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Annals Of Agric. Sc., Moshtohor,
Vol. 34(2):627-640, 1996.

EFFICIENCY OF SOME ORGANIC MANURES AND BIOFERTILIZATION WITH *Azospirillum brasilense* FOR WHEAT MANURING

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ABSTRACT

The efficiency of some organic manures namely biogas manure, sewage sludge manure and garbage manure as well as wheat grains inoculation with (*Azospirillum brasilense*) was studied. Results showed that inoculation of wheat grains with *Azospirillum brasilense* led to the increase of Azospirilla and inorganic phosphate solublizers counts compared with using the organic manures. No difference was observed in Azospirilla and inorganic phosphate solublizers counts according to the type of organic manure. Dehydrogenase activity differed according to the type of organic manure. Sewage sludge manure gave the highest dehydrogenase activity. Organic carbon percentage increased with application of organic manures compared with the biofertilizer and inorganic N-fertilizer. Ammoniacal and nitrate nitrogen as well as total phosphorus increased by wheat bacterization with associative N_2-fixer (*Azos. brasilense*). No differences were observed in total phosphorus by application of different organic manures. Concerning the effect of organic manuring and biofertilization on chemical components of wheat, data showed that the N, P and K concentrations were higher in case of biofertilization compared with the organic manuring. Also, the results showed that the growth characters and yield were significantly increased with wheat grains bacterization compared with the organic manures and inorganic nitrogen fertilization.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second important cereal crop in Egypt. With increasing human demands for food, attempts are made to cultivate desert areas with wheat. Nitrogen fertilization is an important factor in increasing yield of wheat. Makawi (1982) showed that the addition of organic manures to the soil encourages proliferation of soil microorganisms, also dehydrogenase and urease activity, organic carbon and availability of nitrogen and phosphorus were increased with the application of organic manures to wheat plants. Vidyorthy and Nisra (1982) mentioned that the application of organic matter increased the microbial population and microbial activity in the soil, as well as, there was a significant increase in macro and micro-nutrients and yield of wheat crop. Mahmoud *et al.*, (1984) revealed that the application of biogas manure increased the dry matter and grains yield of wheat plants. Gomaa (1991) reported that sewage sludge application to calcareous and sandy soils for wheat plants increased growth and yield and the concentration of heavy metals in wheat grains were under toxic level to plants. For a new source of organic manuring and mean while from an environmental point of view, recycling of treated municipal wastes is suggested. Decreasing the enormous consumption of chemical fertilizers and mean-while minimizing health and environmental risks both are prospectively fufilled (Abou-Bakr and Omar, 1993). Many studies indicated the importance of inoculation with *Azospirillum brasilense* for cereal crops. Ishac *et al.*, (1984) showed that inoculation of wheat grains with *Azospirillum brasilense* significantly enhanced the growth of wheat plants and the yield was increased. El-Haddad *et al.*, (1986) found that seeds inoculation with non-symbiotic N_2-fixing bacteria resulted in higher yields and reduced N-requirements fertilization to 50%. In addition to the beneficial effect of N_2-fixing bacteria associated with roots of cereal crops, these bacteria are also reported to produce growth promoting substances which help in increasing various soil microorganisms and crop yield (De-Freitas and Germida, 1990). Youssef *et al.*, (1993) reported that dry matter, grain yield and protein yield of wheat were significantly increased with inoculation by *Azospirillum brasilense*.

The aim of this study is to investigate the efficacy of organic manures amendment and biofertlization with *Azos. brasilense* on soil fertitlity and wheat growth.

