Biological Journal of Microorganism
Year 11, Vol.11, No.44, Winter 2023
Received: 2022-3-15 - Accepted:Page: 2022-06-27
P:61-75

The Role of Cyanobacteria Isolated from Khabr National Park Biocrust in Modification of Some Soil Characteristics and its Effect on *Secale Montanum* Growth

Zahra Hojjati Bonab
Department of Microbiology, Faculty of Basic Sciences, Islamic Azad University, Bonab, Iran- Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, hojjati_zahra90@yahoo.com
Parisa Mohammadi*
Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, p.mohammadi@alzahra.ac.ir
Azra Saboora
Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, saboora@alzahra.ac.ir
Roghaieh Zarei
Department of Plant Sciences, Faculty of Biological Sciences, Alzahra University, Tehran, Iran, r.zarei@alzahra.ac.ir

Abstract

Introduction: Khabr National Park is one of the valuable natural resources in Iran, and its cyanobacterial population has not been studied yet. Cyanobacteria are the most important nitrogen-fixing bacteria in all aquatic and terrestrial environments, playing a vital role in arid and semi-arid ecosystems. The present study aimed to determine the role of different cyanobacterial species isolated from the grazed and not grazed areas in two cold and warm steppes of Khabr National Park on some soil properties and the effect of the selected cyanobacteria on *Secale montanum* growth.

Materials and Methods: In the present study, cyanobacteria were isolated from a harsh terrestrial ecosystem and cultured on a BG11 medium. Next, the expression of the *nif*H gene was assayed in all nitrogen fixing isolates using the Real Time-PCR. Then, the best nitrogen-fixing cyanobacteria (*Nodularia.* sp.) was selected and its activity was investigated in pot experiments alone and in combination with chemical fertilizer. Three different soils in terms of physical and chemical properties were analyzed.

Results: The results indicated that the treated soils with *Nodularia* sp. as a biofertilizer can significantly increase nitrogen content of both agricultural soil and mixed soil.

*Corresponding Author

This is an open access article under the CC-BY-NC-ND 4.0 License (https://creativecommons.org/licenses by-nc-nd/4.0/)

10.22108/BJM.2022.127281.1460

Consequently, *Nodularia* sp. has the ability to promote some characteristics of the studied soil through improving TN, SOC, SOM, and enzyme activities.

Discussion and Conclusion: It can be concluded that the selected cyanobacterium increase soil stability in terms of mechanical fracture and resistance to desiccation and improve soil structure, which is very important, especially in arid and desert soil.

Key words: Biocrust, Khabr National Park, *Nodularia* sp., Nitrogen Fixation, *Secale Montanum.*

Introduction

The desert has not been able to support the growth of many various plants. Furthermore, the soils of arid and semi-arid regions suffer from major limitations such as weak physical soil properties, low fertility, low organic carbon contents, and high salinity level, which lead to low water penetration and increase soil erosion (1).

Recent investigations have shown that desert ecosystems are relatively complex and rich in terms of biological factors (2, 3, 4). In many arid and semiarid ecosystems, biological soil crusts or biocrusts include algae, cyanobacteria, mosses, hepatica, fungi, and lichens, which cover a few millimeters above the soil and are the most important alive parts of these regions (5). They encompass a specific alive community and have significant effects on key processes of ecosystems (6, 7) such as soil respiration (8), nitrogen fixation, soil stability, and drainage (5, 9). In the two past decades, increasing global attention was observed in the biological soil crusts by scientists, and has improved our knowledge about the structure, composition, physiology, and biogeography of these crusts. These biocrusts cover more than 70% of the soil surface of these regions (10).

Cyanobacteria as photosynthetic microorganisms of biocrusts play a key role in nitrogen fixation, which are capable to feed the soil food (10). These microorganisms can significantly adapt themselves to a vast spectrum of environmental conditions such as humidity, temperature, light exposure, and pH (11). Mostly, cyanobacterial cells are covered by a hygroscopic polysaccharide sheath, which helps the cyanobacteria against drought stress (12). Due to polysaccharides sheaths, soils can stick together in the root area (1, 13). A number of studies focused on increasing crop yield by using cyanobacteria as biofertilizers (14, 15).

In recent studies, the presence of cyanobacteria was detected in semi-arid soils of Iran (4, 16, 17). In this study, we tried to investigate the role of cyanobacteria in the modification of some soil characteristics and its effect on plant growth as a biofertilizer. For this reason, the impact of cyanobacteria on alteration of some growth parameters of *Secale montanum* was assayed. *Secale montanum* is a range plant that can grow in natural ecosystems in poor, non-fertile, and sandy soils such as desert soil.

Materials and Methods

The Site and Soil Sampling: Khabr National Park is an area with 149934 hectares placed in N28°25' to N28°59 latitude and E56°02' to E56°39' longitude near the south of Baft city of Kerman province, Iran. This park was registered as a national park in 2001. Soil sampling was taken from two regions of Khabr National Park, including cold and hot steppe regions. This area encompassed two grazed and not grazed areas. The sampling was carried out from 0-15 cm depth of soil surface in the spring and autumn of 2016.

Culturing of Cyanobacteria: The soil samples were cultured in BG-11 solid medium containing the following: $NaNO_3$ (1.5 g), $NaHCO_3$ (1.7 g), K_2HPO_4 (0.031 g), $MgSO_4.7H_2O$ (0.075 g), $CaCl_2$ .H_2O (0.036 g), Na_2CO_3 (0.020 g), $C_6H_8O_7$ (0.006 g), $C_6H_8FeNO_7$ (0.006 g), EDTA (0.001 g), H_3BO_3 (2.86 g), $MnCl_2.4H_2O$ (1.81 g), $ZnSO_4.H_2O$ (0.220 g), $Na_2MoO_4.2H_2O$ 0.390 g, $CuSO_4.H_2O$ (0.080 g), $CoCl_2.6H_2O$ 0.040 g, and 1L H_2O (18, 19). All materials were purchased from Merck Company.

