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DISTRIBUTION OF HETEROCYSTOUS CYANOBACTERIA IN RICE FIELDS OF CUDDALORE DISTRICT, TAMILNADU

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ABSTRACT

Cyanobacteria forms a large group of structurally complex and ecologically significant gram negative prokaryotes which flourish in rice fields and also known to sustain the fertility of this ecosystem. This study is aimed to characterize the abundance of cyanobacteria in rice field areas in Cuddalore District, Tamilnadu. A total of 30 heterocystous forms from 7 genera comes under a single order with 3 families were recorded. The present study concludes that the density of heterocystous forms which enhances soil fertility.

Key words: Heterocyst, Cyanobacteria, Rice fields.

1. INTRODUCTION

Cyanobacteria are an ancient group of unique prokaryotic organisms with the ability to perform mutually compatible functions like nitrogen fixation and photosynthesis. Information on the diversity of blue green is essential to understand the algal dynamics and interaction with other microorganisms. Studies on cyanobacterial diversity have gained much importance especially after the recognition of their role in the natural environment and their ability to provide an alternate source of energy. The capacity of several cyanobacteria to fix the atmospheric nitrogen is a significant biological process of economic importance (Santra, 1993).

Several reports have indicated a widespread distribution of *Nostoc* and *Anabaena* (Sinha and Mukherjee, 1975; Paul and Santra, 1982). The dominating heterocystous nitrogen fixing blue green algal species of *Aluosira, Cylindrospermum, Nostoc, Anabaena, Tolypothrix* and *Calothrix* were reported from soils of Cuttack and Orissa (Singh, 1961). Distributional profiles of cyanobacterial isolates from soils of West **Bengal** (Saxena et al., 2007). Various workers have studied the cyanobacterial flora of rice fields of our country (Nayak *et al*. 2001, Kaushik and Prasanna, 2002, Mishra and Pabbi, 2004, Choudhury and Kennedy, 2005, Nayak and Prasanna, 2007, Digambar Rao *et al*., 2008) and few attempts have also been carried to explore their diversity in the state of Orissa (Bhakta *et al*., 2006, Dey and Bastia, 2008).But still there are many paddy fields that remain unexplored, at various locations of paddy fields from Keerapalayam and Vallampadugai in the Cuddalore district of Tamilnadu, has also received due attention it deserves. Hence, the present attempt has been made to study the diversity of cyanobacterial population and their nitrogen fixing potential.

2. MATERIALS AND METHODS

2.1 Study area

Vallampadugai which is located 5 Km from Chidambaram towards West (11°24'N Latitude and 79°44'E Longitude) and Keerapalayam is located 4 Km from Chidambaram towards East (11°24'N Latitude and 79°44'E Longitude) Cuddalore District of Tamilnadu.

2.2 COLLECTION, PRESERVATION, AND IDENTIFICATION OF SAMPLES

Samples were collected in two different sites of Cuddalore District. The samplings were done randomly from both soil and water of the paddy fields. The algal samples were preserved in 4% formalin and slides were prepared by staining with methylene blue and mounted in glycerine. Detail studies were made by examining specimens under a compound microscope with Nikon E-200 photo micrographic attachment. The strains were identified based on their morphological features and cell structure following the monograph of Desikachary (1959) and Prescott (1951).

