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DIVERSITY OF THE CYANOBACTERIAL GENUS *NOSTOC* VAUCHER OF GRASSLAND BIOTOPE, MEERUT, UTTAR PRADESH, BHARAT

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ABSTRACT

A Grassland biotope of the Botany Department, Chaudhary Charan Singh University, Meerut, Uttar Pradesh, Bharat was closely monitored to study the diversity of heterocystous cyanobacterium genus *Nostoc* and their observations were recorded during last three years (July 2020 to June 2023). Cyanobacteria containing soil were collected from the Grassland Biotope and their enrichment cultures were raised, and morphological observations were recorded. All species of *Nostoc* have been identified upto the species level with the help of available monographs. Total eleven species of *Nostoc* viz. *N. cycadeae*, *N. paludosum*, *N. microscopicum*, *N. shensiense*, *N. calcicola*, *N. sphaericum*, *N. elliposporum*, *N. linckia*, *N. verrucosum*, *N. gelatinosum* and *N. pruniforme* have been identified. On the basis of observations, it is concluded that Grassland biotope is one of the most suitable habitats which facilitate the growth of *Nostoc* species and the fertility of the soil of Grassland biotope is maintained year after year by their occurrence.

Keywords : Biodiversity, Biotope, Grassland, Nitrogen fixer, Cyanoprokaryotes

Introduction

The Cyanobacteria (Blue green algae / Cyanoprokaryotes) are most ancient autotrophic organisms on our planet Earth (Singh *et al.*, 2016). They contribute significantly as primary colonizer to the almost all the known ecosystems including River, Ponds, Forests and Grassland ecosystem. They have been reported from diverse habitats aquatic, terrestrial and sub-aerial biotopes (Kant *et al.*, 2020a). Traditionally Cyanobacteria have been classified on the basis of their morphological features and growth patterns by several algologists (Gomont, 1892; Geitler, 1932; Desikachary, 1959). The new system of classification was developed based on integration of morphological features with molecular and genetic characters by Bergey's Manual of Systematic Bacteriology (Castenholz, 2001). Algal dynamics particularly their response to environmental changes and nutrient fluctuations have been studied in nature by several algologists on about 15,000 species of algae (Hosmani *et al.*, 2009). The cyanobacteria are primary producer contributing 80% of total oxygen and created oxygenic atmosphere on planet earth.

The range of thallus organization of cyanobacterial forms from unicellular to colonial and multicellular filamentous. Cyanobacteria developed some advanced filamentous morphological features like heterocystous trichomes, false (Nostocales) and true (Stigonematales) branching. Mostly cyanobacterial forms are covered with thick gelatinous sheath. (Banerjee & Pal, 2017). Pandey & Tripathi (1988) suggested the role of cyanobacteria. Cyanobacteria have been good source of several beneficial

bioactive compounds and these are used in Cosmetics, Nutraceuticals and Pharmaceutical industries, Human food and animal feed, biofuels production, as biofertilizers and phycobiliproteins, for control of plant pathogen (Righini *et al.*, 2022), Cyanobacteria also showed antifungal, antibiotic, anticancer, antimicrobial, antibacterial, antiviral, anti-inflammatory, antitumor, anti-malarial, immunosuppressant, and anti-HIV (Zahra *et al.*, 2020), anti-parasitic, hepatoprotective, anti-diabetic, anti-obesity, antioxidant, anti-aging, photoprotective and neuroprotective bioactivities (Perera *et al.*, 2023). About 2000 secondary metabolites have been identified from Cyanobacteria. They are capable to fix atmospheric nitrogen with the help of their special structure heterocysts and sustain the fertility of cultivated and uncultivated land (Stewart *et al.*, 1968). The related research work have previously reported the floral diversity of microplankton cyanobacteria from aquatic ecosystems of West Bengal, and reported more than 149 species of Cyanobacteria (Bose *et al.*, 2016). According to the recent classification of heterocystous cyanoprokaryote, the Nostoclean members have classified into 12 families (Komárek, 2013). The trichomes of the heterocystous cyanobacteria are differentiated into heterocysts, vegetative cells and akinetes and considered as the characteristic features of Nostoclean members. In rice field's soil, Nostoclean members play important role as natural source of nitrogen fertilizers (Singh, 1961).

