



## EFFECT OF CYANOBACTERIA IN COMBINATION WITH REDUCED DOSE OF CHEMICAL FERTILIZERS ON GROWTH AND YIELD OF WHEAT GROWN UNDER SALINITY STRESS

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### Abstract

A pot experiment was conducted to study the effect of to test the effect of cyanobacteria (*Anabaena circinalis* and *Nostoc commune*) applied as extract and grain coating in combination with half of recommended dose of chemical fertilizers (NP) on growth and yield of wheat cv. IPA grown in soil with different salinity levels, i.e.  $EC_e=1.6$  dS/m (control), 6, 12 and 15 dS/m. Pots without cyanobacteria treatments and chemical fertilizer and pots received full rate of chemical fertilizers were used for comparison. Desired salinity levels were obtained by diluting drainage water with  $EC_e$  of 94.2 dS/m to 6, 12 and 15 dS/m. Results indicated that salinity significantly reduced all test growth parameters, yield and yield components of wheat and the reduction increased with increasing salinity level. Application of cyanobacteria as foliar extract together with grains coating with compost significantly improved yield, yield components and other growth parameters over control but it was less than full rate of chemical fertilizer. However, foliar spray together and seed coating with cyanobacteria in combination with 50% of chemical fertilizer provides yield and yield components similar to that achieved by the full dose of chemical fertilizer at all salinity levels tested. The significant application of this approach in sustainable agriculture is briefly discussed.

**Keywords :** Wheat growth and yield, Cyanobacteria, Saline soil, Chemicals fertilizers.

### Introduction

The extent of agricultural land which is affected by high salinity is increasing worldwide, due to both natural phenomena and bad agricultural practices (Munns and Tester, 2008). 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. Different approaches have been established to mitigate the effect of salinity on plant such as use of chemical and organic fertilizers, soil microorganisms (biofertilizers) and growth regulators. Chemical fertilizers are needed to get good crop yields, but their abuse can be harmful for the environment and their cost can be not economic. Cyanobacteria can survive and thrive different soil conditions like extreme pH, salinity and it can be used for the reclamation of calcareous and saline soils (Prasanna *et al.*, 2008). In addition, It could play a significant role in improving plant nutrients supplies, increasing soil pores and production of adhesive substances, producing growth promoting hormones (Venkataraman, 1993), reducing the damages resulting from salt stress (Ashraf *et al.*, 2008), increasing both water holding capacity and soil biomass after their death and decomposition (Alam *et al.*, 2014) and increasing nitrogen by nitrogen fixation and soil phosphate by excretion of organic acids into the soil. The beneficial effects of cyanobacteria inoculation were reported not only for rice, but also for other crops such as wheat, soybean, oat, tomato, radish, cotton, sugarcane, maize, (Saadatnia and Riahi, 2009).

In an earlier work (Burgus *et al.*, 2019) it was 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). However, this combination

was not tested under saline conditions. Therefore the present work was conducted to test the effect of integration of reduced dose of chemical fertilizers with cyanobacteria on growth and yield of wheat grown under different salinity stresses. Wheat is widely cultivated in southern and central regions of Iraq which are affected by higher levels of salinity (Asad and Adnan, 2015).

