Archives of Agriculture Sciences Journal
Print ISSN: 2535-1680
Online ISSN: 2535-1699

ARCHIVES OF AGRICULTURE SCIENCES JOURNAL

Volume 3, Issue 3, 2020, Pages 358–373

Available online at www.agricuta.edu.eg

DOI: https://dx.doi.org/10.21608/aasj.2020.200357

Effect of irrigation treatments and cyanobacteria with different nitrogen fertilization levels on wheat yield and quality grown on sandy soils

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Abstract

A pot experiment was carried out under greenhouse conditions during the two winter seasons 2017/2018 and 2018/2019, at the farm of Agronomy Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt to study the effect irrigation water treatments (a1= tap water and a2= saline water) and cyanobacteria inoculation combined with different nitrogen levels (b1 = 100% N, b2 = 75% N + *Nostoc muscorum*, b3 = 50 % N + *Nostoc muscorum*, b4 = zero % N + *Nosto cmuscorum* and b5 = control) on the yield and quality of wheat (*Triticum aestivum*, L. variety Giza 171) grown on sandy soil. The results indicated that, the studied parameters considered in this study were significantly affected by the utilized irrigation water treatments, cyanobacteria inoculation combined with different nitrogen levels and their interactions. Herein, it was observed that, salinity stress decreased the agronomic and quality attributes of wheat. Moreover, wheat plants retained the best values for plant height (cm), number of spikes per plant, spikes dry weight per pot (g), grains dry weight per pot (g), 1000 grain weight (g) and grain nitrogen, phosphorus and potassium content at the application of tap water compared to those irrigated with saline water. Regarding to cyanobacteria effect under three quarter or half recommended doses of N % improved growth and yield attributes of wheat plant grown under salinity stress via increasing plant tolerance to salinity stress. The obtained results showed that, wheat plants treated with 75% N + *Nostoc muscorum*, recorded the best values for most of studied characters as compared with control.

Keywords: wheat, saline water, cyanobacteria, water holding capacity, nitrogenase activity.

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1. Introduction

Wheat (*Triticum aestivum*, L) is one of the main food crops and cultivated worldwide firstly as food commodity and secondly a strategic commodity. Due to higher protein content of wheat grain over maize or rice, so wheat is considered one of the main leading sources of vegetable protein in human diet (Abd Allah *et al.*, 2015). It is an important cereal crop in Egypt and over the world used in human food and animal feed. It provides 37% of the total calories for the people and 40% of the protein in the Egyptian diet (El-Habbasha *et al.*, 2015). Recently, a great attention has been devoted to cultivating wheat in the newly reclaimed sandy soils to reduce the gap between the production and consumption, especially in Egypt. In general, under unfavorable conditions of such soil which characterized with low fertility, low organic matter content, micronutrients deficiency, high leaching rate and salinity (Abd El-Ghany, 2007; El-Fouly *et al.*, 2011). Moreover, salinity is one of the most serious environmental problems that affect adversely on plant growth and productivity of different crops. According to FAO, about one-third of world cultivated areas is affected by salinity stress. In addition, agricultural practices should become more sustainable, as the use of chemically based fertilizers, pesticides and growth stimulants can pose serious environmental problems and lead to the scarcity of finite resources, such as phosphorus and potassium, thus increasing the fertilizers costs. One possible alternative for the development of a more sustainable and highly effective agriculture is the use of biologically based compounds with known activity in crops' nutrition, protection and growth stimulation. Among these products, cyanobacterial biomass (or their extracts) is gaining particular attention, due to their undeniable potential as a source of essential nutrients and metabolites with different bioactivities, which can significantly improve crops yield. In attempting to develop productive, profitable and sustainable agriculture systems, several agriculturalists have been turned to farming methods, which are based on biotechnologies. One of the several approaches to achieve this goal is using the nitrogen fixing cyanobacteria to improve soil fertility and crop productivity. 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 and N2-fixing microorganisms including cyanobacteria (Spiller *et al.*, 1993). There is a need to test more cyanobacterial strains for artificial symbiosis. Such cyanobacteria may be applied in the field as soil inoculants in combination with fertilizers (Ahmed *et al.*, 2010). Cyanobacteria also have some soil phosphate solubilizing species. Phosphorus is the second important nutrient after nitrogen for plants and microorganisms and cyanobacterial decomposition is also reported to improve zinc nutrition of rice (Hedge *et al.*, 1999). Cyanobacteria excrete a great number of substances that influence plant growth and development. These microorganisms

