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Efficiency Evaluation of Cyanobacterial Strain, Its Extract and Compost Tea as Biofertilizers on Yield of Wheat Plant

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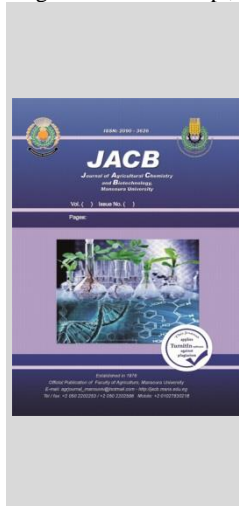
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ABSTRACT

In this study, a pot experiment was carried out in the glass house of the farm at Faculty of Agriculture, Mansoura University, Mansoura, Egypt during the winter season of 2018 to study the effect of inoculation with cyanobacterial strain (*Anabaena cylindrica*), their extracts and compost tea as individual or mixture with doses of inorganic nitrogen on the growth and yield of wheat plant. Obtained results showed that all the treatments as N fertilization rates, compost tea, *Anabaena cylindrica* and its extract addition in single treatment or mixed together on wheat plant through three growth stages (60, 90 and 120 days from planting) are investigation significantly ($p \leq 0.05$) different from one to another. The results indicated that highly significant increase in vegetative/yield growth parameters of wheat, pigments and NPK content (in shoot, root, straw and grain) with the treatment of C₇₅TA (N₇₅% +Compost Tea+*Anabaena*). Also, the biofertilization has a pronounced increase number for viable bacterial count was the highest (7.70×10^3 CFU) with mixture inoculation under 100% nitrogen fertilization after 60 days compared to those recorded by the other treatments. Followed by 75% nitrogen fertilization and compost tea with *Anabaena* after 60 days. The N₂-fixing cyanobacteria found that the highest count was obtained with C₇₅TA after 90 days 3.74×10^3 CFU which was near to 60 day 3.44×10^3 CFU. The cyanobacteria, their extracts and compost tea have been saved the inorganic nitrogen application by 25% compare with recommended nitrogen application.

Keywords: Wheat, *Anabaena*, extracts, compost tea



INTRODUCTION

Wheat is one of Egypt's and the world's most important cereal crops. It is being the most important sources of stable food urban and rural societies used in human nutrition (Gerba *et al.* 2013). Cyanobacteria are the most important microorganisms in terms of soil fertility and bioactivity. These species are important in this system because they provide a constant supply of fixed nitrogen (Roger and watanabe 1986). In addition to Shatta *et al.* (2014) cyanobacteria used as inoculants have numerous functions in the soil, including fixing carbon and nitrogen and generating exopolysaccharides, which improve soil fertility, water retention, and structure and stability. Jaiswal *et al.* (2008) disconnected *Anabaena* and *Nostoc* from the rhizosphere of wheat. The cyanobacterial strains had a close interaction with wheat plants and were able to invade the epidermal layer of wheat roots through root hairs. Essentially higher indole acidic corrosive creation and chlorophyll gathering was seen in application of N₂-fixing cyanobacteria reduce the nitrogen fertilizer to 50 % and enhance growth, length of shoots, and biomass of wheat, could be caused by several physiological factors including growth promoting substances such as auxin, cytokinins, and gibberellin, macronutrients (N, P, K, Ca, Mg), microelements (S, Zn, Fe, Mn, Cu, Mo, Co), amino acids, polyamines, and several other bioactive compounds which can be excreted by cyanobacteria (Chojnacka *et al.* 2010; Sahu *et al.* 2012).

Rana *et al.* (2012) found that *Anabaena* sp. CW1, *Calothrix* sp. and *Anabaena* sp. CW2 improving the nutritional quality of wheat grains, in terms of protein content and important micronutrients (Fe, Cu, Zn, and Mn). A significant promotion in dry weight, total nitrogen, chlorophyll a, chlorophyll b, carotenoids and pigments occurred in wheat plants treated with cyanobacterial biofertilizer (Dhar *et al.* 2015). Compost and its tea are most promising bioproducts responsible for developing different management programmes as plant peast, disease and fertility (Scheuerrell and mahaffe 2002). Since it contains high macro and microelements, vitamins and phytohormones, it could be considered as an efficient bio-organic fertilizer (Mehesen *et al.* 2009). Also, P solubilization by cyanobacteria has been reported by Roychoudhury and Kaushik (1989). Cyanobacterial extracts are fertilisers can be prepared a by different materials such as water (Shariatmadari *et al.* 2013).

Shariatmadari *et al.* (2015) found that extracts of four heterocystous cyanobacteria isolated from wheat field soils (*Triticum aestivum* L.) were used as a promoter of growth and essential oil production in *Mentha piperita* L. Pot experiments were performed by spraying algal extracts on the soil of treated plants on the first day of planting and every 20 days thereafter. Extract improve the plant height, root length, dry and fresh weight of plant, as well as leaf number, leaf area, and ramification after 100 days planting. Therefore, aim of the present study was to determine the effect of inoculation with cyanobacterial strain (*Anabaena cylindrica*), their extracts

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and compost tea as individual or mixture with doses of inorganic nitrogen on the growth and yield of wheat plant.

MATERIALS AND METHODS

Cyanobacterial strain

Anabaena cylindrica as fixed nitrogen Zaki et al., (2021) was isolated and identified by Zaki (2022) in Microbiol. Dept. Fac. of Agric. Mansoura University

Soil samples collection

Soil samples were collected from different sites in El-Dakahlia and Kafr El-Sheikh governorates. Some physio-chemical properties of soil samples collected for cyanobacteria isolation were analysed according to Piper (1950) and Jackson (1958) and obtained data presented in Table (1).

