THE STUDY OF HETEROCYSTOUS CYANOBACTERIA FROM PADDY FIELDS IN KALAT NADERI DISTRICT IN NORTH-EAST OF IRAN

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Received 2013. 12. 25; accepted for publication 2014. 02. 25

Aslani, E., Riahi, H., Shariatmadari, Z. & Bazzi, F. 2014. 12. 31: The Study of Heterocystous Cyanobacteria from Paddy Fields in Kalat Naderi Region in North-East of Iran.- *Iran. J. Bot. 20 (2): 257-264.* Tehran.

Heterocystous cyanobacteria represent an interesting group of photosynthetic prokaryotes which are capable of N_2 fixation and are present abundantly in rice fields. The aim of this study was the identification and purification of cyanobacteria from rice fields in the Kalate Naderi ,which is located in north-east of Khorasan Razavi Province. To approach this aim, four paddy fields were selected to investigate heterocyestous blue-green algae. In total, 30 taxa were identified. These species belong to genera such as *Nostoc*, *Anabaena*, *Cylindrospermum* and *Calothrix*. Among these genera, *Nostoc* was found to be dominant genus in all samples. Six species, including *Anabaena fuellebornii* Schmidle, *Anabaena inaequalis* Bornet & Flahault, *Nostoc pruniforme* C. Agardh ex Bornet & Flahault, *Nostoc amplissimum* Setchell, *Nostoc piscinale* Kützing ex Bornet & Flahault, and *Calothrix fusca* (Kützing) Bornet & Flahault were identified as new records for Iran.

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Key words: Algal flora; Heterocystus cyanobacteria; Kalat Naderi; Paddy field; Khorassan; Iran

مطالعه سیانوباکتری‌های دارای هتروسیست مزارع برنج کلات نادری واقع در شمال شرق ایران

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سیانوباکتری‌های تولیدکننده هتروسیست گروه ویژه‌ای از پروکاریوت‌های فتوسنتزکننده هستند که قادرند نیتروژن اتمسفری را تثبیت کنند و در مزارع برنج به فراوانی یافت می‌شوند. هدف از این مطالعه خالص‌سازی و شناسایی سیانوباکتری‌های مزارع برنج ناحیه کلات نادری از استان خراسان رضوی است. در راستای نیل به این هدف، فلور جلبکی ٤ ایستگاه واقع در این ناحیه مورد بررسی قرار گرفت و در مجموع ۳۰ تاکسون شناسایی شد. گونه‌های شناسایی‌شده مربوط به جنس‌هایی از قبیلNostoc، Anabaena، Cylindrospermum و Calothrix بودند. در میان این جنس‌ها، جنسNostoc به عنوان نمونه غالب موجود در تمام ایستگاه‌ها شناسایی شد. همچنین شش گونه ذیل به عنوان گونه‌های جدید برای ایران گزارش می‌گردند:

Anabaena fuellbornii Schmidle, *Anabaena inaequalis* Borent & Flahault, *Nostoc picinale* Kützing ex Bornet, *Nostoc pruniforme* C.Agardh ex Bornet & Flahault, *Nostoc amplissimum* Setchell, *Calothrix fusca* (Kützing) Bornet & Flahault.

INTRODUCTION

Cyanobacteria are oxygenic photosynthetic microorganisms which are widely distributed over a diverse range of habitats (Geitler 1932; Fremy 1933; Desikachary 1959). Among this group of algae, several taxa with positive and negative environmental impacts have been identified. For example, a significant number of Nostocaceae family are capable of nitrogen fixation,