have been reported to benefit plants by producing growth-promoting regulators (gibberellin and auxin), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity (Zaccaro, 2000). Application of algal biofertilizers is also useful for the reclamation of marginal soils such as saline-alkali and calcareous soils (Hedge *et al.,* 1999). *Nostoc muscorum* can improve the aggregate stability of a saline soil, where the increase in soil aggregation is mainly due to exopolysaccharide secreted by microorganisms or exopolysaccharide added to soil after death and cellular lysis (Zaccaro, 2000). Although several products can be used in the improvement of crop productivities, it is important to understand that different biological compounds may improve the agricultural productivities through different modes of action: (i) soils' improvement; (ii) crops' protection against biotic and abiotic stress factors; and (iii) direct growth stimulation. Considering these roles, microalgal/cyanobacterial products and biomass can be classified as biofertilizers, bio stimulants and biopesticides. the main activities associated to these biologically based products in the development of agricultural practices, highlighting their action mode and effect on crop production (Gonzalez *et al.*, 2016). Therefore, the present study was conducted to determine the effectiveness of irrigation water treatments (tap and saline water) and cyanobacteria inoculation combined with different nitrogen levels on the yield and quality of wheat grown on sandy soils.

2. Materials and methods

2.1 Source of cyanobacterial isolate

The following methods were applied on air-dried soil saline samples collected from El-Gharbia, (Qotor center), Egypt using Modified Watanabe medium (El-Nawawy *et al.,* 1958) for isolation and culturing of cyanobacteria. Semi-solid medium as described by El- Ayouty and Ayyad (1972) were used.

2.2 Purification of cyanobacteria

The unialgal cultures were purified according to Pringsheim (1949), any colored growth was selected, subcultured and streaked several times in new agatized Watanabe medium plate. To get unialgal cultures, the previous technique was repeated many times.

2.3 Bacteria free cyanobacterial cultures

To get bacteria free cultures, each culture was serially purified by washing (Hoshaw and Rosowski 1973) and Ultraviolet irradiation was done (Taha, 1963).

2.4 Identification of the isolated cyanobacteria

For identification of the purified isolated cyanobacteria, 500 ml Erlenmeyer flasks each containing 250 ml of Modified

Watanabe liquid medium and plates of agatized Modified Watanabe medium were inoculated with a loop full of 10 days old culture of each cyanobacterial isolates. For 10 days, Inoculated plates and flasks were incubated at 28-30ºC under continuous illumination (2500 lux). The identification of cyanobacteria was done as follows: Thallus morphology and dimension, thallus color, vegetative and reproductive cells, size of heterocyst. Heterocyst-forming cyanobacteria were also cultured in nitrogen-free Z-medium according to Venkataraman (1981). Plating technique of cyanobacteria enumeration N-free culture medium Watanabe medium (Allen and Stanier, 1968) was used for culturing N_2-fixing cyanobacteria plates.

2.5 Total nitrogen

Total nitrogen in the cyanobacteria were measured using the micro-kjeldahl method according to Jackson (1973). Results were expressed as mg nitrogen/100 ml culture.

2.6 Determination of phytohormones

Separation and determination of phytohormones (auxin, gibberellin and cytokinin) were carried out by gas liquid chromatography (GLC) in Al-Azhar university (the regional center for mycology and biotechnology). HPLC analysis was performed on GBC- germey by winChrome Chromatography Ver. 1.3 which equipped a GBC U.V/vis Detector and Hypercarb (C18, Sum 100x4.6 cm) the detective wavelength was 254nm flow rate of mobilephse was 7 ml/min which 85% Acent: 15% water. The method was according to Van Staden *et al.* (1973).

2.7 Determination of water holding capacity

Determination of water holding capacity (WHC) was determined according to (Richards, 1954).