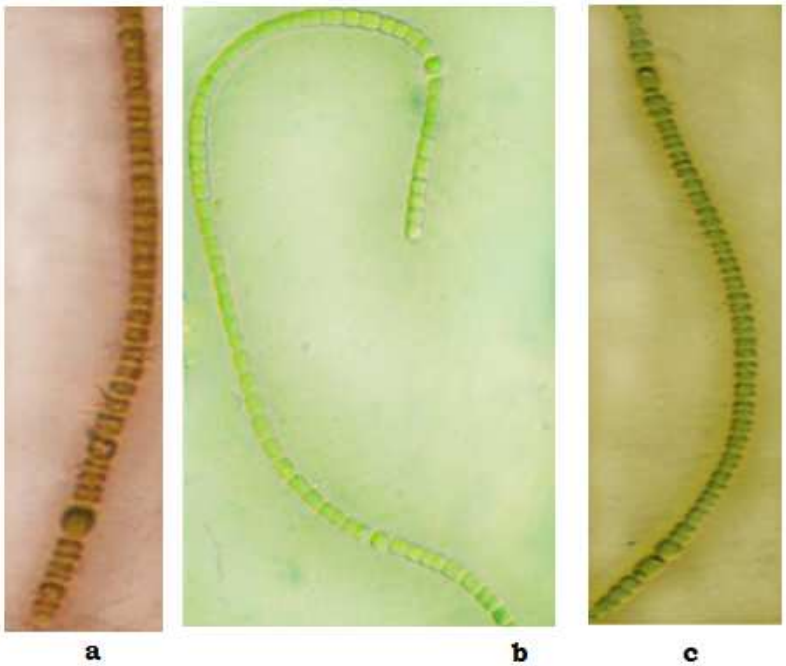
3. RESULT AND DISCUSSION

Anabaena aequalis *Borge* ***plate 2, fig c.***
Order: Nostocales
Family: Nostocaceae
Prescott 1951, Page. 512, Plate 115, Figs 1, 2.
Trichomes straight, forming a small plant mass or scattered among other algae; cells somewhat quadrate 4.5- 5.5µm in diameter, 7-8 µm long; heterocyst ovate to subcylindric, heterocyst 8 µm in diameter, 10 µm long; gonodia cylindrical remote from the heterocyst, the wall smooth and colorless, 5-7 µm in diameter.

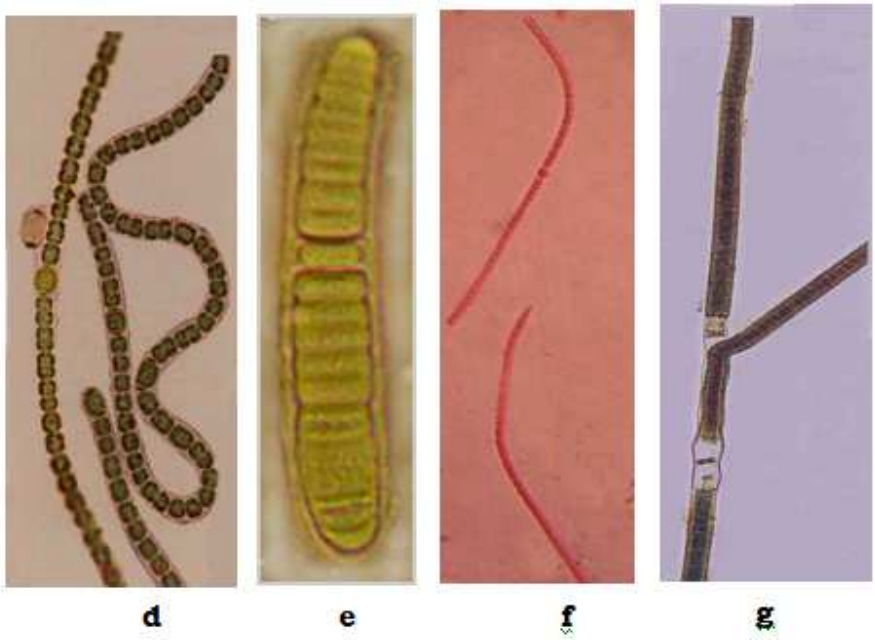
Anabaena constricta (*szafer) Geitler* ***plate 2, Fig a.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, page 394, Plate 71, Figs 1 to 3.
Thallus dull olive or brown; trichome 4.5-5.5 µm broad; cells are constricted, barrel shaped to cylindrical, 5-8 µm long, heterocyst nearly spherical 5-7 µm broad.

Anabaena fertilissima *Rao C.B.* ***plate 2, Fig b.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 398, Plate 74, Fig 1.
Trichome single, bent, with almost rounded end cells, upto 350 µm long, 5-6µm broad, at the apex 4 µm broad, cells barrel shaped, 4-8 µm long, heterocyst almost spherical, 6-8 µm broad, spores in long chains; often making the whole trichome spongenous, adjoining the heterocysts but formed centrifugally, almost spherical, with a smooth hyaline outer wall.