Table 1. Some physio-chemical characteristics of collected soil samples

Character	El-Dakahlia (Mansoura)	Kafr El-Sheikh (Sakha)
Physical analysis (%)		
Coarse Sand	2.24	1.55
Fine Sand	23.51	19.79
Silt	42.00	47.57
Clay	32.26	31.09
Soil Textural class	Clay loam	Clay loam
Chemical analysis		
pH	7.97	7.9
EC (ds/m)	0.7	1.176
Cations (ppm) Ca ⁺⁺	68.01	122.13
Mg ⁺⁺	34.50	65.41
Na ⁺	397.17	646.85
K ⁺	8.33	25.07
Anions (ppm) CO ₃ ⁻	0	0
HCO ₃ ⁻	725.17	792.17
Cl ⁻	143.54	434.22
SO ₄ ⁻	0	18.69

Wheat grain

Wheat grains (*Triticum aestivum* L) cv. Gemmiza12 were kindly obtained from Wheat Research Institute, Agricultural Research Center (ARC), Kafr El-sheikh, Egypt.

Mineral fertilization

The pots soil was plowed three times in perpendicular directions. Phosphate fertilizer at 15 kg phosphorus pentoxide (P₂O₅)/fed was broadcasted as calcium super-phosphate (15% P₂O₅) on the dry soil surface (El-Saadny 2013). Nitrogen fertilizer in the form of urea (46.5%N) at the rate of 37.5, 56.25 and 75 kg N/fed which represent 50%, 75% and 100 % from the final recommended doses was applied in to equal doses, before the first and second irrigation. Potassium fertilizer in the form of potassium sulphate (48% K₂O) at the rate of 24 kg K/fed was applied with the first dose of nitrogen fertilizer.

Compost tea

Compost water extract (Compost Tea) was added three times with foliar spray at a rate of (1:5 v/v) till the end of the experiments and was brought kindly from Agric. Microbiol. Dept., Agric. Res. Center(ARC), Sakha , Kafr El Sheikh, Egypt.

Preparation of cyanobacterial extracts

The selected cyanobacterial strain (*Anabaena cylindrica*) was cultured in 500 ml flasks containing BG-11₀ medium for 14 days under continuous illumination (5000 lux) and at 28-30°C, with constant agitation. Cultures were harvested and cells were washed with distilled water. Cell

extracts were prepared by grinding the cyanobacterial growth in distilled water with a mortar and blender. Cyanobacterial extract containing 5.0 g of fresh cyanobacterial material in 500 ml of distilled water is considered a 1% extract (Shariatmadari et al. 2013). Cyanobacterial extracts were added three times with foliar spray for pots received this treatment.

Standard cyanobacterial inoculum preparation for pot experiment

The standard inoculums of the selected cyanobacterial strains were prepared by inoculating Erlenmeyer flasks (500 ml) containing 250 ml of modified BG11₀ broth medium with a loopfull of 21 days old culture of each ones. Inoculated flasks were incubated at 28-30°C under continuous illumination (5000 lux) for one month before application in the pot experiment. Inoculum was prepared using a sieved clay soil (as a carrier) just before pot application, by adding 100 ml of homogenous cyanobacterial growth to amounts of 1 kg carrier. Mean number of cyanobacteria (as cfu) in the inoculants were determined using the MPN method as recommended by Cochran (1950). Mean numbers of cyanobacteria was found to be 1.5x10⁷ cfu/ml. The soil-based cyanobacterial inocula (SBI) that were prepared as described by Venkataraman (1972), were inoculated to pots 10 days after wheat sowing at the rate of 10 kg SBI fed⁻¹. The cyanobacterial inoculation was carried out only for pots received this treatment.

Pot experiment

A pot experiment was carried out at the glass house in Faculty of Agriculture, Mansoura University, during the winter season of (2018) to study the comparison between bio-fertilization as inoculants of the most efficient cyanobacterial strain that identified (Zaki 2022) as *Anabaena cylindrica* and their extracts as well as tea compost on the growth and yield of wheat. The experiment was arranged as a completely randomized design with three replicates which means three pots for every treatment. Pots with 35 cm height and 30 cm in diameter were filled with 8 kg sandy soil each. Before baking the pots, the soil was thoroughly mixed uniformly with phosphate and potassium fertilizers. The treatments were as recorded in Table (2).

Table 2. List of treatments and abbreviations used in the results

No.	Treatment	Abbreviations
1.	Control 100 (Mineral fertilizer N 100%)	C100
2.	Control 75 (Mineral fertilizer N 75%)	C75
3.	Control 50 (Mineral fertilizer N 50%)	C50
4.	C ₇₅ + <i>Anabaena cylindrica</i>	C ₇₅ A
5.	C ₇₅ + <i>Anabaena cylindrica</i> Extract	C ₇₅ AE
6.	C ₇₅ + Compost Tea	C ₇₅ T
7.	C ₇₅ + Compost Tea + <i>Anabaena cylindrica</i>	C ₇₅ TA
8.	C ₇₅ + Compost Tea + <i>Anabaena cylindrica</i> Extract	C ₇₅ TAE
9.	C ₅₀ + <i>Anabaena cylindrica</i>	C ₅₀ A
10.	C ₅₀ + <i>Anabaena cylindrica</i> Extract	C ₅₀ AE
11.	C ₅₀ + Compost Tea	C ₅₀ T
12.	C ₅₀ + Compost Tea + <i>Anabaena cylindrica</i>	C ₅₀ TA
13.	C ₅₀ + Compost Tea + <i>Anabaena cylindrica</i> Extract	C ₅₀ TAE

Vegetative plant analysis

A plant sample was collected at 60, 90 and 120 day after wheat sowing, transplanting and the harvest stage. Then, different morphological parameters of plant growth [plant height (cm), root height (cm), number of leaves/plant,

number of tillers/plant, number of spikes/plant, fresh weight (g/plant), dry weight (g/plant), root fresh weight (g/plant), root dry weight (g/plant) and weight of 1000-grain (g)] were assessed. A random sample was taken from threshed grains to measure the 1000-grain weight (g). One thousand air dry wheat grains were weighed.

