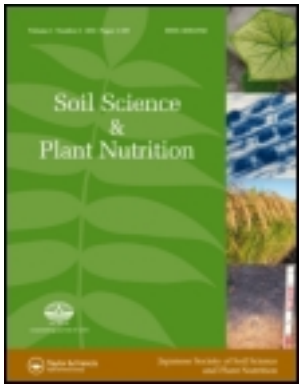

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ORIGINAL ARTICLE

Plant growth promoting cyanobacteria and their distribution in terrestrial habitats of Iran

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Abstract

In this research heterocystous cyanobacteria were isolated from paddy soils and used as algal extracts in a pot culture of vegetable crops. The first aim of this research was to study the distribution of algal species isolated from different rice (*Oryza sativa* L.) cultivation regions of Iran. Soil samples were collected from seven main rice cultivation provinces situated in perhumid and semi-arid regions of the country. In total, 49 heterocystous morphospecies were isolated from 20 locations. Among these taxa, *Anabaena* and *Nostoc* were found to be the dominant genera at most stations. The effect of algal extract on vegetable crops was the second aim of this experiment. After isolation and identification of these taxa, monoalgal cultures of two isolates were carried out by growing the isolates in nitrate-free BG-11 medium. Pot experiments were carried out by spraying algal extract on soil of treated plants. Growth of plants was evaluated by measuring growth parameters such as plant height, root length, dry and fresh weight of plant as well as leaf number after 40 days since planting. Statistical analysis showed that there are significant differences in studied parameters as compared to the control. In complementary studies nitrogenase activity of these taxa was determined by the acetylene reduction technique and identification of phytohormones was performed with the high performance liquid chromatography (HPLC) method. As result of this study, chemical content of algae extracts and production of plant growth-stimulating substances such as phytohormones can be proposed as factors affecting plant growth parameters.

Key words: biofertilizer, cyanobacteria, indole 3-butyric acid, nitrogenase activity, paddy soil.

INTRODUCTION

Cyanobacteria or blue-green algae (BGA) are the largest group of photosynthetic prokaryotes that exist in large diversity and distribution in the world (Stanier and Cohen-Bazire 1977). Application of cyanobacteria in different aspects like food, feed, fuel, fertilizer, colorant, production of various metabolites including vitamins, enzymes, pharmaceuticals and pharmacological probes are increasing day by day. Soil is the habitat of some terrestrial cyanobacteria species which are beneficial organisms for soil fertility by fixing atmospheric nitrogen (N), binding soil particles, helping to maintain moisture and preventing erosion. Increase of essential microelements in soil which are necessary for plant growth and plant ion uptake, increase of N content of the surface soil, as well as production of plant growth promoting substances such as phytohormones and other plant growth regulator substances (PGRs) such as amino acids, sugars and vitamins are the most important factors that are suggested for plant growth stimulating effects of these microorganisms (Misra and Kaushik 1989a, 1989b; Whitton 2000; Irisarri *et al.* 2001; Stirk *et al.* 2002; Karthikeyan 2006; Karthikeyan *et al.* 2007; Obana *et al.* 2007).

The increasing world food demand, and the contamination effect of chemical fertilizers that are used to enhance the crop yield, increase the importance of

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biofertilizers as a proper candidate in sustainable agriculture. Cyanobacteria offer a suitable alternative to chemical fertilizers which can increase the soil productivity both directly and indirectly (Vaishampayan *et al.* 2001; Mishra and Pabbi 2004). Much attention has been paid to the study of cyanobacteria in paddy fields since rice (*Oryza sativa* L.) is a main food for the majority of people in the world. Beneficial effects of cyanobacterial inoculation were reported, not only for rice, but for other crops such as wheat (*Triticum aestivum* L.), soybean [*Glycine max* (L.) Merr.], oat (*Avena sativa* L.), tomato (*Solanum lycopersicum* L.), radish (*Raphanus sativus* L.), cotton (*Gossypium hirsutum* L.), sugarcane (*Saccharum* sp.), maize (*Zea mays* L.), chili (*Capsicum annuum* L.), bean (*Phaseolus vulgaris* L.), muskmelon (*Cucumis melo* L.) and lettuce (*Lactuca sativa* L.) (Venkataraman 1972; Rodgers *et al.* 1979; Singh 1988; Arif *et al.* 1995; Thajuddin and Subramanian 2005; Karthikeyan *et al.* 2007; Maqubela *et al.* 2008; Saadatnia and Riahi 2009). Nevertheless, the beneficial influence of cyanobacteria on other crops such as vegetable crops is little known, whereas these crops are the most important component of many countries' diet.

In order to explore the distribution of these economical microorganisms and their potential as a biofertilizer, we have isolated and identified heterocystous cyanobacteria from paddy soils of Iran. Then, two fast-growing and widespread isolates of them, *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89, were used as monospecies biofertilizers for vegetable crops. The effect of these cyanobacteria on vegetative growth factors of studied plants was analyzed and the suggested reasons for this effect are described in this paper.

MATERIALS AND METHODS

Studied sites

Soil samples were collected from several paddy fields located in the north, center, south, west and east of Iran (Fig. 1), according to the method of Rangaswamy (1996). Soil sampling was done in the summer of three consecutive years, from 2008 to 2010. Among the studied provinces, Guilan and Mazandaran provinces are located on the southern shore of the Caspian Sea with a perhumid climate, and the other

