Response of Maize Crop to Cyanobacteria Applied Under Different Nitrogen Rates

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Abstract: The present investigation was conducted in sandy soil at Ismailia Agricultural Research Station, Agric. Res. Center (ARC) (Latitude 30° 35′ 41.901″ N and Longitude 32° 16′ 45.843″ E), Egypt, during two successive summer seasons of 2011 and 2012.Maize hybrid SC10 was used to study the effect of cyanobacteria (Cyano) and nitrogen fertilizer on maize growth, grain yield, yield components, and their effects on the biological activity of the soil around the rhizosphere of maize plants. Four treatments of cyanobacteria: 1) soaking grains in Cyano filtrate for 24 h then sprayed with Cyano filtrate after 30 d from planting, 2) side dressing along the row (dry) then sprayed at 30 d from planting, 3) soaking grains for 24 h + dry Cyano + Cyano spray, 4) and control (untreated); and three rates of nitrogen: 107 ($^1/_3$ N), 214 ($^2/_3$ N), and 321 kg ha^{-1}(full N rate) were used. Experimental design was split-plot with four replications, where Cyano treatments were assigned to main plots and nitrogen rates in the sub plots. Results showed significant differences among Cyano treatments for days to 50% tasseling and days to 50% silking in 2012 season. Early days to 50% tasseling and days to 50% silking and were associated with the application of Cyano treatment (dry + spray) in 2012 season. However, Cyano did not affect days to 50% tasseling and days to 50% silking in 2011 season. Treatment of Cyano (soaking + dry + spray) was accompanied with the tallest plants and the highest values of ear heights. While, the shortest plants and the lowest values of ear heights were associated with using Cyano (soaking + spray). Effect of Cyano inoculation on grain yield was significant in both years. The highest grain yield was associated with Cyano treatment (soaking + dry + spray).The highest values for any of ear length and ear diameter was recorded as a result of using Cyano treatment (soaking + spray). Number of kernels row^{-1} was significantly affected by Cyano inoculation in the two years. Nitrogen application hastened the time of tasseling and silking. The increase of nitrogen rate from $^1/_3$ N to full N increased significantly the plant height. Application of $^1/_3$ N ha^{-1} was accompanied with the shortest plants and the lowest values of ear height. Grain yield increased as N increased up to the highest rate. Regarding yield components, ear length, ear diameter, and number of kernels row^{-1} were significantly affected by N application. Increasing N levels up to full N rate (the highest N rate) was associated with the tallest ears and the highest values for number of kernel row^{-1} and ear diameter. Nitrogen x Cyano interaction had significant effect on days to 50% tasseling and days to 50% silking in the second season only. Applying full N ha^{-1} with Dry Cyano + Cyano spray accelerated days to 50% tasseling and days to 50% silking. Using Cyano (soaking+ spray) with $^1/_3$ N ha^{-1} gave the shortest plants. The highest grain yield was achieved as a result of applying (full N + soaking in Cyano + dry Cyano and Cyano spray) in the two seasons. Furthermore, the use of cyanobacteria in combination with different nitrogen rates increased the rhizosphere soil biological activity of the maize rhizosphere soil.

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1. Introduction

Maize is considered among the most important cereal crops in terms of grain production in Egypt.The local production is not sufficient to meet the exponential increase in population and to cover the gap between production and local consumption. Therefore, any attempt for increasing maize production is considered a matter of at most importance to face human and animal demands **(Gouda *et al*., 2009).**Maize 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)**. Farmers are used to consume substantial quantities of chemical fertilizers. Nitrogen fertilizer application is one of the major factors that affect maize production and seed quality. It 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, besides making the production costly and expensive **(Badran and Safwat, 2004).** Recently, a real challenge faces the workers in the agricultural research field to stop using high rates of agro-chemicals, which adverse negatively human health and environment. Many attempts have been tried to replace a part of those harmful chemical fertilizers by biofertilizers to get yield of a high quality without loss in its quantity**.** The use of the biological nitrogen fixation through 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 agronimically important plants, particularly cereals such as 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 PGPR **(Malik *et al*., 1994 and Rashid *et al*., 2007)**.

The objective of the current work is to study the impact of cyanobacteria inoculation under different nitrogen fertilizer rates on maize yield grown in sandy soil and its components, as well as, their effect on the biological activity of the soil in the rhizosphere maize plants.