### Materials and Methods

#### Isolation, purification and identification of cyanobacteria

Water samples were collected from different paddy fields in Al-Nagaf Al-Ashraf and Al-Diwaniyah counties during the growing season of 2018 and used to isolate the cyanobacteria (*Nostoc* and *Anabaena*) by the method of Rippka *et al.* (1988). Water samples were transferred to specific growth media to enhance the growth and enrichment of cyanobacteria. One ml from each sample was streaked directly on plates containing BG 11 (Blue Green Algae) medium which consists of  $NaNO_3$ ,  $K_2HPO_4$ ,  $MgSO_4 \cdot 7H_2O$ ,  $CaCl_2 \cdot 2H_2O$ , citric acid, ammonium ferric citrate,  $EDTANa_2$ ,  $Na_2CO_3$  and Trace metal solution ( $H_3BO_3$ ,  $MnCl_2 \cdot 4H_2O$ ,  $ZnSO_4 \cdot 7H_2O$ ,  $Na_2MoO_4 \cdot 2H_2O$ ,  $CuSO_4 \cdot 5H_2O$  and  $Co(NO_3)_2 \cdot 6H_2O$ ). Sodium carbonate at 0.02-0.04% (w/v) was added to the medium to separate blue green algae from green algae (Allen, 1952). The medium was also supplemented with cycloheximide and nystatin at 100  $\mu$ g/ml to inhibit bacteria and fungi organisms (Ferris and Hirsch, 1991). To solidify the medium, agar at 1.5% (w/v) was added. The culture, was incubated at  $28^\circ C \pm 1$  in growth chamber with continuous fluorescent light for two weeks. The cultures were microscopically examined to check the growth of cyanobacteria (Fogg *et al.*, 1973). After the green colonies appeared, a sterile platinum wire loop was used to pick up the green colony and subcultured on new medium to obtain unialgal cultures of cyanobacteria (Pringsheim, 1949). Cyanobacteria isolates were grown in conical flasks containing sterilized liquid BG11 free of nitrogen medium at

pH ranged between 7.8-8.5 (Fogg and Thake, 1987). The cultures were incubated in growth chamber under 16 h light (3000 lux) at  $28 \pm 2$  °C (Strainer, 1971). Identification of cyanobacteria was microscopically carried out based on thickness of the sheath, presence or absence of heterocyst and akintes and gas vacuoles using procedure outlined by Desikachary (1959).

### Biomass cultivation

Cyanobacteria mixture (*Anabaena circinalis* and *Nostoc commune*) was cultivated using photobioreactor (PBR) of 10 liters capacity filled with sterilized liquid BG-11 medium and incubated in incubator under photoperiod 16 h light (3000 lux) at  $28 \pm 2$  °C and initial pH at 8.2 (Dumitru and Laura, 2012) with air bubbling by a commercial aquarium pump. The incubation continued for to 2-3 weeks (until reached the logarithmic growth phase), then fresh weight biomass of cyanobacteria mixture of PBR was collected and washed several times with distilled water to remove impurities, crushed and salt crystals (Svein, 2006)

### Grains coating with compost amended with cyanobacteria

Compost (palm fronds) with some chemical and physical properties were used as incubation medium. Ten ml of mixed culture (*A. circinalis* and *N. commune*) was added to 100 mg of compost moisturized with 50 ml distilled water incubated for 30 days then the activity of cyanobacteria then examined (El-Gamal, 2011; Burjus *et al.*, 2017).

### Preparation of cyanobacteria extract

Fresh biomass of cyanobacteria mixture was harvested by centrifugation at  $2,000 \times g$  for 10 minute. The purified cells pellet was dissolved in sterilized distilled water at a rate of 1% (w/v), extracted by electrical blender and used for a foliar application (Grzesik *et al.*, 2017; Pis and Sabale, 2010).

### Soil sampling and salinization

A bulk of soil samples were taken from the field of Plant Protection Directorate, Ministry of Agriculture, Baghdad, Iraq during 2018 season. The samples were placed in a protected rainfall shelter place, allowed to dry, crushed, sieved through 2mm opening sieves and analyzed for some chemical and physical characteristics (Table1). The soil was packed in 48 plastic pots of 12 kg capacity.

For salinization, drainage water with EC of 94.2dS/m was taken from drainage canal at Al-Diwaniyah county during summer of 2018. Some chemical and physical properties of the drainage water are listed in table 1. The drainage water was diluted to 6, 12 and 15 dS/m and kept until use. Tap water of 1.6 dS/m was used for control treatment. The pots were divided into 4 groups each with 12 pots. Each group was salinized with appropriate salinity level. For each group, enough salinized water of a particular EC was added to their respective pots until the salinity of the water percolated from the pores of the pots becomes similar to that of salinized water level used for salinization. Control pots were made in a similar manner except tap water of 1.6 dS/m level was used. The soil of the pots of each group was taken, air dried under shed, crushed and repacked in plastic

pots of 12 kg soil capacity. The pores of the pots were closed and a thin layer of fine gravels was added to the bottom of each pot, Plastic tube with 5 cm diameter and 30 cm length was inserted in the center of the base of each pot in order to be used for bottom irrigation.