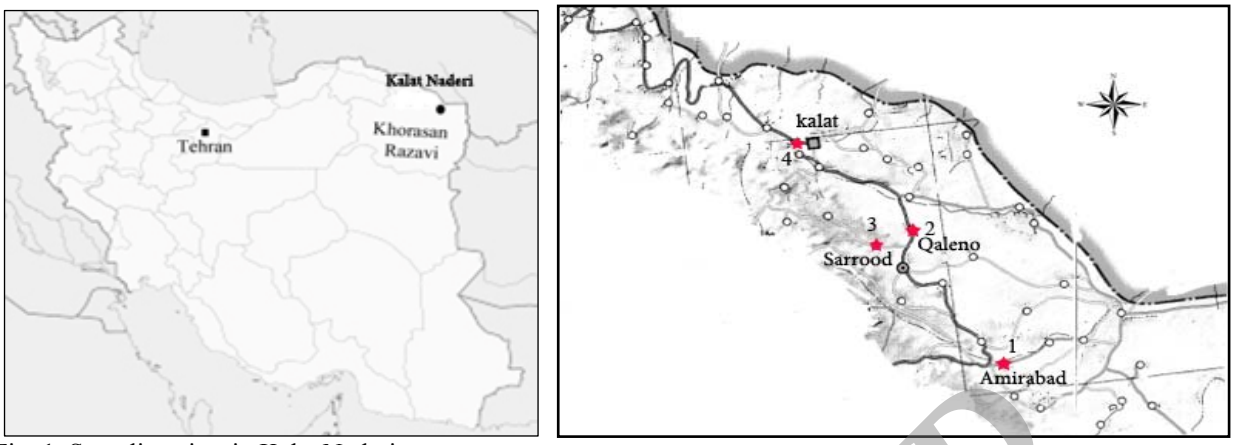

Fig. 1. Sampling sites in Kalat Naderi.

while some of them are known as harmful algae by producing toxins in aquatic ecosystems (Galhano & al. 2011). Nitrogen fixing cyanobacteria are the most important group of these algae with positive environmental effect. They are found in both free living as well as symbiotic forms (Ezhilarasi &Anand 2009). In addition to the nitrogen fixation ability, they appeared to be a rich source of many useful compounds and play an important role in improving soil condition. Consequently, cyanobacteria can increase plant growth and yield as a natural biofertilizer (Song & al. 2005).

The order Nostocales is one of the most important groups of diazotrophic cyanobacteria. This order includes filamentous cyanobacteria that are capable of cell differentiation in heterocysts, akinetes and vegetative cells (Castenholz 1989; Anagnostidis & Komárek 1989). Using morphological characters is considered important way to identify heterocystous cyanobacteria. These include characters such as morphology of filaments, especially different shapes of vegetative cells, heterocysts and akinetes. Up to now, several species from different genera of Nostocacean cyanobacteria were reported from terrestrial habitats of Iran (Nowruzi & Ahmadimoghadam 2006; Saadatnia & Riahi 2009; Siahbalaei & al. 2011; Shariatmadari & al. 2013). Nevertheless, there are limited studies on the cyanobacterial flora of paddy soils of northeast of Iran. The Kalat Naderi district is located in northeast of Iran in Khorasan Razavi Province. The aim of the present work was to study Nostocacean cyanobacteria from rice fields of Kalat Naderi district.

MATERIALS AND METHODS

This study is focused on Kalat Naderi district of Khorasan Razavi Province. The Kalat Naderi township is located in the north-east of Iran and between 59° 9′E to 60° 27′ E longitude and 36° 24′ N to 37° 17′ N latitude (fig.1). Soil samples were collected from four sites (Amirabad, Sarrood, Qaleno, Kalat) in April 2011. The depth of sampling was 5 cm (Stanier & al. 1971). The collected soil samples were transferred to sterilized nitrate free BG-11 medium. The Petri dishes were placed in a culture chamber (Noorsanat Tissue Culture Shelf, Model SB5520) at 25±5°C and a 12/12h light-dark cycle under fluorescent illumination of 2000 to 2500 Lux for two weeks. After colonization, the cyanobacterial strains were studied by optical microscopy (Olympus, Model BM-2) and different taxa were identified based on morphological characteristics using standard key books and articles (Desikachary 1959; Prescott 1970; Komárek 2005). Morphology of filaments, shape and dimensions of vegetative cells, heterocysts and akinetes were some of the characters used for identification of these taxa.

