ANABAENA VARIABILIS VAR. KASHINESIS (NOSTOCACEAE, CYANOPHYTA); A NEW RECORD OF CYANOPHYTA FOR ALGAL FLORA OF IRAN – A MULTIDISCIPLINARY APPROACH

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A new morphospecies, *Anabaena variabilis* var. *kashinesis* (Bharadwaja) Fritsch (Nostocaaceae, Cyanophyta) is reported for algal flora of Iran. The specimens were collected at 2013 from paddy fields of Golestan province. After isolation and uniculture in both liquid and solid media the variety was identified using optical, fluorescence and scanning electron microscope (SEM) techniques based on Morphological characteristics and behavioral analysis under different temperature and carbon dioxide concentration at limited irradiance ($2\mu E\ m^{-2}\ s^{-1}$) which is the first combined method of phytoplankton studies in Iran. Regarding biological versatility of cyanophyta, the study emphasized on most morphological characteristics of the variety and a new description of *Anabaena variabilis* var. *kashinesis* (Bharadwaja) Fritsch has been presented in this paper.

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Key Words: *Anabaena*; Cyanophyta; morphospecies, Iranian algal flora

گزارش Anabaena variabilis var. kashinesis (Bharadwaja) Fritsch به عنوان گونه رکورد از خانواده نوستوکاسه شاخه سیانوفیتا برای فلور جلبکی ایران– نگرش چند وجهی

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Anabaena variabilis var. kashinesis (Bharadwaja) Fritschاز تیره Nostocaceae جلبکهای سبز–آبی برای اولین بار برای فلور جلبکی ایران گزارش می گردد. نمونه برداری این واریته جلبکی در سال ۱۳۹۲ از شالیزار های استان گلستان انجام گرفت و شناسایی نمونه ها بعد از خالص سازی و کشت در دو محیط کشت جامد و مایع با تاکید بر ویژگیهای شاخص موفولوژیک با استفاده از میکروسکوپ نوری، فلورسانس،میکروسکوپ الکترونی (SEM) و نیز رفتار شناسی نمونه ها در محیط کشت تحت شرایط تغییرات دمایی و دی اکسید کربن در محدودیت نوری ($۲\mu E\ m^{-2}s^{-1}$) صورت پذیرفت که روش مطالعه ترکیبی جدیدی در ایران می باشد. نظر به تنوع بیولوژیکی سیانوفیتا، در این مطالعه با تاکید بر ویژگی‌های مورفولوژیک ویژه توصیفی جدید از گونه Anabaena variabilis var. kashinesis (Bharadwaja) Fritsch ارائه شده است.

INTRODUCTION

Anabaena is one of the famous heterocytous cyanophyta with relatively wide distribution on both terrestrial and aquatic habitats. Based on the literatures heterocystous cyanophyta are well adapted for diazotrophic growth (Welsh & al. 2008). This ability helps them to improve their borders and expanding their natural domains even in extreme habitats like paddy-fields and oil polluted soils (Soltani et al. 2012). Various species of *Anabaena* have been reported for Iranian inland areas but there is no clear report on their distribution and ecological diversity (Soltani & al. 2010). It seems in the soils of Northern parts of Iran, especially in Golestan province, some species of *Anabaena* are common and even dominant but there is little data about their morphological characteristics and taxonomy. As field observation cannot provide complete information on morphological plasticity of *Anabaena* strains in response to various environmental conditions, in situ studies seem to be essential. Light is evidently one of the most important factors which affect the natural distribution of cyanophyta. In rice fields, light transmission varies both daily and over the crop cycle because of changes in rice canopy (Jafari & al. 2014). In addition to light, temperature is another factor that clearly affects the distribution of cyanophyta (Shokravi & al. 2012). Most of paddy-fields cyanophyta grow in tropical and subtropical regions, a wide range of adaptation to higher temperature has been observed not only among different genera but also between different isolates of the same species (Valiente & Leganes 1989). In the same way, daily and seasonal DIC (dissolved inorganic carbon dioxide) concentration in the floodwater also varies depending on photosynthetic and respiratory rate (Leganés & Fernández-Valiente 1991). Most of the morphological and taxonomic studies of Cyanophyta usually consider only one or rarely combinations of two environmental factor at the same time (Shokravi & Soltani, 2011). However, there are increasing evidences that the effects of one environmental factor can be modulated by variation of other factors (Leganés & Fernández-Valiente 1991; Prosperi &. al. 1992; Müller & al., 1993; Poza-Carrión & al. 2001). Nowruzi & Ahmadimoghadam (2006) reported four species of *Anabaena* from paddy fields of Golestan province; Saadatnia & Riahi (2009) reported four species and Shariatmadari & Riahi (2010) reported 4 species and one variety of *Anaaena* from Gilan province (Shariatmadari 2011). In this report the taxonomy and morphological characterization of *Anabaena variabilis* var. *kashinesis* (Bharadwaja) Fritsch have been evaluated using multidisciplinary strategy including laboratory studies and morphological characterization combined with behavioral analysis under different temperature at limited carbon dioxide and irradiance conditions. This is the first report about *Anabaena variabilis* var. *kashinesis* (Bharadwaja) Fritsch for Iran.

