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EFFECT OF USING COMPOST, MINERAL NITROGEN AND BIOFERTILIZER ON MICROBIAL POPULATION IN THE RHIZOSPHERE OF WHEAT PLANTS CULTIVATED IN SANDY SOIL.

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ABSTRACT

A pot experiment was carried out on sandy soil at Mansoura Agricultural Rresearch Station during winter 2007-2008 to study the effect of biofertilization, compost and mineral nitrogen fertilization on microbial population and mycorrhizal infection in wheat Gemmeza 69 plants. Compost supplementation (0, 5 and 10 m^3 /feddan), nitrogen mineral fertilization as ammonium sulphate (0, 60 and 120 kg N / fedden) and biofertilization using *Azotobacter Azotobacter* spp. + *Azospirillum lipoferum*, Cyanobacteria, mycorrhiza + *Azotobacter chrococcum* + *Azospirillum lipoferum* and mycorrhiza + *Azotobacter Azotobacter* spp. + *Azospirillum lipoferum* + cyanobacteria as well as the uninoculated treatmens were applied in wheat plants Gemmeza 69. Results indicated that compost supplementation increased total bacterial, *Azotobacter*, *Azospirillum* and Cyanobacteria counts as well as the percent of mycorrhizal infecetion. The same trend was observed with the nitrogen fertilizer levels. Bio-inoculation stimulated the microbial population in the rhizosphere region. The mixed inoculum of (*Azotobacter*, *Azospirillum*, Cyanobacteria and mycorrhiza) caused the highest number of total bacteria, *Azotobacter*, *Azospirillum*, and Cyanobacterial counts as well as the percent of mycorrhizal infection. The counts were high in the first sample which collected after 60 days after sowing (DAS) and in the second sample which collected 90 DAS, but counts decreased at harvesting stage. Mycorrhizal application increased the percent of infection noticed on wheat roots in comparison with non-application treatments. Inoculation with cyanobacteria increased the microbial population in comparison with control.

Keywords: Compost, Mycorrhiza, Cyanobacteria, *Azotobacter* and *Azospirillum*.

INTRODUCTION

In recent years, biofertilizers have emerges as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. However, the application of microbial fertilizers in practice, somehow, has not achieved constant effects. The mechanisms and interactions among these microbes still are not well understood, especially in real applications (Wafaa *et al*., 2006)

The use of biofertilizer in agriculture becomes unavoidable to minimize the nostop addition of high doses of chemical fertilizers in which enormous amounts of deleterious heavy metals and other environmental pollutants might be present, as well as to lower their production costs (Abd Allah *et al*., 2009)

Among cereal crops, wheat (*Triticum Aestivum*, L.) is the major and most important crop in many countries, and it is the main winter cereal crop in Egypt. In order to face the gap between consumption and production, there

are many attemps to increase wheat productivity. Nitrogen fertilizer plays an essential role in improving wheat productivity and it is considered as one of the limiting factors to achieve the high yield of wheat crop. On the other hand, application of mineral nitrogen may be results in environmental pollution. So, many efforts were done to decrease the utilization of chemical fertilizers by using biofertilizer which reduce financial costs. The beneficial effects of *Azotobacter* and *Azospirillum* are related not only to their N2-fixing efficiency but also to their ability to produce antifungal compounds, growth regulators and siderophore (Pandey and Kumar, 1989).

The aim of the work is to study the effect of biofertilization, N-fertilization and compost on some microbial groups in sandy soil.

MATERIALS AND METHODS

A pot experiment was carried out on sandy soil collected from Kalabsho and Zyaan region in Dakahleya governorate during winter 2007-2008 to determine the effect of biofertilization, organic fertilization and nitrogen chemical fertilization on microbial population of the rhizosphere of wheat plants.

Plastic pots (35 cm in diameter and 20 cm in high) were used in this experiment. A ten Kg of air dried soil was put in each pot. All pots were sown in 15/11/2007 and received 15 hat grains. Plans were thinned to reach 10 plants / pot.

Before sown, pots received compost in ratio of either 5 or 10 M^3 / feddan, phosphorus fertilization (3 gm super phosphate of 6.7 % P), 2 gm potassium sulphate (39.8 %K) and 2 gm urea (46 %N) as activated dose were added. All phosphorus and potassium doses for each pot were added at 21 days after sawing, while chemical nitrogen fertilizer (0, 60, 120 Kg N/fedden) were added in 10 equal doses.