RESULTS

In the present study, 30 taxa of heterocystous cyanobacteria were identified and recorded from Kalat Naderi district (figs. 2, 3, 4). Twenty six species belonging to three genera of Nostocaceae and one genus with four species of Rivulariaceae were listed in table 1. The genus *Nostoc* with 15 species were predominant and comprising 50% of all recorded taxa (table 2). Six species of identified taxa, *Anabaena fuellebornii*, *A.inaequlis, Nostoc amplissimum, N. pruniforme, N. picsinale* and *Calothrix fusca* were considered as new records for Iran (fig. 2).

Description of the new records

Nostocaceae

1. *Anabaena inaequlis* (Kütz.) Bornet & Flahault 1888 (fig. 2A)

Table1. List of cyanobacterial species recorded from paddy fields of Kalat Naderi. * indicates new records for Iran.

Taxa	Sites			
	1	2	3	**4**
Anabaena vaginicola F.E.Fritsch & Rich	-	+	+	+
Anabaena torulosa Lagerheim ex Bornet & Flahault	-	+	+	+
Anabaena oscillarioides Bornet & Flahault	-	+	+	+
Anabaena orientalis S.C.Dixit	-	-	+	-
Anabaena portoricensis Gardner	-	-	+	-
Anabaena iyengarii Bharadwaja	-	-	+	-
**Anabaena fuellebornii* Schmidle	-	-	+	-
**Anabaena inaequalis* Bornet & Flahault	-	-	-	+
**Nostoc piscinale* Kützing ex Bornet & Flahault	-	-	+	-
**Nostoc entophytum* Bornet & Flahault	+	-	-	-
Nostoc amplissimum Setchell	-	-	+	-
Nostoc alatosporum Sant'Anna	-	-	-	-
Nostoc muscorum C.Agardh ex Bornet & Flahault	+	-	+	-
Nostoc rivulare Kützing ex Bornet & Flahault	-	-	+	-
Nostoc commune Vaucher ex Bornet & Flahault	-	+	-	-
**Nostoc pruniforme* C.Agardh ex Bornet & Flahault	-	-	+	-
Nostoc sphaericum Vaucher ex Bornet & Flahault	-	-	+	-
Nostoc spongiaeforme C.Agardh ex Bornet & Flahault	-	-	+	+
Nostoc linckia (Roth) Bornet ex Bornet & Flahault	-	-	+	-
Nostoc ellipsosporum Rabenhorst ex Bornet & Flahault	-	-	+	+
Nostoc punctiforme (Kützing) Horjot	-	-	+	-
Nostoc calcicola Brébisson ex Bornet & Flahault	+	+	+	+
Nostoc verrucosum Vaucher ex Bornet & Flahault	+	-	-	-
Cylindrospermum sphaericum B.N.Prasad	-	-	+	-
Cylindrospermum stagnale Bornet & Flahault	-	-	+	-
Cylindrospermum muscicola Kützing ex Bornet & Flahault	-	-	+	-
Calothrix elenkinii Kossinskaja	-	+	+	+
Calothrix dolichomeres Skuja	-	-	-	+
Calothrix marchica Lemmermann	-	-	+	-
**Calothrix fusca* (Kützing) Bornet & Flahault	-	-	+	-

Thrichomes straight, laying parallel and entwined, end cell is conical; cells short, barrel shaped, 4µm broad, 3-4µm long; heterocystes ovate, 5µm broad and 6µm long; akinete developed away from heterocysts, scattered, single or binary, 6-7µm broad and 10-15µm long.

Distribution in studied site*:* Kalat (36̊59′N 59̊43′ E).

2. *A. fuellebornii* Schmidle 1932 (fig. 2B).

Thrichomes straight, blue-green, without distinct mucilaginous envelopes. Vegetative cells quadrate, 4µm broad, 5-6µm long; apical cell rounded at the end. Heterocystes barrel shaped, 5µm broad, 8µm long. Akinetes present in one side of the heterocyst, solitary, ellipsoidal, swollen on both sides, about 8µm broad and 27µm long.

Distribution in studied site: Sarrood (36̊47′N 59̊52′ E).

3. *Nostoc picsinale* Kützing ex Born. et Flahault 1888 (fig. 2D).