2.Materials and Methods

A field trial was conducted in sandy soils at Ismailia Agricultural Research Station, (ARC) (Latitude 30° 35′ 41.901″ N and Longitude 32° 16′ 45.843″ E) in 2011 and 2012, to study the impact of cyanobacteria inoculation under different nitrogen fertilizer rates on maize yield (hybrid SC10) and its components, as well as, their effect on the biological activity of the soil in the rhizosphere maize plants.

This study was practiced in sandy soil.Soil physical and chemical properties are shown in Table (1) according to **Page *et al*. (1982).**

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

pH (1:2.5) Soil suspension	EC dSm^{-1} (Soil paste)	Soluble cations meq L^{-1}				Soluble anions meq L^{-1}			
		Ca^{++}	Mg^{++}	Na^{+}	K^{+}	$CO_3^{=}$	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
8.10	**0.30**	**0.30**	**0.50**	**1.90**	**0.30**	**0.00**	**0.80**	**1.10**	**1.10**

Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	Texture class
83.18	**11.17**	**3.35**	**2.50**	**1.30**	**Sandy**

Available N (mg Kg^{-1})	Available P (mg Kg^{-1})	Available K (mg Kg^{-1})
15	**2.80**	**110**

Cyanobacteria were provided by Agric.Microbiol.Dept., Soils, Water & Environ.Res.Inst., ARC, Giza, Egypt.Cyanobacteria (Cyano) were applied as culture filtrate that contains a mixture of different Cyanobacteria strains, i.e., *Nostoc calcicola, Anabaena oryzae, Tolopothrix tenius and Anabaena laxa.* To obtain the cyanobacteria culture filtrate, each cyanobacterium strain was grown and propagated for 5 weeks on the free nitrogen BG 11_0 medium described by **Allen and Stanier (1968)**.The developed cyanobacteria cultures were centrifuged (3000 rpm min^{-1}) and the supernatant were used as cyanobacteria filtrate by mixing the supernatant for each strain together to have the cyanobacteria culture filtrate (**Aref *et al*., 2009)**.The filtrate was used in soaking treatment for maize grains before planting and to be also used as foliar spray at the rate of 40 L fed^{-1}.As well as, these cyanobacteria strains were prepared as soil based inoculum as described by **Venkataraman (1972)** to be used for maize as seed side dressing (dry inoculum) along the rows. Cyanobacteria are introduced in four treatments, i.e., 1) Soaking grains in Cyano filtrate for 24 h then sprayed with Cyano filtrate at 30 d from planting, 2) Side dressing along the row (dry) then sprayed at 30 d from planting, 3) soaking grains for 24 h + Side dressing along the row (dry) then sprayed at 30 d from planting, and 4) Control (untreated); while nitrogen was introduced in three rates of 107 ($^1/_3$ N), 214 ($^2/_3$ N), and 321 kg ha^{-1} (full N).Experimental design was split-plot with four replications, where Cyano treatments were assigned to main plots and nitrogen rates in the sub plots.Maize hybrid SC 10 was used. Plot size was 5 rows, 6 m in length, 80 cm in width, and 20 cm between hills. One blank row was left between treatments. Nitrogen was added in the form of ammonium nitrate (33.5% N) and split into eight equal doses, the first was added at germination, and the rest were added weekly up to 60 days after planting. Phosphorus at a rate of 71 kg P_2O_5 ha^{-1} in the form of superphosphate (15% P_2O_5) and potassium at a rate of 57 kg K_2O ha^{-1} in the form of potassium sulphate 48% K_2O were added at soil preparation. Soil samples (0.5 kg) were taken from the experimental site before planting for chemical, physical, and biological analysis. Cultural practices were applied as recommended. Data recorded for maize for both tested seasons were number of days from planting to 50% tasseling (DTT) and number of days from planting to 50% silking (DTS), plant height (PHT) and ear heights (EHT) (cm), number of kernels row^{-1} (KPR), ear length (EL) (cm), ear diameter (ED) (cm), and grain yield (t ha^{-1}).Grain yield was adjusted to 15.5% moisture. Statistical analysis of the data was performed according to **Steel and Torrie (1980).**After 70 days from planting, a maize rhizosphere soil samples were collected from maize rhizosphere to determinate total *Azotobacter* and *Azospirillum* counts (**Cochran, 1950**), total cyanobacteria count (**Allen and Stanier, 1968),** total fungi count (**Martin, 1950**), total *Actinomycetes* count (**Williams and Davis, 1965**), total bacterial count (**Allen, 1959**), dehydrogenase activity (**Casida *et al*., 1964**), nitrogenase activity **(Hardy *et al*., 1973)** and CO_2 evolution amount (**Pramer and Schmidt, 1964**).