MATERIAL AND METHODS

Soil samples were collected from Northern parts of Iran (Golestan Province). The collected soils were cultured by usual methods and isolation was carried out by plate agar. The blue- green algae *Anabaena* sp. was purified and incubated after colonization and isolation. At this phase it has been coded as *Anabaena* sp. FS 88 and preserved in algal culture collection of Research Institute of Applied Science, ACECR. Stock cultures were grown in BG110 solid and liquid medium. The cultivation was incubated under extremely limited illumination ($2\mu Em^{-2}s^{-1}$), carbon dioxide limitation (aeration) and different temperatures (20ºC, 25ºC, 30ºC, 35ºC.) using Tris and Hepes for preventing of pH drifts. After 48h of inoculation, when cells were fully adapted to culture condition, culture media were treated with different temperatures. Morphological observations were made in liquid as well as on solid media. Form and color of aggregations, form of cells, filament and trichomes, and finally talus growth and biometrical characteristics were recorded daily using binocular and phase contrast, fluorescence and light microscopy for two weeks. Identification at the species and variety level was done following morphological features comparing to the description of the taxon in John et al. (2003), Komárek & Anagnostidis (1989),Tiffany & Britton (1971) and Desikachary (1959).

RESULTS

In liquid culture, aggregations show clump shape with dense confluent crustose forms specially at the beginning of culture period, tend to thick layer crustose form at the middle of life cycle at all the temperature treatments (20°C to 35°C). Behavior of the variety at liquid cultures tend to centripetal aggregations, but centrifugal configuration may be seen with green, dark green, light and dark brown colors. The color of aggregations also change with temperature and time. Green and brown at 20°C , green and dark green at 25 °C , green and dark green especially on the second days of inoculation. In solid culture, the color of aggregations varies based on temperature and time. For example, dark brown in the beginning and then turn to light brown and at 20 °C (figs. 1A, B, C, D).

Filaments are relatively straight, lightly spiral or bent but not regularly coiled. Conical terminal cells, both as heterocyst or vegetative, may be seen numerously.Vegetative cell shape was squared-