MATERIALS AND METHODS

A pot experiment was conducted under green house conditions. The soil used in this investigation was loamy sand (organic matter 0.7%, total nitrogen 0.07%, total phosphorus 0.04%, $CaCO_3$ 0.23%, pH 7.44 and E.C. 1.76 m mohs/cm). It was obtained from El-Dir Village, Qualubia governorate and the soil samples were collected from 0-15 cm layer, air dried, ground to pass through a 2 mm sieve and thoroughly mixed. 30 cm diameter pots were filled with the soil (6 kg/pot). Organic manures used were biogas manure, composted garbage and composted sewage sludge. They were added before sowing at a rate of 60 kg N/feddan, while the inorganic nitrogen fertilizer was added in two equal doses at tillering and heading stages at the rates mentioned thereafter. All pots were supplemented with calcium super phosphate (15.5 P_2O_5) at a rate of 30 kg phosphorus/feddan. Organic manures analyses are presented in Table (1).

Table (1): Analyses of the organic manures.

Organic manures	O.M %	O.C %	T.N %	T.P %	T.K %	C:N ratio
Composted sewage sludge	56.40	32.71	2.13	1.50	0.82	15.10
Composted garbage manure	56.00	32.48	1.38	0.60	0.36	23.53
Biogas manure	67.00	38.86	1.96	0.98	1.60	19.82

For preparation of Azospirillum inoculum, Dobereiner medium (1978) was inoculated with effective strain of *Azospirillum brasilense* and incubated at 30°C for 7 days till the viable count reached 10^8 cell/ml. Wheat grains were inoculated with *Azospirillum brasilense* at sowing time. There were seven treatments as follows:

1- Control in which no manure and no Azospirillum were applied.
2- Biogas manure (60 kg N) + 30 kg N as ammonium sulphate/fed.
3- Composted garbage (60 kg N) + 30 kg N as ammonium sulphate/fed.
4- Composted sewage sludge (60 kg N) + 30 kg N as ammonium sulphate/fed.
5- Azospirillum inoculum + a quarter dose of inorganic N-fertilizer (22.5 kg N/fed.).
6- Azospirillum inoculum + a half dose of inorganic N-fertilizer (45 kg N/fed.).
7- A full dose (90 kg N) of inorganic N-fertilizer/fed.

Four pots were used as replicates for every treatment in a randomized complete block design. Cultivation process was performed by sowing ten inoculated or uninoculated grains of wheat (*Triticum aestivum* cv. Sakha 8) in every pot, and reduced later to five plants.

Sampling and determinations:

After 45, 90, 120 and 180 days from sowing, rhizosphere soil samples of the developed plants were taken. These periods were considered and referred to in the results discussion as the tillering, heading, grain formation and maturity stages, respectively. The samples were microbiologically and chemically analyzed.

1. Microbiological analyses:

1-1-The populations of *Azospirillum spp.* and inorganic phosphate dissolving bacteria were chosen as representatives of asymbiotic N_2-fixing bacteria and phosphate dissolvers, respectively. Numbers of *Azospirillum spp.* and inorganic phosphate dissolving bacteria were determined on Semi-solid malate medium (Dobereiner, 1978) using the most probable number technique (Cochran, 1950) and (Bunt and Rovira medium modified by Abdel-Hafez, 1966) using the plate count method, respectively.

1-2-Dehydrogenase activity in the soil was assayed by the method described by Casida *et al.*, (1964).

2. Chemical analyses:

2-1-Organic carbon was estimated according to Black *et al.*, (1965).

2-2-Total phosphorus was colorimetrically determined according to Troug and Mayer (1949).

2-3-Ammoniacal and nitrate nitrogen were estimated according to Morkus *et al.*, (1982).

2-4-Total nitrogen was estimated in soil and plant samples using Kjeldahl digestion method as described by Jackson (1973).

2-5-Total potassium was estimated by flame photometer apparatus according to the method described by Brown and Lilliland (1946).

At the end of the experiment, wheat plants were harvested. then plant height, spike length, spike weight, grains weight/spike, number of spikelets/spike, fresh and dry weight of root and shoot system/plant were measured. Also, nitrogen, phosphorus and potassium were determined in shoot system. The obtained data of growth characters were statistically analysed according to Snedecor and Cochran (1982).