Thallus light blue-green, filaments flexuous, loosely entangled, trichome 3-4µm broad. Vegetative cells subspherical, 3-3.5µm broad and 4 µm long. Hetrocysts subspherical or spherical, 4.5-6 µm broad. Akinetes globose, 5-7µm broad, in long chains.

Distribution in studied site: Sarrood (36̊ 47′N 59̊ 52′ E).

4. *Nostoc pruniforme* Ag. ex Bornet et Flahault 1888 (fig. 2F).

Thallus globose or ellipsoid, olivaecous; filaments loosely entangled, 5 µm broad; sheath distinct, hyaline. Vegetative cells short barrel shape, 5µm broad and 2.5-5µm long. Heterocysts spherical, 6-7 µm diameter. Akinetes spherical, 10 µm diameter.

Distribution in studied site: Sarrood (36° 47′N 59° 52′ E).

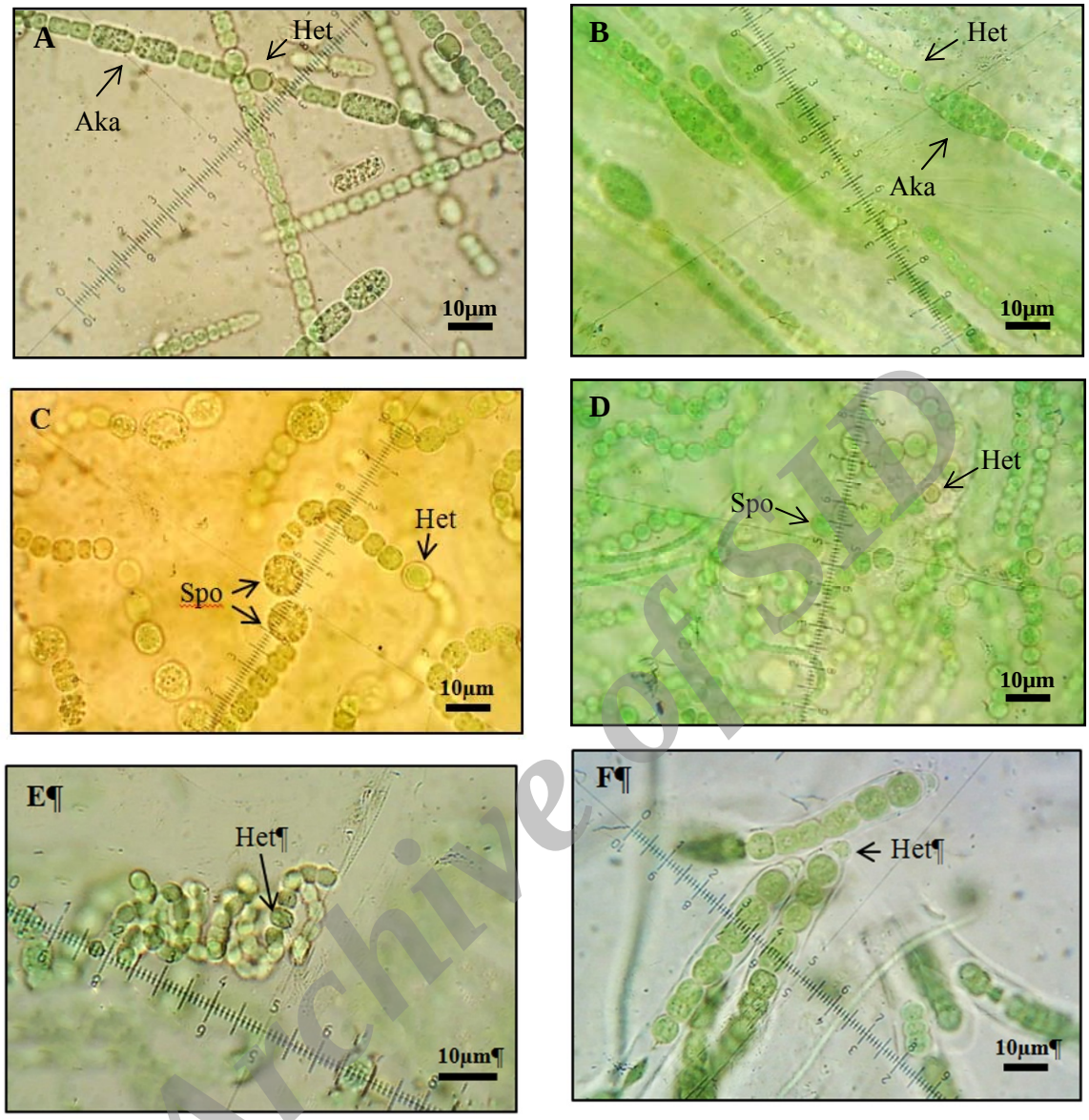

Fig. 2. New records for algal flora of Iran**.** A, *Anabaena inaequalis;* B, *Anabaena fuellborni;* C, *Nostoc pruniforme;* D, *Nostoc picsinale;* E, *Nostoc entophytum;* F, *Calothrix fusca***.** (Het: heterocyst; Aka: akinete; spo: spore)