Biofertilization treatments were included control (untreated pots), *Azotobacter* spp. + *Azospirillum* spp., Cyanobacteria, *Azotobacter* spp. + *Azospirillum* spp. + Mycorrhiza and *Azotobacter* spp. + *Azospirillum* spp. + Mycorrhiza + Cyanobacteria.

Mycorrhiza was added to each pot before sowing while *Azotobacter* and *Azospirillum* were added in two times, the first was after 15 days of sowing while the second dose was after 30 days. Cyanobacteria were added one time after 30 days of sowing (1/2 gm / pot).

Samples were collected three times after 60 days after sowing (DAS), 90 DAS and at harvesting. Samples from the rhizosphere region were obtained to carry out the following microbial analyses: a – Total bacterial count using dilution plate method and nutrient agar medium, b- enumeration density of *Azotobacter* spp. using MPN technique and medium of (Abd El-Malek and Ishak, 1968), c- enumeration density of *Azospirillum* spp. using MPN technique and semi-solid medium (Döbereiner, 1978), d- total Cyaobacterial counts using medium of N_2- fixing cyanobacteria described by (Rippika *et al*., 1979) on nitrogen free medium $BG11_0$ and e- the percent of mycorrhizal infection on wheat roots was calculated.

RESULTS AND DISCUSSION

Data presented in Table (1) show the effect of biofertilization, mineral N-fertilization and compost amendements on the total bacterial counts in the rhizosphere of wheat plant. Data showed that the highest values of bacterial numbers were obtained at 90 DAS under all applications. In all stages of growth, by increasing N-fertilizer and compost levels, the bacterial count increased. Data showed that the maximum values of bacterial counts were recorded under plants inoculated with (*Azotobacter*, *Azospirillum*, Cyanobacteria and Mycorrhizae) and supplemented with 60 kg N/feddan and 10 M^3 compost/fedden.

The high doses of N-fertilizer and /or compost exerted positive effect on the total bacterial counts. This positive effect may be due to the decrease of various nutrients in the used sandy soil, organic matter favored content the reproduction of bacteria and other organisms, it serve as a source of energy for the development of microorganisms and supply certain essential nutrient elements and compounds required by soil microorganisms. Furthermore, organic matter decomposition liberate heat which enhance the growth of microorganism. The obtained results are in harmony with those obtained by El-Sersawy (1997); Moharram (1999) and Khafagy(2003).

Data also show that at the three satges of growth, total bacteria under plant have biofertilizer were higher than those uninculated, the results appear the role of biofertilization in stimulating the growth and reproduction of microorganisms. Data showed that treatments with cyanobacteria either alone or in mixed inoculum caused highly increases in all plants either fertilized with N mineral fertilizer of not.

Results in Table (2) reveal the impact of microbial inoculation, mineral N-fertilizer and compost treatments on *Azotobacter* counts in wheat rhizosphere. Data showed that the highest value was obtained when plants received 10m^3/feddan of compost, 60 kg N/feddan and inoculated with *Azotobacter* + *Azospirillum* + cyanobacteria and mycorrhiza.

Sometimes, the counts decreased in samples of 90 DAS than the first sample at 60 DAS, while at harvest, the values were very low. Results are in accordance with those obtained by Khafagy (2003).

The negative effect of addition 120 kg N/feddan on *Azotobacter* counts is not appeared, it may be related to the decrease in N-content of the used sandy soil. Positive effect of bothe N-fertilizer and compost supplementation on *Azotobacter* counts were observed. Data are in contrast with those obtained by Fayez *et al*. (1985), Monib *et al.* (1982); Zayed (1999) and Nain *et al*. 2000.