RESULTS AND DISCUSSION

Effect of different soil applications on microbial populations and dehydrogenase activity:

1. Changes in *Azospirillum spp.* counts:

Data in Table (2) showed that inoculation of wheat grains with *Azospirillum brasilense* gave the highest counts of *Azospirillum spp.* during all growth stages compared with the organic manuring. While, the lowest counts of *Azospirillum spp.* were recorded in inorganic nitrogen fertilizer application. Generally, *Azospirillum spp.* counts were almost similar when the soil was treated by different organic manures. The counts of *Azospirillum spp.* gradually increased with increasing the growth period to reach their maximal values during the grain formation stage and this was true in all treatments. Similar results were obtained by Makawi (1982) and Vidyorthy and Nisra (1982) who found that the addition of organic manures increased the microbial population and microbial activity in the soil.

2. Changes in populations of inorganic phosphate dissolving bacteria:

Data in Table (3) clearly indicate that inoculation with associative N_2-fixers (*Azospirillum brasilense*) gave the highest counts of inorganic phosphate dissolving bacteria compared with the other treatments in various growth stages of wheat plants. This result may be due to the higher populations of *Azospirillum spp.* in this treatment. Also, the results showed that inorganic nitrogen fertilizer gave higher counts of inorganic phosphate dissolvers than organic manures. The counts of inorganic phosphate dissolvers increased with increasing the growth period to reach their maximal values during the grain formation stage. This increase is likely to be due to the beneficial effect of root exudates and debries

Table (2): Periodical changes in populations of *Azospirillum spp.* ($x10^3$/g dry weight of soil) during various growth stages of wheat.

Treatments	Plant growth stage			
	Tillering	Heading	Grain formation	Maturity
Control	11	30	58	26
Biogas manure	34	67	148	70
Garbage manure	32	87	154	84
Sewage sludge manure	40	96	163	96
Azospirillum + a quarter dose N-fretilizer	64	105	185	102
Azospirillum + a half dose N-fretilizer	78	160	215	125
Complete N-fertilizer	36	60	104	48

Table (3): Periodical changes in populations of inorganic phosphate dissolvers ($x10^6$/g dry weight soil) during various growth stages of wheat.

Treatments	Plant growth stage			
	Tillering	Heading	Grain formation	Maturity
Control	1.3	2.2	6.0	4.0
Biogas manure	3.0	4.5	11.0	6.5
Garbage manure	4.8	9.0	14.0	9.5
Sewage sludge manure	4.6	8.4	15.0	7.0
Azospirillum + a quarter dose N-fretilizer	7.8	15.0	30.0	13.0
Azospirillum + a half dose N-fretilizer	10.0	18.0	34.0	21.0
Complete N-fertilizer	6.0	12.0	16.8	11.0

during grain formation stage. Similar results were observed by many investigators (De-Fretias and Germida, 1990, Mahmoud *et al.*, 1993) who emphasized that, in addition to the beneficial effect of N_2-fixing bacteria associated with roots of cereal crops, these bacteria are also reported to produce growth promoting substances which help in increasing other soil microorganisms and crop yield.

3- Changes in dehydrogenase activity:

Data in Table (4) indicate that dehydrogenase activity varied according to the type of organic manure. Maximum dehydrogenase activity occurred with sewage sludge manuring followed by garbage and biogas manuring. On the other hand, inorganic nitrogen fertilizer showed the lowest values of dehydrogenase activity. Dehydrogenase activity was remarkably high during the first growth stage (tillering) and then gradually decreased with progressive plant growth and this was true in all treatments. These results are in harmony with those obtained by El-Shemi (1976) and Makawi (1982) who reported that dehydrogenase activity increased during the initial period of plant growth and tended to decrease thereafter.

Effect of different soil applications on nutritional elements:

1- Effect on organic carbon:

Data in Table (5) revealed that the application of organic manures clearly increased the organic carbon content of the tested soil compared with the inorganic nitrogen fertilizer treatments either in the presence or without *Azospirillum brasilense* inoculation. This trend was consistent in all soil samples all over the experiment. Soil organic carbon percentage gradually decreased with increasing the growth period and this was true in all treatments. Many earlier investigators revealed that the addition of organic manures increased the carbon content of soil (Khalil, 1979 and El-Huseiny *et al.*, 1988). The results obtained herein confirm these findings.