Pigments plant analysis

The pigments of plant were estimated in fresh leaves: Chlorophyll a, b (mg g⁻¹ F.W) was estimated according to Sadasivam and Manickam (1996). The carotenoids (mg 100g⁻¹) content was determined spectrophotometer by the procedure postulated by Ranganna (1997). And total Chlorophyll content was estimated according to Hoel (1998).

Chemical plant analysis

N, P and K concentrations in plant tissues were determined according to Gotteni et al. (1982). Total Nitrogen was determined using Micro kjeldahl apparatus as described by Jones et al. (1991). Total phosphorus was determined colorimetrically as indicated by spectrophotometer (Mousa et al. 2018). And total potassium was estimated by using Jenway Flame photometer, Model coming 400 according to the modified method of Peters et al. (2003).

Microbiological analysis

Bacterial total counts and total counts of cyanobacteria were determined in soil samples which collected after 60, 90 and 120 day. To enumerate the number of viable bacteria in soil samples, the pour plate method was used (Skerman and Hillard 1967). From each dilution three replicates were prepared. Colony counts of bacteria were counted after incubated for 3 days at 30°C. The total number of colonies was recorded as cfu (colony forming units) per gram of dry soil.

Method used for cyanobacterial count was the most probable number (MPN) technique using the modified Watanabe medium (El-Nawawy et al. 1958. Watanabe et al. 1951). Colony count of cyanobacteria were counted as described by tubes that were incubated at 30°C under continuous light of 120 cm long white fluorescent lamps intensity of 2500 lux after ten days of incubation.

Statistical analysis

Data were subjected to statistical analysis by using COSTAT (2005) Software of analysis of variance according to Gomez and Gomez (1984). Duncan multiple range test have been used to compare means at p=0.05 as outlined by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

In view of the previous study, (Zaki et al., 2021) the present research was undertaken to isolate the prospective cyanobacteria from rhizosphere soil and screen for their potential as N-fixers and plant growth promoters. Then, it could be concluded that the cyanobacteria strain of *Anabaena cylindrica* appeared to be the most efficient strain in nitrogenase activity and Indole Acetic Acid production. Therefore, the strain was chosen to be used in preparing cyanobacterial inoculants as a biofertilizer for the complete of the following experiment.

Pot experiment

Nitrogen-fixing cyanobacterium and bacterial rhizosphere soil content

Total bacterial count in rhizosphere soil was varied according to the inoculated with *Anabaena cylindrica* in

Figs. (1&2). The viable count was the highest (7.70 X10³CFU) with mixture inoculation under 100% nitrogen fertilization after 60 days compared to those recorded by the other treatment. Followed by 75% nitrogen fertilization and compost tea with *Anabaena* after 60 days. Roger and Ardales (1991) reported that the survival of the inoculated species remained for at least 300 days.

As for the N₂-fixing cyanobacteria found that the highest count was obtained with C₇₅TA after 90 days 3.74 X10³CFU which was near to 60 day 3.44 X10³CFU.

This result is similar to those reported by EL-Zawawy (2019) who found that inoculation with mixture cyanobacteria under 60 nitrogen level/fed after 60 days recorded the highest number for viable counts. Similar results were obtained with Dazzo and Yanni (2006) who indicated that foliar fertilization with compost tea an its remarkable nutritional values that are present in soluble chemical components.

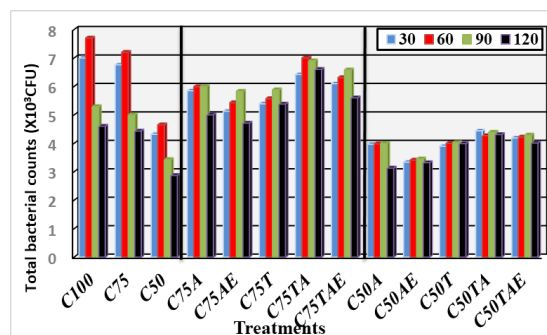


Fig. 1. Total bacterial counts (X10³CFU) in rhizosphere soil affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

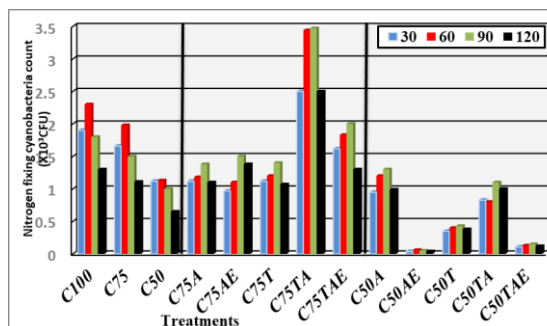


Fig. 2. N₂-fixing cyanobacteria counts (X10³CFU) in rhizosphere soil affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Vegetative growth parameters of wheat

