New morphospecies of *Phormidium* and *Microcoleus* (*Cyanophyta*) in paddy-fields of Iran

گونه‌های جدید *Phormidium* و *Microcoleus* از فلور جلبکی شالیزارهای ایران*

Received: 02.01.2011/ Accepted: 21.04.2011

دریافت: ۱۳۸۹/۱۰/۱۲/ پذیرش: ۱۳۹۰/۲/۱

R. Siahbalaei✉: Former MSc Student, Department of Biology, Isfahan University, Isfahan, Iran
(E-mail: m.siahbala@gmail.com)

S. Afsharzadeh: Assistant Prof., Department of Biology, Isfahan University, Isfahan, Iran

Sh. Shokravi: Assistant Prof., Faculty of Basic Science, Islamic Azad University, Gorgan Branch, Gorgan, Iran

Sh. Nekouei: Former MSc Student, Department of Biology, Isfahan University, Isfahan, Iran

Abstract

Cyanophyta of some paddy-fields of Golestan province (North of Iran, near the Caspian Sea) have been studied during 2006–07. *Phormidium subinerusatum*, *P. tenue*, *P. jadinianum*, *P. purpurascens*, *P. lucidum*, *P. fragile*, *Microcoleus palaodus*, *M. lacustris* are new records for Golestan province and Iran. Results showed that *P. subinerusatum*, *P. tenue* were dominant in all stations. *P. fragile*, in spring and *M. palaodus*, *M. lacustris*, in autumn and winter were not visible in any station. Morphological characteristics of these species are described in detail and information about their ecological distribution is provided.

Keywords: Distribution, Golestan province, rice-field, taxonomy

رقیه سیاه بالایی✉: دانش‌آموخته کارشناسی ارشد، گروه زیست‌شناسی، دانشگاه اصفهان، اصفهان
(E-mail: m.siahbala@gmail.com)

سعید افشارزاده: استادیار گروه زیست‌شناسی، دانشگاه اصفهان، اصفهان

شادمان شکروی: استادیار دانشکده علوم پایه، دانشگاه آزاد اسلامی، واحد گرگان، گرگان

شکوه نکویی: دانش‌آموخته کارشناسی ارشد، گروه زیست‌شناسی، دانشگاه اصفهان، اصفهان

خلاصه

در این تحقیق، تعدادی سیانوفیت به شرح زیر از شالیزارهای استان گلستان طی پاییز ۲۰۰۶ تا تابستان ۲۰۰۷ مورد مطالعه قرار گرفتند: *Phormidium subinerusatum*, *P. tenue*, *P. jadinianum*, *P. purpurascens*, *P. lucidum*, *P. fragile*, *Microcoleus palaodus*, *M. lacustris* برای نخستین بار از ایران گزارش می‌شوند. *P. subinerusatum* و *P. tenue* در تمام فصول و در همه ایستگاه‌ها مشاهده گردیدند ولی *M. palaodus* و *M. lacustris* در دو فصل پاییز و زمستان در هیچ ایستگاهی مشاهده نشدند. ویژگی‌های تاکسونومیک این گونه‌ها و اطلاعات پیرامون پراکنش اکولوژیکی آن‌ها نیز ارایه شده است.

واژه‌های کلیدی: استان گلستان، پراکنش، تاکسونومی، سیانوفیت

* بخشی از پایان‌نامه کارشناسی ارشد نگارنده اول به راهنمایی دکتر افشارزاده ارایه شده به دانشگاه اصفهان

Introduction

Members of *Cyanophyta* (blue-green algae) have characteristics of both simple plants and bacteria (Ahoren 2004). *Cyanophyta* are abundantly found in rice-fields and are important in maintaining rice-fields fertility through nitrogen fixation, nitrogenous and non-nitrogenous compounds liberation and excretion (Shokravi *et al*. 2007). Many rice-field soils contain a high density of Cyanobacteria and over 50% of them that are found in rice-fields are non-heterocystous filamentous forms (Jeong-Dong & Choul-Gyun 2008).

Golestan province is located between the longitudes of 30′ 54° and 30′ 56° E and at latitude of 15′ 38° N in Iran and the paddy-fields contained nearly 62000 hectares of total cultivation fields during the past year. It seems that in north paddy-fields of Iran especially Golestan province, some strains of *Cyanobacteria* especially *Phormidium* spp. are common (Shokravi *et al*. 2006), but there is no detailed report about their taxonomy and morphological characterization.

Members of *Oscillatoriaceae* which are predominately filamentous and photosynthetic members of *Cyanophyta* are frequently found in the paddy-fields. *Oscillatoriaceae* consist of 265 species and more than 14% of the species belong to *Phormidium* and *Microcoleus*. These genera are distinguished on the basis of morphology of filaments, life cycle, trichome structure and type of trichome disintegration (Anagnostidis & Komarek 1990). Approximately 40 species of *Phormidium* and *Microcoleus* occur in oceans, fresh-water and soil. The epilithic and free floating forms of these species are present in the benthos and the water surface (Ullmann & Budel 2001, Belnap *et al.* 2001).

Several morphotypes occur in different habitats which do not correspond to any described species. Our current knowledge of soil algae is based mainly upon enrichment culture studies; however, selective culture conditions may have caused bias in the resulting species lists. This paper reports the algal flora of paddy-fields based on morphological investigations of the *Cyanophyta* of the paddy-fields in Golesten province.

Materials and Methods

Soil samples were obtained from paddy-fields of different stations of Golestan province (N Iran). Five stations were chosen in different areas of the paddy-fields (Fig. 1). Samples were taken from the depth of 2 cm during autumn 2006 and summer 2007. Samples were taken from flooded and non-flooded soils (Kaushik 1987). Samples were brought to the laboratory for culture and detailed morphological studies. The samples cultured in solid BG11 medium ($NaNo_3$, 17.65 mM, $MgSO_4$, 7 H_2O, 0.3 mM, $CaCl_2$, 2 H_2O_2, 0.25 mM, K_2HPO_4, 3 H_2O, 0.18 Mm, Na_2 Mg EDTA, 0.003 mM, ferric citrate ammonium, 0.02 mM Citric acid, 0.029 mM, Na_2Co_3, 0.188 mM and microelements 1 ml). The cultivation was done under illumination 1500–1600 lux, pH 7.2 and temperature ± 20° C. After colonization and isolation, samples were purified by several sub-culturing (Kaushik 1987). Morphological studies of samples were carried out by light microscopy in addition to fluorescence and phase contrast microscopy. Identification of the taxa was accomplished using authentic keys and manuals (John *et al*. 2002, Anagnostidis and Komarek 1990, Prescott, 1962 and Desikachary 1959). Photographs were taken using Nikon microscope, equipped with digital camera. Distribution of species of *Phormidium* and *Microcoleus* in the paddy-fields of Iran are listed in Table 1.