Table 1: Effect of biofertilization, mineral N-fertilizer and compost on the total bacterial counts ×10^4 C.F.U. on the rhizosphere of wheat plants

60 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	Without	34.5	151	165.5	95	154.5	204	156.5	182	218.5
	AZ1+AZ2	53	170	197.5	149	193	243	170	187.5	238
	Cy	51	164.5	191	147	191	239.5	161	178.5	212
	AM+AZ1+Az2	58	188.5	203.5	165.5	193.5	239	193	202	238
	AM+AZ1+Az2+Cy	56.5	177	215	170	197.5	238	194.5	203	217.5
90 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	Without	27.5	78	67	120.5	146.5	146	132	130.5	152
	AZ1+AZ2	60	91	141.5	147	149	160.5	150.5	157	180.5
	Cy	49.5	83	140.5	142.5	161.5	170.5	156.5	163.5	135
	AM+AZ1+Az2	74	92.5	152	158.5	175.5	183.5	147.5	174.5	186.5
	AM+AZ1+Az2+Cy	81	94	154.5	154	172	195.5	153.5	175.5	188
At harvesting		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	Without	3.45	3.75	4.7	3.75	4.05	10.55	8.45	9.6	11.45
	AZ1+AZ2	4	4.35	5.6	4.75	5.45	11.7	9.35	11.25	12.85
	Cy	4	3.65	5.35	4.0	6.4	10.95	8.95	10.4	12.7
	AM+AZ1+Az2	4.3	4.05	5.7	4.9	6.75	12.1	9.3	12	13.3
	AM+AZ1+Az2+Cy	4.9	4.45	6.1	5.8	8.75	12.15	10.2	13.05	13.3

AZ1: *Azotobacter chrococcum*: AZ2; *Azospirillum lipoferum*; AM: mycorrhiza;
Cy: Cyanobacteria; DAS: days after sowing; C.F.U: colony forming unit

Table 2: Effect of biofertilization, mineral N-fertilizer and compost on the *Azotobacter* counts × 10^4 C.F.U. on the rhizosphere of wheat plants

60 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	1.53	13.5	15.1	18.7	25.36	34.51	23.6	23.4	24.7
	AZ1+AZ2	12.60	15.7	22.1	34.4	37.6	43.0	35.14	36.09	61.5
	Cy	8.6	15.4	18.0	18.43	27.3	37.06	23.2	30.14	40.12
	AM+AZ1+Az2	10.3	17.7	30.2	42.7	44.19	59.7	33.87	36.8	43.1
	AM+AZ1+Az2+Cy	13.3	18.4	27.5	36.1	45.8	80.1	36.45	39.45	49.5
90 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	1.19	13.44	15.3	15.2	18.67	37.4	26.9	18.9	22.3
	AZ1+AZ2	19.3	23.6	26.1	36.7	37.1	59.7	36.1	36.4	45.86
	Cy	3.51	18.4	18.6	24.62	32.07	37.5	18.8	15.22	30.5
	AM+AZ1+Az2	15.8	23.6	28.3	36.7	38.7	59.7	35.8	37.1	43.2
	AM+AZ1+Az2+Cy	10.5	26.3	30.6	28.7	39.77	51.9	36.6	37.0	45.0
At harvesting		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	0.126	0.142	0.147	0.097	0.152	0.147	0.063	0.145	0.145
	AZ1+AZ2	0.152	0.207	0.341	0.147	0.604	0.955	0.096	0.218	0.255
	Cy	0.122	0.154	0.143	0.097	0.186	0.147	0.098	0.129	0.083
	AM+AZ1+Az2	0.148	0.217	0.352	0.212	0.871	0.632	0.181	0.153	0.095
	AM+AZ1+Az2+Cy	0.130	0.281	0.351	0.174	0.633	0.942	0.143	0.148	0.294

AZ1: *Azotobacter chrococcum*: AZ2; *Azospirillum lipoferum*; AM: mycorrhiza;
Cy: Cyanobacteria; DAS: days after sowing; C.F.U: colony forming unit

Results in Table (3) show that bio-inoculation of wheat plants increased the counts of *Azospirllum* in the rhizosphere region, compared with control. The first sample 60 DAS achieved the higher numbers but in the 90 DAS and at harvesting the numbers decreased. The triple inoculation with *Azotobacter* + *Azospirillum* + Mycorrhiza caused the highest numbers in the first and second samples. Data clearly showed that the large values of *Azospirillum* numbers were obtained when inoculated or uninoculated plants received 10 m3 of compost and 60 Kg N/feddan.N-fertilizer and compost amendements through the first and the second sample have a positive effect on *Azospirillum* counts. This great response may be due to content of nutrients in compost and plant roots exudates. Similar results were obtained by El-Ghany (1996); El-Sersawy (1997) and Khafagy (2003).