All the treatments as N fertilization rates, compost tea, *Anabaena cylindrica* and its extract addition in single treatment or mixed together on plant of wheat through three growth stages (60, 90 and 120 days from planting) (Table 3) are investigation significantly (p ≤ 0.05) different from one to another. Data at Table (3) presented the maximum growth of wheat in term of plant height (84.71 cm) was measured in treated with C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) which recorded 45.66, 78.53 and 84.71 cm after 60, 90 and 120 days, respectively, from planting. In the case of shoot fresh weight, results are illustrated in Table (3) showed that the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) gave the highest values in shoot fresh

weight and scored 13.21, 15.65 and 27.69 g/plant, respectively after 60, 90 and 120 days from planting, compared to other treatments. Data in the same table mentioned that the treatment C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) gave the highest values in shoot dry weight. Also, results in Table (3) revealed that inoculation with *Anabaena cylindrica* and its extract under different nitrogen fertilization with compost tea on number of leaves/plant ranged from 15.33 to 20.33, 17.33 to 22.67 then decreased 8.67 to 14.00 after 60, 90 and 120 days, respectively. However, the highest mean values recorded with C₇₅TA and results with the number of tillers/plant in wheat showed highest values recorded with the best combination of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*).

While, the data presented in Table (4), showed root length under these treatments showed that all treatments under investigation significantly affected in root length and the highest values recorded with treatments C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*), scored 12.03, 15.63 and 18.26 cm after 60, 90 and 120 days, respectively, from planting. Concerning the data of root fresh weight in Table

(4) it could be revealed that all treatments were significantly affected in root fresh weight. The highest values of root fresh weight after 120 days recorded with C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*), it's recorded 3.46 g/plant. With regard to the data of root dry weight, data in Table (4) showed that the treatment C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) recorded the maximum value of root dry weight .

As for the effect of compost tea enhancing the uptake and accumulation of nutrient elements in the plant (Rodríguez-Ortíz et al. 2006). It is a primary nutrient source, and when applying in conjunction with mineral fertilizers results in high performance (Al-Tahlawy 2018). The increase in vegetative and root synthesis of the studied wheat plant treated with different fertilization treatments can be also due to increase of nutrients added by cyanobacteria and uptake of these nutrients by wheat seeds (Haroun and Hussein 2003). In this context, N fixed by the cyanobacteria and made available to plant can play an important role (Kuhlbusch et al. 1991).

Table 3. Vegetative growth parameters of wheat plants (for 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	Plant length (cm)			Shoot fresh weight (g/plant)			Shoot dry weight (g/plant)			No. leaves/plant			No. tillers/plant		
	60	90	120	60	90	120	60	90	120	60	90	120	60	90	120
C ₁₀₀	45.37a	77.61ab	83.99b	12.96b	15.35ab	27.10ab	3.49b	7.47b	11.35b	19.00abc	22.67a	13.67ab	4.67a	5.00a	6.33a
C ₇₅	39.74d	71.20e	78.17g	10.63e	13.78d	22.73e	2.75g	6.04g	9.86g	18.00cde	21.33a-d	12.00d	4.00ab	4.33a	5.67a
C ₅₀	30.13h	59.70i	66.40m	7.31k	11.05g	14.99j	1.48m	3.76m	7.46m	15.33g	17.33g	8.67f	2.00c	2.67a	3.00b
C ₇₅ A	41.44c	73.18d	79.98e	11.12d	14.26c	24.14d	2.99e	6.43e	10.31e	18.33bcd	21.67a-d	12.67bcd	4.33ab	4.67a	5.67a
C ₇₅ AE	43.39b	75.56c	82.49d	12.36c	14.98b	25.99c	3.30d	7.11d	10.93d	19.33abc	22.00abc	13.33abc	4.67a	5.00a	6.00a
C ₇₅ T	40.57cd	71.74de	79.09f	10.93d	14.36c	23.5d	2.88f	6.23f	10.12f	18.33bcd	21.00a-d	12.33cd	4.00ab	4.33a	5.33ab
C ₇₅ TA	45.66a	78.53a	84.71a	13.21a	15.65a	27.69a	3.61a	7.61a	11.56a	20.33a	22.67a	14.00a	5.00a	5.00a	6.33a
C ₇₅ TAE	44.24ab	76.55bc	83.39c	12.74b	15.19ab	26.62bc	3.41c	7.25c	11.17c	19.67ab	22.33ab	13.67ab	4.67a	5.00a	6.00a
C ₅₀ A	31.59g	61.69h	68.86k	7.96i	11.64f	16.61h	1.72k	4.23k	7.94k	16.00fg	18.67efg	9.67ef	2.67bc	5.00a	4.00ab
C ₅₀ AE	35.00f	65.72g	72.38j	8.86h	12.49e	18.88g	2.09j	4.92j	8.75j	16.33fg	19.67def	10.33e	3.33abc	3.67a	4.33ab
C ₅₀ T	30.70gh	60.72hi	67.80l	7.58j	11.42fg	15.86i	1.56l	4.01l	7.72l	16.67efg	18.00fg	9.00f	2.67bc	3.00a	4.00ab
C ₅₀ TA	36.53e	67.73f	74.24h	9.55f	12.96e	20.06f	2.29h	5.26h	9.14h	17.00def	20.33b-e	10.67e	3.67abc	4.00a	4.67ab
C ₅₀ TAE	35.68ef	66.89fg	73.24i	9.24g	12.74e	19.52fg	2.21i	5.12i	8.94i	16.67fg	20.00c-f	10.33e	3.33abc	3.67a	4.67ab

