Impact of Cyanobacteria, Humic Acid and Nitrogen Levels on Maize (*Zea Mays* L.) Yield and Biological Activity of the Rhizosphere in Sandy Soils

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Abstract: A field trial was conducted in EL-Ismailia Research Station, Agricultural Research Center (ARC), Egypt during two successive summer seasons of 2010 and 2011 on maize crop hybrid single cross 10 to find out the impact of cyanobacteria (CB) and humic acid (HA) forms applied with different methods under three nitrogen fertilizer levels on maize yield and yield attributes, as well as, their effect on the biological activity of the soil around the rhizosphere of maize plants. Results revealed that in both tested seasons, all yield attributes were significantly affected in the first season, whereas, no significant response detected in the second season. The use of either CB or HA accelerated days to 50 % tasseling and silking in the first season, which were earlier than those recorded in the second season. In both tested seasons, the use of CB (dry and spray) along with 120 kg N fed^{-1} (One hectare = 2.4 feddan) gave significantly maize grain yield that was not significantly different from that recorded by the use of 150 Kg N fed^{-1} alone (full recommended N dose). Also, the use of either CB or HA increased the soil biological activity of the plants rhizosphere in terms of total count bacteria, carbon dioxide evolution, dehydrogenase activity (DHA) and nitrogenase activity. Generally, the application of CB and HA may result in the reduction for chemical fertilizers.

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1. Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops grown in Egypt. Maize grain is used for both human consumption and animal feeding. It has a great utility in agro-industrial production. This crop has much higher grain protein content than our staple food rice. Based on area and production, maize is the 3rd most important cereal crop after wheat and rice in the world **(Tollenaar and Dwyer, 1999).** Increasing maize production became one of the most important goals of the world to face human and animal demands. It has a great nutritional value as it contains about 66.7 % starch, 10% protein, 4.8% oil, 8.5 % fiber, 3% sugar and 7 % ash **(Chaudhary, 1983)**. Intensive farming practices that aims to produce higher yield, require extensive use of agro-chemicals which are costly and create environmental pollutions **(Kozdro *et al*., 2004).** Nitrogen is required in large quantities for plants to grow and is mainly provided in the form of synthetic chemical fertilizers. Such products pose a health hazard and microbial population problem in soil, besides making the production cost is high **(Badran and Safwat, 2004).** The use of the conventional chemical farming methods, which substantially increased crop production, was once regarded as a kind of agricultural revolutions, which would solve all problems relating to producing sufficient food for the ever growing world population. However, this belief was later over-shadowed by the emergence of numerous environmental and social problems associated with the heavy use of agrochemicals in intensive farming systems. Conventional farming methods are generally associated with degradation of the environment.

Increasing prices of agrochemicals especially nitrogen often leaves farmers with low profit. Uncertainly the availability of those agrochemicals, especially in the developing countries such as Egypt, is often a serious constraint for the farmers in their attempt to increase crop production. Such problems have directed the attention of the agriculturists world-wide to seek alternative methods of farming. The use of nitrogen fixing cyanobacteria ensures saving entirely or partially the mineral nitrogen required in crop production. Recently, there is a great deal of interest in creating novel association between agronomically important plants, partially cereals such wheat, maize and N_2-fixing microorganisms including cyanobacteria **(Spiller *et al*., 1993).** Biofertilizers are able to fix atmospheric nitrogen in the available form for plants **(Chen, 2006)**. Positive response of maize to nitrogen fertilizer has been reported by **Aflakpui *et al.***

(1997). Many attempts have been tried to replace a part of those harmful fertilizers by biofertilizers in maize to get yield of a good quality without loss in its quantity **(El-Kholy *et al*., 2005)**. Diazotrophs such as *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas* and cyanobacteria frequently colonize the important cereal crops including wheat, rice and maize and promote plant growth by producing certain Plant growth promoting regulators (PGPR) **(Malik *et al*., 1994 and Rashid *et al*., 2007)**. On the other respect, crop yield and its contributing factors are under the influence of different factors as use of a suitable and balanced use fertilizer is one of these. Crop fertilizer needs can be met with the addition of organic fertilizer, which is favorable to be used in poor and marginal soils. **Katkat *et al.* (2009)** illustrated that the application of solid humus at the rate of 1 g kg^{-1} gave the highest wheat dry weight and nutrients uptake in calcareous and greenhouse condition with foliar application. They added that foliar application of humic acid had statistically a positive significant effect on Mg, Fe, and Mn uptake, As well as humic acid raised the wheat dry weight and N, P, K, Ca, Mg,Na, Fe, Cu Zn and Mn uptake of plants. **Aref and EL-Kassas (2006)** in a field experiment on maize found that inoculation with cyanobacteria combined with 50% N increased significantly maize yield and its components over the control treatment (100% N only). They also added that cyanobacteria inoculation enhanced significantly any of soil total bacterial count, cyanobacteria count, amount of CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control treatment without inoculation.