Accordingly, the experiment comprised of 4 salinity levels, 4 treatments and 3 replications. The treatments in each group of salinity level are:

1. Control (without addition).
2. Grains coating with compost amended with *Anabaena circinalis* and *Nostoc commune* mixture (A + N) and foliar extract of cyanobacteria.
3. Recommended dose of chemical fertilizers (NP).
4. Grains coating with compost amended with *Anabaena circinalis* and *Nostoc commune* mixture (A + N) and foliar extract of cyanobacteria with 50% (NP).

Chemical fertilizers (Urea (46% N) and Diammonium phosphate) DAP (18% N and 46% P<sub>2</sub>O<sub>5</sub>) were applied at 50 and 100% of recommended dose for wheat crop (Leaflet of National Program for Wheat Development, 2012). Half of urea and all DAP were applied at planting time while the remaining amount of urea was applied at tillering stage. Grains of wheat cv. IPA 99 were obtained from State Board of Agricultural Researches, Ministry of agriculture, Iraq and coated with compost amended with cyanobacteria before planted in each pot on the second of December of 2018. The pots were irrigated to filed capacity in alternate manner by first applying tap water on the top of the pot (soil surface) and then from the bottom through the tube. This irrigation system was basically run to insure the uniformity of salinity levels within the soil of the pots (William *et al.*, 2013). The seedlings in each pot were allowed to grow for 7 days then thinned to the largest 6 per pots.

For cyanobacteria extract application, tween-20 at 0.1% (V/V) was added to the extract to decrease the surface tension (Khalid, 2016), divided into three equal portions and sprayed on plants of the respective pots at tillering, elongation and flowering stages. Treatment application was done using hand sprayer until all leaf surfaces were fully wetted.

### Measurements

At flowering stage (94 days after planting) two randomly selected plant from each pot were used to measure the plant height (cm), flag leaf area (cm<sup>2</sup>), spike length (cm) and chlorophyll concentration (SPAD). The area of flag leaf (cm<sup>2</sup>) was calculated by the following equation (Thomas, 1975):

$$\text{Flag leaf area (cm}^2\text{)} = \text{the length of leaf (cm)} \times \text{width of leaf (cm)} \times \text{correction factor (0.95)}.$$

At physiological maturity (170 days after planting), plants of each pot were clipped and measured for grains and biological yields, number of spikes, number of grains per spike and weight of 100 grains using standard procedure (Mzhda, 2017). Grain protein was determined by Kjeldahl method Bremner, (1965).

**Table 1 :** Some physical and chemical properties of drainage water and soil.

Parameters	Values*	
	Soil	Drainage water
pH	7.8	7.5
**EC (dS m <sup>-1</sup> )	1.6	94.2
Ca (Meq/l)	15.0	132.5
Mg (Meq/l)	17.5	130.0
Na (Meq/l)	3.4	913.0
Cl (Meq/l)	5.5	922.5
CO <sub>3</sub> (Meq/l)	0.0	0.0
HCO <sub>3</sub> (Meq/l)	2.5	5.0
SO <sub>4</sub> (Meq/l)	10.3	188.5
CaCO <sub>3</sub> (%)	15.0	-
CaSO <sub>4</sub> (%)	0.2	-
N (ppm)	14.0	-
P (ppm)	23.1	-
K (ppm)	85.0	-
Organic matter (%)	0.2	-
Sand (%)	79.2	-
Silt (%)	12.4	-
Clay (%)	8.4	-
Texture	loamy sandy soil	-

\* Each value is an average of three replicates. \*\*EC was measured by saturated soil-water extract (Richards, 1954).

### Statistical Analysis

The experiment was laid out in a complete randomized design (CRD) with the factorial combination of two factors, salinity levels and cyanobacteria treatments. Each treatment was replicated three times. The data were subjected to statistical analysis using analysis of variance (ANOVA). Differences among treatment averages were compared using Least Significant Difference (LSD) at  $\leq 0.05$  probability (Steel and Torrie, 1980).