The *Nostoc* genus is an unbranched filamentous and heterocystous cyanobacterium, found in both terrestrial and aquatic habitat (Potts, 2000), uncultivated and cultivated

moist soils, sub-aerial habitats (tree barks, rocks, walls and roof surfaces) and symbiotic associations with other higher plants (Rasmussen & Nilsson, 2002). The unbranched, heterocystous filaments with akinetes and sheaths are the unique features of genus the *Nostoc* for their characterization. The colonies of the *Nostoc* species appear dark green to black, yellow green to red-brown in nature. The brown colour of *Nostoc* colonies appear due to extracellular pigments Scytonemin and violet due to intracellular pigment nostocine A and mycosporine-like amino acids (MAAs). Scytonemin and MAAs pigments protect the cells from UV radiation (Castenholz & Garcia-Pichel, 2012). The trichomes of the species of *Nostoc* are found immersed in a thick layer of exopolysaccharides. About 250-300 species of *Nostoc* have been reported till now which indicate the huge diversity of heterocystous forms in this genus (Singh, *et al.*, 2016). The genus *Nostoc* is a more complicated cyanobacterium and it needs thorough detailed study in natural habitats as well as culture based observations, physiological and biochemical profiling for assigning proper position in taxonomy (Kant *et al.*, 2020).

The present research work is focused to explore the diversity of *Nostoc* sp of Grassland biotope located at Botany Department, Chaudhary Charan Singh University Meerut, Uttar Pradesh, Bharat.

Materials and Methods

Study area and Sampling Sites

The Site of present study was Grassland of the total area of 585.26 m² and it was established in the Department of Botany, Chaudhary Charan Singh University Meerut, Meerut, Uttar Pradesh, Bharat during 1969-1970. The site is located at 28°58'03" N latitude and 77°44'32" E longitude. Details of study site are given in figure-1. The dominant flora of the present grassland biotope is *Cynodon dactylon* (L.) Pers, Family Poaceae and no any type fertilizer and pesticides are used for the growth of the grasses. Total 110 cyanobacterial growth containing samples were collected from the study site during the year July 2020- June 2023. The details of Meteorological data and soil properties of the study area are given in tables 1 and 2.

Enrichment Culturing

All the collected samples were cultured into nitrogen deficient solid and liquid BG-11 (Stainer *et al.*, 1971) nutrient medium for enrichment culturing. From the mix population of culturing algal samples, total eleven species of *Nostoc* were raised from the collected samples of blue green algae and purified to develop unialgal culture with the help of standard methods (Kant *et al.*, 2005; Kaushik 1987).

Microscopic Observations

The morphological characterization of *Nostoc* species were observed and recorded their details with the help of digital Magnus Magcam camera (DC 10) and Trinocular Olympus Microscope (CH20i). During the observation of isolated species of *Nostoc* of the present study were identify with assistance of available monographs (Geitler 1939; Desikachary 1959; Komárek 2013). Morphological details of the all the eleven species of *Nostoc* are described in the present study based on the Heterocystous cyanoprokaryotes (Komárek 2013)

Results and Discussion

Morphological description of *Nostoc* species

Class: Cyanophyceae

Order: Nostocales

Family: Nostocaceae

Nostoc. Caldicola Brébisson ex Bornet et Flahault (Fig. M) Colonies irregular, flat, gelatinous, but without production of thick slime, without periderm, dirty olive-green, greyish blue-green or yellowish-green, disintegrating, up to 5 cm in diameter in nature. Filaments freely entangled, sheaths usually indistinct, more distinct at colonial margin, Colourless or yellowish.

N. cycadeae A.M. Watanabe et Kiyohara (Figs. F-H)

Colonies flat, olive green, up to 1-2mm in diameter. Sheath gelatinous, pale blue green, up to 6-11.9 µm in diameter. Trichomes curved.

