughly washed in water prior to treatment to get rid of any traces of peticides possibly added for pest control during storage.

Inocula and media:

Dual and triple microbial inocula were used to inoculate wheat and maize crops. The diazotrophic strains as well as one strain of soil yeast were isolated from wheat rhizosphere and purified. According to morphological, cultural and physiological characteristics by Lodder (1970), Dobereiner (1991) and Holt *et al.* (1994), the isolates were identified as *Azospirillum brasilense*, *Bacillus polymyxa* and *Saccharomyces cerevisiae*. These isolates were used :or wheat inoculation. *Azospirillum*, *Bacillus* and *Saccharomyces* were grown on malate N-free medium (Hegazi *et al.*, 1979), nutrient broth and glucose-yeast extract-peptone agar (Lodder, 1970), respecttively. Rhizobacterin (mixed culture of *Azospirillum lipoferum* and *Azotobacter chroococcum*) produced by General Organization for Agricultural Equalization Fund in Egypt (GOAEF) was used for maize seed inoculation.

Field experimentation

Two field trials were designed to evaluated the response of wheat and maize to diazotroph inoculation in combination with organic and inorganic fertilizers.

Field trial 1

All the recommended agricultural practices for wheat cultivation, mainly ploughing and compacting of soil, were initially employed. The selected area was divided into equal plots of 3 x 3.5 m. Liquid cultures of *Azospirillum brasilense*, *Bacillus polymyxa* and *Saccharomyces cerevisiae* were grown on selective media for 4 days at 30^{o}C with continuous shaking. Two integr ated inocula of associative diazotrophs, with and without yeast, were prepared by mixing equal volumes of the individual liquid cultures. The composite inocula were then mixed with Arabic gum and fine peat. Thereafter, seeds of wheat (cvs. Sakha 69 and Giza 163) were coated with the mixture of all. The mineral fertilizers were incorporated into soil as follows: superphosphate 200 Kg fed^{-1}, potassium sulfate 50 Kg fed^{-1}, and urea-form N and ammonium sulfate added at 100 (recommend rate) and 50 Kg N fed^{-1}. Urea-form (UF) N fertilizerr was added once befere planting while ammonium sulfate (AS) was divided into three doses added prior to planting, 45 and 75 days after. Biogas manure was distributed at the rate of 2.5 ton fed $^{-1}$ before bed preparation. Seeds were drilled in rows 30 cm apart and 3.5 m long. The applied treatments for each cultivar were:1) 100 Kg N (UF) fed $^{-1}$, 2) 50 Kg N (UF) fed $^{-1}$ (a), 3) 100Kg N (AS) fed $^{-1}$, 4) 100Kg N (AS) fed $^{-1}$(b) (a) +*Azospirillum* + *Bcillus*, 6) (b) + *Azospirillum* + *Bcillus*, 7) (a) + *Azospirillum* + *Bcillus* + yeast, 8) (b) + *Azospirillum* + *Bcillus*,+ yeast, 9) (a) + *Azospirillum* + Bcillus + yeast + biogas manure.

The experimental design was a complete randomized block design with 4 replicates. Plants were sampled after 83 days of sowing and analyzed for shoot dry weight after drying at 70^{O}C to constant weight. Grain yields were determined at harvest (after 180 days of planting). Population densities of total bacteria, azospirilla and N_2-fixing bacilli were estimated in rhizosphere soil of the difforent rtreatments on appropriate media according to Smibert and Krieg (1981). Dehydrogenase activity in soil and acetylene reducing activity on washed roots were measured adopting the procedures of Skujins (1973) and Hardy *et al.* (1973), respectively.

Field trial 2

After the experimental area being prepared for maize cultivation, the various mineral fertilizers were incorporated into soil. Superphosphate and potassium sulffate were added as 200 and 50 kg fed^{-1}, respectively. Two types of nitrogen fertilizers were tested, urea-form and ammonium sulfate at the rate of (120 Kg N fed^{-1} (recommended rate) and a rational rate of 60 Kg N fed^{-1} of each. Ammonium sulfate was added to soil at three equal doses: before planting, 45 and 75 days after, while urea-form was incorporated into soil at sowing. Maize seeds were thoroughly mixed with rhizobacterin and Arabic gum, then left to dry in shade. The treatments allocated were : 1) O N (control), 2) 120 Kg N (UF) fed^{-1}, 3) 120 Kg N (AS) fed^{-1}, 4) 60 Kg N (UF) fed^{-1} (a), 5) 60Kg N (AS) fed^{-1} (b), 6) (a) + Rhizobacterin, 7) (b) + Rhizobacterin, and 8) Rhizobacterin.

The sward was established by direct seeding with a seeding rate of 25 Kg fed^{-1}. Plot size was 10.5 m^2 and seeds were drilled in rows 50 cm apart. The experimental layout was a completely randomized block design with 3 replicates. Plots were watered with sprinkler irrigation system. Plants were uprooted 75 and 110 days after planting. Shoots were determined for dry weight and nitrogen content (Bremner, 1965). Ear and grain characteristics were determined at harvest. Besides, acetylene reducing activities of soils and washed roots were measured using the procedures described by Dart *et al.* (1972) and Hardy *et al.* (1973), respectively. Dehydrogenase activity in soil (Skujins, 1973) was estimated as well.

Data analysis

Data were statistically analysed by analysis of variance and least significant difference (LSD) according to Steel and Torrie (1980). Correlation coefficients between bacterial numbers and enzymatic activities were also estimated.