2.8 Field experiment

The present investigation was carried out at the greenhouse of Department of Agronomy, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt, during the two successive winter seasons of 2017/2018 and 2018/2019, to investigate the effect of irrigation water treatments (tap and saline water) and cyanobacteria inoculation combined with different nitrogen levels (100 % N, 75 % N + *Nostoc muscorum*, 50 % N + *Nostoc muscorum*, Zero % N + *Nostoc muscorum* and control (without treatment) as well as their interaction on some physical soil properties, yield and its components and grain quality of wheat (Cultivar Giza 171). Plastic pots with a diameter of 30 cm were filled with 10 kg of sandy soil samples collected from Cairo Alexandria's, Desert Road (Sadate City) from surface layer (0-30 cm). Some physical and chemical characteristics of the studied soil before

planting were presented in Table (1) which was determined according to Page *et al.* (1982). Before baking the pots, the soil was thoroughly mixed uniformly with phosphate and potassium fertilizers at rates of 100 and 50 kg feddan^{-1} (feddan = 4200 m² = 0.420 hectares = 1.037 acres) in the form of superphosphates (15% P_2O_5) and potassium sulfate (48% K_2O), respectively, while Nitrogen added in two split doses, the first 2/3 N dose was added prior to wheat sowing. The second (1/3 N) was added after 30 days from sowing. Five wheat grains mixed with 1ml cyanobacteria (1.5×10^7 cfu) then were sowed into each pot and when the wheat seedlings developed, one seedling was taken for doing section of a wheat root and four ones were left in each pot, plant height (cm), spike number, spike dry weight (g), grain dry weight (g), 1000 grain weight (g) and Nitrogen, phosphorus as well as potassium content of wheat grains were determined.

The experiment comprises the following treatment:

(A) Irrigation water treatments:

1. (a1) Tap water.
2. (a2) Saline water (7.69 dS/m EC).

(B) Cyanobacteria inoculation combined with different nitrogen levels:

1. (b1) 100 % N.
2. (b2) 75 %N + *Nostoc muscorum*
3. (b3) 50 % N + *Nostoc muscorum*.
4. (b4) Zero % N + *Nostoc muscorum*
5. (b5) Control (untreated).

Table (1): Experimental soil characteristics.

Soil physical properties					
Particle size distribution	1st Season	2nd Season		1st Season	2nd Season
Coarse sand (%)	52.39	51.77	Field capacity (%)	6.24	6.49
Fine sand (%)	37.20	36.80	Wilting point (%)	1.62	1.78
Silt (%)	4.86	5.13	Available water (%)	4.62	4.71
Clay (%)	5.55	6.30	Water holding capacity (%)	16.78	17.66
Texture class	Sandy	Sandy	Bulk density (Mg m^{-3})	1.72	1.75
HC (m day^{-1})	11.91	11.32	Total porosity (%)	35.09	33.96
Soil chemical properties					
	1st Season	2nd Season		1st Season	2nd Season
Soil pH (1:2.5) *	7.50	7.40	Anions (mmolc L^{-1}):		
EC (dSm^{-1}) **	0.79	0.82	CO_3^{--}	0.00	0.00
Cations (mmolc L^{-1}):			HCO_3^-	0.95	1.15
Ca^{++}	2.18	2.40	Cl^-	4.55	4.75
Mg^{++}	1.18	1.46	SO_4^{--}	2.40	2.30
Na^+	4.13	4.01	Organic matter content (%)	0.44	0.49
K^+	0.41	0.36	$CaCO_3$ content (%)	1.45	1.44

*1:2.5 w/v soil: water suspension, **soil paste extract, HC: Hydraulic conductivity.

Wheat grains cultivar Giza 171 were sown in November 15th and 18th in the two seasons, respectively. Crop was harvested at full maturity and plant growth characteristic as well as grain quality were measured. At harvest, wheat

plants in each treatment were collected to determine, wheat yield and its components, N, P and K contents of grains. All these traits were taken from three plants per pot and their average was taken as a single value. Then the average value of all three replications for each treatment was compared for statistical analysis.

2.9 Statistical analysis

The treatments were statistically arranged in a complete randomized design with three replicates according to Gomez and Gomez (1984). Grain proximate analysis was performed to check the effect of irrigation water treatments (tap and saline water) and cyanobacteria inoculation combined with different nitrogen levels on wheat grain quality.