Results

A total of six species of the genus *Phormidium* and two species of *Microcoleus* were observed (Figs 2 and 3) which are found to be new records (marked by astriks) for Golestan province and Iran. New morphospecies from the *Microcoleus* and *Phormidium* genera were found in different stations.

Phormidium subinerusatum Frisch et Rich*

Filaments more or less straight or curved, green, 3–4 µm broad, sheath very thin, non-lamellated; trichomes 3–3.5 µm broad, not constricted at the cross walls, not granulate, end-cells not attenuated, cross-wall not visible, end-cell rounded, some specimens also truncated (Figs 2/6, 3a, b).

Phormidium tenue (Meneghini) Gomont*

Syn: *Leptotrix subtillisima* Kutz.

Syn: *Hyphaeothrix subtillisima* Rabenh

Filaments more or less straight, curved, loosely intricate, 2–3 µm broad; sheath very thin, non-lamellated; trichomes green, 2.5–3 µm broad, not constricted at the cross walls, not granulate, at the end attenuated and bent, cross wall not visible (Figs 2/7, 3d).

Phormidium jadinianum Gomont*

Filaments single, straight, 4 µm broad; sheath very thin; trichomes green, constricted at the cross walls, not granulate, end-cell distinctly attenuated and straight, cells quadrate, 2–2.5 µm long and 4 µm broad, end-cell conical (Figs 1/2, 3i).

Phormidium purpurascens (Kutz.) Gomont*

Filaments bent, horseshoe-shape, green, 3 µm broad; sheath very thin; trichomes not constricted at the cross walls, granulate, cells nearly quadrate, 2 µm long and 3 µm broad, end-cell cells rounded, apices distinctly attenuated (Figs 2/4, 3c).

Phormidium lucidum Kutz. ex Gomont*

Filaments straight, dull green, 10–12 µm broad; sheath thin, smooth, 1.5–2 µm broad; trichomes 10–10.5 µm broad, constricted at the cross walls, not granulate, at the end attenuated, cells 2.5 µm long and 7–8 µm broad, end-cell conical with calyptras (Figs 2/5, 3f, g).

Phormidium fragile (Meneghini) Gomont*

Filaments more or less flexible, green, 3–4 µm broad; sheath delicate; trichome constricted at the cross walls, not granulate, apical slightly attenuated, cells quadrate, 2 µm long and 3.5 µm broad, end-cell a little attenuated, no calyptras (Figs 2/3, 3e).

***Phormidium* sp.**

Filaments straight or flexible, entirely intricate, 1–2 µm broad; sheath very thin; trichome not constricted at the cross walls, not granulate, not attenuated, not visible (Figs 2/2, 3h).

Microcoleus palaodus (Kutz.) Gomont*

Filaments single, non branch; sheath thin; trichome high and intricate, dull green, rope-shape, not constricted at the cross walls, granulate, not attenuated, cells quadrate, 6 µm long and 4 µm broad, not attenuated, no calyptras (Figs 2/8, 3j).

Microcoleus lacustris (Rabenh) Farlow*

Syn: *M. lacustris* Formalie

Filaments parallel, rarely branch; sheath thin; trichome high, not constricted at the cross walls, not granulate, not attenuated, cells cylindrical, 5–6 µm long and 3 µm broad, end-cell rounded, no calyptras (Figs 2/9, 3k).

Discussion

Our knowledge about *Cyanophyta* of Golestan province is limited. However, until now, a few reports have been published with the highest degree of consideration on stigonematalean species (Sepehri *et al*. 2003, Nowruzi *et al*. 2007). There are no studies on morphological characterization and taxonomic study of *Cyanophyta* especially *Phormidium* and *Microcoleus* species of paddy-fields in the North of Iran, except a few studies on the heterocystous filamentous cyanophytes (Soltani *et al.* 2007) and a few reports on the morphological characterization and taxonomy and geographical variation on *Oscillatoria* (Siahbalaei 2008, Shokravi *et al*. 2007, unpublished data). Morphological versatility and the overlapping characters between the members of *Phormidiaceae* and *Planktothrix*, *Lyngbya*, *Oscillatoria* and *Plectonema* may be the main reason for meager studies. Combination of various factors seems to be a controlling the algal development (Hickmon 1978). In our study on the algal flora in paddy-fields the members of *Cyanophyta* formed the majority with a ratio of 75%, this can be explained by the variety of habitats or may have been the result of physico-chemical or geographical differences between paddy-fields.

Phormidium and *Microcoleus* listed in this paper, are first records for paddy-fields of Iran and other five species has been reported from the lakes and rivers for Iran: *P. corallinae*, *P. nigroviride* (Silva 1992), and

P. inundatum, *P. papyraceum*, *P. retzii* (Afsharzadeh 2003). The flora of algae in paddy-fields varied between stations and months. The number of species at each site ranged from 2–7 with maximum at Gorgan, Azadshahr and Minoodasht stations, and minimum at Kordkoy and Aliabad stations. *Phormidium tenue*, *P. subincrustatum* and *Phormidium* sp. were rarely found dominant in all stations in spring, autumn and winter. *Phormidium fragile* and *P. lucidium* were rarely found in summer and autumn. *Microcoleus* species were found in spring and summer only in Gorgan, Minoodasht and Aliabad. The present study shows that, morphological variation of some *Cyanophyta* such as *Phormidium* and *Microcoleus* in paddy-field is very high and there is a need to improve identification keys. The results draw a preliminary image of the morphological and taxonomical situation of *Cyanophta* especially *Phormidium* and *Microcoleus* species.