RESULTS AND DISCUSSION

Effect of inoculation, biogas manuring and N fertilization on wheat plant growth:

The applied treatments significantly reflected on wheat development. As expected, higher doses of N improved plant growth compared to the lower ones. Diazotrophs together with half the recommended dose almost approached the proper development obtained by full fertilization regime. Plants amended with biogas manure together with inoculation developed well and showed better appearance than those only supplied with N fertilizer. However, biogas manure resulted in no significant stimulation for wheat growth over microbial inoculation combined with 50 Kg fed^{-1} fertilization rate. Expectedly, under the harsh conditions of Ismailia desert soils, the untreated wheat plants showed nitrogen deficiency symptoms as early as 45 days after sward establishment.

Plant biomass:

Higher N fertilization regime, whatever the form, resulted in higher biomass yield of both cultivars (Table 1). Ammonium sulfate was superior in comparison with urea-form N as the reported increases in dry matter yield with the full N dose were 100.0 and 41.9% for cv. Sakha 69 and Giza 163, respectively. The respective increases with 50 Kg N fed^{-1} were 77.4 and 173.7%. Sakha 69 supplied with 50 Kg N fed^{-1} of urea-form did not respond to inoculation with diazotrophs, while Giza 163 recorded 105.3% increase in biomass yield due to inoculation with associative diazotrophs. Strikingly, response of both cultivars to diazotrophs was found to be N form-dependent, whereas increases of 165.6 and 146.2% were obtained due to inoculation together with 50 Kg N fed^{-1} as ammonium sulfate over that in case of the same N level as urea-form. Integrated inoculum of diazotrophs containing yeast showed no additive promotion to biomass yield over yeast-free corresponding, although yields produced were significantly comparable to those obtained by full N fertilized plants. Response to biogas manure amendment was cultivar-depedent. In this respect, cv. Sakha 69 received the organic fertilizer produced comparable yield to those full N supplied ones, while cv. Giza 163 gave an increase of ca. 44% due to organic fertilization.

Grain yield and N content:

Table (1) demonstrates that cv. Sakha 69 recorded 4.5% higher weight of 1000 grains over. Giza 163. The response to the various treatments varied from one cultivar to the other. For example, the highest weight of 1000 grains (45.54 g) for cv. Sakha 69 was produced by 50 Kg N fed^{-1} ammonium sulfate addition, a comparable yield was recorded with cv. Giza 163 due to inoculation with diazotrophs combined with the same dose and form of N fertilizr. Also, biogas manure application more positively affected grain production of Sakha 69 than the other cultivar.

Grain protein percentages varied among the different treatments being 9.30 - 12.83 and 8.24 - 12.52 for cvs. Sakha 69 and Giza 163, respectively (Table1). Generally, the highest estimates were recorded for full N-supplied plants. Inoculated and rational N fertilization plants contained somewhat lower quantities of protein in their grains than those supplemented with the full fertilization regime. Biogas fertilizer slightly stimulated protein accumulation in grains of the gramineous plant. Irrespective of treatment, both cultivars recorded comparable Increases in grain protein yield of ca. 10.0%.

Stress conditions of the experimental site directly reflected on grain yield of both cultivars (Table 1). In general, very low grain yields were attributed to half N fertilizer dose particularly with urea-form whereas 4.3 and 2.6 ardab. fed^{-1} were recorded for cvs. Sakha 69 and Giza 163, respectively. Leveling the N dose to 100 Kg N fed^{-1},

Table 1. Biomass of 80 days-old wheat plant grain yields of various treatments after maturation.

Treatments	Biomass yield (g/10 plants)		Weight of 1000 grains		Grain Protein %		Grain yield ard. fed^{-1}	
	Sakha 69	Giza 163	Sakha 69	Giza 163	Sakha 69	Giza 163	Sakha 69	Giza 163
100 kg UF	43.2	43.2	33.86	28.13	12.83	11.97	7.4	4.8
50 kg UF (a)	31.0	19.4	30.49	25.59	11.79	11.85	4.3	2.6
100 kg As	86.4	61.4	40.53	37.80	11.64	12.37	8.6	5.2
50 kg AS (b)	55.6	5?.1	45.54	38.07	9.30	12.52	7.6	6.0
a+diazotrophs (d)	32.3	39.0	35.25	35.59	10.72	11.30	5.8	5.4
b+d	85.7	96.4	38.40	40.53	10.29	9.48	6.7	8.2
a+d+yeast	48.6	47.2	35.40	35.54	9.78	9.79	6.0	4.8
b+d+yeast	72.3	62.1	38.10	40.28	9.34	8.24	4.9	3.2
a+d+yeast+BM	42.1	68.4	35.59	37.92	9.84	9.40	ND	ND
b+d+yeast+BM	79.0	82.7	40.60	38.02	10.71	9.96	6.2	5.9
L.S.D. 0.05	29.0		6.59		2.17		2.2	
L.S.D. 0.01	39.3		8.82		2.89		2.9	

UF, urea-form; AS, ammonium sulphate; BM, biogas manure; diazotrophs, *Azospirillum brasilense + Bacillus polymyxa*; ND, not determined.

increases of up to 85% in grain yield were scored. Diazotrops had no influence on grain yield of cv. Sakha 69 while considerable increases were recorded for cv. Giza 163. Incorporation of biogas organic ferilizer into soil showed no distinct effect on grain production.