The objective of the current work is to study the impact of cyanobacteria and humic acid forms applied with methods under different nitrogen fertilizer levels on maize yield and its attributes, as well as, their effect on the biological activity of the soil around the rhizosphere maize plants.

2. Materials and Methods

A field trial was conducted in Ismailia Agricultural Research Station (Latitude 30° 35′ 41. 901″ N and Longitude 32° 16′ 45. 843″ E), Agricultural Research Center (ARC), Egypt during two successive summer seasons of 2010 and 2011 on maize crop hybrid single cross 10 which was kindly provided by Maize Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. This study was performed in sandy soil, the soil properties illustrated in Table (1) according to **Page *et al.* (1982)**.

Cyanobacteria were provided by Agricultural Microbiology Department, Soils, Water & Environment Research Institute., ARC, Giza, Egypt. Cyanobacteria were applied as culture filtrate that contains a mixture of different CB strains, i.e., *Nostoc muscorum*, *Anabaena oryzae* , *Tolopothrix tenius and Anabaena fertilissima* to obtain the cyanobacteria culture filtrate, each CB strain was grown and propagated for 5 weeks on BG 11_0 medium described by **Allen and Stanier (1968)**. The developed cyanobacterium cultures were centrifuged (3000 rpm min^{-1}) and the supernatant were used as CB filtrate by mixing the supernatant for each strain together to have the CB culture filtrate. The filtrate was used in soaking treatment for maize grains before planting and to be used also as a foliar spray at the rate of 40 L fed^{-1}. As well as, these CB were also used as dry inoculum prepared according to **Venkataraman (1972)** to be used as side dressing (dry inoculum) along the rows of maize plants. Three levels of nitrogen, i.e., 90, 120 and 150 (Kg fed^{-1}) in the form of ammonium nitrate (33.5% N) were applied in eight equal split doses, the first was added at just after thinning, the rest were added weekly up to 60 days after planting (DAP).

Table (1): Some chemical and physical analyses of the investigated soil

pH (1:2.5) soil suspension	EC dSm^{-1} (Soil paste)	Soluble cations ($mmol_c$ L^{-1})				Soluble anions ($mmol_c$ L^{-1})			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
8.14	1.04	4.56	2.60	3.07	0.36	--	6.60	2.83	1.16

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	Texture class
85.18	10.17	2.35	2.30	1.50	Sandy

Available N (mg Kg^{-1})	Available P (mg Kg^{-1})	Available K (mg Kg^{-1})
20	4	49

Experimental design was split-plot where CB treatments were assigned to main plots and nitrogen rates in the sub plots. Plot size was 5 rows, 6m in length, 80 cm in width, and 20cm between hills. One blank row is left between each treatment. Phosphorus at a rate of 30 Kg P_2O_5 fed^{-1} in the form of superphosphate (15 % P_2O_5) and Potassium at a rate of 24 Kg K_2O fed^{-1} in the form of potassium sulphate 48 % K_2O were added before planting. Soil samples (0.5 Kg) were taken from the experimental site before planting for chemical, physical and biological analysis. All other cultural practices were applied as recommended. The experiment comprises the following treatments:

A. Nitrogen treatments:

1) 90 kg N fed^{-1}.

2) 120 kg N fed^{-1}.
3) 150 kg N fed^{-1}.
1 hectare = 2.4 feddan.

B. Cyanobacteria and humic acid treatments:

1) Soaking grains in CB filtrate for 24 h then sprayed at 30 days from planting.
2) Soaking grains in HAs for 24 h then sprayed at 30 days from planting.
3) Nitrogen levels (90, 120 and 150 Kg N fed^{-1}) + side dressing of CB along the row (dry) then sprayed at 30 days from planting.
4) Soaking grains in a mixture of CB filtrate and humic acid then sprayed at 30 days from planting.
5) Control untreated.