To assay the expression of the *nif*H gene, at first, it was necessary to isolate and identify the isolates. For isolation of cyanobacterial strains, the samples were cultured under aseptic conditions and incubated at 25 ºC under 2500 lux and photoperiod of 16h light/ 8h darkness and incubated for 2 weeks. The morphology of the isolates was compared with the identification keys (20, 21). Then, molecular identification of the isolates was performed via amplification and sequencing of the 16SrRNA genome by using cyanobacterial-specific primers. The forward primer was CYA359F with 5'(GGGGAATYTTCCGCAATGGG) 3' sequences and the reverse primer was CYA781R (CYA781R (a) with 5' (GACTACTGGGGTATCTAATCCCATT) 3' and CYA781R (b) 5' (GACTACAGGGGTATCTAATCCCTTT) 3' sequences (11, 21, 22) which was previously reported (36).

Selection of Nitrogen-fixation Cyanobacteria: All the isolated cyanobacteria were inoculated in 50 ml tubes containing 15 ml BG_0 broth medium and incubated at 25±2˚C. After 48 hours, the nitrogen fixation was determined via acetylene reduction assay. The expression levels of the *nif* H genes for the nitrogen fixation were measured using the real time-PCR technique (17). RT-PCR was performed using Sybr® Premix Ex Taq™II Takara kit. The Applied Biosystems™ StepOne™ Real-Time PCR system was used to record changes in the fluorescence dye. Reactions were performed in a total volume of 12.5 µl, containing 4 µl cDNA template (diluted 1:20), 6.25 µl 2X SYBR green master mix, and 0.1 µM of each forward and reverse primers of *nif*H or 16S rRNA. The relative expression of *nif*H was assessed in comparison to 16S rRNA, as an internal control reference. The experiments were carried out twice and each time in triplicate. All controls were excluded from primers. The RT- PCR was conducted according to this program: initial activation phase at 95 °C in 1 min, denaturation phase at 95 °C in 5 s, annealing phase at 60 °C for 34 s, and was repeated 40 times. The gene expression of *nif*H was determined in different groups using the $2^{-\Delta ct}$ method.

Plant and Pot Experiments: Seeds of *Secale montanum* as a rangeland plant were purchased from Pakan Bazr Company (Esfahan, Iran) in 2017.

At first, *Secale montanum* seeds were soaked and kept in the refrigerator overnight. The seeds were then placed under running water (2 hours), followed by sterilization with 70% ethanol (2 minutes), washed with distilled water (2 times), and embedded in sodium hypochlorite 1% (15 minutes). After that, the seeds were washed with sterile water three times. Finally, the seeds were incubated at room temperature between two layers of filter paper. After germination, 10 seedlings with 2 cm length were planted into the pots (12cm diameter) which were filled with 2kg agriculture or desert soils alone or a mixture of them (1:1) based on the following table.

Table 1- Different Soil Treatments Used for Planting

Treatment number	Sterility of soil	Agriculture soil	Desert soil	Cyanobacterial inoculation
1	Unsterile	+	-	-
2		+	-	+
3		-	+	-
4		-	+	+
5		+	+	-
6		+	+	+
7	Sterile	+	-	-
8		+	-	+
9		-	+	-
10		-	+	+
11		+	+	-
12		+	+	+

The selected cyanobacterial suspension was prepared with concentration of 0.01 g/100 ml in phosphate buffer. Afterward, 100 ml of microbial suspension was poured into each pot. This prepared inoculation was added to each pot on the 1st, 5th, and 10th days. Furthermore, the nutrient solution was also prepared according to Table 2 (30). To prepare 10 liters of nitrogen-free nutrient solution it was added 5 ml of each stock solution to 5 liters of distilled water according to Table 2. After stirring, it was added another 5 liters of distilled water and was set pH between 6-6.8. Irrigation of all pots was carried out with water and nutrient solution (1:1) to reach the farm capacity (Table 2). The temperature of the greenhouse was adjusted to 25 ºC day/ 15 ºC night. After 15 days of planting the germinated seeds, some soil physicochemical characteristics including electrical conductivity (EC), pH, soil organic carbon (SOC), soil organic matter (SOM), total nitrogen (TN), soil texture, dehydrogenase enzyme activity (DHA), alkaline phosphatase activity (ALP), as well as some growth parameters such as length of root, shoot and leaf, fresh and dry weight, and protein content of the plants were measured (9).

Table 2- Components of Nutrient Solution (30)

Stock number	Chemical formula	Concentration (g/l)
1	$CaCl_2.2H_2O$	294.1
2	KH_2PO_4	136.1
3	Fe- Citrate $MgSO_4.7H_2O$ K_2SO_4 $MnSO_4$	5.4 123.3 87.0 0.338
4	H_3B_3 $ZnSO_4.7H_2O$ $CoSO_4.7H_2O$ $CuSO_4.5H_2O$ $Na_2MoO_4.2H_2O$	0.247 0.288 0.056 0.100 0.048

Table 3- Combination of Chemical Fertilizer and Cyanobacterial Inoculation in Soil Treatments

Treatment number	Soil	Cyanobacterial suspension (ml)	Chemical fertilizer (ml)
1	Agriculture soil	10	-
2		-	10
3		7.5	2.5
4		5.0	5.0
5		2.5	7.5

6	Desert soil	10	-
7		-	10
8		7.5	2.5
9		5.0	5.0
10		2.5	7.5
11	Mixed soil	10	-
12		-	10
13		7.5	2.5
14		5.0	5.0
15		2.5	7.5

In the second group of experiments, chemical fertilizer was consumed along with the nitrogen-fixing cyanobacteria. In this regard, the bacterial suspension was prepared by adding 0.01 g of the cyanobacteria to 100 ml of distilled water. Furthermore, a chemical fertilizer including 46% urea fertilizer (CH_4N_2O) and 21% ammonium sulfate fertilizer $(NH_4)_2SO_4$ was mixed in 1:1 (w/ w) and prepared 0.01 g/100 ml of water. Then, the pots were irrigated by 10 ml of a mixture of cyanobacterial suspension and chemical fertilizer solution according to Table 3. After that, irrigation was performed with water and nutrient solution. The plant leaves, stems, roots, as well as soils were sampled 15 days after seed germination for further analysis.