Dehydrogenase and nitrogenase activities:

Nitrogen fertilizer form or application rate had no distinguishable influences on dehydrogenase activity in soil (Fig.1). The highest activities were recorded for full N-fertilized wheat cultivars being 90.0 and 67.0µg TPF $g^{-1}$for cvs. Sakha 69 and Giza 163, respectively. Inoculation with diazotrophs simultaneously with 50Kg N fed^{-1} resulted in lower enzymatic activities compared to full N application, where reductions of 13.1 and 23.8% were recorded. Treatment with yeast-containing inoculum resulted in statistically insignificant increase in dehydrogenase activity particularly with cv. Sakha 69. Biogas manure significantly enhanced the activity in soil cultivated with cv. Sakha 69 by 28.3% increase over that in biogas manure - untreated soil. Giza 163 did not respond to organic fertilization. Regardless of treatment, cv. Sakha 69 supported higher dehydrogenase activity compared to the other cultivar whereas the mean activities were 90.36 µ g TPF g^{-1} for the former and 57.54 µg TPF g^{-1} for the later.

Very little acetylene reducing activities (6.0 - 24.2 nmoles $C_2 H_4$ $g^{-1}h^{-1}$) were estimated for cv. Sakha 69 wheat roots of the various treatments (Fig. 2). The respective activities were higher (6.66 - 116.5 nmoles $C_2 H_4$ $g^{-1}h^{-1}$). Response to mineral N fertilizer differed among the cultivars tested, whereas N fertilized-cv. Giza 163 exhibited 110% higher nitrogenase activity than cv. Sakha 69. Similarly, responses to microbial inoculation and organic fertilization were culivar- dependent. Root systems of cv. Giza 163 revealed > 2 fold increases in nitrogenase ctivity over cv. Sakha 69 when plants were inoculated with diazotrophs in combination with 50 Kg N fed^{-1}as ammonium sulfate. In case of urea-form N, no significant difference was recorded between the test cultivars. Yeast-containing inoculum stimulated the acetylene reducing activity on roots with increases of up to 107 % compared to composite inoculum-devoid of yeast. Unexceptional enzymatic activity was scored for inoculated cv. Giza 163 supplemented with ammonium sulfate and biogas manure (116.2 nmoles $C_2 H_4$ $g^{-1}h^{-1}$). However, the effect of organic fertilization on acetylene reducing activity of other treatments was considerably low (6.0 - 17.5 nmoles $C_2 H_4$ $g^{-1}h^{-1}$).

In 80% of cases, acetylene reducing activity in root-free soil hardly exceeded 10 nmoles $C_2 H_4$ $g^{-1}h^{-1}$(Fig. 3). Generally, cv. Sakha 69 supported higher root- free soil acetylene reducing activity compared to cv. Giza 163, an average percentage increase of 114.9 was estimated.

Bacterial densities:

Rhizosphere soils of the different treatments harboured dense bacterial population (fig.4.) No obvious variations among N fertilizer dose or form were recorded. Inoculation with diazotrophs slightly promoted bacterial multiplication particularly when yeast was incorporated into the inoculum. Such finding were recorded for both wheat cultivars. Further increases in number were attributed to biogas manure application especially in case of cv. Sakha 69 with estimates of $>10^8$ cfu g^{-1}.

As expected, significant positive correlation coefficient betweenn total bacterial number and dahydrogenase activity of soil was scored (r=0.68). Among diazotrophs, bacilli were encountered in higher numbers compared to azospirilla (Fig.4.) No distinct influence on diazotrophs to inoculation or fertilization treatments was observed where numbers hardly fluctuated from 15^5 to 10^6 cfu g^{-1}. Numbers of diazotrophs in rhizosphere soils were positively correlated with acetylene reducing activity, although the relationships were statistically insighificant (r=0.56 and 0.03 for soil and 0.44 and 0.02 for roots, $P<0.05$) for A*Azospirillum* and *Bcillus*, respectively.

Effect of Rhizobacterin and fertilizers on maize

Plant growth and biomass:

Extreme environmental conditions of Ismailia sandy soils severely reflected on growth and development of maize plants. Scant biomass yield was obtained by neither inoculated nor N-fertilized plants (Table 2). Full N fertilization regime did magnify plant vigorousness where increases of up to 213% in plant dry weight were estimated. Such effect was more pronounced with urea- form N. Reducing the quantity of mineral N fertilizer lowered dry matter production, although the differences were statistically insignificant in the majority of cases. Highest biomass yield (average of 815 g/10 plants) was produced by plants inoculated with rhizobacterin and supplemented with a rational N dose. Again, urea-form N supported better plant development compared to a rational N dose. Again, urea-form N supported better er plant development compared to ammonium sulfate. Percentage increase of 11.6 over control was scored for inoculated plant received no N fertilizer. It is noteworthy that the relative increase in dry matter yield of 75-day old plants over the 110-day old ones varied among the different treatments. Highest estimate of 8.5 fold was recorded for inoculated maiza supplied with

60 Kg fed $^{-1}$as ammonium sulfate while 3.4 fold increase was scored for uninoculated plants. At harvest (110 days after planting), superior biomass yields were obtained by either full N fertilized (918.0 g/ 10 plants) or simultaneously half N supplied and inoculated (822.5 g/10 plants) plants. Non-fertilized maize plants, whether inoculated or not, were the inferior (384.3 and 337.6 g/ 10 plants, respectively).