Data recorded for maize crop for both tested seasons were number of days from planting to 50% tasseling and to 50% silking, plant height and ear height (cm), number of row ear^{-1}, ear length (cm), ear diameter (cm), number of grains per row, cob diameter (cm) and grain yield (ardab fed^{-1}).

Data for soil biological activity of rhizosphere maize plants for both tested seasons were determined in soil samples collected from maize rhizosphere soil at 50 and 70 days from planting in terms of nitrogenase activity (**Hardy *et al*., 1973)**, dehydrogenase activity (DHA) (**Casida *et al*., 1964)**, carbon dioxide evolution (**Pramer and Schmidt, 1964)** and total count bacteria (**Allen, 1959).**

Statistical analysis:

Statistical analysis of the data was performed according to **Steel and Torrie (1980)** by using the analysis of variance test (ANOVA) through SAS statistical computer software program**.**

3. Results

Maize growth characters

The effect of CB inoculation, HA application and nitrogen fertilization on some maize growth character under different nitrogen levels in two seasons of 2010 and 2011 is shown in Tables (2 & 3). In both tested seasons, results showed that all the studied parameters were significant in the first season but in the second season they were not. However, days to 50% tasseling and days to 50% silking had not significantly been affected by the tested treatments. Days to 50% tasseling and silking in the first season were earlier than those recorded in the second season. However, days to 50% tasseling ranged from 56.30 to 60 days in the first season against 63.00 to 65.30 days in the second season. Also, days from planting to 50% silking ranged from 59.00 to 62.00 days in the first season against 63.80 to 67.50 days in the second season. For plant height, the effect of the tested treatments was significant in the first season only. Increasing the nitrogen levels up to 150 kg N fed^{-1} without any of CB inoculation or HA increased the plant height to 317 cm in the first season.

Table (2): Effect of cyanobacteria and humic acid treatments under different nitrogen rates on some maize growth characters in 2010 season

Treatments	N Rates (Kg N fed^{-1})	Days to 50% tasseling	Days to 50% silking	Plant height (cm)	Ear height (cm)
Soaking Seeds in *CB. filtrate	90	59.30	61.70	268	145
	120	59.30	61.30	292	152
	150	58.30	61.00	298	158
Seed soaking in humic	90	58.00	60.30	303	160
	120	59.00	61.30	273	143
	150	57.30	58.70	303	167
Dry CB. + CB. spray	90	60.00	62.00	307	162
	120	58.00	60.70	300	160
	150	58.70	60.70	305	160
Soaking in humic + CB. filtrate	90	56.30	58.30	303	163
	120	58.30	60.30	317	165
	150	58.00	60.00	310	163
Control	90	57.00	59.00	285	158
	120	59.00	61.00	310	162
	150	58.70	60.30	317	160
L. S. D.		2.71	2.69	35.51	17.30

***CB = Cyanobacteria.**

Table (3): Effect of cyanobacteria and humic acid treatments under different nitrogen rates on some maize growth characters in 2011 season

Treatments	N Rates (Kg N fed^{-1})	Days to 50% tasseling	Days to 50% silking	Plant height (cm)	Ear height (cm)
Soaking Seeds in *CB. filtrate	90	63.00	63.80	270	133
	120	63.80	65.80	307	153
	150	63.30	65.50	307	160
Seed soaking in humic	90	63.30	64.80	267	130
	120	63.50	64.80	294	140
	150	64.30	65.50	291	145
Dry CB. + CB. spray	90	63.50	64.80	272	128
	120	63.50	65.00	294	139
	150	64.50	66.80	296	146
Soaking in humic + CB. filtrate	90	63.80	65.00	255	127
	120	63.00	64.30	277	136
	150	65.00	67.30	273	128
Control	90	64.00	65.80	283	140
	120	64.00	64.30	309	157
	150	65.30	67.50	303	151
L. S. D.		NS	NS	NS	NS

***CB = Cyanobacteria.**

This value was significantly higher than 268 cm recorded by the treatment received 90 kg N fed^{-1} + soaking in CB filtrate. The tallest was not significantly different from those recorded by the other tested treatments. In the second season, the tallest plant of 309 cm was attained by 120 kg fed^{-1}. Due to ear heights, their values were significantly affected by the tested treatments in the first season only. However, the ear height ranged between 143 cm (120 kg N fed^{-1} + soaking in humic) and 165 cm (120 kg N fed^{-1} + soaking in humic + CB. filtrate) in the first season against 128 cm (150 kg N fed^{-1} + soaking in humic + CB. filtrate) and 160 cm (150 kg N fed^{-1} + CB. filtrate) in the second season. It is worthy to note that the treatments received 90 or 120 kg N fed^{-1} supplemented with CB or HA both applied with different methods gave values slightly less than those given by those recorded by the control treatment (150 kg N fed^{-1}).