## Results and Discussion

### Effect of combination of *Anabaena circinalis* and *Nostoc commune* (A+N) alone or with reduced chemical fertilizer (NP) dose on grain and biological yields of wheat grown under different salinity levels.

Salinity significantly averted grains and biological yields of wheat (Table 2). Application of salinity at 6, 12 and 15 dS/m significantly reduced grains yield by 23, 31 and

57% of control and biological yield by 15, 26 and 36% of control, respectively. The negative effect of salinity observed in this study is compatible with the general effect of salinity on plant. Salinity stress creates osmotic, toxic ion and nutritional disturbance effects which lead to disturb the metabolic activity of the cells and thereby inhibiting growth and development of plant (Levite, 1980; Aziz and Taalab, 2004). The effect of salinity depends on many factors including the dominant ions; therefore using drainage water in this study is more realistic than using single salt (s) for evaluation since the salt composition of drainage water is outcome of salt composition of soil (Ayers and Westcot, 1985).

All treatments significantly improved average grain and biological yields of wheat compared to control (Table 2). Application of cyanobacteria extract and grain coating alone increased grain and biological yields by 76 and 149% of control, respectively. However application of cyanobacteria in combination with 50% chemical fertilizer provides grain and biological yields similar to that of full rate of chemical fertilizer. This result agrees with the result of Peireira *et al.* (2009) who found that that cyanobacteria with decreased dose of NP up to 50% gave grain and biological yields of wheat similar to that achieved by full dose of chemicals fertilizer. The enhanced growth of wheat treated with cyanobacteria under salinity stress may be due to positive effect of cyanobacteria which mitigate the effect of salinity stress on wheat (Sahu *et al.*, 2012). Cyanobacteria is reported to have several plant growth regulators which improve growth and develop of plant (Ördög, 1999). Others found that application of cyanobacteria and NaCl with the irrigation significantly improved the pigments contents, photosynthetic activity and transpiration rate due to the presence of many growth-stimulating substances such as vitamins, hormones and micronutrients (Karthikeyan *et al.*, 2007).

The interaction between salinity stress and the treatments significantly affected grain and biological yields of wheat. The highest grain and biological yields was recorded by combination of cyanobacteria with 50% NP and full dose of chemical fertilizer at 1.6 dS/m, while the lowest grain and biological yields was observed by control treatment at 15dS/m. It is interesting to mention that the treatment of cyanobacteria and 50% NP provides grain and biological yields similar to that full dose of chemical fertilizer at all salinity levels.

**Table 2:** Effect of *Anabaena circinalis* and *Nostoc commune* (A+N) applied as extract and grain coating in combination with chemical fertilizers (NP) on grain and biological yields of wheat grown under different salinity levels.

Treatments (T)	Salinity levels (S)				Average
	1.6	6	12	15	
	<b>Grains yield (g/pot)</b>				
Control	4.1	4.0	3.0	2.0	3.3
A+N	6.5	6.2	6.0	4.6	5.8
100% NP	18.6	13.4	11.2	7.3	12.6
A+N + 50% NP	17.5	13.0	11.1	8.1	12.4
Average	12.6	9.7	8.7	5.4	
LSD $\leq 0.05$	T=0.54 S=0.54 T×S=1.12				
	<b>Biological yield (g/pot)</b>				
Control	12.4	10.3	8.7	7.3	9.7
A+N	28.0	25.7	22.0	20.7	24.1
100% NP	48.5	38.0	31.1	27.7	36.3
A+N + 50% NP	45.3	39.1	35.9	30.0	37.6
Average	33.6	28.5	24.9	21.4	
LSD $\leq 0.05$	T=1.5 S= 1.5 T×S=3.0				

**Effect of combination of *Anabaena circinalis* and *Nostoc commune* (A+N) alone or with reduced chemical fertilizer (NP) dose on yield components of wheat grown under different salinity levels.**