The Soil Physico-Chemical Analysis: Quantitative analysis of various physico-chemical parameters of the soils was carried out for each treatment. EC and pH of saturation extract of soils were measured using a conductivity meter (Lutron CD-4306) and pH meter (Mettler toledo), respectively. TOC was determined by the rapid dichromate titration method (23, 24, 25) and TN was estimated based on the modified Kjeldahl method (23). Soil texture was examined (23, 24, 25).

The Soil Biological Analysis: To evaluate DH activity, 6 g of soil in 1 ml of 3% tri-phenyl tetrachloride was incubated and the absorption was measured at 485 nm by spectrophotometer (26). ALP was assayed in 1 g of soil suspended in 1 ml of modified phosphate buffer (pH 11), along with 1 ml of p-Nitro phenyl phosphate. Next, the absorbance was measured at 440 nm (25).

Plant Growth Analysis: Length of root, shoot, and leaf of plants as well as their fresh and dry weight (48 h at 70 ^{o}C) were measured. The protein content of the plants was determined by using the Bradford method (9).

Statistical Analysis: All of the experiments were conducted in three replicates. The data were presented as mean± standard deviation (SD). The data were performed by one-way variance analysis (ANOVA) followed by the Duncan's Multiple Range Test (DMRT) to compare means and determine the differences between treatments ($p < 0.05$). The SPSS software package version 22.0 was used for statistical analysis.

Results

The Results of Identification of Cyanobacterial Isolates: Molecular identification of the cyanobacterial isolates by using CYA359F and CYA781R primers which were previously carried out, as presented in Table 4 (36).

The Result of RT-PCR: The *nif*H, as a marker gene, was used to study the diversity and ecology of nitrogen-fixing bacteria. The results demonstrated that *nif*H gene expression in *Nodularia* sp. was more than that of the other strains. After that, the highest level of *nif*H gene expression was observed in *Chroococcidiopsis* sp., *Synechococcus* sp., and *Leptolyngbya* sp., respectively. According to the results, the

superior strain, *Nodularia* sp. was selected for pot experiments.

Table 4- The Results of Molecular Identification of Cyanobacterial Isolates

No	Accession number	Region	BLAST match	Query coverage	Identical sites
1	JQ927348	Grazed	*Nodularia* sp.	100	99
2	MF467540.1	Grazed	*Chroococcidiopsis* sp.	99	99
3	MF467540.1	Not grazed	*Chroococcidiopsis* sp.	99	99
4	pRF1-p7	Not grazed	*Leptolyngbya* sp.	100	98
5	AY274622	Not grazed	*Synechococcus* sp.	94	99

The Results of Soil Physico-Chemical Analysis: The characteristics of three types of soils was listed in Table 5. As reported in many studies, the EC depends on the texture of the soil. The agriculture and mixed soil revealed lower EC, while the desert soil with clay loam texture, showed higher EC.

Table 5- The Result of Soil Physico-Chemical Analysis

Soil type	Soil texture	EC (mS/m)	pH
Agriculture	Sandy loam	47.4	8.08
Desert	Clay loam	96.6	8.6
Mixed	Sandy-Clay loam	33.5	8.1

Twenty-first days after inoculation of cyanobacteria in the first culture (Table 1), the EC did not show any significant differences, except for the treatments of 7 (EC 11.4 mS/m) and 8 (EC 6.1 mS/m) (Fig. 1 A).

The acidity of all the tested soils varied from 7.97 to 8.61. The pH of the desert soil was relatively higher (8.59) than the agricultural soil (8.09) and mixed soil (7.98) (Fig. 1 B). In the sterilized soil, cyanobacterial inoculation did not significantly change the soil pH.

Total nitrogen contents of the soil agriculture, desert, and the mixturee of inoculated with *Nodularia* sp. were 1.18, 1.33, and 1.19 fold higher than uninoculated soils, respectively as presented in Table 1. In the sterilized soils, which were inoculated with *Nodularia* sp., TN of the agriculture, desert and mixed soils were 1.22, 1.9, and 1.39 folds more than TN of the treatments of 7, 9 and 11, respectively. Therefore, the differences were not significant (Fig 1 C).

Contents of SOM in the soil of agriculture, desert, and mixed soils which were inoculated with *Nodularia* sp. were 2, 1.33, and 1.77 folds higher than the treatments of 1, 3 and 5, respectively. The inoculation of *Nodularia* sp. to the sterilized soil of agriculture, desert, and mixed resulted to the rise of this parameter to 1.11, 1.27, and 1.07 fold more than the SOM of the treatments of 7, 9, and 11, respectively, and therefore the differences were not significant (Fig 1 D).

The organic carbon of the agriculture, desert, and mixed soils inoculated with *Nodularia* sp. increased 2.05, 1.56, and 1.72 folds in the treatments of 1, 3, and 5, respectively. The inoculation of *Nodularia*sp. to the sterilized agriculture, desert, and mixed soils resulted to the increase of 1.856, 1.9, and 2.04 folds higher than the SOC of the treatments of 7, 9, and 11, respectively, and therefore the differences were significant (Fig 1 E).

There were significant differences between means of ALP activity in sterilized (treatment 1-6) and unsterilized soils (treatments 7-12) (Fig 1 F). Furthermore, the ALP activity differences observed between inoculated and uninoculated soils were the most in treatments of 6 and 2 and the least in treatments 3 and 4.

There were significant differences between means of DH activity in sterilized (treatment of 1-6) and unsterilized soils (treatments of 7-12) (Fig 1 G). Furthermore, the DH activity differences observed

between inoculated and uninoculated soils were the most in treatments of 1 and 2, and the least in treatments 3 and 4.