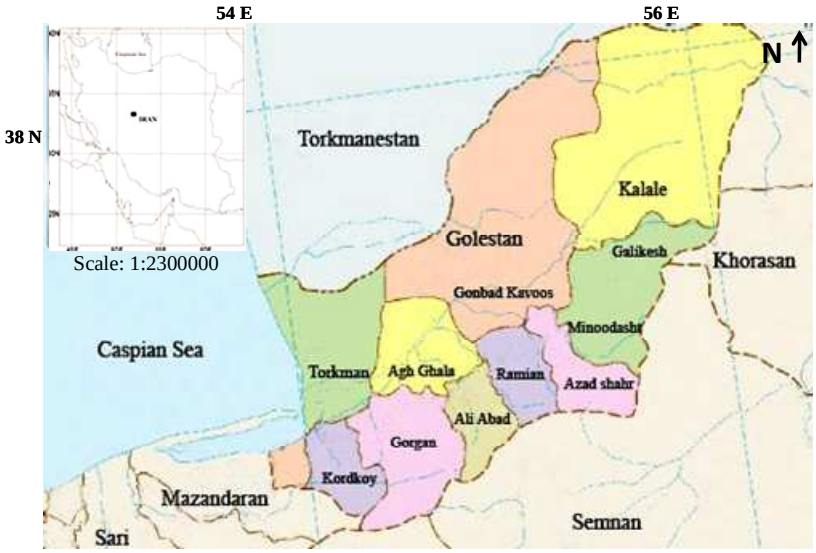

Fig. 1. Location of the study area and research stations.

Table 1. *Phormidium* and *Microcoleus* species and relative frequencies (%) of occurrence on the paddy-fields of Golestan province

Taxa	Spring					Summer					Autumn					Winter				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
P. subincrustatum	A	A	F	F	A	F	-	F	-	A	F	R	F	F	F	F	_	R	R	A
P. tenue	F	F	F	F	F	F	-	F	-	R	R	R	F	F	A	F	R	F	F	A
P. jadinianum	-	-	-	-	R	R	R	A	F	R	-	-	-	A	R	-	-	-	R	-
Phormidium sp.	A	A	A	A	A	-	-	-	-	-	F	F	R	F	A	F	R	F	R	F
P. purpurascens	-	-	-	-	R	R	R	-	F	R	-	-	-	A	R	-	-	-	-	-
P. lucidium	F	-	-	R	F	-	-	-	-	-	-	-	-	-	R	-	-	-	-	-
P. fragile	-	-	R	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
M. paludosus	-	-	R	R	-	R	-	A	F	R	-	-	-	-	-	-	-	-	-	-
M. lacustris	R	-	R	-	R	R	-	-	F	R	-	-	-	-	-	-	-	-	-	-

Stations: 1. Aliabad , 2. Kordkoy, 3. Minoodasht, 4. Azadshahr, 5. Gorgan
A= Abundant (50–75%), F= Frequent (25–50%), R= Rare (<25%)

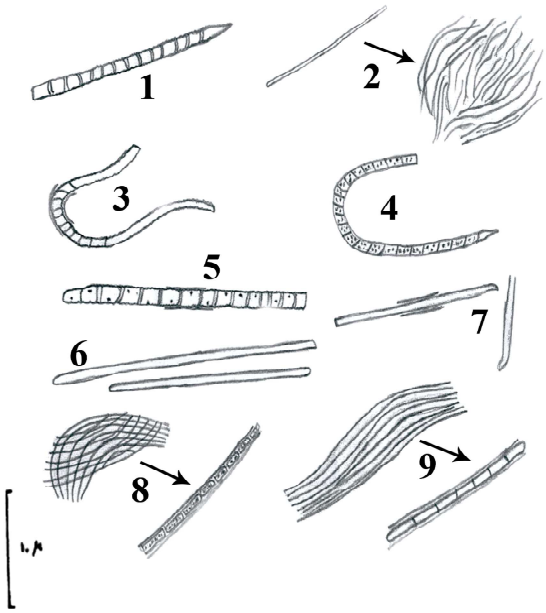

Fig. 2. 1. *Phormidium jadinianum* (apices distinctly attenuated), 2. *Phormidium* sp., 3. *P. fragile* (cross-wall constricted and end-cell attenuated), 4. *P. purpurascens* (terminal part rounded, apices attenuated, granulated), 5. *P. lucidum* (cross wall constricted and end-cell conical with calyptras), 6. *P. subinerrustatum*, 7. *P. tenue* (end of trichom attenuated), 8. *Microcoleus paladus* (not attenuated, granulated), 9. *M. lacustri* (Bar = 10 µm).

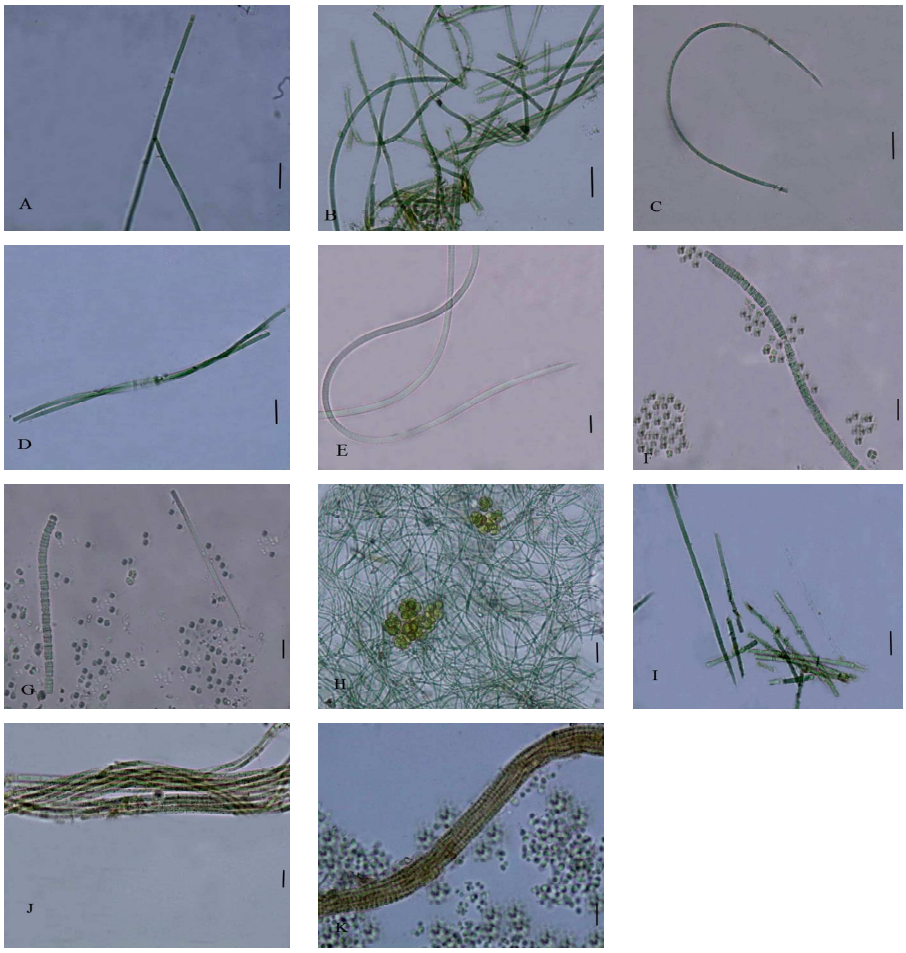

Fig. 3. A & B. *Phormidium subinerrustatum*, C. *P. purpurascens*, D. *P. tenue*, E. *P. fragile*, F & G. *P. lucidum*, H. *Phormidium* sp., I. *P. jadinianum*, J. *Microcoleus palaodus*, K. *M. lacustris* (Bar = 10 µm).