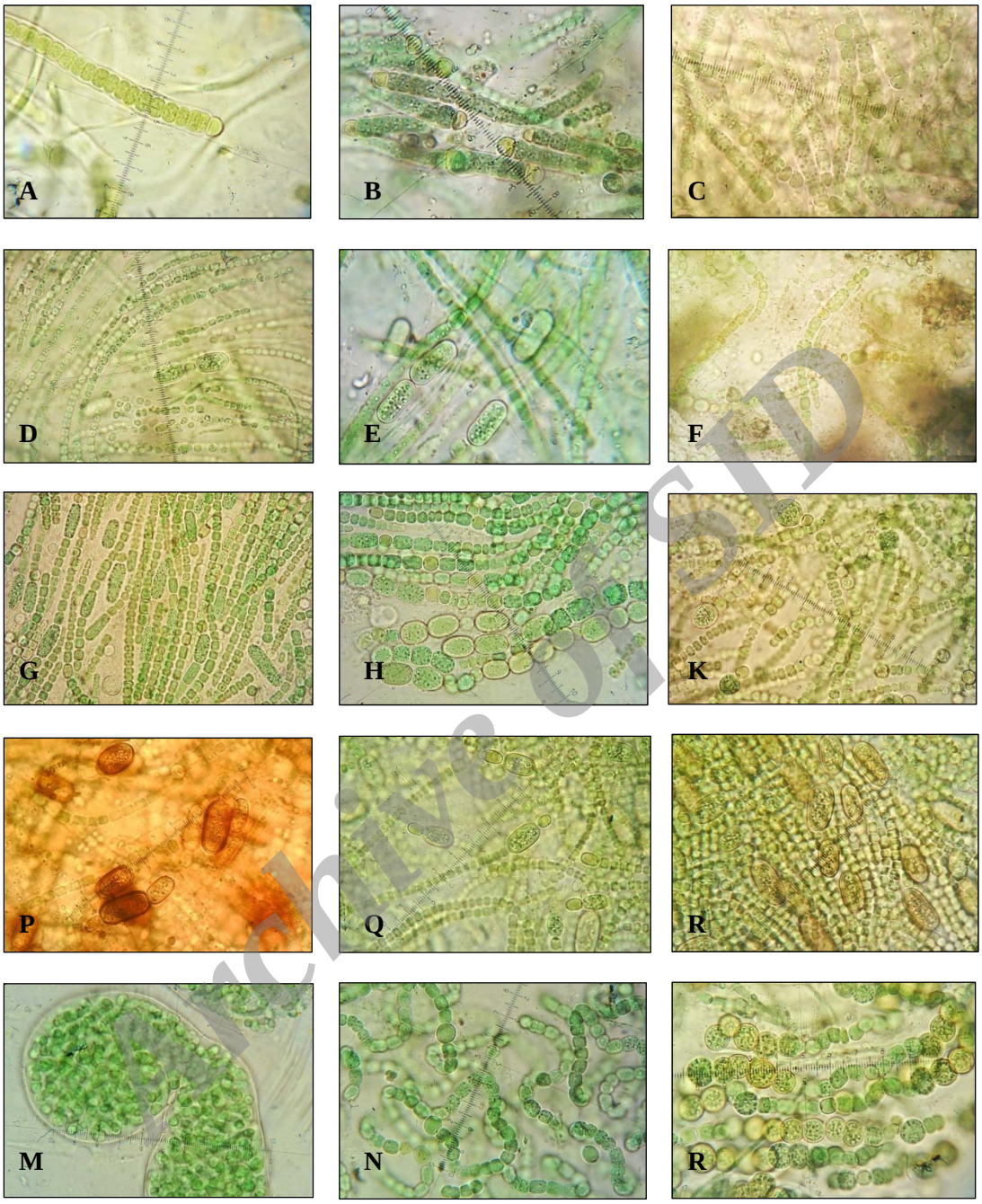

Fig. 3. Heterocystous cyanobacteria observed under optic microscope. A, *Calothrix dolichomeres;* B, *Calothrix elenkinii;* C, *Calothrix marchica;* D, *Anabaena orientalis*; E, *Anabaena torulosa*; F, *Anabaena iyengarii*; G, *Anabaena oscillarioide;* H. *Anabaena vaginicola*; K; *Cylindrospermum sphaericum;*P,Q, R, *Cylindrospermum muscicola*; M, *Nostoc commune;*N, R, *Anabaena portoricensis*.