The Results of Plant Growth Analysis: The effect of the selected cyanobacteriun on *Secale montanum* growth in different soil treatments was assayed and shown in figure 4 (A-F). According to Table 1, the seedling was not grown on the desert soils well (treatment 3, 4, 9, and 10). Among the other 8 treatments, the longest leaf length was observed in the agriculture soil (Fig 4B), which was inoculated by cyanobacteria (Fig 2A, treatment 2). The leaf length of the plants grown on the sterilized agriculture soil did not show significant differences from the control soil (Fig 2A, treatments 5 and 6, Fig 4E). The leaf length of the plants grown on the mixed soil decreased significantly, which was 50% lesser than the control (Fig 2A, treatments of 3, 4, 7, 8).

Among 8 treatments, the highest shoot length was observed in the inoculated agriculture soil (Fig 2B, treatment 2, Fig 4B). The shoot length of the plants grown on the sterilized agriculture soil did not show significant differences from the control (Fig 2B, treatments 5 and 6). The shoot length of the plants grown on the mixed soil decreased significantly, which was 42- 55 % lesser than the control (Fig 2B, treatments of 3, 4, 7 8, Fig 4A, C).

In 8 treatments, the longest root length was observed in the agriculture soil, which was inoculated with cyanobacteria (Fig 2C, treatment of 2, Fig 4B). The root length of the plants grown on the sterilized agriculture soil did not show significant differences in comparision to the control (Fig 2C, treatments of 5, 6). The root length of the plants grown on the mixed soil decreased significantly, which was 13-20 % lesser than the control (Fig 2C, treatments 3, 4, 7, 8).

The average of the relative water content of the seedlings grown in the unsterilized agriculture and mixed soils, inoculated and uninoculated ones was not significantly different. The plants grown on the mixed soil showed slightly higher relative water content in comparison to the control (Fig 2D).

The average of fresh weight of the seedlings showed significant differences between the agriculture and mixed soils in both sterilized and unsterilized treatments (Fig 2E, treatments of 1, 2, 5 and 6 against 3, 4, 7 and 8).

The dry weight of seedlings grown in the unsterilized agriculture soil and mixed soil inoculated with *Nodularia* sp. showed significant differences as presented in Figure 2F, although plants grown on the mixed soil revealed 20-33 % reduction in dry weight.

Among 8 treatments, the Total Nitrogen (TN) contents of the plants grown on the agriculture soil, which was inoculated by cyanobacteria (Fig 2G, treatment of 2) was 1.25% higher than the control. The TN of the plants grown on the sterilized agriculture soil did not show significant differences from the control (Fig 2G, treatments of 5 and 6). The TN of the plants grown on the mixed soil decreased significantly, 31- 43% lesser than the control (Fig 2G, treatments of 3, 4, 7, and 8).

The average of the protein content of seedlings grown in unsterilized agriculture and mixed soils inoculated with *Nodularia* sp. had a significant difference even higher than the control. Furthermore, the protein content of the seedlings grown on both sterilized inoculated and uninoculated soils was significantly less than the control (Fig 2H, treatments of 4-8).

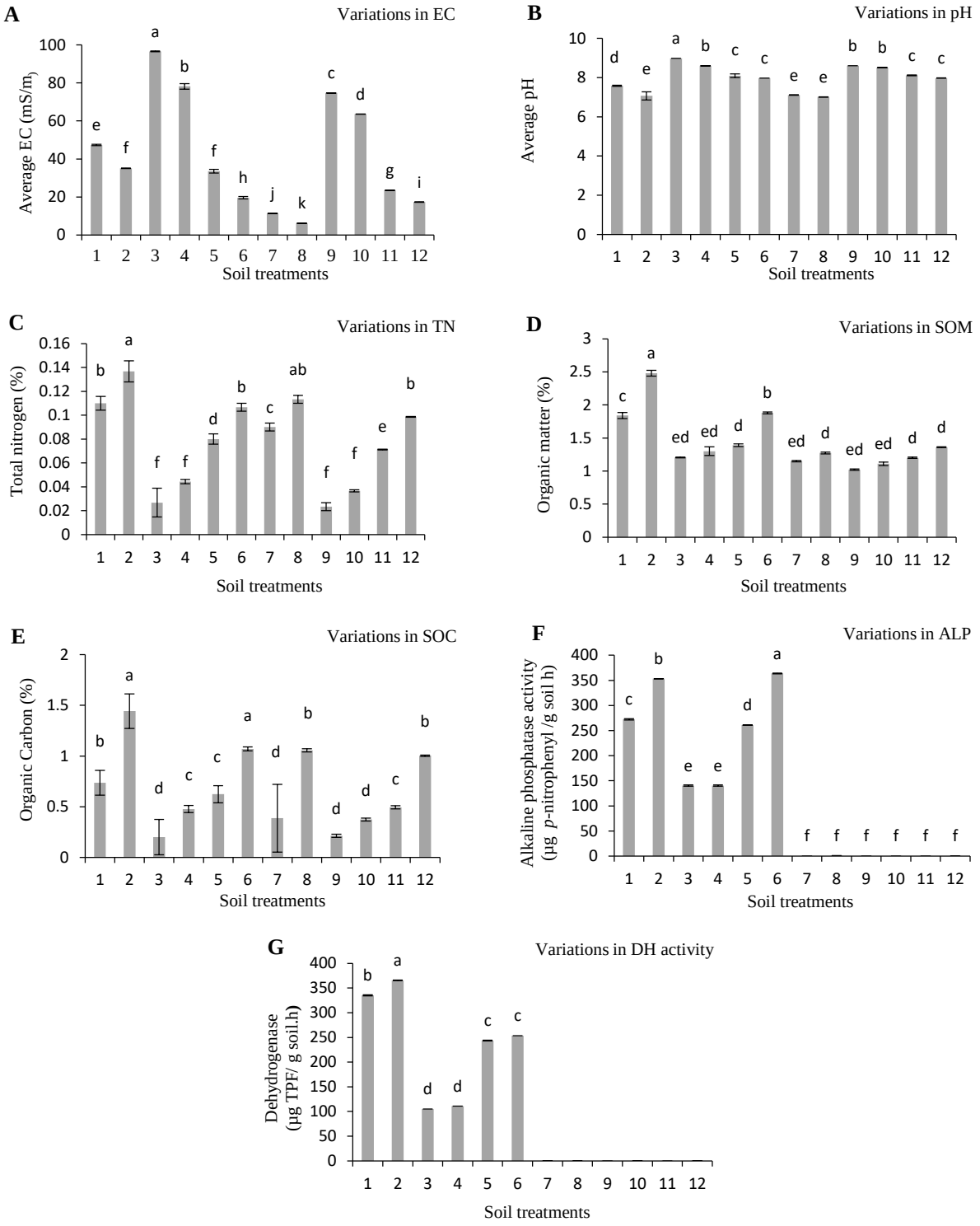

Fig. 1- The changes in the soil physicochemical parameters of EC, pH, TN, SOM, SOC, ALP, and DH with and without cyanobacteria inoculation. The different letters on the columns indicated a significant difference between the average at P <0.05. The treatment number is presented according to Table 1.