References

Afsharzadeh, S., Nejadsatari, T., Rahiminejad, M.R. & Ebrahimnejad, M. 2003. Study of algal flora in Zayanderood River. Iranian Journal of Biology 14: 32–45

Ahoren, O. 2004. A proposal for further integration of the *Cyanophyta* under the bacteriological code. International Journal of Systematics and Evolution 54: 1895–1902.

Anagnostidis, K. & Komarek, J. 1990. Modern approaches to the classification of *Cyanophyta*, *Stigonematales*. Archives fũr Hydrobiology 14: 224–286.

Belnap, J., Budel, B. & Lange, O.L. 2001. Biological soil crust: characteristics and distribution, in biological soil crust: structure, function and management (eds Belnap, J. & Lange, O.L.), Springer, Berlin, pp. 3–30.

Desikachary, T.V. 1959. *Cyanophyta*: Monograph on Algae, Indian Council of Agricultural Research. New Delhi, India.

Hickman, M. 1978. Ecological studies on the epipelic algal community in five prairie parkland lakes in central Alberta, Canadian Journal of Botany 56: 991–1009.

Jeong-Dong, K. & Choul-Gyun, L. 2008. Diversity of heterocystous filamentous Cyanobacteria (blue-green algae) from rice paddy-field and their differential susceptibility to ten fungicides used in Korea. Journal of Microbiology and Biotechnology 16: 240–246.

John, D.M., Whitton, B.W. & Brook, A.J. 2002. The Freshwater Algal Flora of the British Isles, Cambridge University Press.

Kaushik, B.D. 1987. Laboratory Methods for Blue-Green Algae. Associated Publishing Company, New Delhi, India.

Nourowzi, B. & Ahmadi-Moghadam, A. 2007. New records of relationship between soil macro-elements and the distribution of heterocystous Cyanophyta in paddy-fields, wheat fields and woodland in Golestan province. Iranian Journal of Biology 20(1): 80–95.

Prescott, G.W. 1962. Algae of the Western Great Lake Area. W.M.C. Brown Company Publication.

Sepehri, S. & Nejad-Satari, T. 2003. Studies on blue-green algae (*Cyanophyta*) with emphasis on *Stigonemataceae* family from paddy-fields around Gorgan. MSc Thesis. Islamic Azad University, Tehran, Iran.

Shokravi, Sh., Amirlatifi, F., Safaie, M., Ghasemi, Y. & Soltani, N. 2006. Some physiological responses of *Nostoc* sp. JAH 109 to the combination effects of limited irradiance, pH and DIC availability. Quarterly Journal on Plant Science Researche 1(3): 55–63.

Shokravi, Sh., Soltani, N. & Baftechi, L. 2007. Cyanobacteriology. Islamic Azad University Publication

Siahbalaei, R., Afsharzadeh, S. & Shokravi, Sh. 2008. Three new records of Oscillatorian *Cyanophyta* for the paddy-fields algal flora of Iran. Journal of Plant Researche 1(9): 1–5.

Silva, P.C, Basson, P.W. & Moe, R.L. 1996. Catalogue of benthic marine algae of Indian Ocean. University of California Publications series, UC Publication in Botany 79: 1–1259.

Soltani, N., Khavari-Nejad, R.A, Tabatabaei Yazdi, M. & Shokravi, Sh. 2007. Growth and some metabolic features of *Cyanobacterium fischerella* sp. FS18 in different combined nitrogen sources. Journal of Science 18(2): 123–128.

Ullmann, I. & Budel, B. 2001. Ecological determinates of species compositions of biological soil crusts on landscape scale. *In*: Biological Soil Crust: structure, function and management (eds Belnap, J. & Lange, O.L.). Springer.

Study of soil blue-green algae and their effect on seed germination and plant growth of vegetable crops

مطالعه جلبک‌های سبز- آبی خاکزی و تأثیر آن‌ها بر جوانه‌زنی و رشد محصولات زراعی*

Received: 06.10.2010 / Accepted: 21.04.2011

دریافت: ۱۳۸۹/۸/۱۵/ پذیرش: ۱۳۹۰/۲/۱

Z. Shariatmadari: PhD Student, Faculty of Biosciences, Shahid Beheshti University, G.C., Tehran, Iran

H. Riahi✉: Prof., Faculty of Biosciences, Shahid Beheshti University, G.C., Tehran, Iran
(E-mail: h-riahi@cc.sbu.ac.ir)

S. Shokravi: Assistant Prof. Faculty of Basic Science, Islamic Azad University, Gorgan Branch, Gorgan, Iran

زینب شریعتمداری: دانشجوی دکترای دانشکده علوم زیستی، دانشگاه شهید بهشتی، تهران

حسین ریاحی✉: استاد دانشکده علوم زیستی، دانشگاه شهید بهشتی، تهران
(E-mail: h-riahi@cc.sbu.ac.ir)

شادمان شکروی: استادیار دانشکده علوم پایه، دانشگاه آزاد اسلامی واحد گرگان، گرگان

Abstract

Nitrogen fixation blue-green algae make a major contribution to the fertility of soil. It has been suggested that blue-green algae (BGA) assist higher plant growth by supplying growth substances. There are numerous works about roles of blue-green algae in paddy soils and rice growth but little on other plants. In this research, 19 morphospecies belonging to three families of heterocystous and non-heterocystous blue-green algae from several paddy fields in the north of Iran were identified out of which seven species are new to Iran. Among these taxa, three species were used as inoculum in pot culture of cucumber, tomato and squash. The result revealed that addition of all algal extracts can enhance seed germination and plant growth in all treated plants. Statistical analysis showed that there are significant differences in plant height, root length, number of leaves, fresh and dry weight of root, leaf and stem as compared to control.