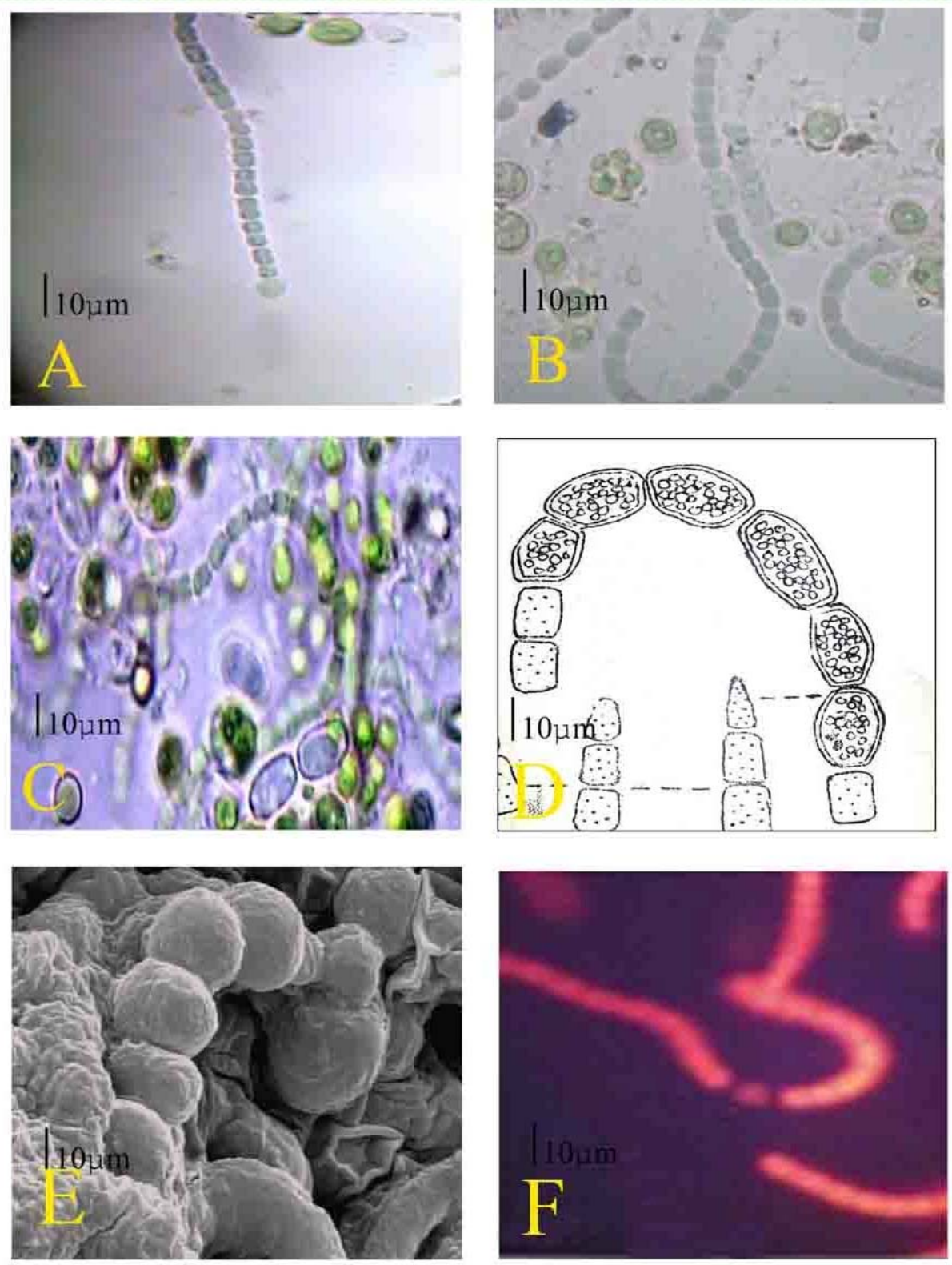

Fig. 1. Spores and filaments (A, B, C, D); Scanning-electron microscopy (E) and Heterocyst situation using fluorescence microscopy (F) of *Anabaena variabilis* var. *kashinesis* (Bharadwaja) Fritsch In control.

cylindrical in all temperature treatments. Vegetative cells were often squared or cylindrical. Electron microscopy (SEM) micrographs showed that increasing of the temperature may cause variations and turning to oval-cylindric or even oval shapes. Constriction of the cross walls which are not sharp and deep may be obvious in SEM micrographs (fig.1). Sometimes degeneration of structure of the cells was observed (fig. 1F). Biometrical analysis (tables 1 and 2), shows that the cells are slightly rectangular. It seems that dimensions of vegetative cells (and spores) are slightly more than that have already been recorded by John et al. (2003) and less than Desikachary (1959). High temperature treatments cause more flexibility and changed of the vegetative cells, from quadrate to oval and oval-cylindrical (and even spherical) in both solid and liquid medium (fig. 1 F)