All yield component parameters were significantly influenced by the test salinity levels, treatments applied and interaction of treatments and salinity levels (Table 3). Salinity at 12 and 15 dS/m significantly reduced average number of spikes by 17 and 24% of control, respectively. Also, the average number of grains per spike is decreased by 17 and 44% of control at salinity levels 12 and 15dS/m, respectively. Weight of 100 grains was reduced by salinity levels applied at 12 and 15dS/m, to 7 and 11% of control, respectively. The reduction in wheat yield is apparently due to all yield components. However, the number of grains per spike was the most effective trait in reducing yield. Maas and Grieve, (1990) showed that NaCl stressed wheat during vegetative stage leading to a shorter spikelet development, which resulted in fewer spikelets per spike, thus reducing the number of grains per spike. Others has been reported that there is a closer relationship between grain yield and the number of grains than between grain yield and grain weight (Shearman, 2005).

All treatments is significantly averted yield components. Application of cyanobacteria alone increased number of spikes per plant by 9% over control. This ratio increased up to 42% when cyanobacteria applied in combination with 50% NP, which was similar to that of sole application of full dose chemical fertilizer. Similarly, application of cyanobacteria on plants received 50% chemical fertilizer increased number of grains per spike and

weight of 100 grains similar or better than that achieved by the label rate of chemical fertilizers.

The interaction between treatments and salinity levels significantly affected all yield components. In most cases, application of cyanobacteria in combination with 50% NP provide yield components similar to that of recommended dose of chemical fertilizers at all salinity levels. It is noteworthy to mention that the trend of effect of combination of cyanobacteria with 50% NP on yield is parallel with the effect of this combination on yield components at all salinity levels. This indicates that increased of yield is mainly due to increase of all yield components.

The results of this study is compatible with the result of Abd El-Baky *et al.* (2008) who found that cyanobacteria spray led to good growth and yield performance of wheat compared to those received 100% N without algal extract. Moreover they found that the algal extracts significantly increased the contents of the total chlorophyll and antioxidant compounds. In another study, foliar spray of cyanobacteria significantly increased plant nutrients content and had a positive effect on plant growth, oxidation behavior and activity of antioxidant enzymes in plants affected by salt stress. Also, he reported that cyanobacteria content cytokines, gibberellins and auxins which promote the plant growth and help to overcome the adverse effect of salinity in saline soil. (Mussa, 2005) .Others indicated that foliar spraying three times at after sowing with cyanobacteria filtrate and grain coating with compost amended with cyanobacteria with reduce chemical fertilizers could be used for maximizing vegetative growth, fruit yield and its components of Cantaloupe (*Cucumis melo* L.) plant (Farrag *et al.*, 2017).

**Table 3:** Effect of *Anabaena circinalis* and *Nostoc commune* (A+N) applied as extract and grain coating in combination with chemical fertilizers (NP) on grain and biological yields of wheat grown under different salinity levels.

Treatments (T)	Salinity levels (S)				Average
	1.6	6	12	15	
<b>Number of spikes /pot*</b>					
Control	6.0	6.0	5.0	4.0	5.3
A+N	6.0	6.0	6.0	5.0	5.8
100% NP	9.3	8.3	6.7	6.7	7.8
A+N+ 50%NP	8.7	7.3	7.0	7.0	7.5
Average	7.5	6.9	6.2	5.7	
LSD $\leq$ 0.05	T=0.36 S=0.36 T $\times$ S=0.72				
<b>Number of grains / spike*</b>					
Control	29.0	24.7	22.0	18.0	23.4
A+N	37.0	38.3	37.0	28.3	35.2
100% NP	70.7	66.0	56.0	34.0	56.7
A+N+ 50%NP	72.2	67.0	57.7	37.7	58.7
Average	52.3	49.0	43.2	29.5	
LSD $\leq$ 0.05	T=2.38 S= 2.38 T $\times$ S=4.76				
* Average of six replicates					
<b>Weight of 100 grains* (g)</b>					
Control	2.5	2.5	2.0	2.0	2.3
A+N	2.8	2.7	2.7	2.4	2.7
100% NP	3.0	3.0	2.7	2.7	2.9
A+N+ 50%NP	3.1	2.9	2.8	2.7	2.9
Average	2.9	2.8	2.6	2.5	
LSD $\leq$ 0.05	T=0.1 S= 0.1 T $\times$ S=0.2				
* Average of three replicates					