Keywords: Algal extract, biofertilizer, *Cyanophyta*, Iran, morphospecies

خلاصه

جلبک‌های سبز-آبی تثبیت‌کننده نیتروژن نقش مهمی در حاصلخیزی خاک ایفا می‌نمایند. این گروه از میکرو-ارگانیسم‌ها قادرند به واسطه تولید ترکیبات تسریع‌کننده رشد موجب افزایش رشد گیاهان عالی گردند. تا به امروز مطالعات متعددی در زمینه بررسی نقش جلبک‌های سبز-آبی در بهبود رشد گیاه برنج صورت پذیرفته است، اما اطلاعات اندکی راجع به تأثیر آن‌ها بر رشد سایر گیاهان موجود است. طی این تحقیق ۱۹ ریخت گونه متعلق به سه تیره از جلبک‌های سبز-آبی دارای هتروسیست و فاقد هتروسیست از شمال ایران مورد شناسایی قرار گرفت که هفت گونه از آن‌ها برای نخستین بار از ایران گزارش می‌شوند. سپس به منظور ارزیابی نحوه اثرگذاری عصاره جلبکی بر رشد گیاهان عالی، سه گونه از جلبک‌های سبز- آبی دارای هتروسیست جداسازی شده به عنوان ماده تلقیحی در کشت گلدانی سه گیاه خیار، گوجه فرنگی و کدو مورد استفاده قرار گرفتند. نتایج نشانگر تسریع جوانه‌زنی بذرها و افزایش معنی‌دار رشد رویشی گیاه در حضور عصاره جلبکی بود.

واژه‌های کلیدی: ایران، سیانوفیتا، ریخت‌گونه، کود زیستی، عصاره جلبکی

* بخشی از رساله دکترای نگارنده اول به راهنمایی دکتر ریاحی ارایه شده به دانشگاه شهید بهشتی

Introduction

Cyanobacteria represent a small taxonomic group of photosynthetic prokaryotes which some of them are able to N_2 fixation and also possess a tremendous potential for producing a wide range of secondary metabolites. Cyanobacteria have drawn much attention as prospective and rich sources of biologically active constituents and have been identified as one of the most promising groups of organisms capable of producing bioactive compounds (Fish & Codd 1994, Schlegel *et al*. 1999). Production of bioactive molecules such as auxins, production of secondary metabolites linked to biocontrol of bacterial and fungal diseases as well as improving soil structure and porosity through secretion of polysaccharides aiding in soil aggregation are the most important functions of these microorganisms (Karthikeyan *et al*. 2007, Sergeeva *et al*. 2002). De (1939) attributed the natural fertility of flooded rice field soil and its maintenance to the process of biological nitrogen fixation by cyanobacteria. This was the first report, which recognized the agronomic potential of cyanobacteria in India. The widespread application of single element fertilizers (especially N in Asian countries) in the cultivation of major crops has led to accelerated exhaustion of other major and minor nutrients leading to nutrient imbalances and poor soil fertility. In the current scenario therefore, an urgent need has been felt to deploy microbial biofertilizer which are multifaceted such as cyanobacterial biofertilizer. As yet for substitution of chemical fertilizers by microbial biofertilizers many studies have been done. Gupta & Shukla (1967) studied the algal influence on growth, yield and protein content of rice plants and showed that pre-soaking rice seeds with BGA cultures or extracts enhances germination, promotes the growth of roots and shoots, and increases the weight and protein content of the grain. Svircev *et al*. (1997) also reported that plant growth was enhanced in the presence of cyanobacterium, even without organic N fertilizer application. Beneficial effects of cyanobacterial inoculation were reported, not only for rice, but for other crops such as wheat, soybean, oat, tomato, radish, cotton, sugarcane, maize, chili, bean, muskmelon and lettuce (Venkataraman 1972, Rodgers *et al*. 1979, Singh 1988, Arif *et al*. 1995, Thajuddin & Subramanian 2005, Saadatnia & Riahi 2009, Maqubela *et al*. 2008, Karthikeyan *et al*. 2007). Several reasons have been proposed for beneficial effects of cyanobacteria on the growth of different plants. The capacity for biosynthesis of growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B_{12}, Folic acid, Nicotinic acid and Pantothenic acid) was reported by Misra & Kaushik (1989 a, b) that can enhance growth of plant. Additionally, cyanobacteria excrete complex organic carbon compounds that bind to the soil particles and improve soil aggregation, hence improve soil structure, soil permeability and water holding capacity of soil (Kaushik 2007). However, to date, the effect of single species cyanobacteria biofertilizer on plant growth has not yet been fully investigated. The primary aim of this research was to study cyanobacteria species isolated from soil and the second aim was pointing out the role of cyanobacteria as a biofertilizer in vegetables such as cucumber, tomato and squash plants.

Materials and Methods

Soil samples were collected from the depth of 0–5 cm on two paddy fields in Gilan and Mazandaran provinces in the north of Iran (Rangaswamy 1996).

- Isolation of cyanobacteria

Soil samples were transferred to sterile Petri dishes and added to them sterilized BG-11 medium with pH: 7.1. The Petri dishes were placed in a culture chamber at 25° C and a 12/12 h light dark cycle at artificial illumination (2000–2500 Lux) for two weeks. After colonization, for purification, identification and multiplication of colonies, a part of each colony was removed by a loop and transferred to a new plate. After purification of taxa, taxonomic determination was carried out by light microscopy and based on Desikachary (1959), Prescott (1970) and Wehr *et al*. (2002), and corrected besed on algaebase website (www.algaebase.org).

In this study, out of 19 morphospecies identified and three dominant species, *Anabaena vaginicola*, *Nostoc* sp. and *Nodularia harveyana*, were selected for further study.

- Preparation of algal extract

Three selected heterocystous cyanobacteria were grown in 500 mL flasks containing nitrate free BG-11 medium for 14 days at artificial illumination (2000–2500 Lux) at 25° C, with constant stirring and aeration. The cultures were harvested and the cells washed with distilled water. Cell extracts were made by grinding the algae in distilled water with a pestle and blender. An algal suspension containing 5.0 g fresh algal material in 500 mL of distilled water is referred to as a 1% extract.

- Germination of seeds

Air-dried seeds of squash, tomato and cucumber plants were soaked in algal extracts for 24h. Seeds, without algal extract, served as control. Percentage of germination was estimated by spreading 30 seeds on filter papers placed in glass Petri-dishes containing 5.0 mL of a cell extract. Petri dishes containing seeds with 5.0 mL of distilled water served as a control (Nanda *et al*. 1991). Four replications were made for each treatment. The Petri-dishes were placed at natural illumination at 25° C.

- Pot culture

Five healthy seedlings from treated and untreated samples were then grown in 1 liter pots for 40 days. No fertilizer was applied, but soil of treated seedlings was sprayed with 200 mL of algal extract every seven day.