2- Effect on nitrogen forms:

Data presented in Table (6) emphasized that both ammoniacal and nitrate nitrogen content of soil varied according to the type of soil applications. Ammoniacal and nitrate nitrogen content were high in case of biofertilization followed by organic manuring and inorganic fertilization. This increase of ammoniacal and nitrate nitrogen in case of biofertilization may be due to nitrogen fixation by *Azospirillum brasilense*. Generally, the nitrogen forms gradually increased with increasing growth period to reach their maximal values during grain formation stage and this was true in all treatments. These results are in harmony with those obtained by Vidyorthy and Nisra (1982), and Ishac *et al.*, (1984) who found that the addition of organic manures and wheat grains inoculation with *Azospirillum brasilense* increased microbial activities as well as macro and micro-nutrients of soil.

Table (4): Periodical changes in dehydrogenase activity during various growth stages of wheat (μl H/g dry soil/24 hrs at 30°C).

Treatments	Plant growth stage			
	Tillering	Heading	Grain formation	Maturity
Control	73	36	22	36
Biogas manure	489	399	319	306
Garbage manure	523	430	324	310
Sewage sludge manure	586	437	367	356
Azospirillum + a quarter dose N-fretilizer	339	317	266	224
Azospirillum + a half dose N-fretilizer	480	387	290	233
Complete N-fertilizer	314	300	218	210

Table (5): Periodical changes in organic carbon percentage during various growth stages of wheat.

Treatments	Plant growth stage			
	Tillering	Heading	Grain formation	Maturity
Control	0.348	0.356	0.338	0.310
Biogas manure	0.954	0.846	0.807	0.765
Garbage manure	0.928	0.821	0.810	0.698
Sewage sludge manure	0.972	0.952	0.887	0.743
Azospirillum + a quarter dose N-fretilizer	0.410	0.372	0.302	0.313
Azospirillum + a half dose N-fretilizer	0.436	0.390	0.368	0.356
Complete N-fertilizer	0.468	0.452	0.338	0.325

3. Effect on total phosphorus:

Data in Table (7) clearly indicate that the total phosphorus increased with inoculation of wheat grains with *Azospirillum brasilense* and this was true in all growth stages. Taking the organic manure source into account. no difference among organic manures was obtained concerning their effect on soil phosphorus content. In addition, total phosphorus decreased with increasing the growth period and this may be due to the phosphorus consumption by cultivated plants.

Effect of organic manuring and biofertilization on chemical components of wheat plants:

Data presented in Table (8) showed the N, P and K concentrations of wheat plants during heading and maturity stages. The results indicate that N, P and K concentrations slightly differed according to the growth stage and this was true in all treatments. The inoculation of wheat grains with *Azospirillum brasilense* in the presence of a half dose of inorganic N-fertilizer gave the highest values of N, P and K concentrations at heading and maturity stages compared with the other investigated treatments. This could be attributed to the important role of *Azospirillum brasilense* in N_2-fixation process which was reflected on the nitrogen supplementation uptake by cultivated plants. With regard to the effect of organic manuring on N. P and K concentrations in wheat plants. data showed that the maximum N, P and K concentrations were obtained with sewage sludge manuring followed by biogas and garbage manuring. This may be attributed to the higher content of sewage sludge manure of N. P and K nutrients, which provides the cultivated plants with their nutritional requirements. These results are in harmony with those reported by El-Haddad *et al.*, (1986) who found that grains inoculation with non-symbiotic N_2-fixing bacteria resulted in higher nutritional contents and reduced N-requirements fertilization to 50%. Also, Gomaa (1991) reported that sewage sludge application to calcareous and sandy soils for wheat plants increased growth and yield of wheat plants.