Plate 2



a b c



a) Anabaena constricta (szafer) Geitler b) Anabaena fertilissima Rao C.B. c) .Anabaena aequalis Borge d) Anabaena oryzae Fritsch f) Nostoc muscorum Ag. ex Born.et Flah e) Portion of scytonema g) Scytonema iyengri Bharadwaja.

Anabaena laxa *(Rabenh.)* ***plate1, Fig g.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 413, Plate 5, Figs 2 to 7.
Thallus floccose, free floating blue green; trichomes 4-5 µm broad, straight, parallel, sometimes free and sometimes with a mucilaginous sheath; cells barrel shaped, 5-6 µm long, apices hardly attenuated, end cells rounded, heterocyst spherical, 6 µm broad, 10 µm long, epispore smooth and yellowish.

Anabaena oryzae *Fritsch* ***plate 2, Fig d.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 396, Plate 72, Fig 3.
Thallus soft green, gelatinous, membranous, trichome short,straight, densly aggregated, generally parallel cells 2.5-3 µm broad, more or less barrel shaped,1½ -2 times as long as broad; heterocysts terminal and intercalary, broader than the vegetative cells.

Anabaena volzii *Lemm.* ***Plate1, Fig f.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 403, Plate 77, Fig 1.
Trichome single, free- swimming bent or curved, seldom very straight, without a mucilaginous sheath, 4-5.5 µm broad, constricted at the cross walls; cells cylindrical, 7-12 µm long, end cell conical, heterocysts cylindrical, 5.5-7 µm broad, 12-15 µm long, with a smooth colorless membrane.

Anabaenopsis arnoldii *Aptekarj* ***plate1, Fig e.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 356, Plate 5, Figs 2 to 7.
Trichome sinle, with a thick, hardly perceptible gelatinous sheath, regularly spirally coiled,spirals 25-50 µm broad, 7-32 µm away from one another, with a heterocyst at one end, at the other one or two heterocyst. Cells adpressed spherical, seldom nearly ellipsoidal, 7-9 broad, 6-8 µm long, with gas vacuoles; heterocyst terminal, two together rarely one, heterocyst spherical in shape 5-7µm broad, 8-10 long.

Cylindrospermum majus *Kutzing ex Born. et Flah.* ***plate1, Fig b.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 360, Plate 80, Fig 1.

Thallus expanded, mucilaginous, blackish green; trichome 4-5 µm broad, cells cylindrical, 5-6 µm long; heterocyst oblong, somewhat broader then the trichome,epispore brownish with distinct papillae.

Cylindrospermum indicum *Rao, C.B., orth. Mut. De Toni.* ***Plate 1, Fig d.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 369, Plate 64, Figs 4, 11.
Trichome single with deep constrictions at the joints, 3.7 µm broad, dark blue green; cells almost quadrate, or more or less barrel shaped, 3-4.5 µm long; heterocyst cylindrical, one at each end of the trichome, 2.8-5.8 µm broad and 3-7.6 µm long.spores almost ellipsoidal.

Cylindrospermum musicola *Kutzing ex Born. et Flah.* ***Plate1, Fig c.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 366, Plate 65, Fig 3.
Prescott 1951, page 531, plate 122, Fig 16.
Thallus expanded, mucilaginous, blackish- green; Trichomes 3-4.7µm broad, constricted at the cross walls, light blue green; cells 4 µm broad, 5-7 µm long; spores oval, 9-12 µm broad,10-20 µm long, epispore smooth, yellowish brown.