- Statistical analysis

Statistical analysis was performed with one way ANOVA, using SPSS software (Package for the Social Sciences, SPSS Inc., Chicago IL) version 15. Means were separated using the Least Significant Difference (LSD) test at $P<0.05$.

Results

In the present study, seven taxa of heterocystous and 12 taxa of non-heterocystous cyanophyta were identified. *Nostocaceae* with four genera and seven species, *Oscillatoriaceae* with three genera and six species and *Chroococcaceae* with four genera and six species were included in the list of isolates (Table 1). The list of these taxa is as follows:

Nostocaceae

Anabaena vaginicola F.E. Fritsch & Rich
Cylindrospermum michailovskoense Elenkin
Nostoc punctiforme (Kützing) Hariot
Nostoc muscorum C. Agardh ex Bornet & Flahault
Nostoc calcicola Brébisson ex Bornet & Flahault
Nostoc sp.
Nodularia harveyana (Thwaites) Thuret

Oscillatoriaceae

Oscillatoria angustissima W. West & G.S. West
* *Oscillatoria chilkensis* Biswas
Phormidium terebriforme (C. Agardh ex Gomont) Anagnostidis & Komárek
* *Phormidium granulatum* (Gardner) Anagnostidis
* *Phormidium articulatum* (Gardner) Anagnostidis & Komárek
Lyngbya sp.

Chroococcaceae

* *Aphanothece gelatinosa* (Hennings) Lemmermann
* *Chroococcus minutus*(Kützing) Nägeli
* *Chroococcus minimus* (Keissler) Lemmermann
* *Chroococcus pallidus* (Nägeli) Nägeli
Gleocapsa sp.
Gloeothece sp.

* New records to Iran

Among these taxa, three species of heterocystous cyanobacteria, *Anabaena vaginicola*, *Nostoc* sp. and *Nodularia harveyana*, which were isolated from paddy field soils, used as a biofertilizer for different vegetables such as cucumber, squash and tomato. The germination of seeds soaked with cyanobacterial extract was faster as compared to seeds soaked in distilled water as control. For untreated seeds of squash and cucumber, germination began after three days, whereas germination of seeds treated with several cyanobacterial inoculum began earlier. Germination of untreated tomato seeds began after six days, whereas treated seeds germinated after four days. In treated seeds, however, seedlings height and roots length were recorded higher than control after 10 days (Table 2, Fig. 1).

In pot culture of squash plant, comparison of control and treatment plants with one way ANOVA showed that treatment groups have a significant difference in root length, plant height, leaf number and weight of fresh and dry root as well as fresh and dry weight of leaf and stem as compared with control (Table 3).

Table 1. Total percent abundance of cyanobacterial genera (summed up over all locations)

Genus	Total No. of species	Percent abundance
Anabaena	1	5.2
Nostoc	4	21
Cylindrospermum	1	5.2
Nodularia	1	5.2
Oscillatoria	2	10.6
Lyngbya	1	5.2
Phormidium	3	16
Chroococcus	3	16
Aphanothece	1	5.2
Gloeothece	1	5.2
Gleocapsa	1	5.2
Total	**19**	**100**

Table 2. The effect of cyanobacterial extracts on seedling height (values are means ± SE)

Species	Control	*Anabaena*	*Nostoc*	*Nodularia*
Squash (*Cucurbita maxima*)	3 ± 0.50	6 ± 0.57*	6 ± 0.00*	5.83 ± 0.44*
Cucumber (*Cucumis sativus*)	5.46 ± 1.23	10.93 ± 0.06*	10.76 ± 0.14*	10.86 ± 0.06*
Tomato (*Solanum lycopersicum*)	11 ± 0.50	14 ± 0.57*	10 ± 1.15	11.93 ± 0.06

* Significant at the 0.05 level

Table 3. The effect of cyanobacterial extracts on squash plant (values are means ± SE)

Growth parameters	Control	*Anabaena*	*Nostoc*	*Nodularia*
Root length (cm)	20.75 ± 1.65	32.50 ± 1.44*	32.25 ± 1.31*	29.75 ± 2.25*
Plant height (cm)	32.25 ± 1.43	46.75 ± 2.25*	48 ± 2.12*	44 ± 2.16*
Leaf number	8.75 ± 0.75	12 ± 0.40*	11.25 ± 0.47*	10.25 ± 0.47
Weight of fresh root (g)	3.85 ± 0.38	9.30 ± 0.56*	6.95 ± 0.85*	4.55 ± 0.24
Weight of dry root (g)	1.77 ± 0.10	5.96 ± 0.57*	3.15 ± 0.43*	2.15 ± 0.08
Weight of fresh stem and leaf (g)	4.17 ± 0.29	7.85 ± 1.39*	6.17 ± 0.26	4.23 ± 0.46
Weight of dry stem and leaf (g)	0.39 ± 0.03	0.76 ± 0.08*	0.63 ± 0.02*	0.46 ± 0.05

* Significant at the 0.05 level

Table 4. The effect of cyanobacterial extracts on cucumber plant (values are means ± SE)

Growth parameter	Control	*Anabaena*	*Nostoc*	*Nodularia*
Root length (cm)	16.50 ± 2.53	34.25 ± 3.37*	34.50 ± 2.62*	31.25 ± 1.25*
Plant height (cm)	25.25 ± 2.83	46 ± 3.24*	45.50 ± 3.12*	41.25 ± 1.62*
Leaf number	5.75 ± 0.25	6 ± 0.40	6.25 ± 0.75	5.75 ± 0.47
Weight of fresh root (g)	0.23 ± 0.01	0.95 ± 0.09*	0.99 ± 0.11*	0.49 ± 0.05
Weight of dry root (g)	0.07 ± 0.01	0.43 ± 0.02*	0.30 ± 0.01*	0.20 ± 0.04*
Weight of fresh stem and leaf (g)	0.65 ± 0.05	1.04 ± 0.01*	0.86 ± 0.04	0.7 ± 0.04
Weight of dry stem and leaf (g)	0.07 ± 0.00	0.11 ± 0.00	0.27 ± 0.14	0.09 ± 0.00

* Significant at the 0.05 level

Table 5. The effect of cyaobacterial extracts on tomato plant (values are means ± SE)