Maize yield and yield attributes

The effect of CB inoculation and HA application on maize yield and yield attributes under different nitrogen levels in two seasons of 2010 and 2011 is shown in Tables (4 & 5). In both tested seasons, results showed that increasing the nitrogen level from 90 to 150 kg N fed^{-1} increased maize yield and its attributes. Grain yield is the main target of crop production. During the first season, the use of 150 kg N fed^{-1} without CB or HA increased significantly maize grain yield (31.03 ardab fed^{-1}) compared to that recorded by the use of 90 kg N fed^{-1} (26.70 ardab fed^{-1}), while this high grain yield was not significantly different from that recorded by 120 kg N fed^{-1} (29.13 ardab fed^{-1}).

Meanwhile, in the second season, no significant differences were recorded for maize grain yield in response to the use of 90, 120 or 150 kg N fed^{-1}. However, the other tested maize yield attributes exhibited the same trend noticed in maize grain yield in response to the use of different tested nitrogen levels in both seasons. For instance, the highest number of rows $ear^{-1}$and number of grains row^{-1} in both tested seasons was recorded by the treatment of 120 kg N fed^{-1} + dry CB + CB spray. The corresponding values were 13.20 and 45.80 in the first season against 12.80 and 41.10 in the second season.

On the other hand, the use of either CB or HA under different nitrogen levels led to increase maize grain yield and its attributes without any significant differences than those recorded by the control treatment (nitrogen levels only) in both tested seasons. The highest maize grain yield of 39.77 and 33.80 ardab fed $^{-1}$ in first and second seasons was corresponded to the use of 150 and 120 kg N fed^{-1} + soaking in CB filtrate and HA, respectively. Generally, the use of CB as seed soaking in addition to seed soaking in HA was better than the use of the other tested treatments under different nitrogen levels in both tested seasons.

Table (4): Effect of cyanobacteria and humic acid under different nitrogen rates on maize grain yield and yield attributes in 2010 season

Treatments	N rates (Kg N fed^{-1})	Grain yield (ard fed^{-1})	Ear length (cm)	Ear diameter (cm)	Cob diameter (cm)	Kernel depth (cm)	Number of rows ear^{-1}	Number of grain row^{-1}
Seed soaking in *CB. filtrate	90	26.18	16.00	4.06	2.43	0.81	12.80	39.40
	120	29.84	17.80	4.33	2.55	0.89	13.10	43.90
	150	30.54	18.40	4.25	2.41	0.92	12.70	44.70
Seed soaking in humic	90	27.16	16.30	4.10	2.39	0.86	12.80	39.70
	120	34.51	18.20	4.17	2.48	0.84	12.50	43.10
	150	36.15	18.50	4. 23	2.39	0.92	12.80	45.00
Dry CB. + CB. spray	90	28.43	16.70	4.14	2.37	0.89	12.40	42.00
	120	34.41	18.90	4.56	2.63	0.97	13.20	45.80
	150	33.98	18.30	4.37	2.53	0.92	13.20	44.70
Seed soaking in humic + seed soaking in CB. filtrate	90	30.37	17.30	4.20	2.43	0.88	12.50	43.10
	120	36.07	18.90	4.30	2.43	0.94	13.00	45.90
	150	39.77	18.60	4.38	2.55	0.92	13.30	45.80
Control	90	26.70	16.90	4.21	2.48	0.86	12.70	39.50
	120	29.13	18.00	4.34	2.50	0.92	12.70	43.30
	150	31.03	18.90	4. 25	2.41	0.92	12.70	44.70
L. S. D.		3.99	1.01	0.22	0.19	0.08	NS	2.17

***CB = Cyanobacteria. 1 ardab maize grains = 140 Kg at 15.5% moisture.**

Table (5): Effect of cyanobacteria and humic acid under different nitrogen rates on maize grain yield and yield attributes in 2011 season