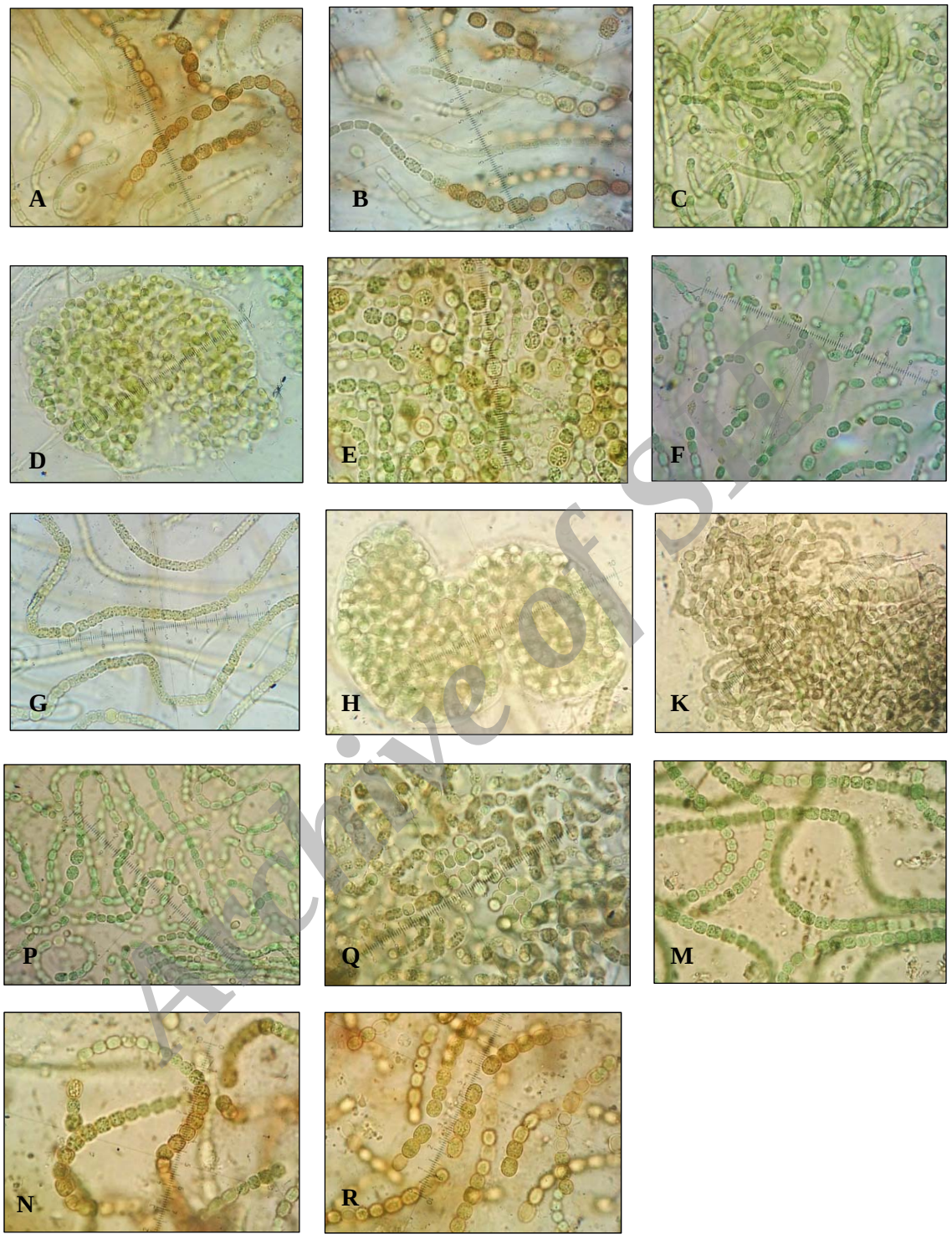

Fig. 4. Heterocystous cyanobacteria observed under optic microscope. A, B, *Nostoc alatosporum*; C, *Nostoc ellipsosporum* var. *violaceum*; D, *Nostoc amplissimum;* E; *Nostoc sphaericum;* F, *Nostoc calcicola;* G, *Nostoc verrucosum;* H, *Nostoc punctiforme*; K**,** *Nostoc linckia*; P, *Nostoc spongiaeforme;* Q, *Nostoc muscorum*; M, N, R, *Nostoc rivulare.*

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

Genus	Total no. of species	Percent Abundance
Nostoc	15	50
Anabaena	8	26.66
Cylindrospermum	3	10
Calothrix	4	13.33
Total	**30**	**100**

5. *Nostoc entophytum* Bornet et Flahault 1888 (fig. 2G).

Thallus small, blue-green, filaments densely entangled; thricomes 2.5-3 µm broad. Vegetative cells short, barrel shap, 2.5 µm broad and 3 µm long. Heterocysts broader than the vegetative cells, 4 µm broad and 3 µm long. Akinetes spherical, 5-6µm broad.

Distribution in studied site: Amirabad (36° 33´N 60° 5´ E).

Rivulariaceae

6. *Calothrix fusca* (Kützing) Bornet & Flahault 1886 (fig 2H).

Filaments single, sheath broad, colorless; trichomes 7-8µm broad. Vegetative cells often shorter than broad, 6µm broad and 3-7µm long. Heterocyst basal, hemispherical, single, smaller than the basal cell of the trichome, 4µm broad.

Distribution in studied sites: Sarood (36° 47´N 59° 52´ E).

DISCUSSION

Heterocystous cyanobacteria have been already studied for their diversity in rice fields of Iran (Shokravi & al. 2002; Siahbalaei & al. 2011), but limited works have been reported on cyanobacteria from paddy fields of northeastern part of this country. In the present study, occurrence of heterocystous cyanobacteria in four study sites of Kalat Naderi district, from Razavi Khorasan Province, was evaluated. The result obtained from this research represent that rice fields of Kalat Naderi contain a rich flora of heterocystous cyanobacteria. Among the identified taxa, members of Nostocacean family were dominant. Similar results were obtained by several researchers and the predominance of Nostocaceae family in rice fields of Iran were reported (Siahbalaei et al. 2011). In our study, *Nostoc* and *Anabaena* from this family had the highest diversity in the soil samples of all sites. Similar to the present study, previous researches reported *Nostoc* as a dominant and most diverse genera of this family in rice fields of Iran (Siahbalaei & al. 2011; Shariatmadari & al. 2013).