Growth parameter	Control	*Anabaena*	*Nostoc*	*Nodularia*
Root length (cm)	11 ± 1.08	18.75 ± 0.47*	17 ± 0.57*	18 ± 1.47*
Plant height (cm)	16 ± 1.47	25.75 ± 0.85*	24 ± 0.91*	24.50 ± 0.86*
Leaf number	5.50 ± 0.28	6.25 ± 0.25	5.50 ± 0.28	6.25 ± 0.25
Weight of fresh root (g)	0.05 ± 0.00	0.24 ± 0.11*	0.16 ± 0.00	0.16 ± 0.04
Weight of dry root (g)	0.02 ± 0.00	0.07 ± 0.00*	0.07 ± 0.00*	0.07 ± 0.01*
Weight of fresh stem and leaf (g)	0.54 ± 0.08	2.27 ± 0.26*	1.60 ± 0.25*	1.65 ± 0.05*
Weight of dry stem and leaf (g)	0.05 ± 0.00	0.21 ± 0.00*	0.14 ± 0.01*	0.16 ± 0.00*

* Significant at the 0.05 level

In pot culture of cucumber plant, comparison of control and treatments with statistic analysis showed that there is a significant difference between control and treatment groups in root length, plant height, weight of fresh and dry root and fresh weight of leaf and stem but no significant difference was observed in leaf number and weight of dry leaf and stem (Table 4). Also in pot culture of tomato plant, comparison of control and treatments with one way ANOVA showed that there is a significant difference between control and treatment groups in root length, plant height, dry weight of root as well as fresh and dry weight of leaf and stem (Table 5).

In other words the results revealed that there was a significant difference in most measurement factors in different plants treated with cyanobacterial inoculum's as compared to controls. However, effect of algal culture is not the same for all parts of plants and in different plants. In addition, effect of different algal inoculum was not the same in different plants. For example among these cyanobacteria genera, *Anabaena* showed more positive effect on most vegetative characters of tomato plant and some characters of other studied plants, whereas *Nodularia* showed less positive effect on vegetative characters of studied plants. Also among several studied

vegetative characters, leaf number showed the least difference in treatments as compared to controls and root length and weight showed the most difference in treatments. Root as an absorptive organ and producer of several substances such as phytohormones is an important part of plants (Hamidi *et al*. 2010). 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 reported by Pan *et al.* (1999). Zahir *et al.* (2000) showed that PGPR increased length and dry weight of maize root. The results of present study also showed that growth parameters of root such as root length, dry and fresh weight of root increased significantly in treated plants. Increase in dry weight of root in treated plants represent that the root growth was increased and as a result water and nutrition uptake to gain strength. Improvement of water and nutritional elements uptake from soil can improve total plant growth.

Discussion

The review of literatures showed that, there are only a few studies on similar subjects, especially on vegetable crops; nevertheless results of other studies on other plants confirm the results of this study. The results obtained in the first part of this work showed that pre-soaking seeds by algal extract accelerates seed germination and seedling height (Fig. 1). Previously, Nanda *et al*. (1991) showed that, pre-soaking of pumpkin and cucumber seeds in *Westiellopsis prolific* extract can accelerate seed germination and spraying extracts of this cyanobacterium to emerged seedling during their subsequent cultivation led to significant increase in growth and development of both crops. They suggested that, the supply of nitrogenous nutrients to the seeds is important. Jacq & Roger (1977) also showed that in addition to N-contributions, pre-soaking of rice seeds in BGA culture has decreased losses from sulphate reducing processes and this has been attributed to the enhancement of germination and faster seedling growth due to algal exudates. The second part of this research revealed that algal extract can enhance plant growth (Fig. 2).

Statistical analysis confirm that there is a significant difference in plant height, root length, number of leaf, fresh and dry weight of root, leaf and stem in treated plants as compared to control. Venkataraman & Neelakantan (1967) showed that the production of growth substances and vitamins by the algae may be partly responsible for the greater plant growth and yield. The capacity for biosynthesis of growth promoting substances such as auxins, amino acids, sugars and vitamins (Vitamin B_{12}, Folic acid, Nicotinic acid and Pantothenic acid) also can enhance plant growth. The other reason that can suggest for increased plant growth by using cyanobacterial extract is that, the growth of BGA in soil seems to influence the physical and chemical properties of soil. The water stable aggregate significantly increase as a result of algal growth and thereby improves the physical environment of the plants. Results of this study showed that these heterocystous cyanobacteria (*Anabaena vaginicola*, *Nostoc* sp. and *Nodularia harveyana*) have ability to promote vegetable growth and they are appropriate candidate for the formulation of a biofertilizer. Study also showed not all heterocystous cyanobacteria can be used as a fertilizer. For example, some species of the genus *Nodularia* may have a negative effect on plant growth since they produce toxins such as nodularin. This genus needed more study for application as a biofertilizer.

Acknowledgments

The authors gratefully acknowledge the financial support of University of Shahid Behashti (Tehran, Iran). Thanks are also due to Dr. Shokri, Ms. Saadatnia, and Ms. Bagheri for their assistance during the research.