Treatments	N rates (Kg N fed^{-1})	Grain yield (ard fed^{-1})	Ear length (cm)	Ear diameter (cm)	Cob diameter (cm)	Kernel depth (cm)	Number of rows ear^{-1}	Number of grain row^{-1}
Seed soaking in *CB. filtrate	90	19.40	17.20	4.25	2.41	0.92	12.40	35.40
	120	21.60	20.00	4.65	2.55	1.05	12.0	44.50
	150	28.90	20.20	4.84	2.57	1.14	12.40	48.90
Seed soaking in humic	90	21.50	17.50	2.28	2.43	0.92	12.20	36.90
	120	26.30	19.90	4.60	2.51	1.04	12.30	43.50
	150	27.50	20.10	4.68	2.52	1.08	12.40	47.0
Dry CB. + CB. spray	90	22.90	17.90	4.36	2.46	0.95	12.20	37.80
	120	25.80	19.70	4.47	2.62	0.93	12.60	42.10
	150	31.20	20.00	4.61	2.56	1.03	12.80	46.0
Seed soaking in humic + seed soaking in CB. filtrate	90	23.30	18.20	4.40	2.39	1.01	12.30	38.30
	120	30.90	19.60	4.58	2.47	1.05	12.10	41.20
	150	33.80	20.00	4.50	2.50	1.00	12.60	45.40
Control	90	18.90	18.10	4.18	2.39	0.90	12.20	33.40
	120	24.30	18.80	4.50	2.50	1.00	12.10	40.60
	150	27.70	19.10	4.47	2.45	1.01	12.10	44.20
L. S. D.		4.08	1.26	0.28	NS	0.14	0.51	3.95

***CB = Cyanobacteria. 1 ardab maize grains = 140 Kg at 15.5% moisture.**

Soil biological activity of maize plants rhizosphere

The effect of CB inoculation and HA application under nitrogen fertilization rates on the soil biological activity of maize plants rhizosphere under different nitrogen levels after 50 and 70 days from planting in two seasons of 2010 and 2011 is shown in Tables (6, 7, 8 & 9). The soil biological activity for maize plants rhizosphere was assessed in terms of total count bacteria, carbon dioxide evolution, dehydrogenase (DHA) and nitrogenase activities**.** Results revealed that any of HA and CB applied in any form under different levels of nitrogen increased the tested soil biological activity items examined either at 50 or 20 days from planting in both tested seasons. However, the values recorded after 50 days were slightly higher than those recorded at 70 days in both seasons. The treatments that received CB soaking, dry or spray forms applied each alone or combined with any form of HA gave higher soil biological activity than those received any form of HA alone. The highest values of the tested soil biological activity items were recorded by the treatment received 120 kg N fed^{-1} + dry CB + CB spray, at 50 and 70 days compared to those received any level of nitrogen alone in both seasons. In correspondence, at 50 days, the highest values of the soil biological activity values were 28 x105 cfu g rhizosphere soil^{-1} (total bacteria count), 490.28 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1} (CO_2 evolution), 390 mg TPF g dry rhizosphere soil^{-1} day^{-1}(DHA) and 30.14 mmol C_2H_2 g dry rhizosphere $soil^{-1}$ day^{-1} (N-ase) in the first season. At 50 days, in the second season, the highest values of the soil biological activity values were 25 $x10^5$ cfu g rhizosphere $soil^{-1}$ (total bacteria count), 480.43 mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1} (CO_2 evolution), 370 mg TPF g dry rhizosphere $soil^{-1}$ day^{-1}(DHA) and 27.65 mmol C_2H_2 g dry rhizosphere $soil^{-1}$ day^{-1} (N-ase).

Due to the soil biological activity at 70 days, the recorded values were slightly lower than those recorded at 50 days. However, in the first season, the highest values of the soil biological activity values were 24 $x10^5$ cfu g rhizosphere $soil^{-1}$ (total bacteria count), 398.45 mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1} (CO_2 evolution), 372 mg TPF g dry rhizosphere $soil^{-1}$ day^{-1}(DHA) and 18.35 mmol C_2H_2 g dry rhizosphere $soil^{-1}$ day^{-1} (N-ase). In the second season, the highest values of the soil biological activity values were 23 $x10^5$ cfu g rhizosphere $soil^{-1}$ (total bacteria count), 365.64 mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1} (CO_2 evolution), 358 mg TPF g dry rhizosphere $soil^{-1}$ day^{-1}(DHA) and 26.12 mmol C_2H_2 g dry rhizosphere $soil^{-1}$ day^{-1} (N-ase).

Generally, the use of 120 kg N fed^{-1} combined with dry CB + CB spray gave the highest maize soil rhizosphere biological activity compared to all tested treatments including all examined nitrogen levels applied alone in both seasons.