N. ellipsoforum [Desmazières] Rabenhorst ex Bornet et Flahault (Fig. O)

N. wollnyanum Richter ex Wittrock et Nordstedt

Colonies gelatinous, flattened, irregular, granular, ± firm, olive green, yellowish green, with filaments densely or loosely entangled. Trichomes flexuous, with sheaths distinct only at the periphery of colonies, brown.

N. gelatinosum Schousboe ex Bornet et Flahault (Fig. S)

Colonies irregular, flat, gelatinous, brown, with freely arranged filaments. Sheaths distinct only at the margin, yellow brown.

N. linckia [Roth] Bornet et Flahault (Fig. P)

N. confusum Agardh ex Bornet et Flahault.; *N. piscinale* Kützing ex Bornet et Flahault.; *N. rivulare* Kützing ex Bornet et Flahault

Colonies fine, thin, mucilaginous, at first ± spherical and freely attached to submersed plants or other substrates, usually brownish, yellow-brownish or olive-green, with gelatinous clusters. Filaments densely entangled and flexuous especially in young colonies. Sheaths colourless, usually visible only in marginal parts of the colonies.

N. microscopium Carmichael ex Bornet et Flahault (Figs. J-K)

N. macrosporum Meneghini ex Bornet et Flahault

Colonies subaerophytic, irregularly spherical or ellipsoidal with smooth, olive-green or brownish surface, enveloped by periderm, up to 1 cm in diameter. Filaments flexuous, densely and later freely entangled, with yellow sheaths, particularly at the colonial margin.

N. paludosum Kützing ex Bornet et Flahault (Fig. I)

N. entophytum Bornet et Flahault; *N. cuticulare* (Brébisson) Bornet et Flahault

Colonies microscopic, in clusters, in form of flattened, irregular, mucilaginous mass, blue-green, without periderm, with freely or densely entangled filaments, sometimes with wide, yellowish, gelatinous sheaths. Trichomes flexuous, irregularly coiled.

N. Pruniforme [Linnaeus] Agardh ex Bornet et Flahault (Fig. T)

Colonies spherical, oval and ovoid, mostly up to 1.5 cm, but also up to 5 (6) cm in diameter, with smooth periderm, inside with soft mucilage, olive-green, bluish, pale blue-green, interior with irregularly entangled trichomes, which are sometimes more concentrated at the peripheral parts. Sheaths thick, gelatinous, bluish up to 5-11 μm in diameter. Individual envelopes around trichomes sometimes clearly visible, particularly in peripheral parts, colourless or yellowish.

***N. shensiense* Jao (Fig. L)**

Colonies floating, small, flat, irregular, gelatinous, and green to blue-green. Sheaths gelatinous and blue-green in colour. Trichomes flexuous or curved, densely entangled.

***N. sphaericum* Vaucher ex Bornet et Flahault (Figs. N & Q)**

Colonies oval or spherical with thin periderm, smooth, yellow-brown or olive-green, up to 2-3 cm in diameter, \pm free living on sandy or muddy bottom, later sometimes irregular

or slightly flattened, with densely entangled trichomes. Sheaths visible only at the periphery, yellowish-brown, but around trichomes in the colonial center mostly lacking.

***N. verrucosum* [Linnaeus] Vaucher ex Bornet et Flahault (Fig. R)**

N. rothii Agardh ex Bornet Flahault; *N. verrucosum* Agardh ex Bornet Flahault

Young colonies spherical, later composed of hemispherical and lobate microscopic formations, warty on the surface, hollow when old, attached firmly to the stony substrate, olive-green, yellow-brown to dark-brown, with firm periderm, usually up to 5 cm in diameter, agglomerations up to 10 cm in diameter. Filaments flexuous, densely entangled, particularly near the colonial margin. Sheaths usually present, thick, colourless and confluent in the center, yellow or yellow-brown in the colonial margin.