3.Results and Discussion

a) Cyanobacteria effect:

Data presented in Tables (2 and 3) indicate that the effect of cyanobacteria inoculation was significant on maize growth attributes and grain yield in 2011 and 2012 seasons. No significant differences among cyanobacteria treatments for days to 50% tasseling and days to 50% silking in 2011 season. But this effect was significant in 2012 season. Early days to 50 % tasseling and days to 50% silking were associated with application of Cyano treatment (dry + spray) in the second season. Application of Cyano (soaking + dry + spray) was accompanied with the tallest plants and the highest values of ear heights in both seasons. While, the shortest plants and the lowest ear heights were associated with using Cyano (soaking + spray) in the two seasons. Effect of Cyano inoculation on grain yield was significant in the two seasons. The highest maize grain yield was achieved when treatments of Cyano (soaking + dry + spray) were used in the first and second seasons, respectively. On the other hand, the untreated plants with Cyano inoculation had the lowest grain yield in both seasons.

Ear length and ear diameter were not affected by Cyano inoculation in 2011 season, but they were significantly affected by Cyano in the second season (Table 3). The highest values for ear length and ear diameter were recorded as a result of using of Cyano (Soaking + spray) in the second season. Number of kernels row^{-1} was significantly affected by Cyano inoculation in the two years and the highest values were associated with the treatment of soaking in Cyano + dry Cyano + Cyano spray in the two seasons.

Table (2): Effect of cyanobacteria on days to 50% tasseling (DTT), days to 50% silking (DTS), plant height (PHT), ear height (EHT), and grain yield (GY) in 2011 and 2012

	DTT	DTS	PHT (cm)	EHT (cm)	GY (t ha^{-1})
Cyano treatments:					
---------- 2011 ----------					
Soaking + spray	60.8	62.3	264	137	8.58
Dry + spray	60.4	62.2	268	135	7.67
Soaking + dry + spray	60.0	61.6	278	145	8.77
Control	61.0	62.6	275	139	7.11
$LSD_{0.05}$	NS	NS	4.00	5.00	1.13
---------- 2012 ----------					
Soaking + spray	64.4	66.2	276	144	8.19
Dry + spray	63.3	65.1	282	150	8.04
Soaking + dry + spray	64.1	65.8	285	153	9.09
Control	63.7	65.6	280	153	7.80
$LSD_{0.05}$	0.70	0.60	6.00	6.00	1.08

NS= not significant at 0.05 level.

B) Nitrogen effect:

Effect of nitrogen fertilization on maize growth, grain yield, and yield components was significant in both years, except for ear diameter in 2011 season (Tables 4 and 5).Nitrogen fertilizer significantly affected DTT and DTS in both years. Increasing Nitrogen rates up to full N dose ha^{-1} hastened the time of tasseling and silking in 2011 and 2012 seasons. However, in the first season, there was no significant differences between $^{1}/_{3}$ and $^{2}/_{3}$ N ha^{-1} for DTT and between $^{2}/_{3}$ N and full N ha^{-1} for DTS. Whereas, significant differences among the three nitrogen rates for DTT and DTS were detected in the second season. Generally, the earliest DTT and DTS were associated with application of full N ha^{-1} in the two seasons.

Effect of nitrogen on plant height was significant in 2011 and 2012.Increasing nitrogen rates up to $^{2}/_{3}$ N ha^{-1} was accompanied with the tallest plants in 2011.However, increasing N rates from $^{2}/_{3}$ to full N ha^{-1} was not associated with a corresponding increase in plant height in the first season. In the second season, plant height increased as N increased up to the highest rate (full N ha^{-1}).On the other hand, the shortest plants were associated with application of $^{1}/_{3}$ N ha^{-1} in the two seasons. Concerning ear height, the effect of nitrogen on ear height was significant in 2011 and 2012.Increasing N up to $^{2}/_{3}$ N ha^{-1} was associated with significant increase in ear height in 2011 season. But no significant difference was detected between $^{2}/_{3}$ and full N ha^{-1} regarding their effect on ear height in the first year. However, increasing N levels up to the highest rate (full N ha^{-1}) gave the highest value for ear height in the second season. Moreover, the lowest values of ear height (136 and 134 cm) were accompanied with application of $^{1}/_{3}$ N ha^{-1} in both years, respectively. Grain yield increased as N increased up to the highest level (full N rate) in both growing seasons (Table 4).