**Effect of combination of *Anabaena circinalis* and *Nostoc commune* (A+N) alone or with reduced chemical fertilizer (NP) dose some agronomic traits of wheat grown under different salinity levels.**

Salinity significantly reduced all test agronomic traits (Table 4). Salinity at 12 and 15 dS/m reduced average shoot length by 7 and 19% of control, average chlorophyll concentration by 11 and 18% of control, respectively. The average of flag leaf area at salinity levels 6, 12 and 15 reduced by 15, 23 and 31% of control, respectively. These findings are in agreement with the results of Karimi *et al.* (2014), who found that Increasing salt concentration in plant gradually decreased the content of photosynthetic pigments. Others reported that the excessive accumulation of salt in plant leaves such as  $\text{Cl}^-$  and  $\text{Na}^+$  causes nutritional imbalance, decreases stomata opening, changes in the photochemical processes and accelerates chlorophyll breakdown (Sherif *et al.*, 2007). The reduction in chlorophyll content due to salinity stress leads to negative impacts on growth and development of flag leaf area and shoot length (Manivannan *et al.*, 2007)

All treatments significantly affected the test agronomic traits (Table 4). Application of cyanobacteria extract and grain coating increased average shoot length, chlorophyll concentration and flag leaf area by 46, 16 and 41% over control, respectively. This ratio increased up to 47, 58 and 110% respectively, when cyanobacteria extract and grain coating applied in combination with 50% NP, which was similar to that of sole application of full dose chemical fertilizer. However combination of cyanobacteria and 50% NP showed more increase in the test growth parameters and

become similar to that achieved by the label rate of full dose fertilizer.

Several investigator found that the cyanobacteria provided the highest plant height, number of tillers/ plant, grain weight/ plant and 1000 grain weight of wheat, due to produce growth promoting hormones such as gibberellin like, cytokinin like and auxin like compounds. These substances increased seed germination, root and shoot growth, weight of grains and their protein contents (Venkataraman, 1993; Ali and Mostafa, 2009)

The interaction between salinity and treatments significantly increased all test agronomic traits (Table 4). In most cases, the highest shoot length, chlorophyll concentration and flag leaf area was recorded by full dose of fertilizer and combination of cyanobacteria and 50% NP when applied at all salinity levels. Also, combination of cyanobacteria and 50% NP provide shoot length, chlorophyll concentration and flag leaf area statically similar to that achieved by label rate of chemical fertilizer.

Application of cyanobacteria have the ability to reduce the chemical fertilizer to 50 % and gave better results to be the best treatment for enhancing wheat growth. The increased 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 secondary metabolites which can be excreted by cyanobacteria (Ördög, 1999; Masojídek and Prášil, 2010; Chojnacka *et al.*, 2010; Sahu *et al.*, 2012).

**Table 4:** Effect of *Anabaena circinalis* and *Nostoc commune* (A+N) applied as extract and grain coating in combination with chemical fertilizers (NP) on grain and biological yields of wheat grown under different salinity levels.

Treatments (T)	Salinity levels (S)				Average
	1.6	6	12	15	
	<b>Shoot length (cm)*</b>				
Control	52.2	51.8	48.6	30.0	45.7
A+N	70.8	72.2	62.2	60.0	66.5
100% NP	72.8	74.6	68.6	65.8	70.5
A+N+ 50%NP	73.8	72.8	74.0	64.6	71.3
Average	67.4	67.9	63.4	55.3	
LSD $\leq$ 0.05	T=2.17 S= 2.17 T $\times$ S=4.34				
	<b>Chlorophyll (SPAD)*</b>				
Control	30.0	30.7	28.7	26.3	28.9
A+N	38.0	34.7	33.1	28.3	33.5
100% NP	50.7	48.4	44.0	42.8	46.5
A+N+ 50%NP	49.5	47.5	44.8	40.8	45.6
Average	42.0	40.3	37.7	34.6	
LSD $\leq$ 0.05	T= 1.2 S= 1.2 T $\times$ S=2.4				
	<b>Flag leaf area (cm<sup>2</sup>)*</b>				
Control	12.1	9.3	8.7	9.5	9.9
A+N	16.9	14.4	14.1	11.0	14.0
100% NP	27.4	22.8	19.4	16.3	21.3
A+N+ 50%NP	23.5	21.6	20.0	18.7	20.8
Average	20.0	17.0	15.5	13.8	
LSD $\leq$ 0.05	T= 1.2 S= 1.2 T $\times$ S=2.4				