Table (6): Effect of CB and HA treatments under different nitrogen rates on soil biological activity in 2010 season (50 days after planting)

Treatments	N Rates (Kg N fed^{-1})	Total count bacteria X 10^5 **cfu g dry hizosphere $soil^{-1}$	CO_2 evolution (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})	***DHA (mg TPF**** g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})	*****N-ase activity (mmol C_2H_4 g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})
Seed soaking in *CB. filtrate	90	15	150.12	134	20.16
	120	21	320.17	230	25.30
	150	17	300.24	146	23.12
Seed soaking in humic	90	13	115.26	123	18.20
	120	18	285.65	290	21.16
	150	15	210.45	232	18.50
Dry CB. + CB. spray	90	21	450.18	315	25.12
	120	28	490.28	390	30.14
	150	23	410.09	290	21.16
Seed soaking in humic + seed soaking in CB. filtrate	90	19	320.25	280	23.25
	120	23	395.76	360	29.17
	150	20	310.22	250	22.25
Control	90	10	100.56	90	16.30
	120	15	140.25	130	20.12
	150	13	110.35	100	17.25

CB = Cyanobacteria. **cfu = Colony formed per unit. ***DHA = Dehydrogenase activity. * TPF= Triphenyl formazan.**
******* N-ase = Nitrogenase activity.**

Table (7): Effect of cyanobacteria and humic acid under different nitrogen rates on soil biological activity in 2011 season (50 days after planting)

Treatments	N Rates (Kg N fed^{-1})	Total count bacteria X 10^5 **cfu g dry hizosphere $soil^{-1}$	CO_2 evolution (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})	***DHA (mg TPF**** g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})	*****N-ase activity (mmol C_2H_4 g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})
Seed soaking in *CB. filtrate	90	13	110.05	120	18.25
	120	18	220.17	215	23.35
	150	15	190.136	106	20.46
Seed soaking in humic	90	11	90.18	110	16.28
	120	14	165.89	270	19.15
	150	13	116.45	210	17.62
Dry CB. + CB. spray	90	19	250.18	300	23.14
	120	25	480.43	370	27.65
	150	20	275.00	245	18.56
Seed soaking in humic + seed soaking in CB. filtrate	90	17	410.56	260	19.12
	120	21	375.89	320	23.44
	150	18	345.14	222	17.15
Control	90	8	112.56	80	14.27
	120	13	145.12	120	17.83
	150	11	118.23	93	12.75

CB = Cyanobacteria. **cfu = Colony formed per unit. ***DHA = Dehydrogenase activity. * TPF= Triphenyl formazan.**
******* N-ase = Nitrogenase activity.**

Table (8): Effect of cyanobacteria and humic acid under different nitrogen rates on soil biological activity in 2010 season (70 days after planting)

Treatments	N Rates (Kg N fed^{-1})	Total count bacteria X 10^5 **cfu g dry hizosphere $soil^{-1}$	CO_2 evolution (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})	***DHA (mg TPF**** g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})	*****N-ase activity (mmol C_2H_4 g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})
Seed soaking in *CB. filtrate	90	13	120.65	123	11.22
	120	18	310.78	215	14.17
	150	16	275.35	137	12.25
Seed soaking in humic	90	10	100.46	98	08.12
	120	15	265.65	283	12.85
	150	12	200.65	217	10.15
Dry CB. + CB. spray	90	19	330.18	312	14.25
	120	24	398.45	372	18.35
	150	20	325.18	265	15.75
Seed soaking in humic + seed soaking in CB. filtrate	90	18	315.25	245	14.12
	120	21	332.45	290	17.13
	150	19	290.35	245	15.12
Control	90	11	110.03	78	10.16
	120	13	125.12	103	13.96
	150	09	98.85	85	11.15

CB = Cyanobacteria. **cfu = Colony formed per unit. ***DHA = Dehydrogenase activity. * TPF= Triphenyl formazan. ***** N-ase = Nitrogenase activity.**

Table (9): Effect of cyanobacteria and humic acid under different nitrogen rates on soil biological activity in 2011 season (70 days after planting)