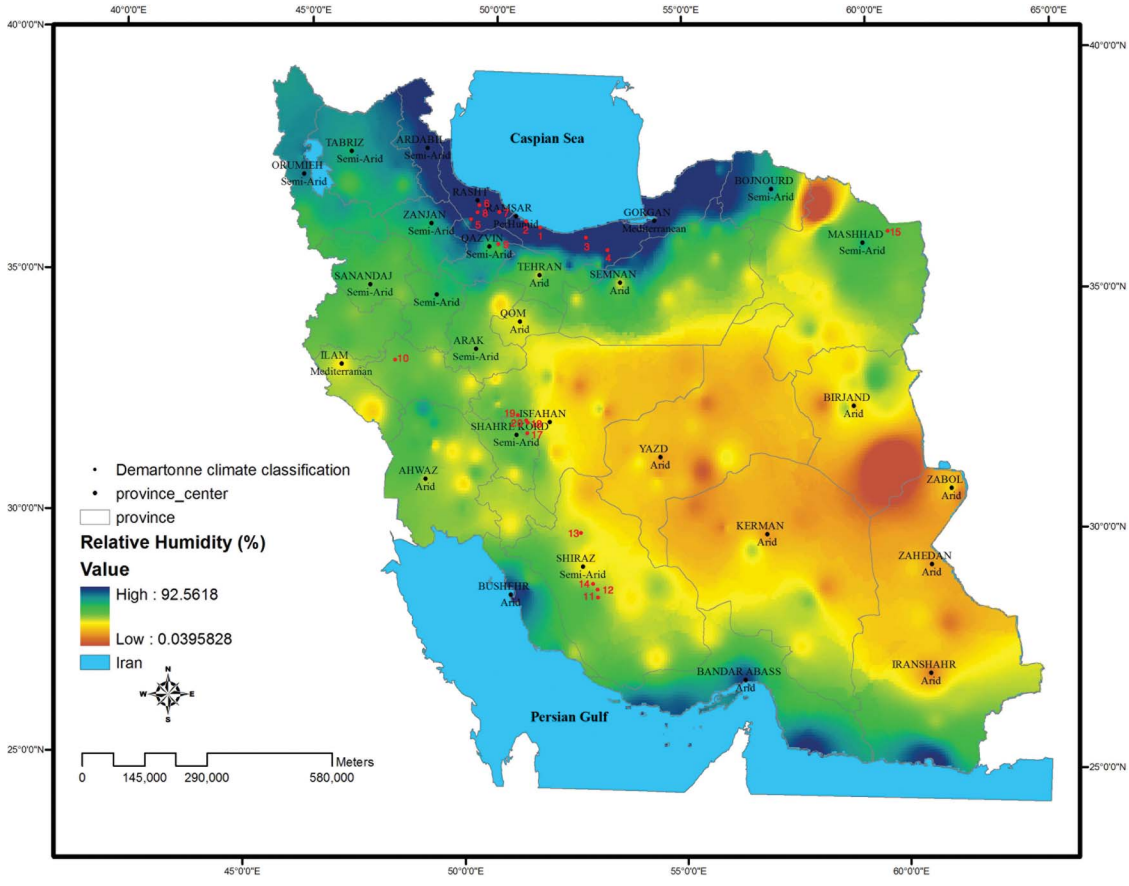

Figure 1 Geographical distribution of studied area and Demartonne climate classification of Iran's map.

provinces (Khorasan Razavi, Esfahan, Fars, Lorestan and Qazvin) are in semi-arid regions of the country. The dominant method of irrigation in studied paddies is continuous flooding.

Measurement of physicochemical parameters of paddy soils

Physical properties such as electrical conductivity (EC) and pH were analyzed for all the soil samples following the methodology outlined by Hayes (1981). The parameters such as phosphorus (P) content and the total N content of soils were determined by the Olsen and Kjeldahl methods as followed by Pauwels *et al*. (1992).

Isolation and identification of cyanobacteria

The sieved soils of different sites were transferred to sterile petri dishes, and sterilized nitrate-free BG-11 medium (Stanier *et al*. 1971) was added. The petri dishes were placed in a culture chamber at 25°C and a 12/12 h light-dark cycle with artificial illumination (2000–2500 µmol photons m^{-2} s^{-1}) for two weeks. After colonization, isolates were transferred to agar plates for purification. Taxonomic determination was carried out by morphometric study of isolates with light microscopy, based on Desikachary (1959), Prescott (1970), Whitford and Schumacher (1973), John *et al*. (2002) and Wehr *et al*. (2002) by prepared semi-permanent slides. The vegetative and reproductive characters used in the taxonomic determination were: shape, color and size of the thallus; width and length of trichomes; shape, size and color of vegetative cells, heterocysts and akinetes; and texture, color and ornamentation of cell walls of the akinetes and heterocysts. Description and pictures of some taxa were reported by Shariatmadari *et al*. (2011) and Shariatmadari and Riahi (2012).

Preparation of algal extract

This study deals with direct fertilization of plants by cyanobacterial extracts as a biofertilizer. For that, two fast-growing and widespread species of heterocystous cyanobacteria, *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89, were grown in 500-mL flasks containing nitrate-free BG-11 medium for 14 d under artificial illumination (2000–2500 µmol photons m^{-2} s^{-1}) and at 25 ± 1°C, with constant stirring and aeration. The cultures were harvested and the cells were washed with distilled water. The cell extracts were made by grinding the algae in distilled water with a pestle and blender. An algal extract containing 5.0 g fresh algal material in 500 mL of distilled water is considered as a 1% extract.

Pot culture

Vegetable seeds were obtained from Enza Zaden and Unigen Seeds Companies of the Netherlands and Italy. Five healthy seedlings of *Cucurbita maxima* Duch. ex Lam. (Squash: UG 5206 F1), *Cucumis sativus* L. (Cucumber: E 32.15720 F1) and *Solanum lycopersicum* L. (Tomato: E 26.32365 F1) were grown in 1-L pots (14 cm diameter) for 40 d. No fertilizer was applied, but algal extracts were sprayed on soil of treated pots and water for control pots every 7 d. The pots were arranged in a completely randomized design in an experimental greenhouse.

Nitrogenase activity measurements

Nitrogenase activity was determined by the acetylene reduction technique according to Asadi *et al*. (2011). Prior to incubation, 10% of the air inside the vial was replaced with the same volume of acetylene. Cells were incubated for 1 h under the same conditions as they were cultured. After incubation, 0.5 mL of gas samples was taken and ethylene concentration was determined. Acetylene reducing activity was determined on a Shimadzu GC-15A gas chromatograph as nmol ethylene mg dry $weight^{-1}$ $hour^{-1}$.

Preparation of auxin standards and extraction procedure

Identification and quantification of the endogenous auxins was performed according to Seyed Hashtroudi *et al*. (2012). The stock solutions were prepared by dissolving 1 mg of each auxin (indole 3-acetic acid, IAA; indole 3-butyric acid, IBA; and indole 3-propionic acid, IPA) in 10 mL of methanol, separately and also as a mixture, and subsequent dilutions were made with methanol:water 80:20 to prepare the desired concentrations. Algal biomass was dried, using an Operon bench top freeze dryer (FDB-5503), and extraction was performed under the commonly used sonication conditions of 20°C for the optimized time of 30 min. The extraction solvents were methanol:water in a ratio of 80:20. The extracts were centrifuged at 7500 rpm for 10 min; the supernatant was filtered through 0.45-µm syringe filter and concentrated to 500–1000 µL using an Organomation N-EVAP.

High performance liquid chromatography (HPLC) performance

Chromatographic separation was performed on an Agilent 1200 series HPLC system including a quaternary pump and a degasser equipped with a G1315D Diode Array Detector and a G1321A *Fluorescence* Detector. The accompanying Agilent LC Chemstation was employed for instrument control, data acquisition and processing.

A Eurosphere RP-column (100-5 C18 column, 250 × 4.6 mm Knauer, Germany) was used for separation of analytes. The column was eluted with a linear gradient (0–5 min, 60% A, 5–20 min, 100%A) at a flow rate of 1 mL min^{-1} of methanol (A) and 0.3% acetic acid (AcOH), and the column temperature was maintained at 25°C. Considering the ultraviolet (UV) maxima of three auxins, UV detection was performed at 225 nm and excitation and emission wavelengths in the fluorescence detector were 280 and 360 nm, respectively.

Both the extracts and standards were injected (injection volume: 20 µL) into the reverse phase column and identifications were carried out using comparison of retention times and UV spectrums of the extracts with standard mixture. Each experiment was repeated at least three times and run in triplicate. Recoveries were calculated by adding a known amount of standards to the microalgae and extracting the auxins by the same method as described above.

Evaluation of chemical contents of algal extracts

Chemical content of the algal extracts, such as total N and inorganic N (nitrite, NO_2^-; nitrate, NO_3^-; and ammonium, NH_4^+), phosphate, sulfate, carbonate and cations (sodium, Na^+; potassium, K^+; magnesium, Mg^{2+}; and calcium, Ca^{2+}) were determined by Arian Fan Azma Institute, Tehran, Iran. Laboratory methods of measurements are summarized in Table 6 in the Results section.