Plant nitrogen:

Very low nitrogen yields (average of 0.36 Kg fed^{-1}) were reported by untreated plants (Table 2) which showed N deficiency symptoms at early stages of growth.The 75- day old maize plants of the various treatments did not significantly differ in their N contents, while older ones variably responded to inoculation and fertilization. Considerable quantities of N were accumulated in plants received full fertilization regime being 48.48 and 46.92 Kg N fed^{-1} for urea-form N and ammonium sulfate, respectively. Superior N yield of 72.84 Kg fed^{-1} was produced by maize inoculated simultaneously with 60 Kg N fed^{-1} of urea-form application. Irrespective of N fertilizer form and plant age, rhizobacterin supported ca. 230% higher N yield over N fertilization alone. Rhizobacterin, in absence of N fertilizer, resulted in low N yield of 0.9 Kg fed^{-1}. Generally, 110-day

Table 2. Biomass (g 10 plants^{-1}) of maize shoots and nitrogen yield (Kg N fed^{-1}) of 75 and 110 day old maize shoots as affected by inoculation with diazotrophs and N-fertilization

Treatments		Biomass yield (g/10 plants)		Nitrogen yield (Kg N fed^{-1})	
		75 days	110 days	75 days	110 days
Control (untreated plants)		404.3	337.6	0.24	0.48
120 Kg AS N fed^{-1}		1183.8	655.4	7.08	48.92
120 Kg UF N fed^{-1}		1265.8	917.8	5.68	48.48
60 Kg AS N fed^{-1}		995.0	711.5	1.68	11.16
60 Kg UF N fed^{-1}		838.1	797.3	1.68	20.88
60 Kg AS N fed^{-1} + Rhizo.		1571.1	822.5	3.51	33.48
60 Kg UF N Fed^{-1} + Rhizo.		1270.5	728.6	7.68	72.84
Rhizobacterin		480.3	384.3	1.08	0.72
L.S.Ds	0.05	364.1		22.71	
	0.01	485.5		31.28	

As, ammonium sulphate; UF, urea-form; Rhizo., rhizobacterin.

old maize plants contained 720% more N in their shoot tissues over the 75-old ones.

Plant yield:

Ear characteristics are summarized in Table (3). Untreated maize plants produced no ears similar to those inoculated but not N fertilized. Irrespective of N fertilizer form, plants received the full dose produced longer ears (average of 16.7 cm) than the correspondings supplied with half N regime (average of 13.3 cm). Inoculation simultaneously with N fertilization resulted in ears of 16.0 cm length. Little fluctuation, of no significancy, in number of rows per ear were recorded among the various treatments, whereas the estimated rows were falling in the range 12-13 per ear.

High ear yield of 3.44 ton $fed^{-1}$was obtained by urea- form full N supplemented maiza (Table 3). Significantly, similar yield (2.80 ton fed^{-1})was produced by plants received half N fertilizer regime togethr with rhizobacterin. Variable yield (1.72 - 2.38 ton fed^{-1}) were scored for the other treatments. Urea-form N supported higher yield compared to ammonium sulphate fertilizer with percentage increases of 17.7 and 34.3 in presence and absence of inoculation, respectively. The respective increase enlarged to 53.6% for full N dose-treated plants.

The complementation of rational N dose of 60 kg $fed^{-1}$with composite inocul-

Table 3. Ear and grain characteristics of maize of the different treatments.

Treatments		No. of ears/ 10 plants	Ear length (cm)	Ear weight (ton fed^{-1})	Cob weight (ton fed^{-1})	No. of grains ear^{-1}	Grain yield (ton fed^{-1})	Grain protein (%)	Shelling (%)
Control*		0	-	-	-	-	-	-	-
120 Kg AS		13	14.7	2.24	0.33	524	1.91	6.44	85.3
120 Kg UF		12	18.7	3.44	0.50	608	2.94	6.04	85.5
60 Kg AS		12	12.8	1.72	0.26	377	1.50	2.44	87.2
60 Kg UF		12	13.7	2.31	0.32	448	1.99	4.22	86.2
60 Kg AS Rhiz.		13	14.7	2.38	0.34	496	2.04	4.34	85.7
60 Kg UF Rhiz.		13	147.2	2.80	0.43	508	2.29	8.06	81.8
Rhizobacterin*		0	-	-	-	-	-	-	-
L.S.Ds	0.05	2	3.0	0.83	0.13	127	0.70	2.08	
	0.01	3	4.2	1.16	0.18	177	0.98	3.15	

um produced cobs yield (averge of 0.29 ton fed^{-1}). Comparable to that attributed to 120 Kg fed^{-1} application (average of 0.42 ton fed^{-1}). Incorporation into soil of 60 kg N fed^{-1}in absence of Rhizobacterin yielded 0.32 and 0.26 ton fed^{-1}cobs for urea- and ammonium-treated maize plants, respectively.

Variable numbers of grains were produced by test plants depending upon treatment (Table 3). Plant of grains were formed by full N fertilized maize compared to others. In this respect, an average of 566 grains $plant^{-1}$were produced due to the application of 120 kg N fed^{-1}regardless of N form. On the other hand, the lowest grain production did result from half N fertilization regime (average of 413 grain $plant^{-1}$).

Inoculation together with 60 Kg N fed^{-1} addition produced 502 grains plant This indicates the importance of inoculation with diazotrophs and reducuing the quantities of mineral N fertilizers to restrict the environmental pollution simultaneously with the appropriate crop production.

Nitrogen fertilizer from showed a unique influence on total grain yield (Table 3). Urea-N was superior, whether the composite inoculum was applied or not. Adequate grain yield (average of 2.2 ton fed^{-1}) was obtained from plants inoculatedwith Rhizobacterin in combination with 60 Kg N fed^{-1} fertilization level. Such yields were 24.0% higher than those obtained from plants left without inoculation with diazotrophs.