Treatments	N Rates (Kg N fed^{-1})	Total count bacteria X 10^5 **cfu g dry hizosphere $soil^{-1}$	CO_2 evolution (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})	***DHA (mg TPF**** g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})	****N-ase activity (mmol C_2H_4 g^{-1} dry rhizosphere $soil^{-1}$ day^{-1})
Seed soaking in *CB. filtrate	90	11	100.85	117	16.30
	120	16	285.25	200	18.17
	150	15	250.45	125	14.25
Seed soaking in humic	90	10	95.55	100	14.35
	120	14	165.65	274	16.27
	150	12	135.45	198	13.15
Dry CB. + CB. spray	90	18	320.18	300	22.16
	120	23	385.64	358	26.12
	150	19	333.26	247	17.15
Seed soaking in humic + seed soaking in CB. filtrate	90	16	325.32	235	16.15
	120	20	347.89	282	19.33
	150	19	300.15	222	14.13
Control	90	09	092.18	68	14.00
	120	11	113.02	96	16.25
	150	10	100.16	65	13.12

CB = Cyanobacteria. **cfu = Colony formed per unit. ***DHA = Dehydrogenase activity. * TPF= Triphenyl formazan ***** N-ase = Nitrogenase activity.**

4. Discussion

Maize yield and yield attributes

The results obtained from this study showed that elevating nitrogen level from 90 to 150 kg fed^{-1} enhanced the grain yield of maize. In this concern, many workers pointed out this phenomenon. **Dahmardeh (2011)** found that increasing nitrogen fertilization up to 300 kg ha^{-1} increased significantly all the studied parameters of maize yield. **Hokmalipour and Darbandi (2011)** confirmed that in maize field trial, increasing nitrogen levels up to 180 kg ha^{-1} increased the harvest index, kernels yield, 1000-kernels weight and numbers of kernels per ear and rows per ear numbers. They explained that increasing nitrogen fertilization rates led to a significant increase in 100- grain weight and grain yield of maize as compared with control treatment. Nitrogen application significantly resulted in increasing the number of grains per ear, 100-grain weight and grain yield. They mentioned that the variation in grain yield due to different levels of nitrogen is related to the differences in size of photosynthetic surface and to the relative efficiency of total sink activity. All treatments received CB soaking, spray or dry inoculation enhanced significantly maize grain yield and its attributes over those soaked in humic only, soaked in a mixture of humic and CB filtrate or control treatments (nitrogen levels only). Cyanobacteria are amongst the array of biofertilizers developed for different crops, cyanobacteria are popularly known as blue green algae, constitute the most important inputs in rice cultivation and recently in maize **(Aref and EL-Kassas, 2006)** and wheat **(EL-Ayouty *et al*., 2012)**. Cyanobacteria form an inexpensive farm grown input, which helps in a better crop nutrient management, while working in perfect harmony with nature. Cyanobacteria also fix the atmospheric nitrogen in soil under extreme conditions and add organic matter, synthesize and liberate amino acids, vitamins and auxins and exo-polysaccharides reduce oxidizable matter content of the soil, provide oxygen to the submerged rhizosphere, ameliorate salinity, buffer the pH, solubilize phosphates and increase the efficiency of fertilizer use in crop plants and in turn, enhance and improve quality and quantity of crop yield and yield attributes **(Kaushik, 2004)**. Maize inoculation with the cyanobacteria resulted in improved maize growth and nitrogen uptake **(Maqubela and Mnkeni, 2009)**. They explained that the improved growth appeared was related to the increase in soil N and favorable mineralization due to the increase of soil carbon and aggregate stability. This led to improve water holding and infiltration capacities of the soil, and potentially the plant water use efficiency from the soil. However, water was not a limiting factor. So the improvement in maize yields could not be attributed to improved water use efficiency but rather to improved soil N levels. Such an effect could, however¸ translate to improved water retention and use efficiency under water scarce condition that often prevail in sandy soil. The use of CB as basal inoculum beside CB as foliar spray as biofertilizer for maize plants **(Subramaniyan and Malliga, 2011)** increased significantly the morphological, biochemical parameters such as plant height, number of leaves, internodes, silk, total length of leaves, shoot, tassel, width of leaves, dry weight of shoot, silk, root and yield. They explained that the presence of micro, macro nutrients and plant growth hormones found in CB spray and CB-basal induced morphological parameters on spray and basal treated plants compared to control.