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

Table 4. Root changes of wheat plants (for 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	Root length (cm)			Root fresh weight (g/plant)			Root dry weight (g/plant)		
	60	90	120	60	90	120	60	90	120
C ₁₀₀	11.65b	15.29b	17.86 b	1.93 d	1.47 j	0.63 l	0.81 a	0.98 b	1.08ab
C ₇₅	9.45 g	13.06g	15.63 g	2.53 b	2.02 g	1.02 k	0.42 d	0.67 e	0.79 e
C ₅₀	5.51m	9.19 m	11.84m	3.41 a	2.76 a	1.32 j	0.10 h	0.12 i	0.23 i
C ₇₅ A	10.15e	13.84e	16.34 e	1.61 g	2.19 e	2.98 c	0.53 c	0.77 d	0.86 d
C ₇₅ AE	11.08d	14.64d	17.26 d	1.80 f	2.38 d	3.25 b	0.71 b	0.88 c	0.96 c
C ₇₅ T	9.85 f	13.49f	15.96 f	1.54 h	2.09 f	2.87 d	0.47cd	0.73de	0.81de
C ₇₅ TA	12.03a	15.63a	18.26 a	1.99 c	2.63 b	3.46 a	0.85 a	1.05 a	1.13 a
C ₇₅ TAE	11.31c	14.98 c	17.66 c	1.88 e	2.48 c	3.41 a	0.76 b	0.94 b	1.04 b
C ₅₀ A	6.31 k	9.94 k	12.54 k	0.82 l	1.24 k	1.62 h	0.12gh	0.20 h	0.33 h
C ₅₀ AE	7.56 j	11.20 j	13.74 j	1.07 k	1.54 i	2.08 g	0.17fg	0.38 g	0.49 g
C ₅₀ T	5.92 l	9.55 l	12.07 l	0.72m	1.14 l	1.47 i	0.11 h	0.14 i	0.29 h
C ₅₀ TA	8.33 h	11.90 h	14.44 h	1.21 i	1.70 h	2.34 e	0.25 e	0.49 f	0.58 f
C ₅₀ TAE	7.95 i	11.54 i	14.05 i	1.14 j	1.60 i	2.22 f	0.22 ef	0.43 g	0.53fg

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

Pigment content

The data in Table (5) on chlorophyll A (mg.g⁻¹) as affected by different levels of nitrogen mineralization with compost tea, *Anabaena cylindrica* and *Anabaena cylindrica*

extract revealed that all the treatments were significantly affected on the chlorophyll A content. The maximum chlorophyll A content of wheat leaves was 0.64, 0.79 and 0.76 mg.g⁻¹ recorded after 60, 90 and 120 days from

planting, respectively were noted with the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*).

Statistical analysis of data on chlorophyll B showed that the treatments means were significantly ($p < 0.05$) different from one another (Table 5). The significantly maximum content of chlorophyll B was 0.42, 0.50 and 0.48 mg.g⁻¹ after 60, 90 and 120 days with the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*). Data on the plant carotenoids content of wheat leaves revealed that treatments means were significantly ($p < 0.05$) different from one another (Table 5). The highest mean values of carotenoids content was realized with the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) which scored 0.32, 0.45 and 0.36 mg.g⁻¹.

Application of nitrogen directly increased the chlorophyll content and leaf surface area resulting in increased photosynthesis process leading to more sugar formation (Dikshit and Paliwal 1989). The increase in growth owing to the application of N-fertilizers may be attributed to the fact that these nutrients being important constituents of nucleotides, proteins, chlorophyll and enzymes, involve in various metabolic processes which have direct impact on vegetative and reproductive phases of plants (Mengel and Kirkby 1996).

Verma *et al.* (2004) recorded that the N content in the third leaf and chlorophyll a content increased with

increasing nitrogen rate. Chlorophyll is essential for leaf photosynthesis, and its content in wheat leaves reflects their photosynthetic capacity (Hlavacova *et al.* 2018). Nitrogen affects and participates in chlorophyll synthesis (Gaju *et al.* 2014). Moderate nitrogen fertilizer application has been shown to increase leaf chlorophyll content, which promotes the growth of aboveground parts, such as leaves, and thus leads to an increase in biomass (Yang *et al.* 2017; Tian *et al.* 2020). Chlorophyll content was significantly correlated with nitrogen content (Bojović and Marković 2006).

Cyanobacterial filtrates can stimulate synthesis of GA3 (Drazkiewicz 1994) and auxin and cytokinin (Stirk *et al.* 2002) in germinated wheat. Ördög (1999) showed that cyanobacterial contain a special set of biologically active compounds including plant growth regulators, which can decrease senescence and transpiration and increase the content of leaf chlorophyll.

Also, the increase in leaf area in response to the different fertilization treatments may have accumulated chlorophyll in leaves. In this respect, Pereira *et al.* (2009) found that the use of bio-fertilizers based on local strains of cyanobacteria was effective to increase the nitrogen use efficiency in rice. It seems reasonable to suggest that, significant increase in photosynthetic pigments a result of bio-fertilizer treatments may be due to the role of nitrogen in the increase of photosynthetic activity of the chloroplast.