Table 1 : Showing the meteorological data of the study site Grassland Biotope during January-December-2022:

Parameters	Seasons			
	Winter	Summer	Monsoon	Post-monsoon
Temperature ($^{\circ}\text{C}$)	8.3-25.5	13.8-39.4	27.2-32.2	11.1-32.2
Rainfall (mm)	5.08-20.32	15.24-45.72	48.26-220.98	5.08-55.88
Humidity (%)	0-1	1-36	37-99	75-01
Solar energy (kWh/m^2)	3.6-5.3	5.3-7.6	7.6-5.7	5.7-3.9

Table 2 : Showing the Soil parameters of the study site Grassland Biotope:

Soil Parameters				
pH	Conductivity	Nitrate	Nitrite	Ammonia
7.4	35 mS/m	4.3 ppm	0.3 ppm	0.5 ppm

Table 3 : Showing the morpho-metric details of studied *Nostoc* sp.

S. N.	Species	Trichomes										
		Vegetative Cell				Colour	Heterocysts			Akinetes		
		Apical		Intercalary			Shape	Size, Position (diameter)	Colour	Shape	Size (μm) (diameter)	Colour
		Shape	Size (μm) Wide	Shape	Size (μm) Wide							
1.	<i>Nostoc calcicola</i>	Rounded	2-3.5	barrel	2-3.8	Pale blue-green	Spherical	4-5	Pale blue-green	Spherical	6-7 x 4-5	Yellow cell wall
2.	<i>N. cycadaeae</i>	Oval shape	4.5-6.5	Oval	5.9-6.8	Yellowish	Oval	8.2-13.8 x 9.4-11.8	Yellowish green	Widely oval	5-9 x 6-10.7	Pale blue green, greenish brown
3.	<i>N. ellipsosporum</i>	Cylindrical	4-5	Cylindrical	4-5	Olive-green	Spherical	6-14 x 6-7	Olive-green	Ellipsoidal, cylindrical	11-19 x 6-8	Colorless cell wall
4.	<i>N. gelatinosum</i>	Cylindrical	4.5-9	Cylindrical	± 4	Yellowish brown	Oval	6-10 x ± 5	Yellowish brown	Elongated oval	8-14 x 6-8	Pale brown cell wall
5.	<i>N. linckia</i>	Oval shape	3-4.5	Barrel	3.5-5	Pale blue-green	Spherical	4.5-6	Yellow-brownish	Spherical-oval	7-8 x 6-7	Brownish
6.	<i>N. microscopicum</i>	Rounded	5.5-8	Barrelshaped	5-8	Blue-green	Spherical	6.5-10	Yellowish	Spherical	9-15 x 6-7	Olive-green
7.	<i>N. paludosum</i>	Rounded	2.5-6.1	Barrelshape, shorter than wide	3-5	Greyish blue-green	Spherical	(3.5) 4-8.5	Yellowish brown	Oval,	5-9 x 4-6.5	Colorless cell wall
8.	<i>N. Pruniforme</i>	Rounded	4.5-6.5	Barrel	4-6.7	Pale blue-green	Spherical	6-7	Pale blue-green	Rare, but Spherical	10	Pale blue-green
9.	<i>N. shensiense</i>	Oval shape	3-5	Spherical	4.5-5.5	Blue-green and granular content	Sub Spherical	5.5-7 6.8 x 7	Pale blue-green	Sub spherical	6.5-7.5	Pale blue-green
10.	<i>N. sphaericum</i>	Rounded	3-4.5	Barrel	3.5-5	Yellowish green	Spherical or oval	6-8 x 4-6	Yellowish	Oval	6-8 x 4-6	Yellowish
11.	<i>N. verrucosum</i>	Cylindrical	3-5	Barrel	3-4.5	Yellow-brown	Spherical	± 6	Yellowish green	Oval	± 7 x 5	Yellow cell wall