Table (3): Effect of cyanobacteria on ear length (EL), ear diameter (ED) and number of kernels row^{-1} (KPR) in 2011 and 2012

	EL (cm)	ED (cm)	KPR^{-1}
Cyano treatments:			
------------------------------------- 2011 -------------------------------------			
Soaking + spray	19.0	4.57	43.6
Dry + spray	19.3	4.55	45.1
Soaking + dry + spray	19.5	4.57	45.7
Control	19.1	4.60	43.2
$LSD_{0.05}$	NS	NS	1.10
------------------------------------- 2012 -------------------------------------			
Soaking + spray	18.1	4.52	44.2
Dry + spray	17.9	4.42	44.5
Soaking + dry + spray	18.3	4.47	45.7
Control	17.1	4.25	42.8
$LSD_{0.05}$	0.40	0.08	0.90

Table (4): Effect of nitrogen fertilizer on DTT, DTS, PHT, EHT, and GY in 2011 and 2012

	DTT	DTS	PHT (cm)	EHT (cm)	GY (t ha^{-1})
N rates(ha^{-1})					
------------------------------------- 2011 -------------------------------------					
$^{1}/_{3}$ N	61.1	62.8	264	136	6.38
$^{2}/_{3}$ N	60.7	62.0	276	141	7.89
Full N	59.9	61.7	275	140	9.83
$LSD_{0.05}$	0.50	0.40	4.00	4.00	0.98
------------------------------------- 2012 -------------------------------------					
$^{1}/_{3}$ N	64.8	66.7	249	134	6.54
$^{2}/_{3}$ N	63.8	65.6	288	148	8.75
Full N	62.9	64.7	306	168	9.56
$LSD_{0.05}$	0.03	0.40	5.00	5.00	0.94

DTT=days to 50% tasseling, DTS=days to 50% silking, PHT= plant height, EHT= ear height, GY= grain yield.

This increase in grain yield was more pronounced when full N ha^{-1} was applied in 2011.But the difference between $^{2}/_{3}$ and full N treatments were not significant in 2012 season. This result revealed that application of $^{1}/_{3}$ N ha^{-1} was linked to the lowest grain yield (6.38 and 6.54 t ha^{-1}) in the two seasons, respectively. Regarding yield components, ear length, ear diameter, and number of kernels row^{-1} were significantly affected by N fertilizer treatments in both seasons, except for ear diameter in 2011 season (Table 5).Increasing N up to the highest rate (full N ha^{-1}) was associated with the tallest ears and the highest number of KPR in both seasons as well as the highest value for ED in the second season. But no significant difference was detected between $^{2}/_{3}$ and full N treatments for EL in 2011 season. In this respect, **Gouda *et al.*(2009)** found that increasing nitrogen rates up to full N produced the highest values of grain yield per unit area. **Dahmardeh (2011)** confirmed that increasing N up to 300 kg ha^{-1} significantly increased all the studied parameters of maize yield. **Hokmalipour and Darbandi (2011)** showed that in maize field trail, increasing nitrogen levels up to 180 kg ha^{-1} increased the harvest index, kernels yield, 1000 kernels weight, number of kernels per ear, and number of rows per ear. They reported that increasing nitrogen fertilization rates led to significant increase in 100 grain weight and grain yield of maize compared with control treatment. They explained 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. **Ghazal *et al.* (2013)** mentioned that elevating nitrogen level from $^{2}/_{3}$ N to full N rate enhanced grain yield of maize.

Table (5): Effect of nitrogen on ear length (EL), ear diameter (ED), and number of kernels row^{-1} (KPR) in 2011 and 2012

	EL (cm)	ED (cm)	KPR
N rates (ha^{-1})			
------------------------------------- 2011 -------------------------------------			
$^{1}/_{3}$ N	18.5	4.53	43.1
$^{2}/_{3}$ N	19.4	4.57	44.1
Full N	19.8	4.61	46.0
$LSD_{0.05}$	0.50	NS	1.00
------------------------------------- 2011 -------------------------------------			
$^{1}/_{3}$ N	16.2	4.28	41.3
$^{2}/_{3}$ N	17.8	4.40	44.9
Full N	19.6	4.56	46.8
$LSD_{0.05}$	0.30	0.07	0.80

EL= Ear length, ED= Ear diameter, and KPR= Number of kernels row^{-1}.