Statistical analysis

Statistical analysis was performed with one-way analysis of variance (ANOVA), using the procedure of SPSS (Package for the Social Sciences, SPSS Inc., Chicago IL) version 16. Means were separated using the Tukey honestly significant difference (HSD) test at $P < 0.05$. Also, Microsoft Office Excel 2007 was used to create graphs to study the correlation between physicochemical parameters of paddy soils and cyanobacteria diversity.

RESULTS

Diversity and distribution of heterocystous cyanobacteria in paddy soils

Rice is the most important cereal crop, which has been cultivated for many years in Iran. Rice fields are temporary wetland ecosystems which represent a favorable environment for the growth of several microorganisms such as cyanobacteria. In this study, representative soil samples were collected from seven different rice cultivation provinces of Iran (Fig. 1). In total, 49 heterocystous morphospecies were isolated from several sites (Table 1). Total percentage abundance of the identified heterocystous cyanobacteria is summarized in Table 2. Overall, 10 genera were identified, among which *Anabaena* and *Nostoc* were found to be the dominant genera in most stations. The geographic and some ecological details of the sampling locations are shown in Table 3. Correlation between some physicochemical parameters of paddy soils and general diversity of heterocystous cyanobacteria are shown in Fig. 2. The results indicated that the evaluated physicochemical factors did not show significant correlation with the species diversity of paddy soils. In other word, species diversity in studied sites was not influenced by physicochemical characteristics such as soil pH, EC, total N, P and organic carbon (C).

Cyanobacterial extracts as inoculants for vegetable crops

The second aim of this study was to investigate the effect of cyanobacterial extracts as inoculants for vegetable crops. The two fast-growing and widespread species of heterocystous cyanobacteria in the studied sites, *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89, were used as monospecies extracts for different vegetables such as cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*) and squash (*Cucurbita maxima*). Comparison analysis showed that there is a significant difference between treated plants and controls, especially in vegetative growth factors such as root length and plant height as well as fresh and dry weight of roots ($P < 0.05$; Table 4). In other words, the results revealed that there was a significant difference in most measurement factors in different plants treated with cyanobacterial extracts as compared to controls. However, effect of algal culture is not the same for all parts of plants or in different plants (Fig. 3). For instance, leaf number showed the lowest difference between treatments as compared to controls, whereas root length and root weight showed the greatest difference.

Determination of some plant growth stimulating factors in algal extracts

In the complementary studies, N fixation rate of these two heterocystous species was evaluated and presence of some growth promoting substances such as phytohormones in algal biomasses was studied. The HPLC chromatograms of the two microalgal samples under the optimized HPLC conditions are shown in Fig. 4. The use of HPLC equipped with a fluorescence detector showed the presence of two endogenous auxins including IAA, and its homologue, IBA in cyanobacterial biomass. Mass spectrometric data and their comparison with standard compounds confirmed this result (Seyed Hashtroudi *et al.* 2012). The results also

Table 1 Cyanobacterial diversity in different rice (*Oriza sativa* L.) cultivated regions of Iran

	Species	1	2	3	4	5	6	7
1	*Anabaena pseudoscillatoria* Bory de Saint-Vincent	•	•	•		•	•	
2	*Anabaena sphaerica* Bornet et Flahault	•		•	•		•	
3	*Anabaena iyengarii* Bharadwaja	•		•			•	
4	*Anabaena vaginicola* Fritsch et Rich	•	•	•	•	•	•	
5	*Anabaena ambigua* C.B. Rao.				•	•	•	
6	*Anabaena portoricensis* N. L. Gardner							•
7	*Anabaena viguieri* Denis et Frémy						•	
8	*Anabaena orientalis* Dixit							•
9	*Anabaena torulosa* Lagerheim ex Bornet & Flahault	•						•
10	*Anabaena* sp.	•						
11	*Trichormus fertilissimus* (C.B. Rao) Komárek et Anagnostidis				•			
12	*Trichormus ellipsosporus* (Fritsch) Komárek et Anagnostidis	•						
13	*Nostoc oryzae* (Fritsch) Komárek et Anagnostidis	•			•	•		
14	*Nostoc linckia* var. *arvense* Rao. C.B.			•		•		
15	*Nostoc paludosom* Kützing ex Bornet & Flahault	•		•				
16	*Nostoc punctiforme* Hariot	•						
17	*Nostoc ellipsosporum* var. *violaceum* C.B. Rao	•			•			
18	*Nostoc muscorum* C. Agardh ex Bornet & Flahault	•	•	•	•	•	•	
19	*Nostoc calcicola* Brebisson ex Born. et Flah.	•	•		•	•	•	•
20	*Nostoc alatosporum* Sant' Anna *et al.*			•		•		
21	*Nostoc spongiaeforme* Agardh ex Born. et Flah.		•					
22	*Nostoc sphaericum* Vaucher ex Born. et Flah.	•						
23	*Nostoc verrucosum* Vaucher ex Born. et Flah.	•						
24	*Nostoc* sp.$_1$					•		
25	*Nostoc* sp.$_2$		•					
26	*Nostoc* sp.$_3$						•	
27	*Nostoc* sp.$_4$						•	
28	*Cylindrospermum michailovskoense* Elenkin	•	•			•		
29	*Cylindrospermum majus* Kützing	•						
30	*Cylindrospermum catenatum* (Ralfs) Bornet & Flahault					•		•
31	*Cylindrospermum sphaericum* B.N. Prasad					•		
32	*Cylindrospermum stagnale* (Kütz.) Born. et Flah.					•		
33	*Cylindrospermum minutissimum* Collins						•	
34	*Cylindrospermum marchicum* Lemmermann					•		
35	*Cylindrospermum muscicola* Kützing ex Bornet & Flahault		•	•	•		•	•
36	*Nodularia harveyana* (Thwaites) Thuret	•				•	•	
37	*Nodularia spumigena* Mertens ex Bornet & Flahault	•				•		
38	*Tolypothrix distorta* Kützing ex Born. et Flah.	•			•			
39	*Tolypothrix bouteillei* (Brébisson & Desmazières) ex Bornet & Flahault Lemmermann	•						
40	*Scytonema* sp.$_1$	•						
41	*Scytonema* sp.$_2$				•			
42	*Aulosira fertilissima* Ghose	•						
43	*Calothrix elenkinii* Kossinskaja	•						
44	*Calothrix marchica* Lemm.	•		•				
45	*Calothrix stagnalis* Gomont						•	
46	*Calothrix thermalis* (Schwabe) Hansgirg	•						
47	*Calothrix* sp.$_1$	•						
48	*Calothrix* sp.$_2$							•
49	*Hapalosiphon welwitschii* West & G.S. West		•					

1. Guilan province, 2. Mazandaran Province, 3. Qazvin Province, 4. Lorestan Province, 5. Fars Province, 6. Esfahan Province, 7. Khorasane Razavi Province.

showed that the concentration of phytohormones in the *Nostoc calcicola* ISC89 was higher than in *Anabaena vaginicola* ISC90, whereas nitrogenase activity of *Anabaena* species was observed to be higher than that of the other species (Table 5).