3. Results and Discussion

Nostoc muscorum was isolated and purificated from soil saline samples to obtain a bacterial free cyanobacteria according to Geitler (1932), who showed that it must be purified from any contaminant. Results indicated that on solid medium the 21-day-old culture of the isolate gave a localized growth with fibrous appearance on the agar surface. Culture is dark green trichomes had no ramifications. They were uniseriate, single, aggregated, and showed neither polarity nor tapering. No sheath was formed. Trichomes composed of three sizes and shapes of cells; 1) barrel cells (5-5.5 × 5.3-6.9 µ); 2) granular, ellipsoidal cells (4.9 × 5.5-7.3 µm); 3) yellowish- brown rounded cells of 8.9 µ in diameter. Few heterocysts were observed. They were of single occurrence with 2 positions, intercalary and terminal According to Roger and Ardales (1991). Data in Table (2) show the efficiency of *Nostoc muscorum* infixed nitrogen (intracellular and extracellular) and dry weight during 30 days growth period for the *Nostoc muscorum.* The data revealed that fixed nitrogen and dry weight increased with increasing incubation periods. These results are in harmony with those obtained by Taha (2000).

Table (2): The efficiency of *Nostoc muscorum* in fixed nitrogen (intracellular and extracellular) and dry weight during 30 days growth period.

Incubation periods (days)	Nitrogen fixation (mg N/100 ml-culture)			Dry weight (mg/100 ml)
	Intracellular	Extracellular	Total	
6	0.520	0.140	0.660	42
12	1.211	0.233	1.444	122
18	2.231	0.421	2.652	212
24	2.316	0.472	2.788	237
30	2.927	0.611	3.538	301

Data in Table (3) show the efficiency of *Nostoc muscorum* on production of phytohormones (Indole 3 acetic acid (6.22), Gibberellic acid (7.31) and Cytokinin (4.12)) µg/100 ml. The cyanobacterial filtrates in suspensions significantly increased the IAA, GA_3 and cytokinin (Tantawy and Atef, 2010).

Hormone indole-3-acetic acid (IAA) was demonstrated. A colorimetric (Salkowski) screening of 34 free living and symbiotically competent cyano-bacteria, that represent all morphotypes from the unicellular to the highly differentiated, showed that auxin-like compounds were released by about 38% of the free-living as compared to 83% of the symbiotic isolates.

Table (3): The efficiency *Nostoc muscorum*on production of phytohormones (Indole 3 acetic acid, gibberellic acid and cytokinin) µg/100 ml.

Indole 3 acetic acid	Gibberellic acid	Cytokinin
6.22	7.31	4.12

The endogenous accumulation and release of IAA were confirmed immunologically (ELISA) using an anti-IAA antibody on 10 of the Salkowski-positive strains, and the chemical authenticity of IAA was further verified by chemical characterization using gas chromatography mass spectrometry in Nostoc PCC 9229 (isolated from the angiosperm Gunnera) and in Nostoc 268 (free-living). Addition of the putative IAA precursor tryptophan enhanced IAA accumulation in cell extracts and supernatants (Sergeeva *et al.*, 2002).

3.1 Colonization of cyanobacteria with the roots of d wheat plant

Data in Figure (1) show the section of a *Nostoc muscorum* filaments colonization on wheat root. The ability of *Nostoc muscorum* to form associations with the roots of wheat seedlings was high. The cyanobacterial strains formed close associations with wheat plants and were able to enter through root and penetrate the epidermal layer of wheat roots. In freshly cut sections of wheat roots with cyanobacterial association, a thick cyanobacterial growth was observed outside the epidermis. The entry of the cyanobacterium was through the tip of root and the progressive stages of this process are depicted. *Nostoc muscorum* strains were able to penetrate the epidermal layer of wheat root celled filaments, resembling hormogonia could be located below the epidermal layer, in transverse sections. Cyanobacterial filaments were also visible in the cortical region of the wheat root (Goñi *et al*., 2016)**.** On the other hand, Adams *et al.* (2006) and El-Zemrany (2017) reported that five distinct phases were clearly seen in the association between filamentous cyanobacteria strain *Leptolyngbya* sp. and plant roots. In the first step, filaments enter into root cells. The exact mechanism of cyanobacterial penetration into root cells is still not known but it is generally believed to be facilitated by hydrolytic enzymes of bacterial origin. In the second step, after entering the plant cell, cyanobacterial filaments start spreading to surrounding cells, and in the third step they grow and multiply within

the plant cell. In the fourth step, it seems that cyanobacteria rupture the plant cell wall by exerting mechanical pressure, and thus enter the neighboring cells.