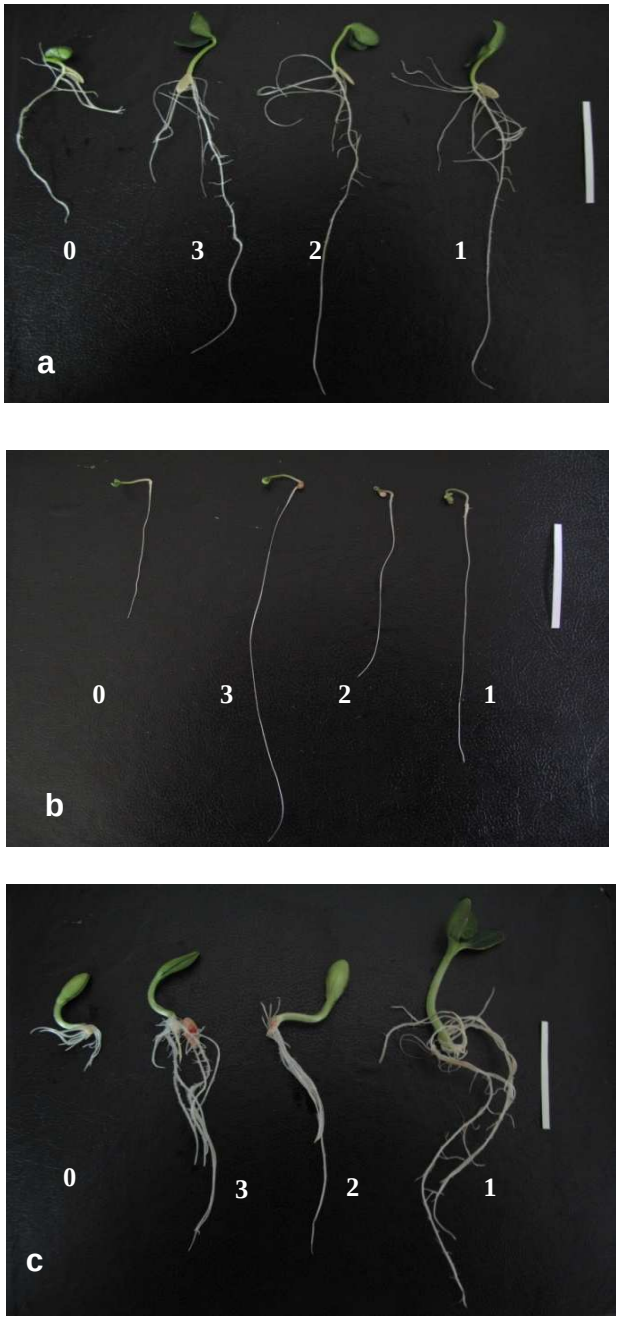

Fig. 1. Seedling height of control and treated plants: a. Cucumber seedling, b. Tomato seedling, c. Squash seedling (0. Control, 1. Plant treated by *Anabaena*, 2. Plant treated by *Nostoc*, 3. Plant treated by *Nodularia* (Bar = 5 cm).

Fig. 2. Plant height and root length of control and treated plants: a–c. Cucumber seedling, d–f. Squash seedling, g–i. Tomato seedling (0. Control, 1. Plant treated by *Anabaena*, 2. Plant treated by *Nostoc*, 3. Plant treated by *Nodularia* (Bar = 5 cm).

References

Arif, M., Gupta, R. & Joshi, M.C. 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. Department of ocean development. Technical publication 9: 243–246.

Desikachary, T.V. 1959. Cyanophyta. New Delhi: Indian Council of Agricultural Research. 686 pp.

De, P.K. 1939. The role of blue-green algae in nitrogen fixation in rice fields. Proceeding of the Royal Society of London. Series B 127: 121–139.

Fish, S.A. & Codd, G.A. 1994. Bioactive compound production by thermophilic and thermo tolerant cyanobacteria (blue-green algae). World Journal of Microbiology and Biotechnology 10: 338–347.

Gupta, A.B. & Shukla, A.C. 1967. Studies on the nature of algal growth promoting substances and their influence on growth, yield and protein content of rice plants. Labdev Journal of Science and Technology 5: 162–163.

Hamidi, A., Asgharzadeh, A., Chaokan, R., Dehghanshoar, M., Ghalavand, A. & Malakouti, M.J. 2010. Effects of PGPR application on dry matter partitioning and some growth characteristics of maize (*Zea mays* L.) hybrids under greenhouse conditions. Iranian Journal of Soil Research 24(1): 55–67.

Jacq, V. & Roger, P.A. 1977. Decrease of losses due to sulphate reducing processes in the spermosphere of rice by presoaking seeds in a culture of blue-green algae. Cahiers ORSTOM se´rie Biologie 12: 101–108 (in French).

Karthikeyan, N., Prasanna, R., Nain, L. & Kaushik, B.D. 2007. Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. European Journal of Soil Biology 43: 23–30.

Kaushik, B.D. 2007. Cyanobacterial biofertilizer technology. Pp. 53–59. *In*: S. Kannaiyan, K. Kumar & K. Govindarajan (eds). Biofertilizers technology. Scientific Publishers. India.

Maqubela, M.P., Mnkeni, P.N.S., Malamissa, O., Pardo, M.T. & Acqui, L.P.D. 2008. *Nostoc* cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility and maize growth. Plant and Soil 315: 79–92.

Misra, S. & Kaushik, B.D. 1989a. Growth promoting substances of cyanobacteria I. Vitamins and their influence on rice plant. Proceeding of the Indian Science Academy B55: 295–300.

Misra, S. & Kaushik, B.D. 1989b. Growth promoting substances of cyanobacteria II. Detection of amino acids, sugars and auxins. Proceeding of the Indian Science Academy B55: 499–504.

Nanda, B., Tripathy, S.K. & Padhi, S. 1991. Effect of algalization on seed germination of vegetable crops. World Journal of Microbiology and Biotechnology 7: 622–623.

Pan, B., Bai, Y.M., Leibovitch, S. & Smith, D.L. 1999. Plant growth promoting rhizobacteria and kinetin as ways to promote corn growth and yield in a short growing season area. European Journal of Agronomy 11: 179–186.

Prescott, G.W. 1970. Algae of the western great lakes area. WM. C. Brown Company Publishers. 977 pp.

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

Rangaswamy, G. 1996. Agricultural microbiology. Asia Publishing House, Bombay, p. 54–76.

Saadatnia, H. & Riahi, H. 2009. Cyanobacteria from paddy-fields in Iran as a biofertilizer in rice plants. Plant Soil Environment 55(5): 207–212.

Schlegel, I., Doan, N.T., De Chazol, N. & Smit, G.D. 1999. Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. Journal of Applied Phycology 10: 471–479.

Sergeeva, E., Liaimer, A. & Bergman, B. 2002. Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta 215: 229–238.

Singh, P.K. 1988. Biofertilization of rice crop. pp. 109–114. *In*: S.P. Sena. & P.C. Palit (eds). Biofertilization polentialities and problems. Plant Physiology Forum.

Svircev, Z., Tomas, I., Nenin, P. & Drobac, A. 1997. Co-cultivation of N2-fixing cyanobacteria and some agriculturally important plants in liquid and sand culture. Applied Soil Ecology 6: 74–81.

Thajuddin, N. & Subramanian, G. 2005. Cyanobacterial biodiversity and potential application in biotechnology. Current Science 89: 47–57.

Venkataraman, G.S. 1972. Algal biofertilizer and rice cultivation. Today and Tomorrows Printer and Publishers. New Delhi. 71 pp.

Venkataraman, G.S. & Neelakantan, S. 1967. Effect of the cellular constituents of the nitrogen fixing blue-green algae *Cylindrospermum muscicola* on the root growth of rice seedlings. Journal of General and Applied Microbiology 13: 53–61.

Wehr, J.D., Sheath, R.G. & Thorp, J.H. 2002. Freshwater algae of North America: Ecology and classification. Aquatic Ecology Press. 917 pp.

Zahir, A.Z., Abbas, S.A., Khalid, A. & Arshad, M. 2000. Substrate dependent microbially derived plant hormones for improving growth of maize seedling. Pakistan Journal of Biological Science 3: 289–291.

http://www.algaebase.org