Chemical content of the algal extracts was another factor which was analyzed. Table 6 shows the chemical contents of these two cyanobacterial extracts. The results of this analysis showed that, except for the total N content, other chemical parameters such as content of phosphate, ammonium and cations such as K^+ and Ca^{2+} in *Anabaena vaginicola* ISC90 extract are higher than *Nostoc calcicola* ISC89.

Table 2 Total percent abundance of heterocystous cyanobacterial genera (Summed up over all locations)

Genus	Total No. of species	Percent abundance
Nostoc	15	31
Anabaena	10	21
Trichormus	2	4
Cylindrospermum	8	16
Nodularia	2	4
Aulosira	1	2
Tolypothrix	2	4
Scytonema	2	4
Calothrix	6	12
Hapalosiphon	1	2
Total	**49**	**100**

Table 3 Geographical data and some ecological details of the sampling locations

No.	Location	Latitude/Longitude	pH	EC (dSm^{-1})	Total nitrogen %	P ($mgkg^{-1}$)	O.C.%
1	Mazandaran: Tonkabon,Tazehabad village	36°39'N 51°25´E	8.1	1.16	0.14	19.60	1.69
2	Mazandaran: Tonkabon city	36°48'N 50°52'E	8.1	1.52	0.09	12.90	1.07
3	Mazandaran: Gharakheil	36°27'N 52°46'E	7.9	0.73	0.29	12.10	2.95
4	Mazandaran: Savadkoh	36°08'N 53°02'E	7.8	1.87	0.11	13.80	1.17
5	Gilan: Rostamabad	36°53'N 49°20'E	8.1	3.18	0.12	11.60	1.03
6	Gilan: Sangar, Omsheh village	37°16'N 49°35'E	8.2	2.39	0.19	16.30	2.05
7	Gilan: Rodsar, Rahimabad village	36°51'N 50°13'E	8.0	1.47	0.53	12.10	6.05
8	Gilan: Rasht, Saravan village	37°05'N 49°24'E	8.1	2.79	0.16	13.80	1.97
9	Qazvin: Alamut village	36°23'N 50°33'E	8.1	2.47	0.07	4.80	0.94
10	Lorestan: Visian village	33°49'N 48°07'E	8.4	1.03	0.14	18.20	1.52
11	Fars: Firuzabad	28°59'N 52°55'E	8.1	9.55	0.10	18.20	1.17
12	Fars: Marv dasht, Esmaeilabad village	28°60'N 53°60'E	8.3	2.38	0.15	35.80	1.77
13	Fars: Marv dasht, Kamfiroz village	30°15'N 52°17'E	8.3	1.51	0.09	17.30	1.15
14	Fars: Fathabad	29°19'N 52°37'E	8.0	18.92	0.06	20.30	0.82
15	Khorasan razavi: Kalat village	36°59'N 59°47'E	8.1	2.93	0.06	6.30	0.39
16	Esfahan: Flavarjan	32°32'N 51°30'E	8.4	2.48	0.06	17.90	0.58
17	Esfahan: Lenjan, Zarrinshahr village	32°22'N 51°22'E	8.3	3.31	0.19	31.20	2.27
18	Esfahan: Varnamkhast	32°21'N 51°22'E	8.1	3.53	0.15	35.70	1.50
19	Esfahan: Ghahdarijan	32°30 N 51°30'E	8.3	1.26	0.12	23.60	1.25
20	Esfahan: Falavarjan, Jujil village	32°34'N 51°28'E	8.1	3.53	0.15	35.70	1.50

EC, electric conductivity; P, phosphorus; O.C., organic carbon.

DISCUSSION

Review of the literature showed that there are a few reports regarding the study of cyanobacteria in paddy soils of Iran, though rice is the second major crop and is cultivated in 20 provinces of the country. Representative soil samples were collected from seven different rice cultivation provinces. The maximum number and variation of species was recorded in paddy fields of Guilan Province with 27 species. Guilan Province is one of the largest producers of rice in Iran and it is located near the Caspian Sea with favorable climate conditions or with perhumid climate conditions (Fig. 1).

Among the heterocystous isolates, members of Nostocaceae with six genera (*Anabaena*, *Aulosira*, *Cylindrospermum*, *Nodularia*, *Nostoc* and *Trichormus*) and 38 species showed the highest algal richness (Table 2). *Anabaena* and *Nostoc* were found to be the dominant genera in most stations whereas *Aulosira* and *Hapalosiphon* species were limited to paddy fields of Guilan and Mazandaran Provinces, and showed the least diversity and occurrence in paddy soils of Iran. Similar results were reported by Prasanna and Nayak (2007) in paddy field soils of India.

The study of the correlation between species diversity and physicochemical parameters of soil samples showed that the environmental factors such as soil pH, EC, as well as total nitrogen, phosphorus and organic carbon contents did not show significant correlation with species diversity (Fig. 2). Among soil properties, pH is the most

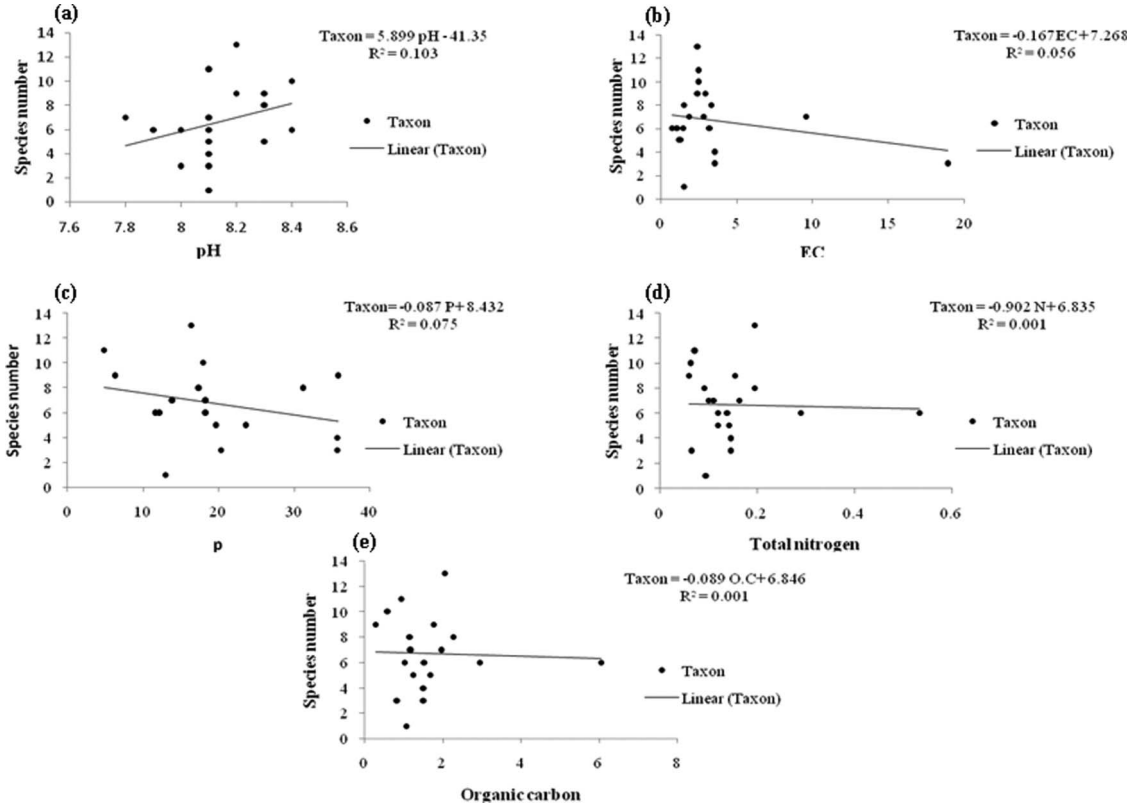

Figure 2 Correlation between physicochemical parameters of paddy soils and diversity of terrestrial heterocystous cyanobacteria. (a) Correlation between soil pH and diversity, (b) correlation between soil electrical conductivity (EC) and diversity, (c) correlation between soil phosphorus content and diversity, (d) correlation between soil total nitrogen content and diversity, (e) correlation between soil organic carbon content and diversity.