Cylindrospermum sphaerica *Prasad, B.N.* ***plate 1, Fig a.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 363, Plate 64, Figs 7 to 8.
Plant mass soft ,mucilaginous, pale brown, and forming a mat; trichome single, curved, often entangled with each other,4.8-5.6µm broad, cells barrel shaped, 4-8 µm long, constricted at the septa; heterocyst subconical to ellipsoidal, rounded at the distal ends, one at each end of the trichome, 4.8-5.6 µm broad and 7.2-11.2 µm long; spores spherical, 16-19.2 µm broad, sub terminal, at the both end of the trichome, formed singly, occasionally in pairs, the sub-terminal ones maturing first, thick brown exosphere and then, smooth and hyaline endospore.

Nostoc calcicola *Brebisson ex Born.et Flah.* ***Plate1, Fig h.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 384, Plate 68, Fig 1.
Thallus mucilaginous, slightly diffluent, expanded, olive, grey or blue green, often up to 5 cm in diameter filament loosely entangled; sheath mostly indistinct, or distinct only at the periphery of the thallus, colorless or yellowish brown; trichome 2.5µm broad, pale blue green; cells barrel shaped, subspherical, rarely longer than broad; heterocysts subspherical, 4 µm broad; spores subspherical, 4-5 µm broad, with smooth yellowish membrane.

Nostoc muscorum *Ag. ex Born.et Flah.* ***plate 2, Fig e.***
Order: Nostocales
Family: Nostacaceae
Desikachary 1959, Page 385, Plate 70, Fig 2.
Thallus gelatinous, membranous, irregularly expanded, attached by the lower surface, tuberculate, dull olive 2-5 µm in diameter.; filaments densely entangled; sheath distinct only at the periphery of the thallus, yellowish brown; trichome 3-4 µm broad; cells short barrel shaped to cylindrical, up to twice as long as broad; heterocyst nearly spherical,6-7 µm broad; spores oblong, many in series, 4-8 µm broad, 8-12 µm long, epispore smooth and yellowish.

Scytonema hofmanni *Ag.ex Born et Flah* ***plate 3, Fig a.***
Order: Nostocales
Family: Scytonemataceae
Desikachary 1959, Page 476, Plate 91, Fig 2.
Stratum cushion-like, broadly expanded,1-3 mm height, blackish blue green, sometimes impregnated with calcium carbonate, amethyst green or bluish-grey; filaments 7-12 µm broad, aggregated in vertical fascicles; false branches aggregated; sheath firm, membranaceous; trichome 5-10 µm broad, olive to blue green , cells unequal in length; heterocystous oblong.

Scytonema iyengari *Bharadwaja* ***plate 2, Fig g.***
Order: Nostocales
Family: Scytonemataceae
Desikachary 1959, Page 465, Plate 89, Fig 7.
Thallus thick, dirty green filaments irregularly bent false branch short, single and germinate in equal numbers. Filament 15 -20 µm broad. Sheath firm 2-

4 µm thick, gradually thinning towards the growing apices. hetrocysts cylindrical.

Calothrix castellii *(Massal.) Born. et Flah* ***plate4, Fig b.***
Order: Nostocales
Family: Rivulariaceae
Desikachary 1959, Page 529, Plate 114, Fig 7.
Thallus spongy, cushion shaped, widely expanded, dull blue green, surface pubescent by projection ends of filaments; filaments bents, erect, densely aggregated, 12-13 µm broad, swollen at the base and prostrate, 4-8 µm long; sheath thin close to the trichome, firm, uniform, hyaline, trichome 8-10 µm broad, attenuated into a long hair, heterocyst basal.

Calothrix fusca *(kuetz.) bornet & flahaut* ***plate3, Fig e.***
Order: Nostocales
Family: Rivulariaceae
Desikachary 1959, Page 527, Plate 107, Fig 10.
Prescott 1951,Page 553, Plate 132, Figs 4,5.
Filaments strongly curved from short horizontal basal portions, attached in the mucilage of other algae, bulbous at the base,11-14µm in diameter, tapering to a long hair; vegetative cells 7-11µm in diameter,1/3 as long as wide;heterocysts basal, hemispherical,9-10µm in diameter.