Among studied sites, Sarood (Fig. 1, Station 3) with 24 species and Galeno (fig. 1, Station 2) with four species possess the most and the least species variation respectively. Due to the necessity of thorough soil analysis for conclusions about the species diversity and its reasons, the authors do not have permission to comment definitively about the reason of this diversity in several sites. Nevertheless, in respect to the same weather condition and a slight difference in latitude and longitude of all sites, difference in species composition may be the result of difference in physico-chemical properties of soil in several sites. In the other studies, physical characters such as pH and Electrical conductivity are proposed as the most important factors determining soil floristic composition (Nayak and Prasanna 2007). But the results of some researches indicated that in some of cases, physicochemical factors such as soil pH, EC, total nitrogen, phosphorus and organic carbon, have not significant correlation with the species diversity of cyanobacteria in paddy soils (Shariatmadari & al. 2013). Due to different opinions about this issue, and according to the insufficient data around our studied sites, certain comments require further analysis and ecological studies.

ACKNOWLEDGMENTS

This study was supported by University of Shahid Beheshti. The authors would like to express their thankfulness to all colleagues in laboratory of phycology for their help.

REFRENCES

Anagnostidis, K. & Komárek, J. 1985: Modern approach to the classification system of cyanophytaes 1- Introduction.- Arch. Hydrobiol./algolog. Stud. 38-39:291-302.

Castenholz, R. W. 1989: Subsection IV. Order Nostocales. In Staley, J. T., M. P. Bryant, N. pefennig & J. G. Holt (eds.). –Bergey's Manual of Systematic Bacteriology 3: 1780-1793.

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

Ezhilarasi, A. & Anand, N. 2009: Phylogenetic analysis of Anabaena spp. (cyanobacteria) using sequnces of 16SrRNA gen. –Australian Journal of Basic and Applied Sciences 3(4): 4026-4031.

Galhano, V., De Figueiredo, D.R., Alves, A., Correia,

A., Pereira, M. J., Gomes-Laranjo, J. & Peixoto, F. 2011: Morphogical, biochemical and molecular characterization of Anabaena, Aphanizomenon and Nostoc strains (Cyanobacteria, Nostocales) isolated from Portuguese freshwater habitats. –Hydrobiologia 663: 187-203.

Komárek,J. & Anagnostidis, K. 1989: Modern approach to the classification system of cyanophytes, 4-Nostocales. –Arch. Hydrobiol. Suppl. 82/Algological Studies 56: 247-345.

Nowruzi, B. & Ahmadimoghadam, A. 2006: Two new Records of heterocyctus cyanobacteria (Nostocaceae) from paddy fileds of Golestan Province. ─ Iran. Journ. Bot. 11(2): 170-173.

Prescott, G.W. 1970: Algae of the western great lake area. WM. C. Brown company publishers, 977pp

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

Shariatmadari, Z., Riahi, H., Seyed Hashtroudi, M., Ghassempour, A.R. & Aghashariatmadary, Z. 2013: Plant growth promoting cyanobacteria and their distribution in terrestrial habitats of Iran.- Soil Science and Plant Nutrition 59: 535-547.

Shariatmadari, Z & Riahi, H. 2013: A taxonomic study on soil taxa of apoheterocytic cyanoprokaryota from Nostocaceae family in Iran. ─ Iran. J. Bot. 19 (2): 143-152.

Shokravi, S. 2002: Applied research management of cyanobacteria in Iran: problems and solutions. The first Iranian Congress on Applied Biology, Islamic Azad University, Mashhad, Iran.

Siahbalaei, R., Afsharzade, S. & Shokravi, S. 2011: New Records of Nostocalean Cyanobacteria from rice fields in the Golestan Province In North-East of Iran. –Biological Sciences. 1(2): 50-55.

Song, T., Martensson, L., Eriksson, T., Zheng, W. & Rasmussen, U. 2005: Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. –The Federation of European Materials Societies Microbiology Ecology 54:131-140.

Stanier, R.Y., Kunisawa, R., Mandel, M. & Cohen-Bazire, G. 1971: Purification and properties of unicellular blue- green algae (order Chroococcales). ─Bact. Rev. 35: 171-205.