In comparison with maize supplied with 120 kg N fed^{-1} as ammonium sulfate, increases of 53.9 and 19.9% in grain yield were estimated for plants recevied the full N dose as urea- form and those inoculated simulaneously with the application of half urea-N fertilization regime, respectively (Table 3). Reduction of 21.5 % in yield was attributd to reducing the quantity of ammonium to 60 Kg N fed^{-1}.

Grain protein percentage ranged from 2.44 to 8.06 being the highest with inoculated maize and fertilized with 60 Kg N fed^{-1}as urea- form (Table3).

As shown in Table (3) shelling percentage hardly fluctuated and ranged from 81.8 to 87.2 being highest with maize supplemented with 60 Kg N fed^{-1}. Nitrogen fertilizer form had no remarkable influence on shelling percentage particularly in absence of diazotrophs.

Dehydrogenase and nitrogenase activities:

Dehydrogenase activity of soil, determined as an indirect measure for microbial population and activities, considerably varied from one treatment to another (Fig.5).

At the 75-day growth period, inoculation with diazotrophs simultaneously with 60 Kg N fed^{-1} application revealed that the superior activity of 124.1 µg TPF g^{-1}. Full dose of urea-form N supported higher dehydrogenase activity compared to ammonium sulfate whereas 38.3 and 18.8 µg TPF 9^{-1} were estimated, respectively. As expected, soils cultivated with non-fertilized uninoculated maiza showed the lowest enzymatic activity (average of 16.4 µg TPF g^{-1}).

No measurable acetylene reducing activity was scored on roots of 45-day old piants. Thereafter, high activities was detected by 75-day old ones (Fig. 6). The absence of nitrogenase activity for young plants including the inoculated ones might be attributed to the failure of soil diazotrophs to colonize root systems successfully or to insufficient photosythates necessary to provide N_2-fixers with their energy and nutrient requirements. The N fertilizer form did greatly affect the activity of associative diazotrophs. In this respect, inoculation in presence of ammonium sulfate resulted in nitrogenase activity of 571.0 nmoles C_2 H_4 $g^{-1}h^{-1}$. The corresponding activity in case of urea-N was 808 nmoles C_2 C_4 $g^{-1}h^{-1}$. The exceptional high nitrogenase activity recorded for the 75-day old plants supplemented with high N-fertilization regime (ca. 350 nmoles C_2H_4 $g^{-1}h^{-1}$) is ce tainly attributed to the depletion of most of applied nitrogen due to plant consumption throughout such prolonged growth period.

Incorporation of 120 kg N fed^{-1} into soil did inhibit nitrogenase activity in soil at the early growth period (Fig. 7). Rational N-fertilization regime promoted the aactivity by 66.5 and 32.0% for ammonium-and urea-form N fertilized plants, respectively. Such stimulative effect magnified with Rhizobacterin inoculation whereas the respective increases raised to 69.3% and 76.2%, respectively. Inoculation with integrated group of diazotrophs in absence of N-fertilizer resulted in acetylene reducing activity of 192.7 nmoles C_2H_4 $g^{-1}h^{-1}$ representing 103% increae over untreated soil. In 50% of cases, acetylene reducing activity significantly increased with plant age. The highest increases of 47.5 and 130.2% were recorded in inoculation treatments together with 60 kg N fed^{-1} of ammonium-and urea-N fertilizers, respectively. Acetylene reduction decreased to the lowest extent (64.1%) with inoculated non-fertilized plants.

Assuming a C_2H_4 reduced/N_2 fixed factor of 3 (Hardy *et al.*, 1968), the maximum acetylene reducing activity of 385.0 nmoles $C_2H_4g^{-1}h^{-1}$ recorded in root free soil ioculated with Rhizobacterir in combination with 60 Kg N fed^{-1} as urea-form represents a gain of 9.2 Kg N fed^{-1} (Fig. 8). Raising the N fertilizer level decreased the N-gains. As expected, the lowest N-gain was obtained with untreated soil. Irrespective of treatment, N-gain increased by 22.3% with plant age.

Desert, as marginal soils with their conditions and detrimental factors, necessitates intensive agricultural practices to secure a reasonable productivity. A unique approach is to introduce BNF systems to such soils. But in fact, inoculation with diazotrophs does not meet all nutritional requirements and supportive N-fertilization is indispensable particularly under extreme and stress conditions (Kennedy *et al.,* 1997). To optimize crop productivity in the experimental site of Ismailia sandy soils, the complementation of diazotrophic inoculation and adequate supply of mineral and/or organic fertilizers was the major target of the present study. As the experimental field harboured relatively low densities of diazotrophs (ca. 10^5 cells g^{-1}), inoculation simultaneously with rational N fertilization resulted in good development and biomass production of both wheat and maize. Such promoting effect extended to nitrogen, grain and straw yields.

Results recorded in this study and of other researchers (Hegazi *et al.,* 1979; Shahaby, 1981; Fayez and Vlassak, 1984; Fayez, 1990) indicated that inoculation with diazotrophs enhanced nitrogenase activity in root regions of Egyptian cereals. Such findings support the view that these bacteria produce growth-regulating compounds mainly indole acetic acid, gibberillin and cytokinine-like substances which may improve plant productivity by hormonal stimulation besides N_2 -fixation (Tien *et al.*, 1979, Shahaby *et al.*, 1994 and Hirsch *et al.*, 1997). It is well established that inoculation with diazotrophs induced the proliferation of lateral roots and root hairs which increase nutrient-absorbing surfaces and many more root tips available for infection.