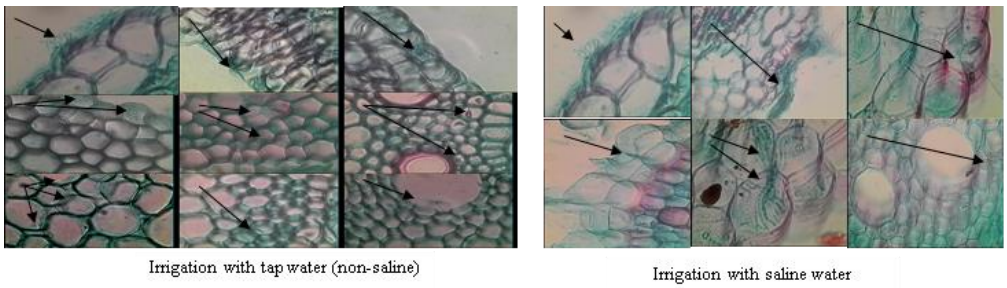

Figure (1): *Nostoc muscorum* association with roots of wheat seedlings under salinity and non-salinity conditions after 30 days of culture.

In the fifth and last step, cyanobacterial filaments completely occupied the plant cell. cyanobacteria associated with plant cells are in direct contact with each other and both organisms synthesize a variety of biologically active substances that initiate and maintain the association or hormogonia-promoting factor / bioactive compounds present in root exudates. Long cyanobacterial filaments were found to be closely attached to the root tip surface in all the four cases, but inside the root epidermis, 3-4 celled filaments were only observed. Two out of three *Nostoc* strains tested triggered lateral root formation, leading to increased surface area of wheat roots coming in contact with cyanobacteria, thereby aiding in better colonization. In the co-culturing experiment, all the strains showed significantly higher amounts of IAA, which is reflective of the plant growth-promoting role of the cultures. The strain also showed higher nitrogenase in association with wheat seedlings.

3.2 Wheat yield and its components

The results presented in Tables (4 and 5) show the mean values of the measured parameters (plant height (cm), number of spike per plant, spikes dry weight per pot (g), grains dry weight per pot (g), 1000 grain weight (g) and grain total nitrogen, phosphorus and potassium content) of wheat as affected by irrigation water treatments (tap and saline water) and cyanobacteria inoculation combined with different nitrogen levels as well as their interaction cultivated in sandy soil in 2018/2019 and 2019/2020 seasons.

3.2.1 Effect of irrigation treatments

It has been observed that all the studied characters were significantly affected by irrigation treatments. Irrigation of wheat plants with diluted sea water (7.69 dS/m) caused significant decreases in the studied parameters. Results in Tables (4 and 5) show that wheat plants retained

the highest mean values of (plant height 69.77 and 69.16 cm, number of spikes per plant 6.2 and 6.13, spikes dry weight per pot 16.47 and 16.40 g, grains dry weight per pot 14.24 and 14.06 g, 1000 grain weight 43.25 and 43.13 g and grain nitrogen 1.67 and 1.68 %, phosphorus 0.46 and 0.45 % and potassium 1.62 and 1.63 % content) when irrigated with tap water compared with those irrigated by saline water in the two seasons, respectively. Rodríguez *et al*. (2006) mentioned that Salt stress is one of the most serious factors limiting the productivity of plant, gibberellic acid and extracellular products increased 5-aminolevulinate dehydratase activity over the control (without salt). When coincident with high salinity, exposure to either EP or GA3, resulted in a reversal of shoot-related responses to salt stress. they proposed that *Scytonema hofmanni* extracellular products may counteract altered hormone homeostasis of seedlings under salt stress by producing gibberellin-like plant growth regulators. Also, salinity poses three major threats to plant growth, namely osmotic stress, ionic stress and nutritional disturbance (Flower and Colmer, 2008). In addition, it also manifested an oxidative stress, thus the deleterious effects of salinity affect different physiological and metabolic processes of plants.