important factor determining soil floristic composition (Nayak and Prasanna 2007). Due to the limited range of pH in the studied sites (7.9–8.4), soil pH as an influencing factor that affects the diversity of cyanobacteria populations had no significant impact. EC is another environmental factor that can affect diversity of these microorganisms. Among the studied sites, the soil samples of Ebrahimabad and Fathabad from Fars province exhibited high ECs and low species diversity, while the other soil samples exhibited low or moderate EC. No significant difference was observed in chemical parameters of soil such as total N and organic C in the studied sites (Table 3).

In the other section of this study, comparison analysis in pot experiments showed that there is a significant difference between the treated plants and controls in vegetative growth factors, especially root growth parameters. A positive effect of PGPR (Plant growth promoting rhizobacteria) on root growth parameters such as root length, total surface of root, dry weight of root and rootlet density was previously reported (Pan *et al.* 1999; Zahir *et al.* 2000). The results of the present study also showed that the growth parameters of root such as its length and dry and fresh weight increased significantly, which can improve uptake of water and nutrition from soil.

Stimulation of plant growth in the treated plants can be affected by several factors, especially chemical content of algal extracts. N fixing ability of these heterocystous cyanobacteria can increase the content of easy available N or the ammonium content of algae extracts. According to the influential role of N fixation in the N content of algal extract, the N fixation rate of these two species was evaluated. Evaluation of N fixation rate of these two species showed that these heterocystous cyanobacteria were capable of fixing atmospheric N in natural conditions (Table 5). This capacity can effect an improvement of plant growth (Gantar *et al.* 1995; Irisarri *et al.* 2001; Nilsson *et al.* 2002). Our results also revealed that the presence of some growth-promoting substances may be responsible for the beneficial effect of algal extract on plant growth parameters. The obtained results with HPLC equipped with a fluorescence detector showed the presence of these compounds, especially Indole 3-butyric acid (IBA) and Indole 3-acetic acid (IAA), in cyanobacterial biomass which was then homogenized

Table 4 Effect of algal culture on growth of vegetable plants (Mean ± SE)

Plant	BGA	Root length (cm)	Plant height (cm)	Leaf number	Fresh root (g)	Dry root (g)	Fresh stem & leaf (g)	Dry stem & leaf (g)
Cucumber	***Nostoc***	34.50 ± 2.62*	45.50 ± 3.12*	6.25 ± 0.75	0.91 ± 0.16*	0.30 ± 0.01*	0.73 ± 0.11	0.12 ± 0.01
	Anabaena	34.25 ± 3.37*	46.00 ± 3.24*	5.25 ± 0.85	0.83 ± 0.15*	0.40 ± 0.04*	1.04 ± 0.01*	0.11 ± 0.00
	Control	16.50 ± 2.53	25.25 ± 2.83	5.75 ± 0.25	0.23 ± 0.08	0.08 ± 0.00	0.65 ± 0.02	0.11 ± 0.00
Tomato	***Nostoc***	17.25 ± 0.47*	24.00 ± 0.91*	5.50 ± 0.28	0.24 ± 0.10*	0.07 ± 0.00*	1.49 ± 0.18	0.14 ± 0.01*
	Anabaena	18.75 ± 0.47*	25.75 ± 0.85*	6.25 ± 0.25*	0.16 ± 0.00	0.10 ± 0.00*	2.27 ± 0.26*	0.19 ± 0.01*
	Control	10.75 ± 1.10	16.00 ± 1.47	4.25 ± 0.47	0.05 ± 0.00	0.03 ± 0.01	0.77 ± 0.19	0.06 ± 0.01
Squash	***Nostoc***	32.25 ± 1.31*	48.00 ± 2.12*	11.25 ± 0.47*	6.95 ± 0.85*	3.15 ± 0.43	5.79 ± 0.46	0.64 ± 0.27
	Anabaena	32.50 ± 1.44*	46.75 ± 2.25*	12.00 ± 0.40*	9.30 ± 0.56*	5.70 ± 0.71*	7.33 ± 1.63	0.86 ± 0.14*
	Control	20.75 ± 1.65	32.25 ± 1.43	8.75 ± 0.75	3.85 ± 0.38	1.86 ± 0.15	4.51 ± 0.38	0.42 ± 0.03

*Significant at the 0.05 level. SE, standard error; BGA, blue-green algae.

with distilled water for the pot experiments (Table 5). The dominant auxin observed in these two isolates was IBA in the range of 1.275–2.958 μg g^{-1} dry weight and only trace amounts of IAA (0.020–0.046 μg g^{-1} dry weight) were detected. Auxins, especially IBA, are commonly applied to stimulate root initiation and used as plant hormones for the induction and improvement of rooting. Before to this study, increase in lateral root production in the seedlings of several plants with IBA was reported (Lee and Hackett 1976; Simpson 1986; Mahmood Khavar and Özcan 2002; Mobli and Baninasab 2009). Li *et al.* (2009) also showed that phytohormones can promote root elongation and ion uptake in rice seedlings. The results of our study showed that improvement of rooting in the studied plants may be affected by phytohormones such as IBA and IAA.

In a recent work, a positive effect of heterocystous cyanobacteria on plant growth and nutrient uptake was reported by Obana *et al.* (2007). They showed that *Nostoc* application increased the organic C and N content of the surface soil and enhanced plant growth and plant ion uptake. They believed that the microelements necessary for plant growth can be supplied by these microorganisms. In our study, estimation of chemical content of the microalgal extracts supported this fact (Table 6). In addition, polysaccharides secreted by cyanobacteria contribute to the structural stability of the soil, to increased soil C and N levels, and to the promotion of plant growth (Foth 1990).