Effect of organic manuring and biofertilization on growth characters and yield of wheat:

It is clearly observed from data in Table (9) that inoculation of wheat grains with *Azospirillum brasilense* in the presense of a half dose of inorganic N-fertilizer significantly increased all studied growth characters except the fresh weight of root system, compared with the other investigated treatments. With respect to organic manures, no significant differences were observed in spike length and number of spikelets/spike. On the other hand, there was significant differences among organic manuring treatments concerning other investigated characters. Also, data showed that wheat fertilization by inorganic-N fertilizer gave the lowest values of growth characters and grain yield. Similar results were obtained by many investigators (Ishac *et al.*, 1984; El-Haddad *et al.*, 1986; De-Freitas and Germida, 1990; Youssef *et al.*, 1993) who found that dry matter.

Table (6): Periodical changes in nitrogen forms during various growth stages of wheat (concentration, ppm).

Treatments	Plant growth stage							
	Tillering		Heading		Grain formation		Maturity	
	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Control	10	12	13	17	12	19	10	14
Biogas manure	18	22	16	24	23	48	18	26
Garbage manure	20	23	22	26	25	41	21	32
Sewage sludge manure	21	24	18	26	29	47	13	28
Azospirillum + a quarter dose N-fretilizer	26	19	25	30	34	50	28	32
Azospirillum + a half dose N-fretilizer	33	20	26	31	38	56	30	38
Complete N-fertilizer	24	20	18	24	27	36	22	24

Table (7): Periodical changes in total phosphorus during various growth stages of wheat (concentration, ppm).

Treatments	Plant growth stage			
	Tillering	Heading	Grain formation	Maturity
Control	470	440	421	366
Biogas manure	832	816	809	794
Garbage manure	842	819	810	710
Sewage sludge manure	863	860	817	783
Azospirillum + a quarter dose N-fretilizer	1732	1700	1683	1512
Azospirillum + a half dose N-fretilizer	1872	1806	1793	1680
Complete N-fertilizer	1032	1019	993	889

Table (8): N, P and K concentration (ppm) during heading and maturity stages of wheat plants.

Treatments	Ingredients					
	Nitrogen		Phosphorus		Potassium	
	Heading stage	Maturity stage	Heading stage	Maturity stage	Heading stage	Maturity stage
Control	8207	8977	892	989	11534	12790
Biogas manure	14582	14890	1745	1784	22634	22133
Garbage manure	12124	12630	1535	1598	19650	20470
Sewage sludge manure	14990	15413	1897	1951	23195	23980
Azospirillum + a quarter dose N-fretilizer	12436	12836	1574	1645	20156	20809
Azospirillum + a half dose N-fretilizer	15863	16471	1881	1958	24089	25075
Complete N-fertilizer	16688	17190	2112	2177	22047	22860

Table (9): Effect of organic manuring and biofertilization on growth characters and yield of wheat.

Treatment	Growth characters								
	Plant height (cm)	Spike length (cm)	No. of spikelets/ spike	Spike weight (gm)	Grain weight of spike (gm)	Fresh weight of root system (gm)/plant	Dry weight of root system (gm)/plant	Fresh weight of shoot system (gm)/plant	Dry weight of shoot system (gm)/plant
Control	42.33	5.00	9.00	2.36	1.46	2.19	1.70	6.10	4.77
Biogas manure	58.00	9.33	13.00	3.20	2.27	3.36	2.36	8.50	7.00
Garbage manure	64.00	8.66	14.33	3.96	3.19	3.00	2.34	9.22	6.67
Sewage sludge manure	60.66	7.66	13.00	4.53	3.44	5.24	4.37	9.36	7.46
Azospirillum + a quarter dose N-fretilizer	68.66	10.33	15.66	5.13	3.96	4.70	2.46	10.60	8.23
Azospirillum + a half dose N-fretilizer	70.66	11.33	18.33	5.46	4.36	4.33	2.31	12.01	8.80
Complete N-fertilizer	56.33	7.50	13.00	2.63	1.70	3.05	2.12	7.76	6.26
L.S.D. at 5%	3.88	1.48	2.49	0.26	0.39	0.95	0.05	0.60	0.38
L.S.D. at 1%	5.44	2.07	3.49	0.37	0.55	1.40	0.07	0.84	0.53