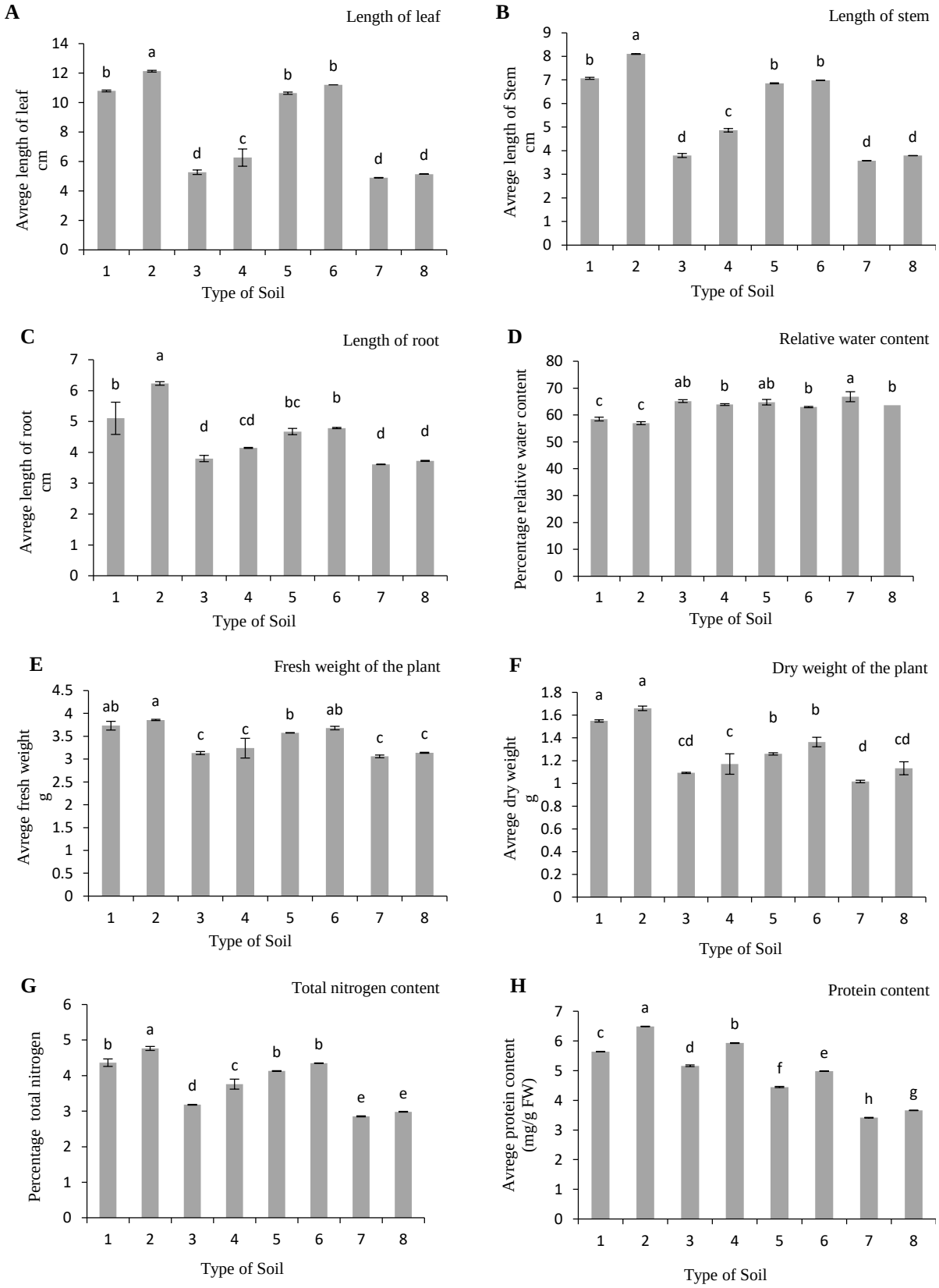

Fig. 2- The plant growth analysis of *Secale montanum* cultured on the inoculated and uninoculated soils. The different letters on the columns indicated significant differences at P <0.05. The treatment number is presented according to Table 1.

Evaluation of the statistical results regarding to the changes in the measured factors due to cyanobacterial inoculation (*Nodularia* sp.) and chemical fertilizer showed that the most average electrical conductivity was obtained at 7.8 and 6.9 dS/m for the soil treated by cyanobacteria (100%) and cyanobacteria-chemical fertilizer (25:75%), respectively. While as shown in Figure 3A, the soil treated with 100% chemical fertilizer led to less electrical conductivity (1.6 dS/m).

Furthermore, the acidity of the treated soils varied from 7.98 to 8.6 (Fig 3B). The highest pH belongs to the desert soils treated with cyanobacteria-chemical fertilizer whereas the lowest pH belongs to the mixed and agriculure soils. No significant difference was observed within three groups of soils given the average acidity.

The average total nitrogen content of the soil in treatments 2 and 4 of agriculture soil with 100% and 50% fertilizer was 5.90 and 3.63 times greater than the control, respectively. Moreover, the average of total nitrogen in soils treated with different ratios of cyanobacteria-chemical fertilizers was not significant. However, nitrogen content increased in the treatment of 11-15 in the mixed soils (Fig 3C).