Table 3: Effect of biofertilization, mineral N-fertilizer and compost on the *Azospirillum* counts counts × 10^4 C.F.U. on the rhizosphere of wheat plants

60 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	3.11	2.24	13.2	15.7	15.4	23.2	26.9	10.1	23.6
	AZ1+AZ2	18.7	12.9	18.4	19.2	16.3	49.7	22.8	23.9	23.8
	Cy	13.3	16.0	27.6	15.7	18.5	37.0	18.7	21.7	33.7
	AM+AZ1+Az2	23.8	27.1	37.6	22.2	42.7	50.6	30.3	36.6	49.0
	AM+AZ1+Az2+Cy	15.4	24.0	30.67	19.9	39.6	51.5	35.6	35.2	41.5
90 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	3.51	1.52	23.6	13.64	18.67	23.6	10.5	15.2	3.74
	AZ1+AZ2	11.9	19.3	18.4	36.7	37.5	36.7	26.1	38.7	25.3
	Cy	5.97	16.8	15.8	26.99	28.3	28.7	22.07	22.3	30.6
	AM+AZ1+Az2	18.6	23.6	26.1	37.1	39.7	45.8	25.0	24.62	35.8
	AM+AZ1+Az2+Cy	18.8	21.9	26.3	37.1	38.9	43.2	29.7	27.0	30.5
At harvesting		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	0.178	0.123	0.211	0.248	0.063	0.095	0.176	0.213	0.095
	AZ1+AZ2	0.127	0.224	0.099	0.415	0.174	0.147	0.173	0.248	0.098
	Cy	0.579	0.152	0.095	0.101	0.186	1.011	0.098	0.274	0.096
	AM+AZ1+Az2	0.223	1.158	0.143	0.265	0.424	0.179	0.174	0.223	0.151
	AM+AZ1+Az2+Cy	0.227	0.175	0.214	0.176	0.100	0.22	0.218	0.097	0.181

AZ1: *Azotobacter chrococcum*: AZ2; *Azospirillum lipoferum*; AM: mycorrhiza; Cy: Cyanobacteria; DAS: days after sowing; C.F.U: colony forming unit

Results in Table (4) show that cyanobaterial counts in wheat rhzosphere were affected by biofertilizer, N-fertilization and compost supplementation. Data showed that cyanobacteria inoculation either in single form or with onother inocula caused marked increase in its counts at sample of 60 and 90 DAS. Also slightly increases were observed with bioinoculation with *Azotobacter* + *Azospirillum* or Mycorrhiza + *Azotobacter* + *Azospirillum*. The highest counts were observed in plants treated with the previous inoculation and received N and compost doses. As noticed with other microorganisms, the counts of cyanobacteria sharply decreased at the harvesting stage. Obtained results are in harmony with those obtained by Abd-El-Rasoul *et al.* (2004) who found that cyanobacteria treatments enhanced the numbers of total bacteria, total cyanobacteria, Actinomycetes and soil fungi and caused increase in soil biological activity.

Table 4: Effect of biofertilization, mineral N-fertilizer and compost on the cyanobacterial counts counts × 10^2 on the rhizosphere of wheat plants

60 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	2.5	3.5	1.5	8	4.5	10.5	5	7	11
	AZ1+AZ2	3.5	0.5	4	7	6.5	7	4	4	7
	Cy	11.5	17.5	21.5	33.5	34.5	29.5	35.5	35	41
	AM+AZ1+Az2	5	4.5	2.5	5	1	10.5	12.5	9.5	7
	AM+AZ1+Az2+Cy	18.5	14.5	13.5	28	35.5	37.5	33.5	38.5	40
90 DAS		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	3.5	7	3	11.5	1	4	13	10.5	7.5
	AZ1+AZ2	4.5	7	1.5	10	7	5.5	6.5	8	3.5
	Cy	27.5	22	18	34.5	33.5	34	30	37	38.5
	AM+AZ1+Az2	10	7	8	12.5	6.5	4	3.5	5.5	7.5
	AM+AZ1+Az2+Cy	21.5	24.5	19.5	29.5	32	31.5	34.5	37	39
At harvesting		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	0.5	0.95	0.6	0.55	0.85	0.75	0.75	1.15	0.4
	AZ1+AZ2	1	0.65	0.95	1.15	1.05	1.05	1.45	0.75	0.7
	Cy	1.25	1.35	2.45	2.95	2.85	2.65	3.95	3.55	3.1
	AM+AZ1+Az2	0.65	0.35	0.6	0.8	0.9	0.95	1.05	0.6	0.65
	AM+AZ1+Az2+Cy	1.65	1.85	2.35	3.55	2.6	2.4	4.5	3.75	3.55