Table (4): Effect of irrigation treatments and cyanobacteria combined with nitrogen levels on plant height (cm), spike number, spike dry weight (g), grain dry weight (g) and 1000 grain weight (g) of wheat during 2017/2018 and 2018/2019 seasons.

Treatments		2017/2018 Season					2018/2019 Season				
Irrigation (A)	Nitrogen and Cyanobacteria (B)	Plant height (cm)	spike number plant^{-1}	Spike DW (g pot^{-1})	Grain DW (g pot^{-1})	1000 grains weight (g)	Plant height (cm)	spike number plant^{-1}	Spike DW (g pot^{-1})	Grain DW (g pot^{-1})	1000 grains weight (g)
a_1	b1	75.10	8.00	19.72	17.33	44.18	74.93	8.00	19.78	17.23	44.13
	b2	74.60	8.00	20.73	18.13	44.23	74.20	8.00	20.65	17.97	44.23
	b3	71.97	6.33	17.43	15.30	43.90	70.50	6.00	17.17	14.98	43.80
	b4	68.40	5.67	14.22	12.33	42.60	68.00	5.67	14.10	12.03	42.43
	b5	58.80	3.00	10.25	8.10	41.33	58.17	3.00	10.30	8.07	41.07
Mean		69.77	6.20	16.47	14.24	43.25	69.16	6.13	16.40	14.06	43.13
a_2	b1	70.17	7.00	16.85	14.97	44.03	70.53	6.67	16.80	14.60	43.70
	b2	72.00	8.00	18.72	16.77	44.07	71.70	8.00	18.63	16.30	43.93
	b3	69.33	6.00	16.47	14.70	42.30	68.93	5.67	16.53	14.40	42.03
	b4	65.67	4.67	12.83	10.90	41.77	65.00	4.67	12.67	10.52	41.52
	b5	54.90	2.00	7.37	5.50	39.77	52.83	2.00	7.17	5.57	39.80
Mean		66.41	5.53	14.45	12.57	42.39	65.80	5.40	14.36	12.28	42.20
Over all means for B	b1	72.64	7.50	18.28	16.15	44.11	72.73	7.33	18.29	15.92	43.92
	b2	73.30	8.00	19.73	17.45	44.15	72.95	8.00	19.64	17.13	44.08
	b3	70.65	6.17	16.95	15.00	43.10	69.72	5.83	16.85	14.69	42.92
	b4	67.04	5.17	13.53	11.62	42.18	66.50	5.17	13.38	11.28	41.98
	b5	56.85	2.50	8.81	6.80	40.55	55.50	2.50	8.73	6.82	40.43
L.S.D. at 0.05%	A	0.14	0.06	0.07	0.07	0.04	0.16	0.07	0.06	0.05	0.02
	B	0.36	0.16	0.18	0.19	0.11	0.40	0.18	0.15	0.12	0.06
	A*B	0.72	0.31	0.37	0.37	0.22	0.79	0.36	0.30	0.25	0.11

a1= tap water, a2= saline water. b1=100 % N, b2= 75% N + *Nostoc muscorum*, b3= 50% N + *Nostoc muscorum*, b4 = Zero % N + *Nostoc muscorum* and b5= control.

Table (5): Effect of irrigation water and cyanobacteria combined with nitrogen levels on nitrogen, phosphorus and potassium content of wheat grains during 2017/2018 and 2018/2019 seasons.

Treatments		2017/2018 Season			2018/2019 Season		
Irrigation (A)	Nitrogen and Cyanobacteria (B) treatment	N	P	K	N	P	K
a_1	b1	1.86	0.55	1.97	1.86	0.51	1.97
	b2	1.90	0.58	2.04	1.91	0.57	2.06
	b3	1.64	0.45	1.89	1.66	0.45	1.87
	b4	1.53	0.40	1.42	1.55	0.41	1.44
	b5	1.43	0.35	0.79	1.41	0.32	0.81
Mean		1.67	0.46	1.62	1.68	0.45	1.63
a_2	b1	1.67	0.46	1.90	1.66	0.46	1.89
	b2	1.73	0.50	1.99	1.74	0.50	2.01
	b3	1.46	0.41	1.75	1.47	0.42	1.77
	b4	1.27	0.38	1.40	1.23	0.38	1.41
	b5	1.13	0.24	0.73	1.12	0.25	0.71
Mean		1.45	0.40	1.55	1.45	0.41	1.56
Over all means for B	b1	1.76	0.51	1.94	1.76	0.49	1.93
	b2	1.82	0.54	2.02	1.83	0.54	2.03
	b3	1.55	0.43	1.82	1.56	0.43	1.82
	b4	1.40	0.39	1.41	1.39	0.39	1.42
	b5	1.28	0.29	0.76	1.27	0.29	0.76
L.S.D. at 0.05%	A	0.01	0.01	0.01	0.01	0.01	0.01
	B	0.01	0.01	0.01	0.01	0.01	0.01
	A*B	0.03	0.02	0.02	0.03	0.02	0.02