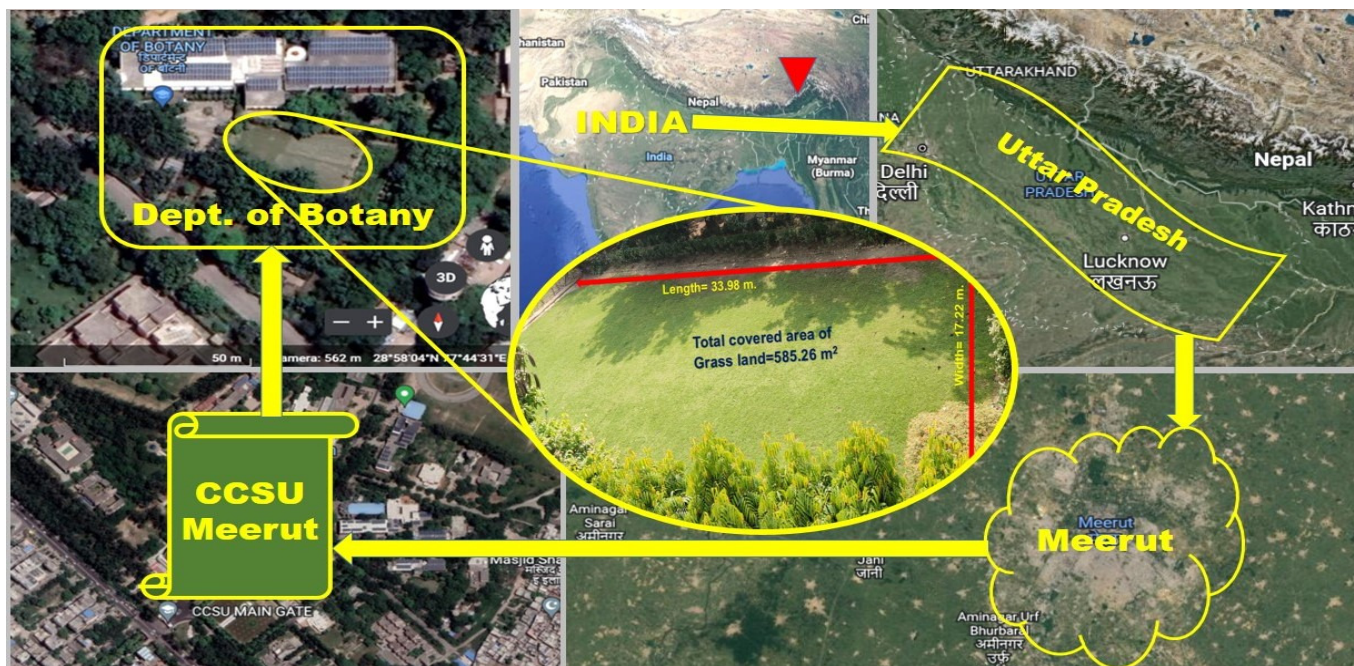


Fig. 1 : Showing the location map of the study area

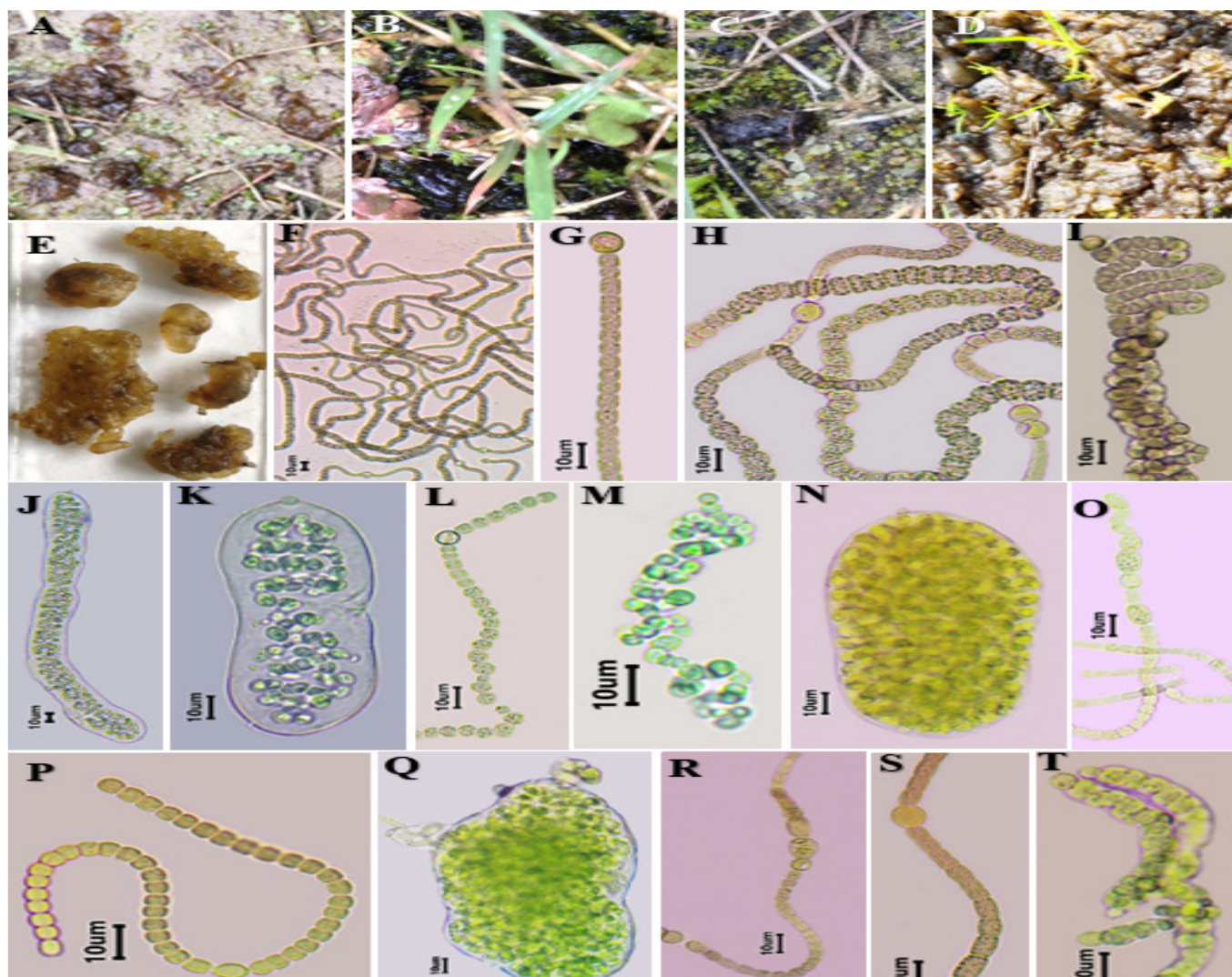


Fig. 2 : (A-E): Growth of *Nostoc* species in nature: A. *Nostoc calcicola*, B. *N. linckia*, C. *N. paludosum*, D&E. *N. gelatinosum*, (F-T): Morphological details of *Nostoc* species in culture condition: F-H. *N. cycadeae*, I. *N. paludosum*, J-K. *N. microscopicum*, L. *N. shensiense*, M. *N. calcicola*, N&Q. *N. sphaericum*, O. *N. ellipsosporum* P. *N. linckia*, R. *N. verrucosum*, S. *N. gelatinosum*. T. *N. pruniforme*.

Discussion

Cyanobacteria occur in all the terrestrial as well as aquatic habitats including tropical paddy fields, damp soil, in north polar zones and south polar zones. They play very important role in almost every ecosystem as primary colonizer and particularly heterocystous forms including *Nostoc* contribute significantly as nitrogen fixer and maintain the soil fertility year by year for the sustainability of the growing vegetation. The present study site was Grassland and it is dominated by the grass vegetation of *Cynodon dactylon*. The study area was closely observed for last three years (Junly-2020 to June-2022) for the diversity and annual recurrence of the *Nostoc* species in the grassland biotope.