Interestingly, response of graminea to diazotrophs was reported to be N fertilizer form-dependent (Bockman, 1997). In the present work, two forms of N were investigated, urea-form as a slow-release N fertilizer and ammonium sulfate as a soluble form of N. Ammonium sulfate was the superior N combined with diazotroph's inoculation for supporting better biomass yields compared to urea-form. The response to N-form not only differed from plant type to another but even among the cultivars of the same plant type.

The relative low acetylene reducing activities in soil (<600 nmoles $C_2H_4g^{-1}h^{-1}$ representing N-gains of less than 6.0 Kg N fed^{-1}) reported in the majority of diazotrophic inoculated soils confirm that production of plant growth-altering substances (PGAS) by associated N_2-fixing bacteria is the main explanation of how these bacteria may affect plant growth in the absence of measurable N_2-fixation (Smith *et al.*, 1984; Hirsch *et al*, 1997 and Shahaby, 1997). This does not say that the action of diazotrophs is all due to PGAS, but they may be part of the overall effect. Certainly, many studies reported increases in plant N-uptake, and N^{15} dilution experiments introduce that the bacteria contribute to the plant nitrogen budget (Vose, 1983 and Shantharam and Mattoo, 1997).

In the present wheat field trial, limited quantity of organic fertilizer, as biogas manure (2.5 ton fed^{-1}), was added to soil in an attempt to accelerate BNF and consequently to improve soil biofrtility. The insignificant promoting influence of such fertilization regime on crop yield indicates that the applied level is insufficient for the appropriate production particularly under harsh environmental conditions prevailling in Ismailia sandy soils. A finding proves that heavy organic dressing of poor sandy soils is compulsory. For better management of desert environments, it is strongly recommended to adopt application of crop refuse and nitrogen fertilization to sandy soils together with diazotroph's inoculation. This, certainly, results in a potential N_2-fixation which, in turn, reflects on the final crop outputs.

In conclusion, it could be stated that adoption of integrated BNF-fertilization systems is unavoidable for better management of infertile sandy soils. Selection for potent bacterial symbionts and proper plant genotypes for such environments should be considered as well.

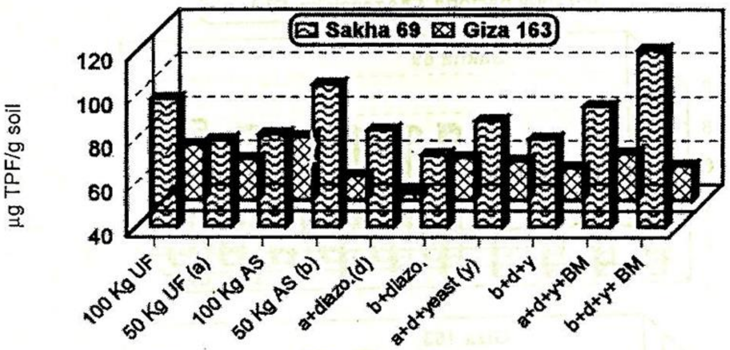

Fig. 1. Dehydrogenase activity of soil of the different treatments after 80 days of planting. UF, urea-form; AS, ammonium sulphate; d, diazotrophs; y, yeast; BM, biogas manure.

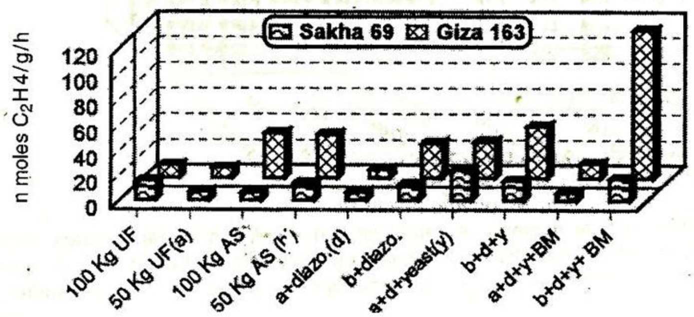

Fig. 2. Nitrogenase activity of roots of inoculated and fertilized wheat cultivars, UF, urea-form; AS, ammonium sulphate; d, diazotrophs; Y, yeast; BM, biogas manure.

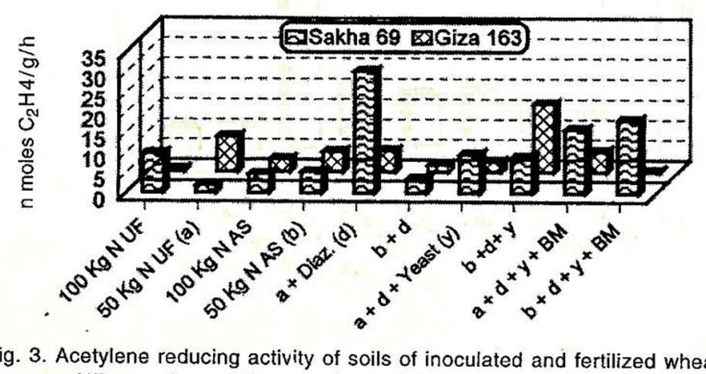

Fig. 3. Acetylene reducing activity of soils of inoculated and fertilized wheat cultivars. UF, urea-form; AS, ammonium sulphate; d, diazotrophs; y, yeast, BM, biogas manure.