a1= tap water, a2= saline water. b1=100 % N, b2= 75% N + *Nostoc muscorum*, b3= 50% N + *Nostoc muscorum*, b4 = Zero % N + *Nostoc muscorum* and b5= control.

3.2.2 Effect of cyanobacteria inoculation combined with different nitrogen levels

As demonstrated in Tables (4 and 5), results revealed that cyanobacteria inoculation can compensate partially the nitrogen fertilizer required for wheat cultivation. cyanobacteria amended to soil significantly increased the studied parameters of wheat plant compared with those plants cultivated in absence of cyanobacteria. However, results showed that the inoculation with cyanobacteria *Nostoc muscorum* combined with 75% N dose significantly attained the superior effect on wheat yield and its components with mean values of (plant height 73.30 and 72.95 cm, number of spike per plant 8 and 8, spikes dry weight per pot 19.73 and 19.64 g, grains dry weight per pot 17.45 and 17.13 g, 1000 grain weight 44.15 and 44.08 g and grain Nitrogen 1.82 and 1.83 %, phosphorus 0.54 and 0.54 % and potassium 2.02 and 2.03 % content) compared to that achieved by the other tested treatments in both seasons, respectively. Meanwhile, the lowest mean values were observed in the control plants (without treatment). Also, insignificant difference was found between *Nostoc muscorum* + 75% N and 100% N for 1000 grain weight (44.15 and 44.11 g) in the first season and plant height (72.95 and 72.73 cm) in the second season. Most paddy soils have a natural population of Cyanobacteria, prokaryotic photosynthetic micro-organisms, which synthesize and liberate

plant growth regulators such as gibberellins that could exert a natural beneficial effect on salt stressed plants. El-Ayouty *et al.* (2012) found that combination of cyanobacteria with less chemical fertilizers was a potential approach in providing wheat growth and yield similar to that achieved by the label rate of chemical fertilizers consisting of urea (46% nitrogen) and diammonium phosphate (DAP). This result is in agreement with those reported by Abdoli *et al.* (2013) and Sadak and Ahmed (2016).

3.2.3 Effect of interaction between irrigation water treatments and Nostoc muscorum combined with different nitrogen levels

The analysis of variance for the effect of interaction between irrigation water treatments and cyanobacteria inoculation combined with different nitrogen levels on agronomic and quality characters of wheat were significant in both seasons. Results in tables (4 and 5) revealed that the corresponding highest significant values for all studied characters were found at wheat plants irrigated with tap water and treated with *Nostoc muscorum* + 75% N except for plant height (cm) that achieved by 100 % N without significant difference between them in both seasons. At the same time, cyanobacteria amended to soil partially or completely alleviated the reduced effect of diluted sea water on yield attributes of wheat plants by increasing significantly these studied parameters compared with those cultivated without cyanobacteria. Data clearly show that, cyanobacteria in soil with three quarter and half recommended dose of N significantly increased yield attributes of wheat plant compared with those without cyanobacteria. The maximum mean values of (plant height 75.10 and 74.93 cm, number of spikes per plant 8 and 8, spikes dry weight per pot 20.73 and 20.65 g, grains dry weight per pot 18.13 and 17.97 g, 1000 grain weight 44.23 and 44.23 g and grain nitrogen 1.90 and 1.91%, phosphorus 0.58 and 0.57 % and potassium 2.04 and 2.06 % content) compared to that achieved by the other tested treatments in both seasons, respectively. Also, insignificant difference was found between *Nostoc muscorum* + 75% N and 100% N for 1000 grain weight (44.23 and 44.18 g) and (44.23 and 44.18 g) in the two seasons, respectively. However, the increases recorded by the treatment of *Nostoc muscorum* + 50% N was significantly less than those recorded by both treatments of *Nostoc muscorum* + 75% N and 100% N in both seasons. In this concern, El-Ayouty *et al*. (2012) studied the effect of cyanobacteria inoculation on wheat in a greenhouse experiment under different levels of nitrogen. They found that inoculation with cyanobacteria enhanced the growth of wheat plants and the treatment of 75% N + cyanobacteria gave the highest wheat grain and straw yields. Moreover, they observed that the results due to the