Previously, Nanda *et al.* (1991) showed that spraying extracts of *Westiellopsis prolific* Janet on pumpkin (*Cucurbita pepo* L.) and cucumber (*Cucumis sativus* L.) seedlings during their subsequent cultivation led to a significant increase in growth and development of both crops. They suggested that the supply of nitrogenous nutrients to the seeds is important. A study on cyanobacterial extract effect on potato (*Solanum tuberosum* L.) tissue culture by Shanab *et al.* (2003) confirms that the increase in crop yields can not only be attributed to the nitrogen-fixation potency of cyanobacteria, but may be largely due to the growth-regulating substances endogenously produced by these algae. This assumption is greatly supported by the fact that non-N fixing species such as *Oscillatoria* sp. and *Phormedium* sp. stimulated the growth of plants such as rice (Gupta and Shukla 1967; Gupta and Gupta 1970). The production of growth-promoting substances and vitamins (Vitamin B_{12}, folic acid, nicotinic acid and pantothenic acid) by the algae may be another reason for the greater plant growth and yield in treated plants (Venkataraman and Neelakantan 1967). Moreover, cyanobacteria can enhance the production of secondary metabolites and these

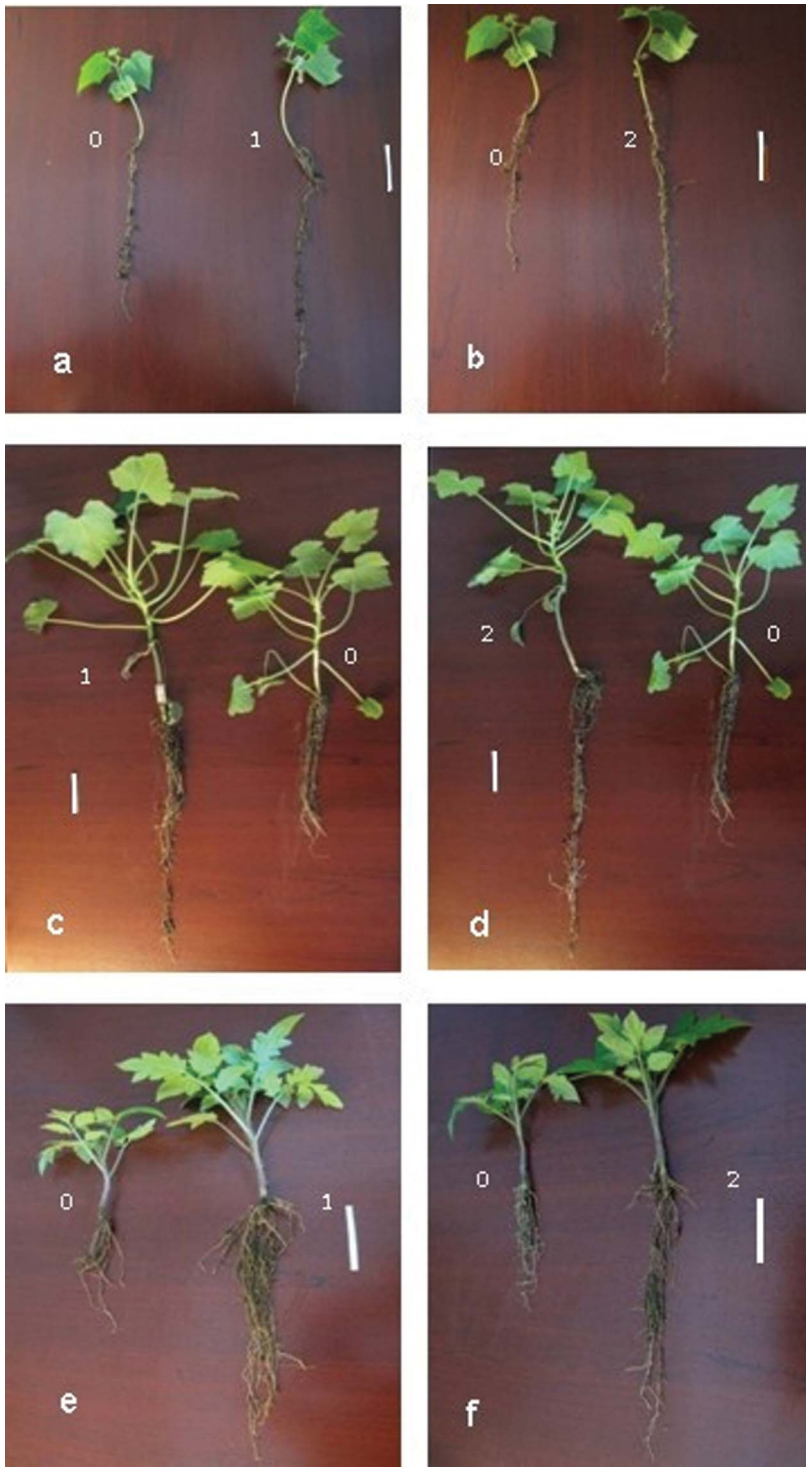

Figure 3 Plant height and root length of control and treated plants: (a, b) cucumber (*Cucumis sativus* L.) plant, (c, d) squash (*Cucurbita maxima* Duch. ex Lam.) plant, (e, f) tomato plant (*Solanum lycopersicum* L.). (0. Control, 1. Plant treated by *Anabaena* (*Anabaena vaginicola* Fritsch et Rich.), 2. Plant treated by *Nostoc* (*Nostoc calcicola* Brebisson ex Born. et Flah.), bar = 5 cm).