Table 5. Pigment content (mg.g⁻¹) of wheat plants (for 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	Chlorophyll A			Chlorophyll B			Carotenoids		
	60	90	120	60	90	120	60	90	120
C ₁₀₀	0.63ab ab	0.78ab ab	0.75 b	0.41 b	0.50 b	0.47 b	0.31 b	0.44 b	0.35 b
C ₇₅	0.57 e	0.71 f	0.68 g	0.35 g	0.43 f	0.41 g	0.25 g	0.37 g	0.30 g
C ₅₀	0.46 i	0.60 k	0.57 l	0.23m	0.31 l	0.29 m	0.15 l	0.26m	0.20 m
C ₇₅ A	0.59 d	0.75 d	0.71 e	0.37 e	0.45 d	0.42 e	0.26 e	0.39 e	0.31 e
C ₇₅ AE	0.61 c	0.76 cd	0.73 d	0.40 d	0.48 c	0.45 d	0.29 d	0.42 d	0.34 d
C ₇₅ T	0.58 e	0.73 e	0.69 f	0.36 f	0.44 e	0.41 f	0.25 f	0.38 f	0.30f
C ₇₅ TA	0.64 a	0.79 a	0.76 a	0.42 a	0.50 a	0.48 a	0.32 a	0.45 a	0.36 a
C ₇₅ TAE	0.62 b	0.77 bc	0.74 c	0.40 c	0.49 b	0.46 c	0.30 c	0.43 c	0.35 c
C ₅₀ A	0.48 h	0.62 j	0.60 j	0.26 k	0.33 j	0.32 k	0.16 k	0.28 k	0.21 k
C ₅₀ AE	0.51 g	0.66 i	0.63 i	0.29 j	0.37 i	0.35 j	0.19 j	0.31 j	0.25 j
C ₅₀ T	0.46 i	0.61 k	0.59 k	0.24 l	0.32 k	0.30 l	0.15 l	0.27 l	0.20 l
C ₅₀ TA	0.53 f	0.68 g	0.56m m	0.31 h	0.39 g	0.37 h	0.21 h	0.33 h	0.25 i
C ₅₀ TAE	0.52 g	0.67 h	0.64 h	0.30 i	0.38 h	0.36 i	0.20 i	0.32 i	0.25 h

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

Nitrogen, phosphorus and potassium content in shoot

The data of nutritional status of plant (at 60, 90 and 120 DAS) are presented in Table (6) as affected by nitrogen mineralization with compost tea, *Anabaena cylindrica* and *Anabaena cylindrica* extract. From observed data found that all treatments significantly affected in N, P and K percent in shoot during the three stages.

The highest mean values of N, P, K were obtained with the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) which recorded 2.74, 3.30 and 2.42% for N, 0.32, 0.42 and 0.28% for P and 2.72, 3.26 and 2.56% for K after 60, 90 and 120 days.

Nitrogen, phosphorus and potassium content in root

Data in Table (7) showed the values of N, P and K percent in root as affected by nitrogen mineralization with compost tea, *Anabaena cylindrica* and *Anabaena cylindrica* extract. Regarding the varietal differences, the data showed

that all treatments significantly affected the root content of NPK and the different application from all treatments increase the nutritional root content. From the data found the highest value recorded with the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*).

Nitrogen, phosphorus and potassium content in straw and grain

Concerning the effect of nitrogen mineralization with compost tea, *Anabaena cylindrica* and *Anabaena cylindrica* extract on content of N, P and K percent in straw and grain, data in Table (8) showed a significant ($P \leq 0.05$) predictable improvement in N, P and K concentration in both straw and grain of wheat. The increment obtained by the best interaction was with the treatment of C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) which recorded 1.85, 0.24 and 1.83% in straw and 2.84, 0.36 and 2.80% in grain for N, P and K%.

Table 6. Nitrogen, phosphorus and potassium content (%) in shoots of wheat plants (for 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	N			P			K		
	60	90	120	60	90	120	60	90	120
C ₁₀₀	2.69 b	3.23b	2.37b	0.31 b	0.42 b	0.27 b	2.63 b	3.17 b	2.50 b
C ₇₅	2.25 e	2.82e	1.97 f	0.25 g	0.36 f	0.22 g	2.21 g	2.78 e	2.06 f
C ₅₀	1.48 k	2.02 j	1.30 l	0.16m	0.25 k	0.15m	1.29m	1.95 j	1.22 l
C ₇₅ A	2.35 d	2.94d	2.08d	0.27 e	0.38 d	0.24 e	2.33 e	2.86 d	2.20e
C ₇₅ AE	2.56 c	3.13 c	2.26 c	0.29 d	0.40 c	0.26 d	2.51 d	3.09 c	2.37d
C ₇₅ T	2.31 d	2.90d	2.02e	0.26 f	0.37 e	0.23 f	2.27 f	2.82de	2.09 f
C ₇₅ TA	2.74 a	3.30a	2.42a	0.32 a	0.42 a	0.28 a	2.72 a	3.26 a	2.56a
C ₇₅ TAE	2.60 c	3.17 c	2.32b	0.30 c	0.41b	0.26 c	2.57 c	3.13bc	2.42 c
C ₅₀ A	1.64 i	2.16h	1.45 j	0.18 k	0.28 i	0.16 k	1.54 k	2.19 h	1.46 j
C ₅₀ AE	1.90h	2.39g	1.63 i	0.16 l	0.26 j	0.15 l	1.77 j	2.45 g	1.70 i
C ₅₀ T	1.57 j	2.09 i	1.40k	0.21 j	0.31 h	0.18 j	1.39 l	2.09 i	1.32k
C ₅₀ TA	2.04 f	2.55 f	1.75g	0.23 h	0.33 g	0.20 h	1.92 h	2.56 f	1.82g
C ₅₀ TAE	1.96 g	2.44g	1.69h	0.22 i	0.31 h	0.19 i	1.85 i	2.49 g	1.75h