The manifold significance of HA application to plants is now well established. The treatment received seed soaking in HA + seed soaking in CB filtrate in both seasons gave days of 50 % to tasseling, 50 % to silking, plant height, ear length and grain yield as well as the other yield attributes were not significantly different than those recorded by the treatments received either 120 kg N fed^{-1} + seed soaking in CB + CB spray or 150 Kg N fed^{-1} only. These results are similar to those obtained by **Youssef *et al*. (2011)** who revealed that application of HA enriched with CB increased significantly yield components of barley and faba bean crops as well as their total contents of macro and micro nutrients. They explained that the use of HA enriched with CB was superior in decreasing values of EC in soils and increased values of both organic matter and available macronutrients in the soil. These effects led to increase yields of both barley and faba bean crops. Also, HA effects on plant physiology are mainly positive, and they include enhancement of biomass yields, induction of lateral roots emergence, ATPase activity, increase of cell respiration and membrane uptake of nutrients, and exertion of hormone-like activities **(Puglisi *et al*., 2009). Celik *et al.* (2010)** explained that humus had beneficial effects on nutrient uptake, transport and availability to maize plant that enhances the maize plant growth and increases maize yield. They also added that the use of humus in combination with mineral fertilizers benefits agricultural yield and improve plant growth as well as the uptake of nutrients. **Albayrak (2005)** reported that HA significantly affected most of the yield components of *Brassica raya.* **Chris *et al.* (2005)** reported that both the foliar and soil application of HA significantly improved seed yield and oil content of mustard. **MacCarthy *et al.* (2001)** concluded that humates enhance nutrient uptake, improve soil structure, and increase the yield and quality of various oilseed crops. Researchers such as **Salt *et al.* (2001)** also found lower dose of HA equally effective to their higher levels in increasing plant growth and enhancing the nutrient uptake. Humic acid influences plant growth both direct and indirect ways. Indirectly, it improves physical, chemical and biological conditions of soil. While, directly, it increases chlorophyll content, accelerates plant respiration and hormonal growth responses, increases penetration in plant membranes, etc. These effects of HA operate singly or in integration. Generally, **Kulikova *et al.* (2005)** believed that humic substances can be useful for living creatures in developing organisms (as a substrate material or a food source, or by enzyme-like activity); as a carrier of nutrients; as catalysts of biochemical reactions; and in antioxidant activity.

The above discussion clearly validates the suitability of HA as a beneficial fertilizer product.

Soil biological activity of maize plants rhizosphere

In the present work the use of CB and HA combined with nitrogen enhanced the maize soil rhizosphere biological activity in terms of nitrogenase activity, dehydrogenase activity (DHA), carbon dioxide evolution and total count bacteria. In this concern, **Zulpa *et al*. (2008)** studied the effect of CB products of *Tolypothrix tenuis* and *Nostoc muscorum* on the microbiological activity and the nutrient content of the soil. The biomass and extracellular products of both strains increased the soil microbial activity. *N. muscorum* and *T. tenuis* biomasses increased the soil oxidizable C (15%; 14%), total N

(10%; 12%) and available P (22%; 32%), respectively. *T. tenuis* extracellular products increased by 28% oxidizable carbon and *N. muscorum* extracellular products increased by 15% the available phosphorus. These are caused the soil biological activity to be increased also because they are a continuously renewable carbon source. **Caire *et al.* (2000)** established that CB can increase the soil enzymatic activity. **Aref and EL- Kassas (2006)** found that CB inoculation to maize field enhanced significantly any of total count bacteria, CB count, CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control treatment received no inoculation. They explained that biofertilization with CB led to increase microorganisms' community and in turn soil biological activity in soil through increasing the organic matter and microbial activity. **Saruhan *et al.* (2011)** revealed that humic compounds added to soil increased the soil fertility through increasing the soil microbial population including beneficial microorganisms. They explained that humic substances are major components of organic matter, often constituting 60 to 70% of the total organic matter, thus they may enhance the plant nutrients uptake through stimulation of microbiological activity **Ulkan (2008)** postulated that addition of HA to soil in wheat cultivation stimulated the soil microbiological activity that led to increase the soil fertility.

In conclusion, results from the present study indicate that the application of CB and HA fertilizer can positively affect the maize yield and its attributes, especially for the treatment received 120 kg N fed^{-1} + dry CB + CB spray, which recorded a maize yield that was not significantly different from that recorded by the use of 150 Kg N fed^{-1} alone (full recommended N dose). In general, the application of CB and HA can reduce the need for chemical fertilizers and subsequently reduce environmental pollution compared with other mineral chemical fertilizers, they are affordable. However, further studies are required to determine economically feasible application levels of HA and/or CB under different field conditions.

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