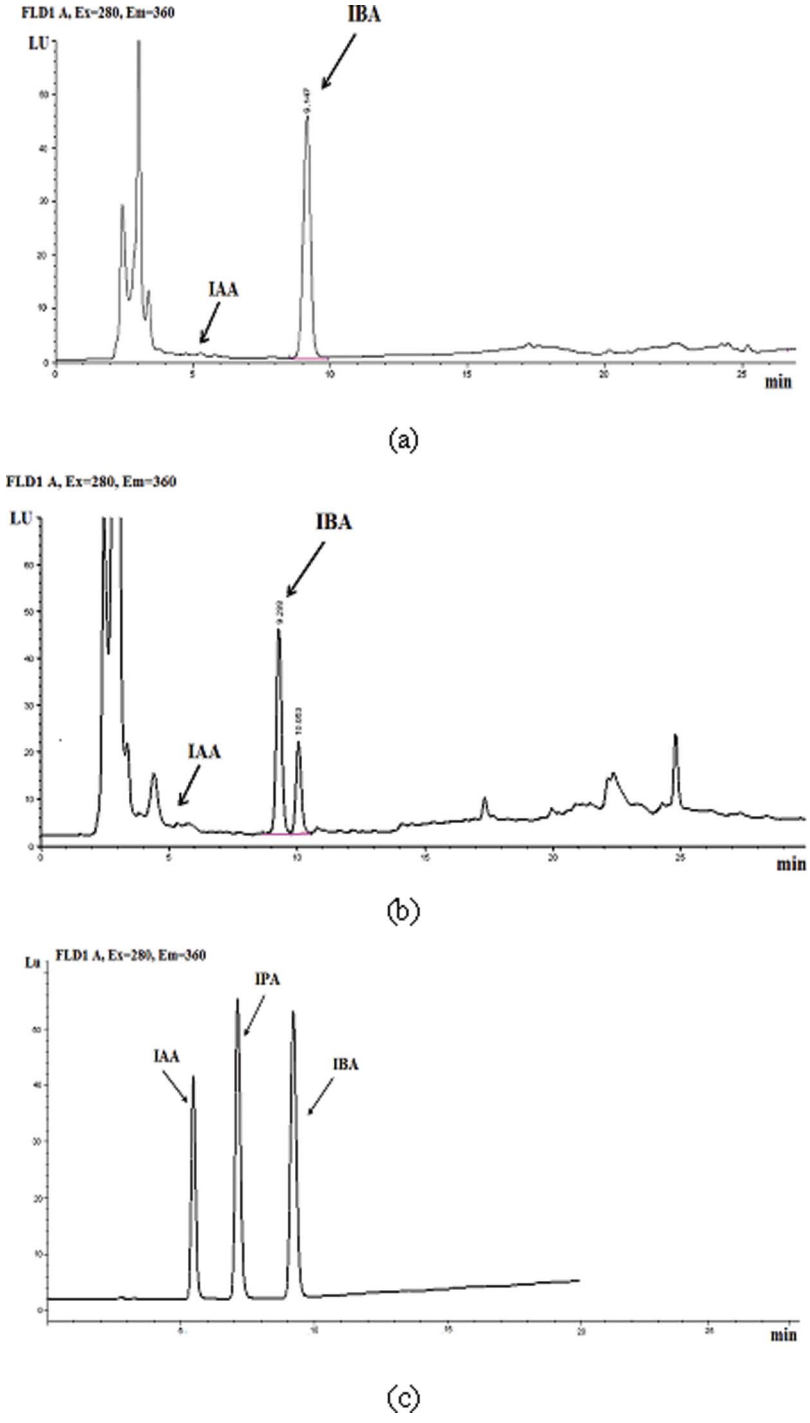

Figure 4 (a) High performance liquid chromatography (HPLC) chromatograms of the ultrasonicated sample of *Nostoc calcicola* ISC89 for 30 min, (b) HPLC chromatograms of the ultrasonicated sample of *Anabaena vaginicola* ISC90 for 30 min, (c) HPLC chromatograms of a 250 ng mL^{-1} standard of three auxins with fluorescence detectors.

mechanisms may be controlled with or mediated by hormones (Saker *et al*. 2000; Shanab 2001). In conclusion, we report here that heterocystous cyanobacteria, *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89, from paddy soils of Iran have the ability to promote vegetable growth and

Table 5 Nitrogenase activity and estimated concentrations of three auxins in the microalgal samples

Microalgae	Nitrogenase activity (nmol mg^{-1} h^{-1})	Estimated concentration (μg g^{-1}) in DW		
		IAA	IPA	IBA
Anabaena vaginicola Fritsch et Rich	896.0	0.020	Nd	1.275
Nostoc calcicola Brebisson ex Born. et Flah.	714.6	0.046	Nd	2.958

DW, dry weight; IAA, Indole 3-acetic acid; IBA, Indole 3-butyric acid; IPA, Indole 3-propionic acid.

Table 6 Chemical contents of *Anabaena vaginicola* ISC90 and *Nostoc calcicola* ISC89 extract (1% water extract)

	Analytical method	*Anabaena* extract	*Nostoc* extract
pH	Electrometric	6.62	7.18
EC (μS cm^{-1})	Platinum electrode	63.00	17.00
Total nitrogen (mgL^{-1})	Macro kjeldahl	162.00	220.00
NO_2^- (mgL^{-1})	Colorimetric	0.02	0.01
NO_3^- (mgL^{-1})	Ultraviolet spectrophotometric	<1.00	<1.00
NH_4^+ (mgL^{-1})	Nesslerization	80.20	2.30
Phosphate (mgL^{-1})	Vanadomolybdophosphoric acid colorimetric	10.40	0.50
SO_4^{2-} (mgL^{-1})	Gravimetric	50.00	0.00
CO_3^{2-} (mgL^{-1})	Titrimetric	0.00	0.00
HCO_3^- (mgL^{-1})	Titrimetric	250.00	100.00
Ca^{2+} (mgL^{-1})	EDTA titrimetric	280.00	80.00
Mg^{2+} (mgL^{-1})	EDTA titrimetric	0.00	0.00
Na^+ (mgL^{-1})	Flame emission photometric	12.00	9.00
K^+ (mgL^{-1})	Flame emission photometric	6.10	2.10

NO_2^-, nitrite; NO_3^-, nitrate; NH_4^+, ammonium; SO_4^{2-}, sulphate; CO_3^{2-}, carbonate; HCO_3-, bicarbonate; Ca^{2+}, calcium; Mg^{2+}, magnesium; Na^+, sodium; K^+, potassium; EDTA, ethylenediaminetetraacetic acid.

they are appropriate candidates for the formulation of a biofertilizer.

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REFERENCES

Arif M, Gupta R, Joshi MC 1995: Studies on the use of cyanobacteria as biofertilizer for vegetable cultivation in hydroponic system. *In* Schirmacher Oasis Region, East Antarctica. Eleventh Indian Expedition to Antarctica. Scientific Report, Ed. Dhar, A, pp. 243–246. Department of Ocean Development, Technical Publication 9, New Delhi, India.

Asadi A, Khavari-Nejad RA, Soltani N, Najafi F, Molaie Rad A 2011: Physiological variability in cyanobacterium *Phormidium* sp. Kützing ISC31 (Oscillatoriales) as response to varied microwave intensities. *Afr. J. Agric. Res.,* **6**, 1673–1681.

Desikachary TV 1959: *Cyanophyta*, Indian Council of Agricultural Research, New Delhi.

Foth HD 1990: *Fundamentals of Soil Science*, 8th ed. John Wiley, New York.

Gantar M, Kerby NW, Rowell P, Obreht Z, Scrimgeour C 1995: Colonization of wheat (*Triticum vulgare* L.) by N_2-fixing cyanobacteria: IV. Dark nitrogenase activity and effects of cyanobacteria on natural ^{15}N abundance in the plants. *New Phytol.*, **129**, 337–343.

Gupta AB, Gupta KK 1970: The effect of *Phormedium fovealarum* extract on growth and development of pea seedlings. *Labdev J. Sci. Technol.,* **8**, 151.

Gupta AB, Shukla AC 1967: Studies on the nature of algal growth substances and their influence on growth, yield and protein contents of Rice plants. *Labdev J. Sci. Technol.*, **5**, 162.

Hayes WA 1981: International studies of physical, chemical and biological factors in casing soils. *Mushroom Sci.,* **XI**, 103–120.

Irisarri P, Gonnet S, Monza J 2001: Cyanobacteria in Uruguayan rice fields: Diversity, nitrogen fixing ability and tolerance to herbicides and combined nitrogen. *J. Biotechnol.*, **91**, 95–103.

John DM, Whitton BA, Brook A 2002: *The Freshwater Algal Flora of the British Isles: An Identification Guide to Freshwater and Terrestrial Algae*. Cambridge University Press, Cambridge.

Karthikeyan N 2006: Characterization of cyanobacteria from the rhizoshere of wheat (M.Sc thesis). Division of Microbiology, Post Graduate School, Indian Agricultural. Research Institute, New Delhi, India.

Karthikeyan N, Prasanna R, Nain L, Kaushik BD 2007: Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *Eur. J. Soil Biol.,* **43**, 23–30.

Lee CI, Hackett WP 1976: Root regeneration of transplanted *Pistacia chinensis* Bunge seedlings at different growth stages. *J. Amer. Soc. Hort. Sci*., **101**, 236–240.

Li X, Suzuki T, Sasakawa H 2009: Promotion of root elongation and ion uptake in rice seedlings by 4,4,4-trifluoro-3-(indole-3-) butyric acid. *J. Soil Sci. Plant Nutr.,* **55**, 385–393.