Calothrix gloeocola *skuja* ***plate 4, Fig d.***
Order: Nostocales
Family: Rivulariaceae
Desikachary 1959, Page 542, Plate 109, Figs 13 to 18.
Filaments solitary, simple, flexuous, in the mucilage of other algae, gradually attenuated into a moderately long hair, up to 650 µm long, base dilated, sheath thin, sheath about 1 µm thickness, colorless, more or less homogenous, sometimes diffluent, cells at the base 5-6 µm in the middle about 3 µm broad, slightly shorter than broad; heterocyst basal, solitary, hemispherical. Heterocyst 6-7 µm broad, 7-8 µm long, partly enclosed by the sheath.

Calothrix linearis gardneri *J.De Toni* ***plate 4, Fig a.***
Order: Nostocales
Family: Rivulariaceae
Desikachary 1959, Page 535, Plate 115, Fig 5.
Filament erect, for the major part straight, 350-500 µm long, cylindrical, but swollen at the base and attenuated at the apex, sheath 2-2.5µm thick, somewhat slimy, colorless, not lamellated; trichome cylindrical,5-7µm broad, in the basal part constricted at the cross-walls, ending in short hair; cells quadrate to ½ as long as broad; heterocysts basal, mostly hemispherical.

Calothrix simplex *Ag. ex Born et Flah* ***plate 3, Fig d.***
Order: Nostocales
Family: Rivulariaceae
Wehr and Sheath, Page 165, Fig.25 (A) d .
The thallus is filamentous, attached to the substratum with heterocyst and occasionally an associated akinite. Heterocyst develops basally; trichomes are unconstructed at the crosswalls and always taper terminally. Sheath are always present, firm, cells barrel shaped elongated towards the end. Heterocysts are ellipsoidal mainly basal.

Calothrix tenella *Ag. ex Born et Flah* ***plate 4, Fig c.***
Order: Nostocales
Family: Rivulariaceae
Wehr and Sheath, Page 165, Fig.25 (A)b.
The thallus is filamentus, filaments are heteropolor, with a wider basal part. Heterocyast develops basally; trichomes are unconstructed at the crosswalls and always taper terminally. Sheath are always present, firm, cells may be narrowly elongated towards the end. Heterocysts are spherical in shape and basal. Akinites are ellipsoidal appearing above basal heterosis and developing from a vegetative portion of a trichome.

Gloeotrichia raciborskii *Woloszynska* ***plate 4, Fig f.***
Order: Nostocales
Family: Rivulariaceae
Desikachary 1959, Page 562, Plate 118, Fig 14.
Thallus spherical, soft, trichome 7-12 µm broad, ending in long hair, sheath at base lamellated, dull brown; cells at the base of the trichome shorter than broad, higher up as long as broad, pale blue green; heterocyst spherical 7-10 µm broad; spores long ellipsoidal.

Homoeothrix hansgirgi *(schmidle) lemm.* ***Plate 3, Fig b.***
Order: Nostocales
Family: Rivulariaceae
Desikachary 1959, Page 521, Plate 106, Fig 4 and Plate 112, Fig 4.
Filaments many growing with other algae, unbranched, erect, about 4µm broad, 20-60 µm long; sheath thin, colourless; trichomes ending in a short hair; cells very short.
Homoeothrix varians (after Komarek) **plate 3, Fig c.**
Order: Nostocales
Family: Rivulariaceae
Wehr and Sheath, page 136, Fig.7 (A)c,
Well developed trichome narrowed towards ends. Terminal hairs may be present, sometimes separating.