C) Cyanobacteria × nitrogen interaction effect:

Effect of Cyano × N interaction on DTS and DTS was not significant in 2011, but this effect was positively significant in 2012 (Table 6).Application of full N ha^{-1} combined with dry Cyano + Cyano spray was associated with the earliest DTT and DTS in 2012 season. In contrast, application of $^{1}/_{3}$ N ha^{-1} with soaking seeds in Cyano filtrate + Cyano spray was accompanied with the latest DTT and DTS in 2012 season. Effect of Cyano × N interaction on plant height was significant in 2011, but this effect was not significant in 2012 season. Application of $^{2}/_{3}$ N ha^{-1} plus soaking in Cyano filtrate + Dry Cyano + Cyano spray was associated with the tallest plants (285 cm), but with no significant difference with increasing N up to full N ha^{-1} without Cyano inoculation (control) in the same season. In contrast, the shortest plants (254 cm) were attained by application of $^{1}/_{3}$ N ha^{-1} (the lowest rate of N fertilizer) + soaking in Cyano Filtrate + Cyano spray in the first season. No significant difference was detected between the tallest plants (285cm), which received ($^{2}/_{3}$ N ha^{-1} + soaking in Cyano filtrate + dry Cyano + Cyano spray) and the untreated plants with Cyano that received the highest rate of N (full N ha^{-1}).Effect of Cyano × N interaction on grain yield was positively significant in both seasons (Table 7).There was no significant difference between $^{2}/_{3}$ N and full N ha^{-1} (without Cyano) for grain yield in both tested years. The highest grain yield (11.11 and 10.03 t ha^{-1}) was associated with application of full N ha^{-1} + soaking in Cyano + dry Cyano and Cyano spray in 2011 and 2012 seasons, respectively. However, no significant difference was detected between the rate of $^{2}/_{3}$ N and full N ha^{-1} + seed soaking in Cyano filtrate and the treatment of full N + Dry Cyano + Cyano spray and between the rate of $^{1}/_{3}$ and $^{2}/_{3}$ N ha^{-1} combined with Dry Cyano + Cyano spray in 2011 season. In contrast, significant differences amongst the three nitrogen levels were detected with seed soaking in Cyano + Cyano dry +Cyano spray in 2011 season. While, in the second season, there were no significant differences between $^{2}/_{3}$ N and full N ha^{-1} for all treatments of Cyano, and between $^{1}/_{3}$ N and $^{2}/_{3}$ N ha^{-1} with Dry Cyano + Cyano spray.

D) Effect of nitrogen and cyanobacteria on rhizosphere soil biological activity:

Data in Tables (8, 9 and 10) indicate the rhizosphere soil biological activity of maize rhizosphere soil samples for 2012 seasons under the effect of different nitrogen rates and cyanobacteria. The soil biological activity was expressed in terms of the total count of *Azotobacter*, *Azospirillum*, cyanobacteria, fungi, actinomycetes and bacteria. Dehydrogenase activity, nitrogenase activity and carbon dioxide evolution were also considered. Results noted that increasing nitrogen rate from $^{1}/_{3}$ N to full N rate increased significantly the biological activity due to the soil maize rhizosphere area with priority to $^{2}/_{3}$ N rate. This nitrogen rate gave significantly the highest significant mean total counts numbers of *Azotobacter* (10 x 10^{4} cfu dry rhizosphere soil^{-1}), *Azospirillum* (8.25 X 10^4 cfu dry rhizosphere soil^{-1}), cyanobacteria (8.78 X 10^3 cfu dry rhizosphere soil^{-1}), fungi (44.90 X 10^3 cfu g dry rhizosphere soil^{-1}), actinomycetes (52.03 X 10^3 cfu g dry rhizosphere soil^{-1}) and bacteria (79.90 X 10^4 cfu dry rhizosphere soil^{-1}).Similar trend was true for dehydrogenase activity, nitrogenase activity and CO_2 evolution for the same nitrogen rate of $^2/_3$ N. The corresponding mean values were 465.45 mg TPF dry rhizosphere soil^{-1} day^{-1}, 196.45 mmole C_2H_4 dry rhizosphere soil^{-1} day^{-1} and 853.90 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1}, respectively. Moreover, results also revealed that the use of cyanobacteria (Cyano) as seed soaking, dry and\or spray increased significantly all the tested terms of the biological activity due to the soil maize rhizosphere area. Nevertheless, the use of cyanobacteria as seed soaking + dry + spray gave significantly the highest significant mean values for biological activity of the maize rhizosphere soil compared to the other applied cyanobacteria treatments. The corresponding significant mean values were *Azotobacter* (7.97 x 10^4 cfu dry rhizosphere soil^{-1}), *Azospirillum* (8.67 X 10^4 cfu dry rhizosphere soil^{-1}), cyanobacteria (10.73 X 10^3 cfu dry rhizosphere soil^{-1}), fungi (43.25 X 10^3 cfu g dry rhizosphere soil^{-1}), actinomycetes (43.25 X 10^3 cfu g dry rhizosphere soil^{-1}) and bacteria (79.40 X 10^4 cfu dry rhizosphere soil^{-1}).Similar trend was observed for dehydrogenase activity, nitrogenase activity and CO_2 evolution in response to the same cyano treatment. The corresponding mean values were 652.35 mg TPF dry rhizosphere soil^{-1} day^{-1}, 287.17 mmole C_2H_4 dry rhizosphere soil^{-1} day^{-1} and 1152.47 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1}, respectively.