AZ1: *Azotobacter chrococcum*: AZ2; *Azospirillum lipoferum*; AM: mycorrhiza; Cy: Cyanobacteria; DAS: days after sowing; C.F.U: colony forming unit

Results in Table (5) show the effect of biofertilization, nitrogen and compost supplementation on percent of mycorrizal colonization on roots of wheat plants. Data reveal that biofertilization caused increase in root infection of plants compared with the un-inoculated plants. *Azotobacter* and *Azospirillum* as well as cyanobacteria increased the level of colonization under different levels of nitrogen or / and compost supplementation. The mixed inoculum caused over-increase in the percent of colonization. The essential synergestic effect of biofertilization and mycorrhiza inoculation increased the percent of mycorrhizal infection. The highest percent of infection were obtained in plants inoculated with Mycorrhiza + *Azotobacter* + *Azospirillum* + cyanobacteria.

Table 5: Effect of biofertilization, mineral N-fertilizer and compost on the percent of mycorrhizal infection on the roots of wheat platns

		0 kg N/feddan			60 kg N/feddan			120 kg N/feddan		
Compost m^3/ feddan		0	5	10	0	5	10	0	5	10
Biofertilizer	without	6.66	22.22	31.11	24.44	15.55	31.11	22.22	24.44	33.33
	AZ1+AZ2	8.88	17.77	24.44	31.11	31.11	26.66	24.44	37.77	35.55
	Cy	13.33	17.77	35.55	20	26.66	24.44	13.33	31.11	17.77
	AM+AZ1+Az2	64.44	71.11	77.77	77.77	71.11	86.66	91.11	73.33	84.44
	AM+AZ1+Az2+Cy	60	86.66	82.22	73.33	68.88	84.44	71.11	80	75.55

AZ1: *Azotobacter chrococcum*, AZ2; *Azospirillum lipoferum*; AM: mycorrhiza; Cy: Cyanobacteria

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تأثير إستخدام الكمبوست، التسميد النيتروجينى والتسميد الحيوى على التعداد الميكروبى فى ريزوسفير القمح المنزرع فى أرض رملية

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أجريت تجربة أصص فى مزرعة كلية الزراعة – جامعة المنصورة على أرض رملية جمعت من منطقة قلابشو وزيان بمحافظة الدقهلية لدراسة تأثير التلقيح الحيوى والتسميد المعدنى النيتروجينى وكذلك إضافة الكمبوست على الكائنات الحية الدقيقة فى منطقة الريزوسفير لنبات القمح جميزة 69. وقد استخدمت فى التسميد الحيوى المعاملات التالية :

١ - بدون تسميد حيوى .
٢ - لقاح الأزوتوباكتر ولقاح الأزوسبيريللم.
٣ - لقاح السيانوبكتيريا.
٤ - لقاح الميكوريزا مع الأزوتوباكتر والأزوسبيريللم.
٥ - لقاح الميكوريزا مع الأزوتوباكتر والأزوسبيريللم والسيانوبكتيريا.

واستخدم التسميد المعدنى بإضافة كبريتات الأمونيوم على 10 دفعات بمعدلات صفر و 60 و 120 كجم نيتروجين للفدان. وكانت معدلات الكمبوست صفر و5 و 10 متر مكعب للفدان.

أوضحت النتائج زيادة فى أعداد البكتيريا فى منطقة الريزوسفير مع كل المعاملات حيث أدى التسميد الحيوى وكذلك المعدنى والكومبوست إلى زيادة الأعداد.

أيضا لوحظت زيادة ملحوظة فى أعداد الأزوتوباكتر والأزوسبيريللم نتيجة التلقيح الحيوى بالسيانوبكتيريا. كما زادت أعداد المجاميع البكتيرية المختلفة خلال 60 و 90 يوم بعد الزراعة بينما انخفضت فى مرحلة الحصاد.

زادت نسبة العدوى بالميكوريزا فى جذور نباتات القمح أثر معاملة التربة بالميكوريزا وأدت المعاملة الميكروبية لزيادة هذه النسبة.

قام بتحكيم البحث

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