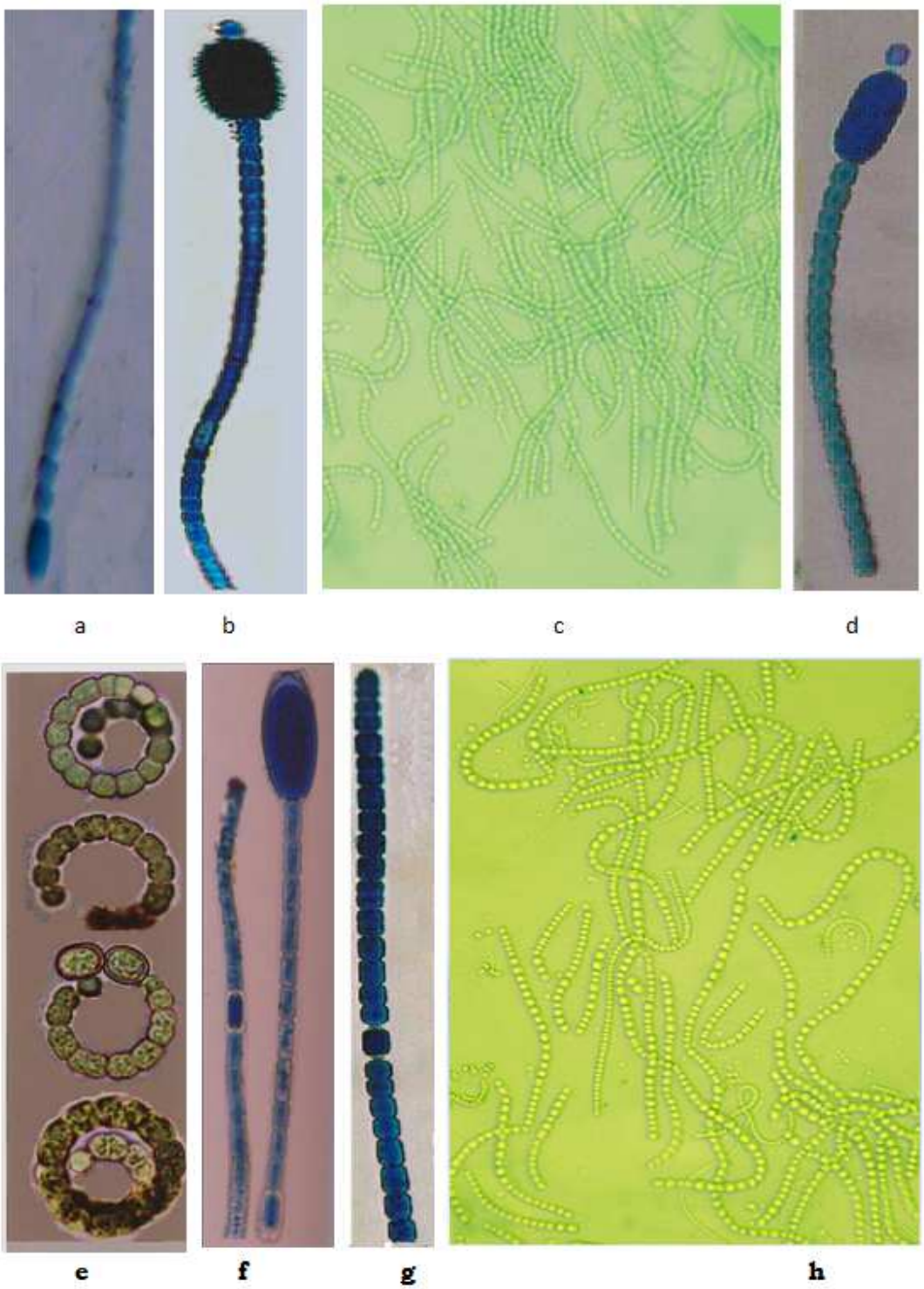
Diversity of cyanobacteria is expressed by their morphological, biochemical, and physiological properties, which enable them to settle and persist in a wide range of habitats. Their structural complexity, unusual for prokaryotes, prompted their taxonomic distinction based on phenotypic, mostly morphological properties. Heterocystous forms of cyanobacteria have been extensively studied for their diversity in rice fields (Choudhary, 1999). The occurrence of lesser number of forms during early cultivation stage might be attributed to the inhibitory effect of high light intensity, whereas fewer forms in the later part might be due to loss of nutrients as well as low light intensity reaching to the surface due to increased rice covering.