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

Table 7. Nitrogen, phosphorus and potassium content (%) in roots of wheat plants (for 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	N			P			K		
	60	90	120	60	90	120	60	90	120
C ₁₀₀	1.64 b	2.51 b	2.18 b	0.25 b	0.33 b	0.24 b	1.91 b	2.40 b	2.20 a
C ₇₅	1.33 e	2.14 f	1.88 e	0.21 g	0.29 f	0.21 g	1.56 g	1.98 g	1.77 f
C ₅₀	0.79 k	1.48 l	1.26 k	0.13m	0.19 l	0.14m	0.91m	1.26m	1.04 k
C ₇₅ A	1.41 d	2.27 d	1.98 d	0.22 e	0.30 e	0.22 e	1.66 e	2.10e e	1.88 d
C ₇₅ AE	1.54 c	2.41 c	2.11 c	0.24 d	0.31 d	0.24 d	1.82 d	2.27 d	2.07 c
C ₇₅ T	1.38 d	2.20e e	1.91 e	0.21 f	0.29 e	0.21 f	1.62 f	2.05 f	1.82 e
C ₇₅ TA	1.69 a	2.56 a	2.23 a	0.26 a	0.33 a	0.25 a	1.96 a	2.45 a	2.24 a
C ₇₅ TAE	1.61 b	2.49 b	2.14bc	0.24 c	0.32 c	0.24 c	1.85 c	2.34 c	2.15 b
C ₅₀ A	0.90 i	1.64 j	1.39 i	0.15 k	0.21 j	0.15 k	1.09 k	1.37 k	1.15 i
C ₅₀ AE	1.08 h	1.81 i	1.56 h	0.17 j	0.24 i	0.18 j	1.28 j	1.62 j	1.42 h
C ₅₀ T	0.85 j	1.57 k	1.34 j	0.13 l	0.20 k	0.14 l	0.98 l	1.31 l	1.09 j
C ₅₀ TA	1.18 f	1.97 g	1.68 f	0.18 h	0.26 g	0.19 h	1.39 h	1.76 h	1.51 g
C ₅₀ TAE	1.14 g	1.91 h	1.62 g	0.18 i	0.25 h	0.18 i	1.33 i	1.67 i	1.47 g

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

Table 8. Nitrogen, phosphorus and potassium content (%) in straw and grains of wheat plants (for 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	Straw			Grains		
	N	P	K	N	P	K
C ₁₀₀	1.81 b	0.23 ab	1.78 b	2.78 b	0.36 b	2.73 b
C ₇₅	1.58 e	0.20 f	1.56 e	2.43 e	0.31 f	2.39 e
C ₅₀	1.13 j	0.14 k	1.09 j	1.74 j	0.22 k	1.68 j
C ₇₅ A	1.65 d	0.21 d	1.60 d	2.53 d	0.32 d	2.46 d
C ₇₅ AE	1.75 c	0.22 c	1.73 c	2.69 c	0.34 c	2.66 c
C ₇₅ T	1.62 d	0.20 e	1.58 de	2.49 d	0.32 e	2.42 de
C ₇₅ TA	1.85 a	0.24 a	1.83 a	2.84 a	0.36 a	2.80 a
C ₇₅ TAE	1.78 bc	0.23 b	1.75 bc	2.73 c	0.35 b	2.69 bc
C ₅₀ A	1.21 h	0.15 i	1.23 h	1.86 h	0.24 i	1.89 h
C ₅₀ AE	1.34 g	0.17 h	1.37 g	2.06 g	0.27 h	2.11 g
C ₅₀ T	1.17 i	0.15 j	1.17 i	1.80 i	0.23 j	1.79 i
C ₅₀ TA	1.43 f	0.18 g	1.44 f	2.19 f	0.28 g	2.21 f
C ₅₀ TAE	1.37 g	0.18 h	1.39 g	2.1 g	0.27 h	2.14 g

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

The highest mean values of N, P, K % recorded with nitrogen fertilization may be return to the elements availability for plant and improving root growth, hence increasing the absorbing area of root, these results were consistent with finding of El-Sobky and Desoky (2016)

resulted that *Anabaena* and spraying with *Anabaena* extract and mineral nitrogen fertilizer significantly increased N, P, and K content of shoots of rice plant.

Results of Abed EL-Rasoul *et al.* (2004) and Mussa *et al.* (2003) indicated that spraying nitrogen fixing bio-fertilizers individually significantly increased N, P, and K concentration by grains and straw over the control treatments (without spraying bio-fertilizers) confirmed increasing the nutrient concentration by wheat grain and straw in response to the use of cyanobacterial as an activator bio-fertilizer separately. In this respect, Safinaz and Ragaa (2013) found that inoculation by *Laurencia obtusa*, *Jania rubens*, *Corallina elongata* either singly, and their combinations have significant potential as biofertilizers on NPK content of maize plant. Also, El-Sobky and Desoky (2016) suggested significant differences among bio-fertilizer treatments in N, P, and K content while the best result of bio-fertilizer was obtained for treatment inoculated with *Anabaena* and spraying with *Anabaena* extract treatment followed by that inoculated with *Anabaena* treatment.

Yield parameters

Results in Table (9) indicated the effect of inoculation under nitrogen fertilization with compost tea on weight of 100 grain g. Results revealed that with increasing nitrogen fertilization the weight of 100 grain increased especially under inoculation *Anabaena cylindrica* and

Anabaena cylindrica extract and compost tea. While the highest mean values recorded with C₁₀₀ (100% N fertilization) and scored 5.59 g followed by C₇₅TA (N 75 % + compost tea + *Anabaena cylindrica*) and scored 5.47 g.