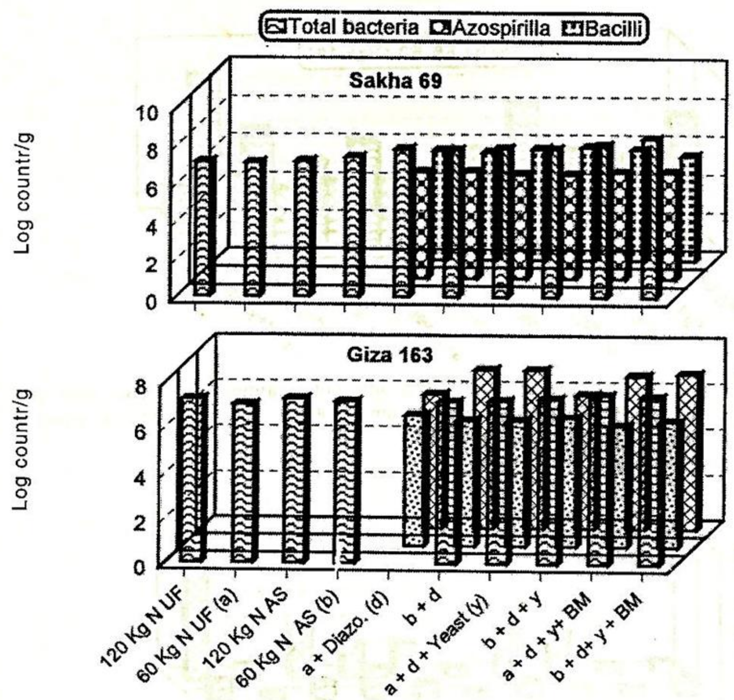

Fig. 4. Densities of total bacteria, *Azospirillum* and *Bacillus* in wheat cultivars rhizosphere soils of the different treatments (after 80 days of planting). UF, urea-form; AS, ammonium sulphate, d, diazotrophs; Y, yeast; BM, blogas manure.

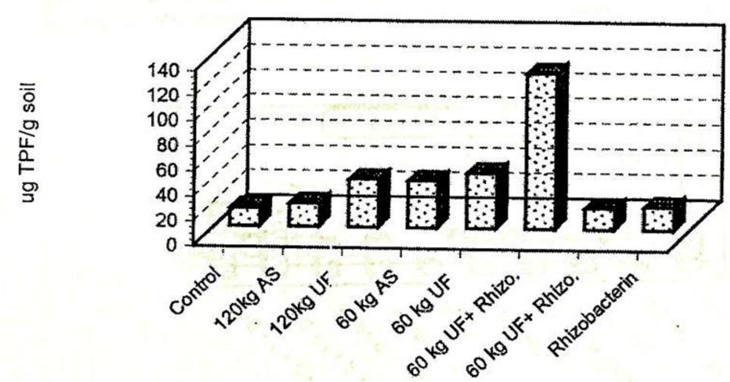

Fig. 5. Dehydrogenase activity of maize soil of the various inoculation and N-fertilization treatments after 75 days of planting. AS, ammonium sulphate; UF, urea-form.

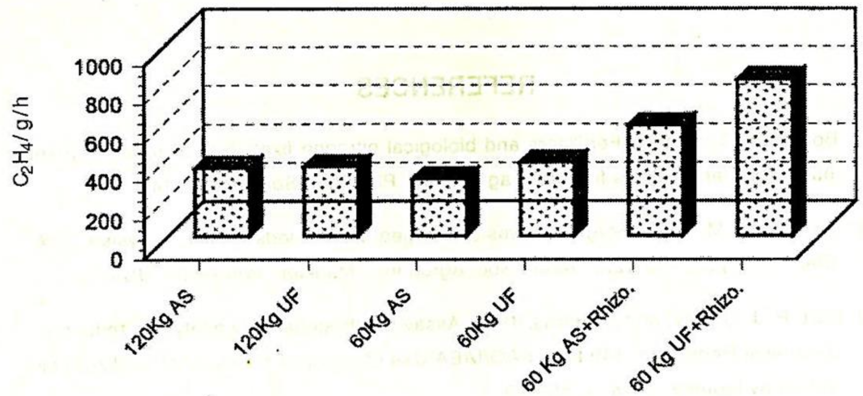

Fig. 6. Nitrogen activity on 75-day old maize roots due to diazotrophs inoculation and N fertilization. UF, urea-form; AS, ammonium suphate; Rhizo., Rhizobacterin.

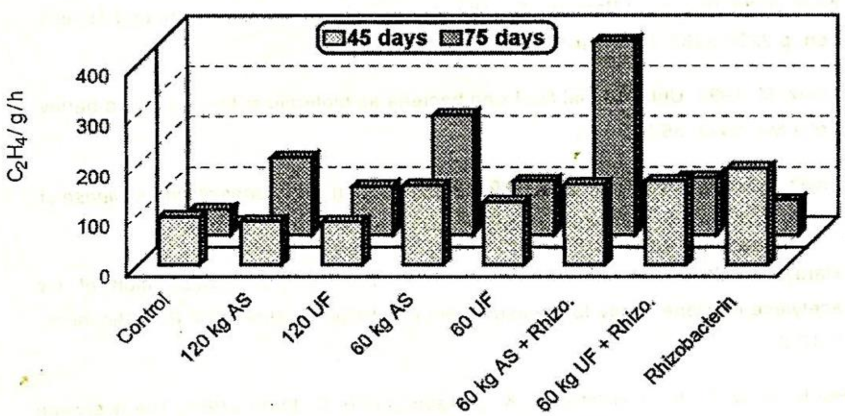

Fig. 7. Nitrogenase activity in maize soil of different treatments. UF, urea-form; AS, ammonium sulphate; d, diazotrophs; y,yeast; BM, biogas manure.