The average soil organic carbon content in treatment of 2 and 4 of agriculture soil with 100% and 50% fertilizer, respectively was the most and in treatments of 6 and 10 was the least. Furthermore, the average of soil organic carbon content of treatments of 2, 4, 12, and 14 exhibited a significant difference. As shown, there was a significant difference among treatments of 6-10 compared to other treatments (Fig 3D).

The average soil organic matter content was maximum in treatments of 2, 4, and 12 with 100% and 50% of fertilizers. Moreover, the minimum average soil organic matter content was in treatments of 6, 8 and 10 (Fig 3E).

The average alkaline phosphatase acitivity of soil treatments was significantly different. The average alkaline phosphatase was maximum in treatments 12, 2, 4, and 14 with 100% and 50% of fertilizers. Moreover, the average soil alkaline phosphatase was minimum in treatments 6, 8, and 10 (Fig 3F).

The average dehydrogenase acitivity of soil treatments was also significantly different. The average dehydrogenase was maximum in treatments 2, 12, 4, and 14 with 100% and 50% of fertilizers. Moreover, the average soil dehydrogenase was minimum in treatments 6-10 (Fig 3G).

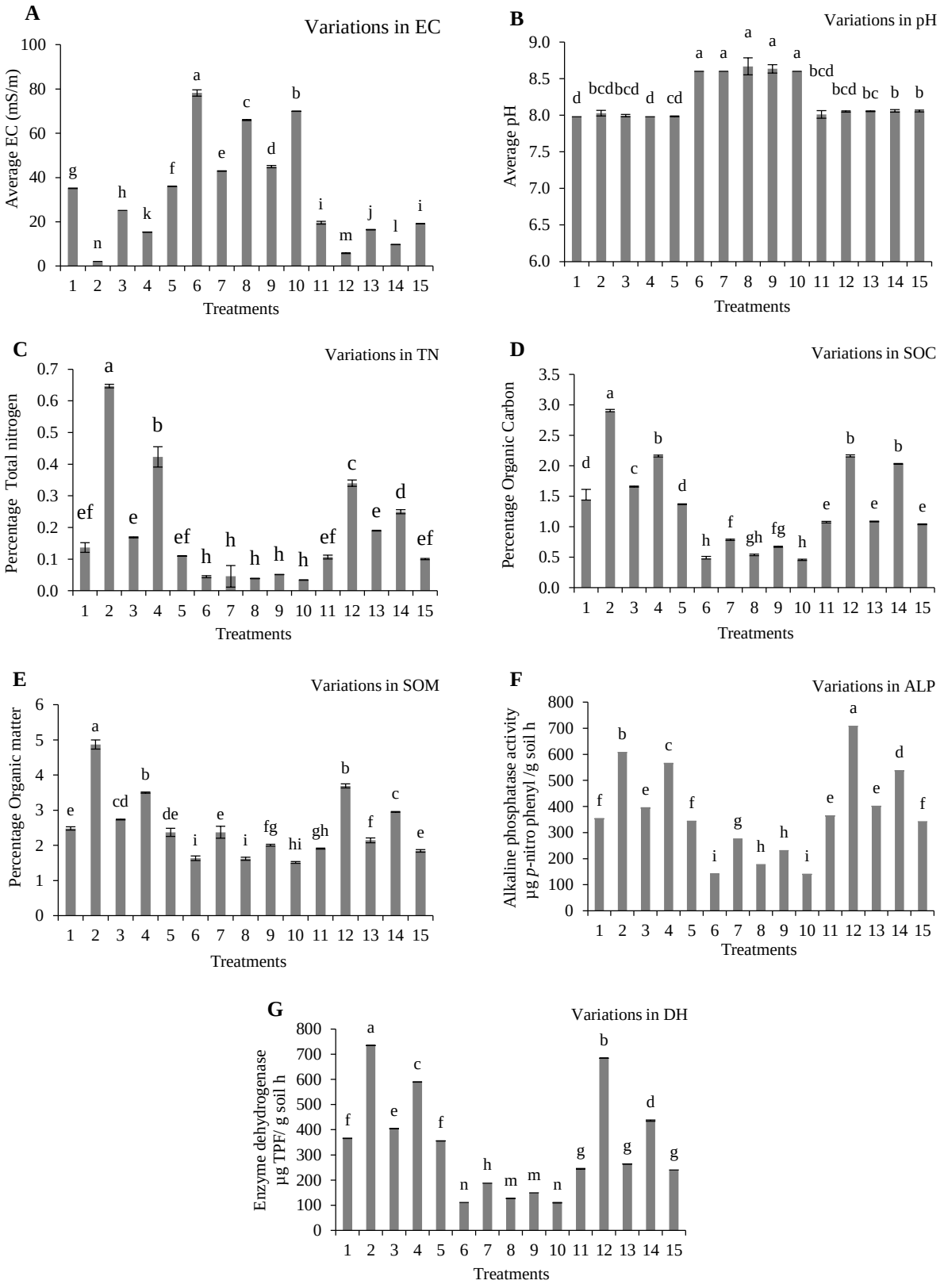

Fig. 3- Changes in the average physicochemical parameters of different soils with and without inoculation of cyanobacteria and chemical fertilizer based on Table 3. The different letters on the columns indicated the significant differences between the average at P <0.05.