treatment of 75% N + cyanobacteria were not significantly different from those recorded by 100% N treatment. Additionally, cyanobacteria addition to soil combined with different NPK rates (R & ½ R NPK) under normal and diluted sea water stress caused an enhancement effect on photosynthetic pigments of wheat plant. Also, the enhancing role of cyanobacteria amended to soil might be due to its important role on increasing the availability of water and minerals to plant cells. These results of cyanobacteria enhancing role are in harmony with those obtained by Abou-Zeid (2014) and Abbas *et al.* (2015).

Table (6): Effect of irrigation water and cyanobacteria combined with nitrogen levels on water holding capacity (WHC) %, nitrogenase activity (mmole C2 H4/L/hr), viable counts cyanobacteria ($\times 10^4$ g dry soil^{-1}) during 2017/2018 and 2018/2019 seasons.

Treatments		2017/2018 Season			2018/2019 Season		
Irrigation (A)	Nitrogen and Cyanobacteria (B) treatment	(WHC) %	Nitrogenase activity	Total cyanobactera	(WHC) %	Nitrogenase activity	Total cyanobactera
a_1	b1	9.02	150	0.001	9.02	150	0.001
	b2	9.25	540	12	9.26	543	13
	b3	9.21	360	11	9.22	361	12
	b4	9.14	210	7.7	9.14	215	7.7
	b5	9.02	9	0.001	9.02	11	0.001
Mean		9.128	253.8	6.1404	9.132	256	6.5404
a_2	b1	9.02	153	0.001	9.02	153	0.001
	b2	9.27	549	13	9.27	570	13
	b3	9.22	367	12	9.22	371	12.1
	b4	9.16	213	7.7	9.16	219	7.5
	b5	9.02	12	0.001	9.02	13	0.001
Mean		9.138	258.8	6.5404	9.138	265.2	6.5204

a1= tap water, a2= saline water. b1=100 % N, b2= 75% N + *Nostoc muscorum*, b3= 50% N + *Nostoc muscorum*, b4 = Zero % N + *Nostoc muscorum* and b5= control.

Our obtained results in Tables (4 and 5) demonstrates that diluted sea water (7.69 dS/m EC) decreased significantly the studied agronomic parameters of wheat plant cultivar Giza 171 compared with control plant that irrigated with tap water. These results are in good agreement with those on wheat plant (Karthikeyan *et al.*, (2007) and faba bean (Abdelhamid *et al.*, 2013). Effect of irrigation water and cyanobacteria combined with nitrogen levels on water holding capacity (WHC) %, Nitrogenase activity N2_ase activity (mmole C2 H4/L/hr), viable counts cyanobacteria (x10^4 g dry soil^{-1}) during 2017/2018 and 2018/2019 seasons. The data presented in Table (6) showed differences in WHC values Nitrogenase activity N2_ase activity, viable counts *Nostoc muscorum* in response to *Nostoc muscrum*, soil inoculation during the growing season compared to uni-noculated controls. The results showed slight increases in WHC values due to inoculation with cyanobacteria species, and the best results were saline water irrigation with 75 %N + *Nostoc muscorum*. These results are similar to

those reported by Caire *et al.* (2000) who cleared that Polysaccharides are polymers of carbohydrate molecules, which correspond to monosaccharide chains bonded by glycosidic interactions. A great diversity of polysaccharides can be obtained, being their characteristics determined by the building blocks, that is, the monosaccharides (e.g., hexose, pentose, uronic acid and methyl pentose). These polysaccharides are used by other organisms as carbon and energy sources, being excreted by algae under both normal and stress conditions.

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