Table (6): Effect of Cyanobacteria x nitrogen interaction on DTT, DTS, and PHT in 2011 and 2012 seasons

Treatments	N rate (ha^{-1})	DTT	DTS	PHT (cm)
		2011		
Soaking seeds in Cyano filtrate + CYANO spray	$^1/_3$ N	61.0	63.0	254
	$^2/_3$ N	61.0	62.0	275
	Full N	60.3	62.0	264
Dry Cyano + Cyano spray	$^1/_3$ N	61.0	62.5	261
	$^2/_3$ N	60.5	62.0	273
	Full N	59.8	62.0	271
Soaking in Cyano filtrate + dry Cyano + Cyano spray	$^1/_3$ N	60.5	62.3	268
	$^2/_3$ N	60.3	61.5	285
	Full N	59.3	61.0	283
Control	$^1/_3$ N	61.8	63.5	274
	$^2/_3$ N	61.0	62.5	271
	Full N	60.3	61.8	281
$LSD_{0.05}$		NS	NS	7.60
		2012		
Soaking seeds in Cyano filtrate + Cyano spray	$^1/_3$ N	65.8	68.0	243
	$^2/_3$ N	64.5	65.8	285
	Full N	63.0	64.8	300
Dry Cyano + Cyano spray	$^1/_3$ N	64.5	66.3	254
	$^2/_3$ N	63.0	65.0	285
	Full N	62.3	64.0	308
Soaking in Cyano filtrate + dry Cyano + Cyano spray	$^1/_3$ N	64.5	66.0	251
	$^2/_3$ N	64.3	66.0	289
	Full N	63.5	65.3	316
Control	$^1/_3$ N	64.5	66.5	249
	$^2/_3$ N	63.5	65.5	291
	Full N	63.0	64.8	301
$LSD_{0.05}$		0.60	0.90	NS

Cyano = Cyanobacteria, DTT= Days to 50% tasseling, DTS= days to 50% Silking, PHT= Plant height.

Due to the interaction effect of both cyanobacteria and nitrogen fertilizer rate on the biological activity of maize rhizosphere soil, results revealed that all the treatments received any rate of nitrogen combined with any cyanobacteria treatments gave significantly higher values of the biological activity of maize rhizosphere soil compared to those received any of the nitrogen rates only. Moreover, the treatment of $^2/_3$ N rate + Cyano seed soaking + Cyano dry + Cyano spray gave significantly the highest values for the terms of the biological activity of maize rhizosphere soil compared with the other treatments received the other nitrogen rates combined with cyanobacteria treatments. The corresponding biological activity of maize rhizosphere soil were *Azotobacter* (9.30 x 10^4 cfu dry rhizosphere soil^{-1}), *Azospirillum* (11.80 X 10^4 cfu dry rhizosphere soil^{-1}), cyanobacteria (12.30 X 10^3 cfu dry rhizosphere soil^{-1}), fungi (67.3 X 10^3 cfu g dry rhizosphere soil^{-1}), actinomycetes (84.10 X 10^3 cfu g dry rhizosphere soil^{-1}) and bacteria (121.40 X 10^4 cfu dry rhizosphere soil^{-1}).In addition, similar trend was observed for dehydrogenase activity, nitrogenase activity and CO_2 evolution in response to the same interaction cyano x N treatment. The corresponding mean values were 830.86 mg TPF dry rhizosphere soil^{-1} day^{-1}, 460.61 mmole C_2H_4 dry rhizosphere soil^{-1} day^{-1} and 1708.67 mg CO_2 100 g dry rhizosphere soil^{-1} day^{-1}, respectively.