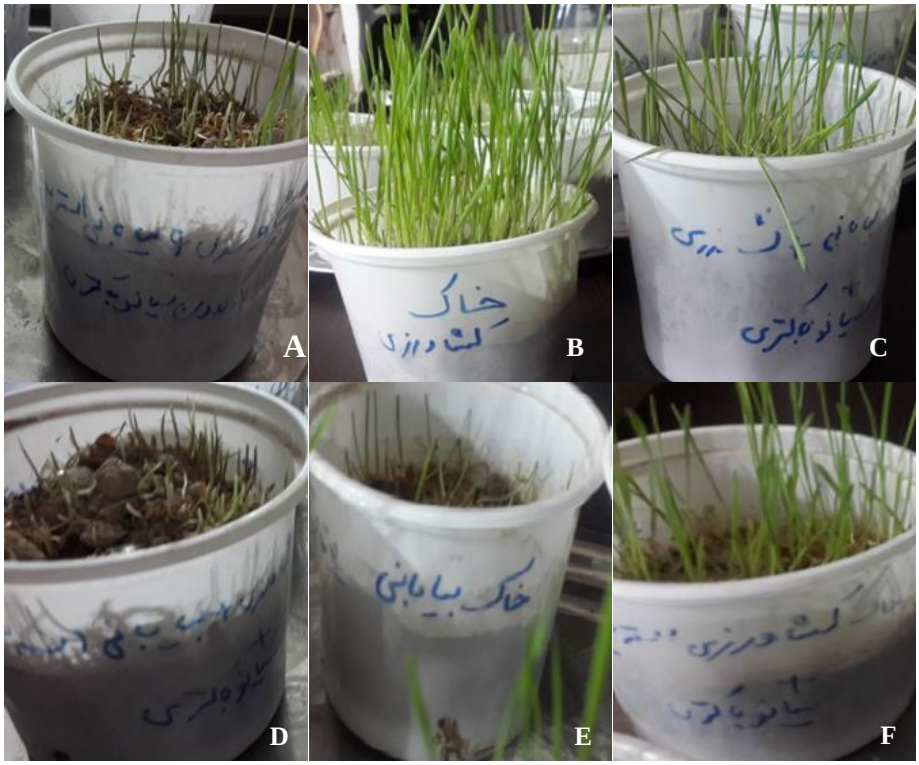

Fig. 4- The effect of the selected cyanobacterium on *Secale montanum* growth in different soil treatments. A) Mixed soil without cyanobacterial inoculation (control), B) Agriculture soil with cyanobacterial inoculation, C) Mixed soil with cyanobacterial inoculation, D) Sterilized desert soil with cyanobacterial inoculation, E) Desert soil with cyanobacterial inoculation, F) Sterilized agriculture soil with cyanobacterial inoculation.

Discussion and Conclusion

Biological crusts have a crucial role in the soil structures, and cyanobacteria areone of the important microorganisms in these crusts. Due to the presence of cyanobacteria, the crusts are able to do nitrogen and carbon fixation and studies were shown that natural cycles of nitrogen and carbon are very essential for soil fertility. Thus, improvement of the soil structure with microorganisms and at several centimeters depth can increase fertility, especially in poor soil (3, 27, 28).

The electrical conductivity parameter can be used as a marker of soil salinity. It was reported that cyanobacteria can decline the soil EC. This occurs *via* adsorption and consequently they will alter the acidity of soils (29). Our experiments indicated that EC has been reduced the presence of cyanobacteria in the sterilized agricultural soil remarkably.

The increased pH in the soil can be considered an undesirable factor for soil quality (30, 31) and desert soil showed alkaline properties, and its pH improved when mixed with agricultural soil.

The results of all experiments showed that *Nodularia* sp. significantly increased TN. In this regard, it was reported that cyanobacteria are the most important nitrogen-fixing microorganisms in agricultural soils. Moreover, this bacterium can fix carbon in the soil, and carbon fixation enhances soil fertility too (32). The clay loam soil such as agricultural soil has more TN than the desert silty soil (33).

Between chemical and biological properties of the soils, enzymes are more sensitive to changes in soils. Based on the results, DH activity was significantly increased in the mixed soil which can be attributed to the presence of diverse microorganisms in this soil. One of the

most important biological source of enzymes is the microorganism (26, 34).

The increase of TN, protein, fresh and dry weight, lengths of leaf, root, and stem was achieved in the inoculated unsterilized agricultural soil, which can be attributed to the impact of the presence of cyanobacteria and other microorganisms. This can promote the plant growth. Furthermore, *Nodularia* sp. can synthesize and secrete growth regulator factors such as auxin, various amino acids, and antibiotics resulting to the growth of the plant. Moreover, cyanobacteria can rise up the accessibility of nutrients through optimizing pH and increasing their solubilities too (34). Although chemical fertilizers can increase the amount of nitrogen in soils, their impacts on other organic matters are very low (35).

The results showed that *Nodularia* sp. had a significant impact on soil properties quantitatively and qualitatively, as well plant growth. It can be concluded that *Nodularia* sp. have the ability to progress some characteristics of the studied soil through improving TN, SOC, SOM, and enzyme activities. Consequently, this cyanobacterium can promote plant growth through better availability of nitrogen sources and some soil properties. According to these results, the combination of biofertilizers and chemical fertilizers is suggested to decline the used dosage of hazardous chemical fertilizers, as a friendly environmental approach.

Acknowledgment

This project was financially supported by office of research chancellor of Alzahra University. The authors would like to thank all the staff of Shayesteh Sepehr Laboratories of Industrial Microbiology for their cooperation.

Reference

(1) Nisha K., Shahi DK., Sharma A. Effect of endosulfon and monocrotophos on soil enzymes in acid soil of Ranchi. *Pestology* 2006; 30 (11): 42-4.

(2) Polis GA. Complex trophic interactions in deserts: An empirical critique of food-web theory. *American Naturalist* 1991; 138 (1): 123-155.

(3) Langhans TM., Storm C., Schwabe A. Biological soil crusts and their microenvironment: Impact on emergence, survival and establishment of seedlings. *Flora* 2009; 204 (2): 157–168.

(4) Teimouri M., Mohammadi P., Jalili A., Dick WA. Assessing soil quality through soil chemical properties and enzyme activities in semiarid area Iran. *Journal of Applied Ecology and Environmental Research* 2018; 16 (3): 2113-27.

(5) Belnap J., Lange OL. Structure and functioning of biological soil crusts: A synthesis. In Biological soil crusts: structure, function, and management. *Springer Berlin Heidelberg* 2003; 471-479.

(6) Lázaro R., Mora JL. Sediment content and chemical properties of water runoff on biocrusts in drylands. *Biologia* 2014; 69 (11): 1539-54.

(7) Belnap J. The potential roles of biological soil crusts in dryland hydrologic cycles. *Hydrological Processes* 2006; 20 (15): 3159-78.