Maqubela MP, Mnkeni PNS, Malam Issa O, Pardo MT, Acqui LPD 2008: Nostoc cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility and maize growth. *Plant Soil,* **315**, 79–92.

Mahmood Khavar K, Özcan S 2002: Effect of Indole-3-butyric acid on in vitro root development in Lentil (*Lens culinaris* Medik). *Turk. J. Bot.,* **26**, 109–111.

Misra S, Kaushik BD 1989a: Growth promoting substances of cyanobacteria I. Vitamins and their influence on rice plant. *Proc. Indian Natl Sci. Acad*., **B55**, 295–300.

Misra S, Kaushik BD 1989b: Growth promoting substances of cyanobacteria II. Detection of amino acids, sugars and auxins. *Proc. Indian Natl Sci. Acad*., **B55**, 499–504.

Mishra U, Pabbi S 2004: Cyanobacteria: A potential biofertilizer for Rice. *Reson*, 6–10.

Mobli M, Baninasab B 2009: Effect of Indole butyric acid on root regeneration and seedling survival after transplanting of three *Pistacia* species. *J. Fruit Ornam. Plant Res*., **17**, 5–13.

Nanda B, Tripathy SK, Padhi S 1991: Effect of algalization on seed germination of vegetable crops. *World J. Microbiol Biotechnol.,* **7**, 622–623.

Nayak S, Prasanna R 2007: Analysing diversity among Indian isolates of *Anabaena* (Nostocales, Cyanophyta) using morphological, physiological and biochemical characters. *World J. Microbiol. Biotechnol.,* **23**, 1575–1584.

Nilsson M, Bhattacharya J, Rai AN, Bergman B 2002: Colonization of roots of rice (*Oryza sativa*) by symbiotic Nostoc strains. *New Phytol*., **156**, 517–525.

Obana S, Miyamoto K, Morita S, Ohmori M, Inubushi K 2007: Effect of *Nostoc* sp. on soil characteristics, plant growth and nutrient uptake. *J. Appl. Phycol*., **19**, 641–646.

Pan B, Bai YM, Leibovitch S, Smith DL 1999: Plant growth promoting rhizobacteria and kinetin as ways to promote corn growth and yield in a short growing season area. *Eur. J. Agron.,* **11**, 179–186.

Pauwels JM, Van Ranst E, Verloo M, Mvondo ZEA 1992: *Manuel de laboratoire de pedologie. Methodes d'analyse de sols et de plantes, equipement, gestion de stocks de verrerie et de produits chimiques*, Publications Agricoles 28. Brusells, Belgium.

Prasanna R, Nayak S 2007: Influence of diverse rice soil ecologies on cyanobacterial diversity and abundance. *Wetlands Ecol. Manage*., **15**, 127–134.

Prescott GW 1970: *Algae of the Western Great Lakes Area*, WMC Brown Company Publishers, Dubuque, IA.

Rangaswamy G 1996: *Agricultural Microbiology*, Asia Publishing House, Bombay, India.

Rodgers GA, Bergman B, Henriksson E, Udris N 1979: Utilization of blue-green algae as biofertilizers. *Plant Soil,* **52**, 99–107.

Saadatnia H, Riahi H 2009: Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. *Plant Soil Environ.,* **55**, 207–212.

Saker M, Shanab S, Khater M 2000: In vitro studies on *Ambrosia maritime*. I: Morphogenic responses and algal toxins elicitation. *Arab J. Biotechnol.,* **3**, 217–224.

Seyed Hashtroudi M, Ghassempour AR, Riahi H, Shariatmadari Z, Khanjir M 2012: Endogenous auxins in plant growth promoting cyanobacteria – *Anabaena vaginicola* and *Nostoc calcicola*. *J. Appl. Phycol*., doi: 10.1007/s10811-012-9872-7

Shanab S 2001: Effect of fresh water cyanobacterialracts on alkaloid production of the in vitro *Solanum elaeagnifolium* tissue culture. *Arab J. Biotechnol.,* **4**, 129–140.

Shanab SMM, Saker MM, Abdel-Rahman MHM 2003: Crude extracts of some fresh water cyanobacteria have auxin-like activity on potato tissue culture. *Arab J. Biotechnol.,* **6**, 297–312.

Shariatmadari Z, Riahi H 2012: A taxonomic study on soil taxa of *Cylindrospermum* Bory ex Bornet & Flahault (Nostocaceae) in Iran. *Iran. J. Bot.,* **18**, 130–140.

Shariatmadari Z, Riahi H, Shokravi S 2011: A taxonomic study on soil taxa of *Anabaena* Bory ex Bornet & Flahault (Nostocaceae) in Iran. *Iran. J. Bot.,* **17**, 105–117.

Simpson DG 1986: Auxin stimulates lateral root formation of container-grown interior Douglas-fir seedlings. *Can. J. For. Res*., **16**, 1135–1139.

Singh PK 1988: Biofertilization of rice crop. *In* Biofertilization Potentialities and Problems, Eds. Sena SP, Palit PC, pp. 109–114. Plant Physiology Forum, Calcutta, India.

Stanier RY, Cohen-Bazire G 1977: Phototrophic prokaryotes, the cyanobacteria. *Ann. Rev. Microbiol.,* **31**, 225–274.

Stanier RY, Kunisawa R, Mandal M, Cohen-Bazire G 1971: Purification and properties of unicellular blue-green algae (Order Chroococcales). *Bacteriol Rev.,* **35**, 171–305.

Stirk MA, Ördog V, Van Staden J, Jäger K 2002: Cytokinin and auxin-like activity in cyanophyta and microalgae. *J. Appl. Phycol*., **14**, 215–221.

Thajuddin N, Subramanian G 2005: Cyanobacterial biodiversity and potential application in biotechnology. *Cur. Sci.,* **89**, 47–57.

Vaishampayan A, Sinha RP, Hader DP, Dey T, Gupta AK, Bhan U, Rao AL 2001: Cyanobacterial biofertilizers in rice agriculture. *Bot. Rev.,* **67**, 453–516.

Venkataraman GS 1972: *Algal Biofertilizer and Rice Cultivation*, Today and Tomorrows Printer and Publishers, New Delhi, India.

Venkataraman GS, Neelakantan S 1967: Effect of the cellular constituents of the nitrogen fixing blue-green algae

Cylindrospermum muscicola on the root growth of rice seedlings. *J. Gen. Appl. Microbiol.* **13**, 53–61.

Wehr JD, Sheath RG, Thorp JH 2002: *Freshwater Algae of North America: Ecology and Classification*, Aquatic Ecology Press, San Diego, CA.

Whitford LA, Schumacher GJ 1973: *A Manual of Fresh-Water Algae*, Sparks Press, Raleigh, NC.

Whitton BA 2000: Soil and rice-fields. *In* The Ecology of Cyanobacteria, Their Diversity in Time and Space, Eds. Whitton BA, Potts, M, pp. 233–255. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Zahir AZ, Abbas SA, Khalid A, Arshad M 2000: Substrate dependent microbially derived plant hormones for improving growth of maize seedling. *Pak. J. Biol. Sci.,* **3**, 289–291.