grain yield, plant height of wheat were significantly increased by inoculation with *Azospirillum brasilense*. In addition, Vidyorthy and Nisra (1982). Mahmoud *et al.*, (1984) and Abou-Bakr and Omar (1993) reported that the application of organic manures increased the dry matter and grains yield of wheat as well as decreasing the enormous consumption of chemical fertilizers and meanwhile minimizing health and enviromental risks both are prospectively fulfilled.

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كفاءة بعض الأسمدة العضوية والتلقيح بالأزوسبيريللام فى تسميد نبات القمح

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** معهد بحوث الأراضى والمياه والبيئة - مركز البحوث الزراعية - وزارة الزراعة - مصر.

أجرى هذا البحث بصوبة قسم النبات الزراعى بكلية الزراعة بمشتهر عام ١٩٩٤ لدراسة كفاءة إستخدام بعض الأسمدة العضوية (وهى سماد البيوجاز، سماد حمأة المجارى، سماد القمامة) والتلقيح بالأزوسبيريللام على نمو نباتات القمح صنف سخا ٨. وقد اسفرت الدراسة عن النتائج التالية:

- أدى تلقيح تقاوى القمح عند الزراعة بواسطة البكتريا المثبتة لأزوت الهواء الجوى *Azospirillum brasilense* إلى زيادة ملحوظة فى أعداد الأزوسبيريللا والبكتريا المذيبة للفوسفات فى التربة بالمقارنة بالتسميد العضوى.
- لم يكن هناك فرق كبير فى أعداد الأزوسبيريللا والبكتريا المذيبة للفوسفات بإختلاف نوع السماد العضوى.
- لوحظ إختلاف فى نشاط إنزيم الديهيدروجينيز بالتربة بإختلاف نوع السماد العضوى حيث لوحظ أعلى نشاط للإنزيم فى حالة سماد حمأة المجارى.
- أوضحت النتائج أن نسبة الكربون العضوى بالتربة زادت بإضافة الأسمدة العضوية مقارنة بالأسمدة الحيوية والمعدنية.
- بالنسبة لصور النيتروجين فى التربة (الأمونيا والنترات) فقد أسفرت الدراسة على أن التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى يزيد من محتوى التربة من الأمونيا والنترات مقارنة بالأسمدة العضوية.
- بالنسبة للفوسفور الكلى فقد أظهرت الدراسة أن التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى أدى إلى زيادة محتوى التربة من الفوسفور الكلى ولم يشاهد أى إختلافات واضحة فى محتوى التربة من الفوسفور بإختلاف نوع السماد العضوى.
- بخصوص تأثير التسميد العضوى والحيوى على المحتوى الكيماوى لنباتات القمح من العناصر فقد أوضحت الدراسة أن التلقيح بالبكتريا المثبتة لأزوت الهواء الجوى مع إضافة نصف جرعة سماد أزوت معدنى أعطى أعلى محتوى من العناصر تحت الدراسة وهى النيتروجين والفوسفور والبوتاسيوم. أما الأسمدة العضوية فقد أخذت هذا الترتيب سماد حمأة المجارى > سماد البيوجاز > سماد القمامة من حيث تأثيرها على محتوى نباتات القمح من العناصر.
- أظهرت الدراسة كذلك أن التلقيح البكتيرى فى وجود نصف جرعة سماد معدنى أدى إلى زيادة معنوية فى صفات النمو التى قدرت (طول النبات، طول السنبلة، عدد السنيبلات فى السنبلة، وزن السنبلة، وزن حبوب السنبلة، الوزن الخضرى والجاف لكل من المجموع الجذرى والخضرى لكل نبات) وكذلك المحصول مقارنا بالأسمدة العضوية والمعدنية.