The results in Table (9) illustrated the effect of nitrogen mineralization with compost tea, *Anabaena cylindrica* and *Anabaena cylindrica* extract on weight of straw g/plant.

On average the maximum weight of straw 12.28 g/plant was obtained with the application of 75% N and compost tea with inoculation with *Anabaena cylindrica* and *Anabaena cylindrica* extract over the other treatments.

Data in Table (9) showed significant differences among bio-fertilization and N fertilization with compost tea. On the average maximum weight of spike (3.91 g) was obtained with C₇₅TA (75% nitrogen with compost tea, *Anabaena cylindrica* and *Anabaena cylindrica* extract). According to results in Table (9) the number of grain/spike recorded that the highest increase was obtained with C₇₅TA treatments.

The increase in yield with increasing N rates up to adequate level might be due to the role of N in increasing the leaf area and promote photosynthesis efficiency which promote dry matter production and increase yield. In line with this, improvements in wheat yield and its components under the acceptable increasing N rates were reported by Sticksel *et al.* (2000). As for the effect of cyanobacteria, this result is similar to those reported by Karthikeyan *et al.* (2007) and El-Zemrany (2017) who reported the effects of inoculant cyanobacterial strains on wheat involving (single or in combination) showed visible differences in terms of the appearance of plants. This was accompanied by enhancement in plant height, dry weight and grain yield of wheat crop.

Table 9. Yield parameters of wheat plants (at 120 days) affected by *A. cylindrica*, its extract and compost tea comparing with different levels of nitrogen mineralization.

Treatments	100 grain weight (g)	Straw weight (g/plant)	Spike weight (g)	No. of grain/spike	Harvest Index %
C ₁₀₀	5.59 a	11.75 b	3.82 ab	70.67 ab	47.57
C ₇₅	4.68 e	8.98 g	3.22 e	60.00 c	52.11
C ₅₀	3.03 j	4.24 l	2.39 i	42.00 e	71.46
C ₇₅ A	4.90 d	9.74 e	3.37 d	63.33 c	50.30
C ₇₅ AE	5.34 c	11.08 d	3.65 c	68.00 b	48.19
C ₇₅ T	4.83 d	9.45 f	3.32 de	61.67 c	51.11
C ₇₅ TA	5.40 bc	12.28 a	3.91 a	72.33 a	43.97
C ₇₅ TAE	5.47 b	11.41 c	3.72 bc	69.67 ab	47.94
C ₅₀ A	3.35 h	4.69 k	2.51 h	46.00 e	71.42
C ₅₀ AE	3.88 g	6.38 j	2.79 g	52.33 d	60.81
C ₅₀ T	3.19 i	4.42 l	2.42 hi	44.67 e	72.17
C ₅₀ TA	4.17 f	7.24 h	2.94 f	55.00 d	57.59
C ₅₀ TAE	3.98 g	6.82 i	2.86 fg	53.00 d	58.35

C₁₀₀: 100% N; C₇₅: 75% N; C₅₀: 50% N; A: *Anabaena*; E: Extract, T: Compost tea. Different letters in each column indicate significant differences between treatments at 5% level of significance

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تقييم كفاءة سلالة السيانوبكتيريا ومستخلصها وشاى الكمبوست كأسمدة حيوية على محصول نبات القمح

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الملخص

تعتبر الطحالب الخضراء المزرقة من مثبتات النتروجين الجوى حيث تقوم بتزويد نباتات القمح باحتياجاتها من النتروجين الذى تقوم بتثبيتته فى حقول القمح . فى هذه الدراسة أجريت تجربة زراعة القمح فى البيوت الزجاجية بمزرعة كلية الزراعة جامعة المنصورة بالمنصورة مصر خلال موسم شتاء ٢٠١٨ بغرض دراسة تأثير التلقيح بسلالة السيانوبكتيريا *Anabaena cylindrica* ومستخلصها وشاى الكمبوست منفردة أو كمخاليط مع جرعات من السماد النتروجينى المعدنى وذلك على نمو ومحصول القمح . وقد أشارت النتائج المتحصل عليها خلال ثلاثة مراحل من نمو النبات (٦٠ - ٩٠ - ١٢٠ يوم من الزراعة) إلى وجود فرق معنوى من معاملة لأخرى وسجلت النتائج أن هناك معنوية فى نمو النبات الخضرى وأيضاً فى المحصول وكذلك فى محتوى العناصر (النتروجين والفوسفور واليوتاسيوم) فى المجموع الخضرى والجذرى والقش بالإضافة إلى محصول الحبوب وذلك مع المعاملة C₇₅TA *Anabaena cylindric* + شاى الكمبوست + ٧٥% من الجرعة الموصى بها من التسميد النتروجينى) . كما أشارت النتائج إلى أن معاملات التسميد الحيوى أظهرت زيادة فى أعداد البكتيريا الحية فى التربة بالإضافة إلى زيادة أعداد سلالة السيانوبكتيريا المستخدمة وذلك مع المعاملة C₇₅TA بعد ٦٠ يوم من الزراعة فى كلتا الحالتين . من هذه الدراسة يمكن القول أن التلقيح بسلالة السيانوبكتيريا ومستخلصها وشاى الكمبوست أدى إلى توفير ٢٥% من معدلات التسميد النتروجينى المعدنى الموصى بها.