(8) Maestre FT., Cortina J. Small-scale spatial variation in soil CO2 efflux in a Mediterranean semiarid steppe. *Journal of Applied Soil Ecology* 2003; 23 (3): 199–209.

(9) Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Journal of Analytical Biochemistry* 1976; 72 (1-2): 248-254.

(10) Evans RD., Lange OL. Influence of biological soil crusts on ecosystem nitrogen and carbon dynamics. Biological Soil Crusts: Structure, Function and Management 2001; 150: 263-79.

(11) Genua DB. Morphological and molecular characterization of cyanobacteria from a Brazilian facultative wastewater stabilization pond and evaluation of microcystin production. *Hydrobiologia*

2009; 627 (1): 195–209.

(12) Malam Issa O., Défarge C., Le Bissonnais Y., Marin B., Duval O., Bruand A., d'Acqui LP., Nordenberg S., Annerman M. Effects of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical soil. *Journal of Plant and Soil* 2007; 290 (1): 209-19.

(13) Kaushik BD., Krishna Murti GS. Effect of blue-green algae and gypsum application on physico-chemical properties of alkali soils. *Phykos* 1981; 20: 91–4.

(14) Acea MJ., Prieto-Fernández Á., Diz-Cid N. Cyanobacterial inoculation of heated soils: Effect on microorganisms of C and N cycles and on chemical composition in soil surface. *Journal of Soil Biology and Biochemistry* 2003; 35 (4): 513-24.

(15) Kaushik BD. Reclamative potential of cyanobacteria in salt-affected soils. *Phykos* 1989; 28 (1): 101-9.

(16) Khani JF., Mohammadi P., Zarrabi M. The identification of some phothotrophic microorganism from a semi-arid ecosystem in Iran. *Comptes rendus de l'Academie bulgare des Sciences* 2018, 71 (7), 907-15.

(17) Hojatti Bonab Z., Mohammadi P., Asgarani E., Ghorbanmehr N. The evaluation of nitrogen fixation activity of soil cyanobacteria via reduction assay and real time PCR. *Comptes rendus de l'Academie bulgare des Sciences* 2018, 72 (6), 751-7.

(18) Andersen RA. Algal culturing techniques. 1st ed. Amsterdam*:* Elsevier; 2005.

(19) Prescott GW. *Algae of the western great lakes area*. Iowa: Brown Company Publishers; 1970; 977.

(20) Wehr JD., Armonk YF. *Freshwater algae of North America*. 1st ed. Elsevier; 2003.

(21) Evans RD., O. L. Lange. *Reviews in Environmental Science and Bio/Technology* 2001; 11: 71–104.

(22) Karen LK., Videla HA. Surface analysis and materials characterization for the study of biodeterioration and weathering effects on cultural property. *International Journal of Biodeterioration & Biodegradation* 2009; 63 (7): 813-822.

(23) Allen SE., Grimshaw HM., Rowland AP. Chemical analysis. In: Moore, P.W., Chapman, S.B. (Eds.), Methods in Plant Ecology. *BlackwellScientific Publication, Oxford* 1986: 285–344.

(24) Phogat VK., Agarwal RP., Oswal MC., Kuhad MS. *Laboratory manual for soil physical analysis*. Hisar: CCS HAU Publication; 1999.

(25) Tabatabai MA. Soil enzymes. In: Miller A L., Keeney R H. *Methods of soil analysis*. Part 2. American Society of Agronomy, Soil Science Society of America, Madison, Wisconsin. 1982: 903–947.

(26) Casida, JR., Klein DA., Santoro T. Soil dehydrogenase activity. *Journal of Soil Science*, 1964; 98 (1): 371–376.

(27) Malam Issa O., Defarge C., Le Bissonnais Y., Marin B., Duval O., Bruand A., d'Acqui L P., Nordenberg S., Annerman M. Effect of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical soil. *Journal of Plant and Soil* 2007; 290 (1): 209-19.

(28) Turk-Kubo KA., Achilles KM., Serros TR., Ochiai M., Montoya JP., Zehr JP. Nitrogenase (nifH) gene expression in diazotrophic cyanobacteria in the Tropical North Atlantic in response to nutrient amendments. *Journal of Frontiers in Microbiology* 2012; 3: 386-7.

(29) Yang W., Wang F., Liu LN., Sui N. Responses of membranes and the photosynthetic apparatus to salt stress in cyanobacteria. *Journal of Frontiers in Plant Science* 2020; 713.

(30) Broughton WJ., Dilworth MJ. Plant nutrient solutions: In Somasegaran P., Hoben H J (eds). *Handbook for rhizobia; Methods in Legume-Rhizobium technology*. Niftal Project, *University of Hawaii, Hawaii*. 1970: pp: 245-249.

(31) Maqubela MP., Mnkeni PN., Malam Issa O., Pardo MT., D'acqui LP. Nostoc cyanobacterial inoculation in South African agricultural soils enhances soil structure,

fertility, and maize growth. *Journal of Plant and Soil* 2009; 315 (1), 79-92.

(32) Grant F., Roger P.A., Watanabe I. Ecosystem manipulation for increasing biological N2 Fixation by blue-green algae (cyanobacteria) in lowland rice fields. *Biological Journal of Agriculture & Horticulture* 1986, 3 (2-3): 299-315.

(33) Xue Z., Cheng M. Soil nitrogen distributions for different land uses and landscape positions in a small watershed on Loess Plateau, China. *Journal of Ecological Engineering* 2013; 60: 204-13.

(34) Han HS., Lee KD. Effect of coinoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Journal of Plant Soil and Environment* 2006; 52 (3): 130-6.

(35) Lal R. Enhancing crop yields in the developing countries through restoration of soil organic carbon pool in agricultural lands. *Journal of Land Degradation and Development* 2006; 17 (2): 197–209.

(36) Hojjati Bonab Z., Mohammadi P., Asgarani E. Identification of some cyanobacterial isolates of Khabr National Park of Kerman using classic and molecular methods. *Biological Journal of Microorganism* 2018; 7 (27): 25-35.