In the present study rich diversity of heterocystous cyanobacteria was recorded from rice fields of Cuddalore District. 30 species, represented with 7 genera comes under single order and three families have been reported from this area. The distribution of these cyanobacteria forms might be indicating the lower nitrogen status in rice fields. Nayak and Prasanna (2007) recorded more heterocystous forms while studying cyanobacterial abundance and diversity in rice field soils of India. Choudhary (2009) observed that the enumeration of cyanobacteria revealed the maximum diversity during the mid cultivation cycle of the rice fields. In conclusion, the present study documented a remarkable biodiversity of cyanobacteria.

Choudhary (2011) reported that documentation on nitrogen-fixing cyanobacteria and their application in the rice fields can be used for management of nitrogen fertilizer at different stages of paddy cultivation for sustainable agricultural practices by making the field environment supportive for nitrogen-fixers. Dey et al., (2010) have described occurrence of nitrogen-fixing cyano bacteria in rice fields of Orissa, India. They also reported 58 taxa belonging to 20 genera were obtained in which 19 forms were heterocystous forms.

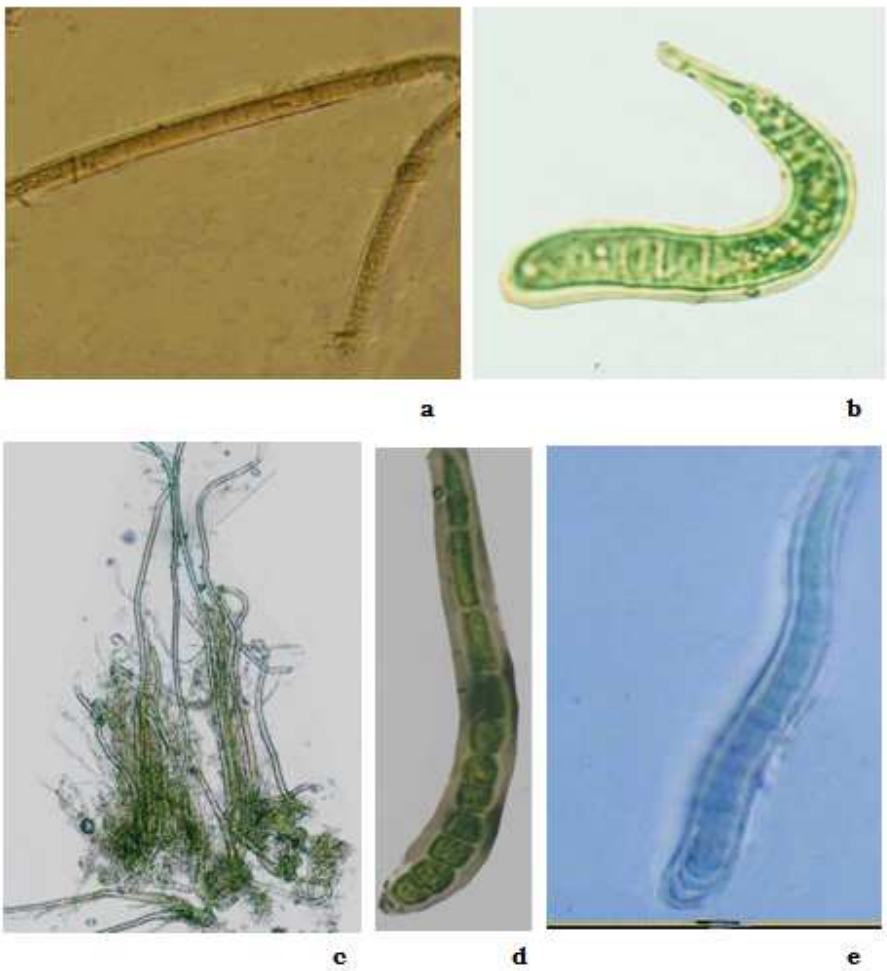
Similar results were obtained in a study where 166 cyanobactarial isolates were purified which included maximum heterocystous genera followed by non heterocystous forms in diverse rice soil (Prassanna and Nayak, 2007). Similar assumption was made by several workers and reported predominance of heterocystous form during dry periods (Roger and Kulasooriya, 1980). The diversity of cyanobacteria in the rice fields were studied by Shannon index in two different seasons. Rich diversity was observed in all the study sites. Many workers have also reported both richness and evenness of cyanobacterial diversity from different rice fields of India (Prasanna *et al.* 2009).