The genus *Nostoc* is a filamentous, unbranched, with gelatinous sheathed and heterocystous cyanobacterium (Dodds *et al.*, 1995). They can fix atmospheric nitrogen into the soil to improve fertility, due to presence of their specialized cells called heterocysts (Whitton *et al.*, 1979). The trichomes of *Nostoc* are differentiated into vegetative cells, akinetes and with terminal or intercalary heterocysts (Komárek and Anagnostidis, 1989). A lot of work on cyanobacterial diversity has been done in Bharat. Many algologists explored the diversity of different groups of cyanobacteria from different habitats of Bharat and they include Parukuty (1940); Mitra (1950); Desikachary (1959); Singh (1961); Kamat (1974); Tiwari (1972); Bendre and Kumar (1975); Tiwari *et al.* (1979, 2007, 2009); Tiwari and Pandey (1976); Roger and Kulasooria (1980); Anand (1989); Santra (1993); Kant *et al.* (2004, 2005, 2006, 2008, 2012, 2014, 2020b); Tiwari, O.N. *et al.* (2004); Tiwari and Chauhan (2006); Dhingra and Ahluwalia (2007); Singh *et al.* (2008); Dwivediet *al.*(2010); Kant (2011, 2013); Roy and Keshri (2014); Adhikari and Baruah, (2015); Sarma *et al.* (2020); Tandon *et al.* (2021); Singh *et al.* (2022) and Neha *et al.*(2021). Many algologists investigated Nostocales, mainly Brühl and Biswas (1922); Sen (2005) and Keshri and Chatterjee (2010). On the basis of Morphology and 16s rRNA gene sequence Singh *et al.* (2014) identified total 20 algal species belonging to eleven genera of cyanoprokaryotes from different region of Himachal Pradesh. Minj *et al.* (2017) explored cyanobacterial diversity and isolated 22 heterocystous algal strains from Rice fields of Chhattisgarh, Bharat. Das and Adhikary (2012) explored algal diversity from Odisha, Bharat and reported total 75 algal strains including 15 Cyanobacteria. Samad and Adhikary (2008) reported 57 species of blue green algae from different monuments and sub-aerial habitats of India. Keshari and Adhikary (2014) reported 24 species of cyanobacteria on the basis of 16S rRNA gene sequencing from different sub-aerial habitats of Bharat. Richa and Sinha (2015) worked on two strain of *Nostoc* (HKAR-2 and HKAR-6) for biochemical screening and enhance the production of sunscreening pigment mycosporine-like amino acid (MAA). Total 677 algal taxa reported by Desikachary (1959) from Bharat. Srinivasan (1965) reported 326 algal strains from Bharat. Komárek (2013) listed 85 taxa of algae including 23 species of *Nostoc*. Desikachary (1959) listed 23 strains of *Nostoc* from Bharat. Tiwari *et al.* (2007) reported 55 taxa of the genus *Nostoc* from Bharat. Kant *et al.* (2020a) reported total 17 species of *Nostoc* from Tripura, Bharat and they revealed that more than 66% species of *Nostoc* grow on moist soil or uncultivated lands. Our observation of the present study and recurrence of the *Nostoc* species in the Grassland biotopes

also supports the findings of Kant *et al.* (2020a). Although a lot of work has been undertaken by the phycologists in Bharat but there are many unexplored regions and still have too much scope for biodiversity exploration in the Western Uttar Pradesh of the country. In the investigation, we report total eleven species of *Nostoc*, *N. cycadeae*, *N. paludosum*, *N. microscopicum*, *N. shensiense*, *N. calcicola*, *N. sphaericum*, *N. elliposporum*, *N. linckia*, *N. verrucosum*, *N. gelatinosum* and *N. pruniforme* from the ignored Grassland Biotope of Meerut, Uttar Pradesh, Bharat. Study also suggested that the uncultivated grassland biotopes have rich flora of Nostocales, Cyanoprokaryotes .

Conclusion

The present study contributes to our knowledge on diversity of the genus *Nostoc* from grassland biotopes of Meerut. The soil fertility of grassland biotopes can be maintained for long time by inoculation of the bio-inoculants of *Nostoc* strains.

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