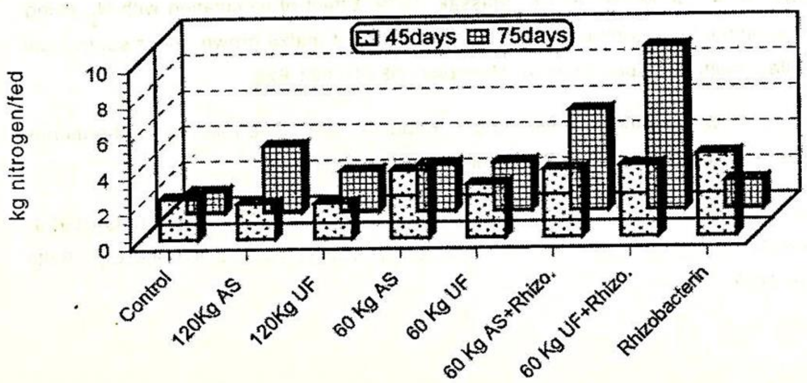

Fig. 8. Soil N-budget of maize field of the different inoculation and N fertilization treatments.

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التكامل بين التلقيح بالبكتريا المثبتة للنيتروجين الجوى والتسميد العضوى والمعدنى لتحسين إنتاجية محصول القمح والذرة فى الاراضى الرملية

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في هذا البحث تم تقدير قياسات محصول الوزن الجاف والنتروجين والحبوب للقمح صنفي سخا ٦٩ وجيزة ١٦٣ وكذلك الذرة صنف هجين فردي ١٢٢ وذلك لتقيم مدي إستجابة المحاصيل النجيلية للتلقيح بالبكتريا المثبتة للنيتروجين الجوي مع التسميد العضوي والمعدني في أراضي محافظة الاسماعلية بمصر. حيث ظهر بوضوح تأثير الظروف البيئية القاسية علي الموقع التجريبي وبالتالي علي نمو وتطور النباتات غير الملقحة والتي ظهر عليها اعراض نقص النتروجين في اولي مراحل النمو.

كان سماد سلفات الامونيوم اكثر فعالية وتأثيرا علي نمو النباتات مقارنة بسماد اليوريا وخاصة عند استخدام الجرعة الكاملة الموصي بها حيث سجل زيادة مقدارها ١٠٠ و ٤١,٩٪ لصنفي القمح سخا ٦٩ وجيزة ١٦٣ علي التوالي. وكانت استجابة صنفي القمح للتلقيح البكتيري تعتمد علي التسميد النتروجيني حيث كانت الزيادة تمثل ١٦٥,٦ ، ١٦٤,٢٪ عند التلقيح والتسميد معا بمقدار ٥٠ كجم نتروجين/ للفدان كسلفات أمونيوم وكانت أعلي منة في حالة استخدام سماد اليوريا بنفس الجرعة. وبغض النظر عن المعاملة اعطي صنف القمح سخا ٦٩ نشاط أعلي لأنزيم الديهيدروجنيز مقارنة بالصنف جيزة ١٦٣ وكان متوسط النشاط للانزيم ٩٠,٤ ، ٥٧,٥ ميكروجرام TPF/ جرام تربة علي التوالي. كذلك فان صنف القمح سخا ٦٩ شجع حدوث نشاط أعلي لأنزيم النتروجينيز مقارنة بالصنف جيزة ١٦٣ معطية زيادة مقدارها ١١٤,٩ نانومول ايثلين/ساعة/جرام جذور وكانت النباتات الملقحة والمسمدة معا بجرعة معقولة من السماد النتوجيني تحتوي علي كميات أقل من البروتين نوعا ما في الحبوب عند مقارنتها مع النباتات المسمدة بالجرعة الكاملة من النتروجين وبدون تلقيح. وكذلك شجعت اضافة سماد البيوجاز بمعدل ٢,٥ طن / فدان تراكم النتروجين في الحبوب.

كان لإضافة ١٢٠ كجم نتروجين / فدان تأثير كبير علي نباتات الذرة حيث زاد محصول الوزن الجاف حتي ٢١٣٪ وباضافة الريزوباكترين زاد محصول النترجين بمقدار ٢٣٠٪ عنه في حالة استخدام التسميد النتروجيني. سجل تسميد محصول الذرة بالجرعة الكاملة من السماد

النتروجيني محصول كيزان وفير مقداره ٣,٤٤ طن / فدان وكان نشاط انزيم الديهيدروجينيز في التربة (١٢٤,١ ميكروجرام TPF / جرام تربة) اكثر فاعلية عند التلقيح بالبكتريا المثبتة للنتروجين الجوي مع التسميد النتروجيني بمقدار ٦٠ كجم نتروجين /فدان. وكان أعلي نشاط لأنزيم النتروجينيز ٣٨٥ نانومول ايثلين/ساعة/جرام تربة، عند التلقيح بالريزوباكترين مع التسميد بمقدار ٦٠ كجم نتروجين/فدان كسماديوريا. وهذا يمثل زيادة مقدارها ٩,٢ كجم /فدان. وأدت زيادة معدلات التسميد الي نقص النتروجين المكتسب. وكما هو متوقع كانت أقل كمية نتروجين مقدرة في التربة الغير معاملة.