Plate 1



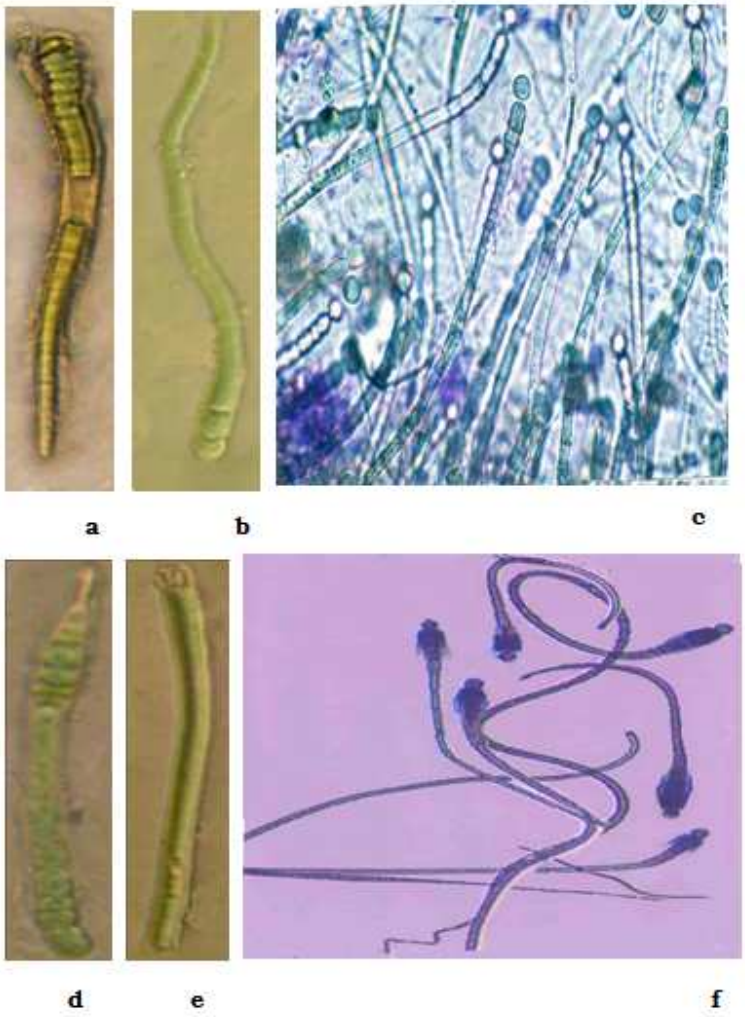
a)Cylinrospermum spharica prasad B.N. b)Cylindrospermum majus kutzing ex Born et Flah. C)Cylindrospermum musicola kutzing ex.Bornet Flah d) Cylindrospermum indicum Rao C.B. e) Anabaenopsis arnoldi Aptekarj f) Anabaena valzii lemm g) Anabaena laxa h) Nostoc calcicola Breb ex Born .et Flah.

Plate 3



a) Scytonema hofmanni Ag.ex.Born et Flah b) Homeothrix hansgirgi (schmidle) Lemm c) Homeothrix varians after komarek d) Calothrix simplex Ag.ex.Born et Flah e) Calothrix fusca (kuetz.)

Plate 4



a) Calothrix linearis gardner J.De Toni b) Calothrix castelli (massal) Born. et Flah c) Calothrix tenella Ag.ex Born et Flah d) Calothrix gloeocola Skuja e) Gloeotrichia sps.f) Gloeotrichia raciborskii woloszynska

4. CONCLUSIONS

The present study concludes that the heterocystous cyanobacteria present in the paddy fields of Cuddalore District. The presence of heterocystous cyanobacteria which fix atmospheric nitrogen in paddy soils and also enhances the soil fertility.

5. ACKNOWLEDGEMENT

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