\* Average of six replicates

### Effect of combination of *Anabaena circinalis* and *Nostoc commune* (A+N) with reduced chemical fertilizer (NP) dose on protein of wheat grown under different salinity levels.

Salinity significantly reduced rate on grain protein (Table 5). Salinity significantly averted grains protein in wheat. Salinity at 6.12 and 15 dS/m reduced average of protein by 3, 8 and 16% of control, respectively. These findings are in agreement with the results of Maqsood *et al.* (2008) who reported that a decrease in protein content accumulation in grains are associated with salt stress due to high interferes of external  $\text{Na}^+$  with nitrogen absorption resulting in low protein concentration in the grains. Others found that in many plant species grow under salinity, the protein content decreased may be attributed to salinity that causes physiological disorders due to the elimination of  $\text{K}^+$  ions by roots of plant where  $\text{K}^+$  is the key element in the protein biosynthesis (Mahboobe and Akbar, 2013).

Application of cyanobacteria alone increased average protein grains by 19 % over control. However average of protein content was increase by 60% when cyanobacteria applied in combination with 50% NP, which was better than that achieved by the label rate of chemical fertilizers

The foliar applications of cyanobacteria extract and grains coating with cyanobacteria are practiced whenever

roots are restricted to uptake nutrients due to salt stress (Singh *et al.*, 2013). The stimulatory responses of cyanobacteria was observed when spraying wheat plants with liquid cyanobacteria gave the greatest values of crude protein percent per wheat grains. Protein hydrolysates are also included among the active ingredients of plant biostimulants (Calvo *et al.*, 2014) and their use in a foliar spray application might enhance the biological activity in crops growth and development. Microalgae contains also amino acids that are a well-known biostimulant with positive effects on plant growth and crop yield. Moreover, amino acids can contribute to mitigate the injuries caused by abiotic stresses (Kowalczyk *et al.*, 2008). Water extract of cyanobacteria improved wheat tolerance to salinity, and enhanced the antioxidant capacity and protein content of the whole grains produced by treating plants with cyanobacteria extracts (Abd El-Baky *et al.*, 2010).

The interaction between salinity and treatments significantly increased rate of protein (Table 5). In most cases, the highest protein concentration was recorded by full dose of fertilizer and combination of cyanobacteria with 50% NP when applied at all salinity levels. Also, combination of cyanobacteria with 50% NP provide protein statically similar or better to that achieved by label rate of chemical fertilizer.

**Table 5 :** Effect of *Anabaena circinalis* and *Nostoc commune* (A+N) applied as extract and grain coating in combination with chemical fertilizers (NP) on grain and biological yields of wheat grown under different salinity levels.

Treatments (T)	Salinity levels (S)				Average
	1.6	6	12	15	
	Protein %*				
Control	7.15	6.81	6.15	5.63	6.44
A+N	8.77	7.89	6.98	6.94	7.64
100 % NP	10.04	10.06	9.98	8.96	9.76
A+N+ 50 % NP	10.58	10.67	10.59	9.25	10.27
Average	9.14	8.86	8.42	7.70	
LSD <sub>≤0.05</sub>	T= 0.2 S=0.2 T×S=0.4				

\* Average of three replicates

### Conclusion

Salinity adversely effected all wheat growth and yield parameters tested and cyanobacteria applied as extract and grains coating mitigated the salinity effects. The combined application of cyanobacteria extract and grain coating with 50% of chemical fertilizer (NP) provided growth and yield of wheat similar to that achieved by full dose of chemical fertilizer at all salinity levels tested.

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