Table (7): Effect of Cyanobacteria x nitrogen interaction on maize grain yield in 2011 and 2012 seasons

Treatments	N rate ha^{-1}	Grain yield (t ha^{-1})	
		2011	2012
Seeds Soaking in Cyano filtrate + Cyano spray	$^1/_3$ N	6.71	6.08
	$^2/_3$ N	8.69	8.54
	Full N	10.35	9.96
Dry Cyano + Cyano spray	$^1/_3$ N	6.58	6.69
	$^2/_3$ N	7.27	8.17
	Full N	9.17	9.26
Seed soaking in Cyano + dry Cyano + cyano spray	$^1/_3$ N	6.47	7.24
	$^2/_3$ N	8.73	10.00
	Full N	11.11	10.03
Control	$^1/_3$ N	5.75	6.14
	$^2/_3$ N	6.89	8.28
	Full N	8.69	8.98
$LSD_{0.05}$		1.96	1.87

Table (8): Effect of cyanobacteria inoculation and N-fertilization on N_2-fixers (*Azotobacter*, *Azospirillum* and total cyanobacteria) counts in maize rhizosphere soil (Data are a mean of two seasons)

N- rate (ha^{-1})	Treatments				
	Control	Cyanobacteria			Means
		Soaking + spray	Dry + spray	Soaking + dry + spray	
	Azotobacter x10^4 cfu g dry rhizosphere soil^{-1}				
$^1/_3$ N	2.60	4.40	5.80	7.50	6.77
$^2/_3$ N	5.40	6.70	8.60	9.30	10.00
Full N	5.00	5.30	6.30	7.10	7.90
Means	4.33	5.47	6.90	7.97	
$LSD_{0.05}$					
N:	2.22				
Cyano:	1.23				
N X Cyano:	0.80				
	Azospirillum x 10^4 cfu g dry rhizosphere soil^{-1}				
$^1/_3$ N	1.40	2.20	4.30	5.70	3.40
$^2/_3$ N	5.20	6.80	9.20	11.80	8.25
Full N	4.60	5.30	7.20	8.50	6.40
Means	3.73	4.77	6.90	8.67	
$LSD_{0.05}$					
N:	1.88				
Cyano:	1.78				
N X Cyano:	2.63				
	Total cyanobacteria x 10^3 cfu g dry rhizosphere soil^{-1}				
$^1/_3$ N	2.70	4.70	6.20	9.70	2.83
$^2/_3$ N	6.00	8.20	9.00	12.30	8.87
Full N	5.43	6.30	7.60	10.20	7.38
Means	4.71	6.40	7.60	10.73	
$LSD_{0.05}$					
N:	1.19				
Cyano:	2.14				
N X Cyano:	2.24				

Table (9): Effect of cyanobacteria inoculation and N-fertilization on total fungi, actinomycetes and total bacterial counts in maize rhizosphere soil (Data are a mean of two seasons)

N-rate (ha^{-1})	Treatments				
	Control	Cyanobacteria			Means
		Soaking + spray	Dry + spray	Soaking + dry + spray	
	Total fungi $x10^3$ cfu g dry rhizosphere $soil^{-1}$				
$^1/_3$ N $^2/_3$ N Full N	7.20 22.60 15.60	10.10 36.00 18.50	15.70 53.70 33.20	28.90 67.30 42.80	15.48 44.90 27.53
Means	14.93	21.53	24.20	46.33	
$LSD_{0.05}$ N: Cyano: N X Cyano:	 17.52 23.10 13.19				
	Actinomycetes x 10^3 cfu g dry rhizosphere $soil^{-1}$				
$^1/_3$ N $^2/_3$ N Full N	8.00 18.70 11.30	15.20 46.70 31.60	20.10 58.60 35.80	31.80 84.10 57.10	18.78 52.03 33.95
Means	12.67	31.17	38.17	43.25	
$LSD_{0.05}$ N: Cyano: N X Cyano:	 17.13 5.02 23.20				
	Total bacteria $x10^4$ cfu g dry rhizosphere $soil^{-1}$				
$^1/_3$ N $^2/_3$ N Full N	16.10 38.20 28.50	18.70 77.60 34.70	40.20 82.00 39.80	65.10 121.40 51.70	35.03 79.80 38.78
Means	27.60	43.67	54.00	79.40	
$LSD_{0.05}$ N: Cyano: N X Cyano:	 41.00 24.80 38.40				