Heterocytes were apical and most intercalary, intercalary heterocytes are numerous and obvious. Fluorescence microscopy analysis, showed cylindrical to rarely oval-cylindrical forms. It seems that temperature has not visible effect on the dimension of heterocyst and their dimensions are between 8-10 microns Spores (akinetes) have more or less oval form, athough, rarely, sub-spherical spores may be seen but oval and oval-spherical forms are dominant forms. (figs 1 C, D; tables**.** 1 & 2.**).**

Regarding the above finding; a new description of this variety is described as the followings:

Anabaena variabilis var. ***kashinesis*** (Bharadwaja) Fritsch

Aggregations clumps at the beginning of culture period tend to crustose form at the middle of life cycle specially at limited carbon dioxides and various temperature level (20°C to 35°C), sometimes centripetal after carbon dioxide enrichment but mostly centrifugal configuration in liquid culture; Trichomes green, dark green, light and dark brown in colour; entangled, unsheathed; filaments straight, lightly spiral or bent but not regularly coiled, irregularly curved and more or less attenuated at the ends, the terminal cell curve and dependent to temperature pointed; cell cylindrical to oval-cylindrical depends on temperature, constriction of the cross wall not so deep specially at the higher temperatures; heterocyst single, cylindrical to rarely oval-cylindrical, intercalary or rarely apical and distributed at more or less regular intervals throughout the trichome length; spores green, spores oval to oval-spherical dependent to temperature, bilayered, not always contiguous but contiguous spores are common; maximum and minimum dimensions of the vegetative cells may not affected by environmental fluctuations specially at relatively non limited carbon dioxide concentrations; survival, growth, nitrogenase activity may be remained at relatively low temperature (20°C) and high (35°C) at different carbon dioxide; phycoerythrocyanin present and highly correlated to combination of temperature and irradiances; vegetative cells (micron): 5.7-6.2 (20°C); 5.5-8.02 (25°C); 6.1-6.8 (30°C); 6.6-6.08(35°C); spores (micron): 9.3-12.1 (20°C); 9.9-14.5 (25°C); 10.4-13.8 (30°C); 9.9-12.6 (35-40°C); heterocyst: (micron) 8-10 at all temperatures.

Specimen studied: Gorgan, paddy-field soils of Golestan Province, Safaee, FS88, cultures from culture collection of Algae at Research Institute of Applied Science, ACECR

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Table 1. Biometrical analysis (µm) at different temperature in solid medium of *Anabaena variabilis* var. *kashinesis*. Data shows X±SE. L: Length; D, Diameter; VC, Vegetative Cell; S, Spore; H, Heterocyst.

Cells	Temperature(°C)			
	20 ºC	25 ºC	30 ºC	
VC (D)	5.07±0.3	4.7±0.4	4.5±0.5	6.8±0.6
S (L)	12.6±0.5	12.6±0.3	5.5±0.6	11.9±0.8
S (D)	12.2±0.2	12.1±0.5	4.5±0.4	10.8±0.4
H (L)	7.6±0.8	11.8±0.8	11.3±0.9	10.5±0.6
H .(D)	5.07±0.6	6.6±0.6	7.7±0.4	7.4±0.3

Table 2. Biometrical Analysis (µm) at different temperature in liquid medium of *Anabaena variabilis* var. *kashinesis*. Data shows X±SE. L: Length; D: Diameter; VC: Vegetative Cell; S: Spore; H: Heterocyst.

Cells	temperature(°C)			
	20ºC	25 ºC	30 ºC	35 ºC
VC (L)	5.7±0.5	8.02±0.6	6.8±0.5	6.60±0.5
VC (D)	6.2±0.3	5.5±0.3	6.1±0.3	6.08±0.8
S (L)	12.1±0.2	14.5±0.8	13.8±0.4	12.6±0.4
S (D)	12±0.6	13.9±0.4	13.2±0.5	12.2±0.7
H (L)	10.1±0.5	9.1±0.3	7.7±0.6	8.3±0.3
H (D)	9.2±0.4	7.7±0.6	5.2±0.4	7.9±0.4

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