In the present work the use of cyanobacteria combined with different nitrogen rates ($^1/_3$, $^2/_3$ and full N recommended rates) enhanced maize soil rhizosphere biological activity in terms of total count of *Azotobacter*, *Azospirillum*, cyanobacteria, fungi, actinomycetes and bacteria, dehydrogenase activity, nitrogenase activity and carbon dioxide evolution. In this concern, **Zulpa *et al*.(2008)** found that the the biomass and extracellular products of *Tolypothrix tenuis* and *Nostoc muscorum* increased significantly the soil microbial activity and its nutrients availability. *Nostoc muscorum* and *T. tenuis* biomasses increased the soil oxidizable C (15%; 14%), total N (10%; 12%) and available P (22%; 32%), respectively. In addition, *Tolypothrix tenuis* extracellular products increased oxidizable carbon by 28% and *N. muscorum* extracellular products increased the available phosphorus by 15%.These increases caused the soil biological activity to be increased also because they are a continuously renewable carbon source. Production of bioactive substances, which accelerate the decomposition process in the soil due to the increase of microbial activity and because they are a continuously renewable organic matter source (**Caire *et al*., 2000).** They also added that cyanobacteria can increase the soil enzymatic activity. Besides, exopolysaccharide secreted by cyanobacteria are a source of organic carbon for the soil microflora increasing microbial activity **(Storni de Cano *et al*., 2002)**.Cyanobacteria inoculation to maize field enhanced significantly any of total count bacteria, cyanobacteria count, CO_2 evolution, dehydrogenase and nitrogenase activities compared to the control treatment received no inoculation. They explained that biofertilization with cyanobacteria led to increase microorganisms' community and in turn soil biological activity in soil through increasing the organic matter and microbial activity. **Ghazal *et al.* (2013)** stated that the use of cyanobacteria to maize increased the soil biological activity of the maize plants rhizosphere in terms of total count bacteria, carbon dioxide evolution, dehydrogenase activity and nitrogenase activity.

Table (10): Effect of cyanobacteria inoculation and N-fertilization on nitrogenase, nitrogenase and CO_2 evolution in maize rhizosphere soil (Data are a mean of two seasons)

N- rate (ha^{-1})	Treatments				
	Control	Cyanobacteria			Means
		Soaking + spray	Dry + spray	Soaking + dry + spray	
	Dehydrogenase activity (mg TPF* g dry rhizosphere $soil^{-1}$ day^{-1})				
$^{1}/_{3}$ N $^{2}/_{3}$ N Full N	75.10 110.12 91.27	125.23 230.55 145.42	420.08 690.28 520.74	510.75 830.86 615.43	282.79 465.45 343.22
Means	92,16	167.07	543.67	652.35	
$LSD_{0.05}$ N: Cyano: N X Cyano:	120.23 138.58 106.68				
	Nitrogenase activity (mmole C_2H_4 g dry rhizosphere $soil^{-1}$ day^{-1})				
$^{1}/_{3}$ N $^{2}/_{3}$ N Full N	30.45 52.15 41.62	42.56 82.18 50.12	65.78 190.85 81.36	170.16 460.61 230.74	77.24 196.45 100.96
Means	41.41	58.29	112.66	287.17	
$L.S.D_{0.05}$ N: Cyano: N X Cyano:	93.49 165.75 170.50				
	CO_2 Evolution (mg CO_2 100 g dry rhizosphere $soil^{-1}$ day^{-1})				
$^{1}/_{3}$ N $^{2}/_{3}$ N Full N	130.12 180.58 145.06	268.15 510.67 318.43	660.25 1015.66 730.45	835.33 1708.67 913.40	473.46 853.90 526.84
Means	151.92	365.75	802.12	1152.47	
$L.S.D_{0.05}$ N: Cyano: N X Cyano:	125.06 190.40 145.25				

In conclusion, results from the present study indicate that the application of cyanobacteria and nitrogen fertilizer rate can positively affect the maize yield and its components, especially for the treatment received $^{2}/_{3}$ N (214 kg N ha^{-1}) + Cyano seed soaking + dry Cyano + Cyano spray, which recorded a maize yield that was not significantly differed from that recorded by the use of full N dose alone (321 kg N ha^{-1}).In general, application of cyanobacteria along with nitrogen can reduce the demands for chemical fertilizers and subsequently reduce environmental pollution. However, further studies are required to determine economically feasible application cyanobacteria under different field conditions.

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