

**Morphological, biochemical and molecular
characterization of *Nostoc* spp. and their
assessment as biofertilizer for rice**

Thesis submitted to the
University of Calicut in partial fulfillment of the
requirements for the Degree of

DOCTOR OF PHILOSOPHY IN BOTANY

By

FARHAD V. P.



**BIOTECHNOLOGY DIVISION
DEPARTMENT OF BOTANY
UNIVERSITY OF CALICUT
2024**

**UNIVERSITY OF CALICUT
DEPARTMENT OF BOTANY**



Dr. A. Yusuf
Professor
Biotechnology Division

Calicut University (P.O.)
673635
Kerala, India
Mob: 9497192730

Date:

CERTIFICATE

Certified that the Ph.D. thesis entitled “**Morphological, biochemical and molecular characterization of *Nostoc* spp. and their assessment as biofertilizer for rice**” is an authentic record of the original research work accomplished by **Ms. Farhad V. P.**, under my supervision at the Biotechnology Division in the Department of Botany, University of Calicut and that no part of has been published earlier for the award of any other degree or diploma. Also certified that the contents in the thesis are subjected to **Plagiarism Check** using the software **iThenticate** and that no text or data is reproduced from other’s work.

Dr. A. Yusuf

DECLARATION

I hereby declare that the work presented in the thesis entitled **“Morphological, biochemical and molecular characterization of *Nostoc* spp. and their assessment as biofertilizer for rice”** is based on the original work done by me under the guidance of Dr. A. Yusuf, Professor, Department of Botany, University of Calicut and has not been included in any other thesis submitted previously for the award of any degree. The contents of the thesis are undergone plagiarism check using iThenticate software at C.H.M.K. Library, University of Calicut, and the similarity index found within the permissible limit. I also declare that the thesis is free from AI generated contents.

Calicut University
Date:

Farhad V. P.

Dr. A. Yusuf
Professor
Department of Botany
University of Calicut

ACKNOWLEDGEMENT

Nothing comes worthwhile in life without struggle. It would not have been possible to write this doctoral thesis without the help and support of the kind people around me who, in their ways, have helped me to overcome difficult situations.

*It is my utmost pleasure to express deep sense of gratitude to my Ph.D. supervisor **Professor A. Yusuf**, Department of Botany, University of Calicut, Kerala for his constant support, intellectual guidance and continuous motivation. It would never have been possible for me to take this work to completion without his incredible support and encouragement. I am extremely privileged to be his student and express my deep-hearted gratitude to him for his patience and for contributing his valuable time and energy without which completing this research would have remained a distant dream. It was a great privilege and honour to work and study under his guidance. I was able to carry out my work entirely due to his unflinching support, enlightening discussion and the drive to go ahead by surpassing all hurdle. I could not have imagined having a better advisor and mentor for my Ph.D. study. I would give my warmest thanks to him for his patience, motivation, enthusiasm and immense support he extended throughout my research work and also during this thesis writing. I shall always be indebted to you my beloved Sir.*

*I am also grateful to **Prof. C.C. Harilal, Prof. Jose T. Puthur, Prof. V.V. Radhakrishnan, Prof. Santhosh Nampy, Prof. John E. Thoppil and Prof. K.M. Jayaram**, Heads, Department of Botany, University of Calicut for providing necessary facilities and support throughout my research period.*

*I gratefully extend my sincere thanks to **Prof. K. V. Mohanan, Prof. P. Manimohan** and all the other teaching and non-teaching staff members of Department of Botany, University of Calicut for providing facilities during the research period.*

*I extend my thanks to **Dr. P.M. Prakasan** (Former Librarian) and **Mrs. Soni Krishnan**, Librarian, Department of Botany, University of Calicut for providing*

library facilities. My heartfelt gratitude to **Mr. Santhosh Mithra** and **Mr. Shaji N.B.**, Art and Photography Division, University of Calicut, for their valuable assistance in imaging the photographs used in this study.

This could have never happened without the unending co-operation of my fellow researchers. I was fortunate enough to enjoy wonderful moments with my colleagues. It's my great pleasure to express my heartfelt thanks to my former and present fellow lab-mates, **Dr. Showmy K. S**, **Dr. Aparna M. B**, **Dr. Pravishya P**, **Dr. Santhoshkumar R**, **Dr. Lins Simon**, **Mr. Habeebmon K. C**, **Mr. Jishin Prakash T. S**, **Mr. Ranjith N. K** and **Ms. Sreena K.** for their support and encouragement. I would like to thank **Mrs. Raseena M.** for her tremendous help, love, care and support, **Dr. Deepti M.** the most down to earth Postdoctoral fellow I had ever met in life, for her sisterly love, care, encouragement, innumerable and unflinching help at in my thesis writing period, **Ms. Nimisha**, **Ms. Devika**, **Ms. Theertha**, and **Ms. Abhinna**, for their wholehearted co-operation, joyful moments and love.

I am deeply indebted to **Dr. Maya R.**, for all her generous support, constant endorsement, help and inspiration throughout my work. I will always oblige to her for the fruitful discussions, camaraderie and the moral support which helped me successfully complete my research. She created a friendly atmosphere and provided me with all her scientific expertise, which helped me carry out my work with ease. I am also grateful to **Ms. Avani A. Suresh**, **Mrs. Fathima Nasreen Azhar** and **Mrs. Sreelakshmi** (my lovely sisters) for their affection and everlasting help and irrevocable support. You people make my research period more happy and funny. A special word of thanks to **Habeeb** and **Jishin** for their ambient support while preparing plates of images. It's my pleasure to acknowledge **Mrs. Bindhu**, Staff, ICPB for her extensive help, moral support and tasty food.

I fondly cherish the friendship I had and take this opportunity to thank each one of them. I express my heartfelt gratitude to my best friends who have been my pillars of strength, **Dr. Surekha Y. Pawar** and **Dr. Neethu K.** who always stood with me and helped me to overcome all the difficulties I had to face while completing my work. I am also thankful to them for their emotional and financial supports during

*my Ph.D. period. Without their help, the journey would have been more difficult. I am extremely happy to express my wholehearted love and gratefulness to my best friend **Dr. Manu Philip** for the constant caring, love and encouragement. You accompanied me during all the collection trips and made every journey a memorable one. Thanks for your unconditional efforts. You are the most thoughtful person to work with. Accept my hearty gratitude for your understanding and support during my difficult times. Thank you for being my best friend.*

*A big thanks to **Mrs. Swetha K., Dr. Veena V., Mr. Aneesh, and Dr. Arun T. Ram** for their support, assistance, love, encouragement and moral support. I am taking this opportunity to express my gratitude to my friends **Mrs. Saji Das, Mr. Sajin Babu, Dr. Resmi, Dr. Geethika and Mrs. Nimly**, for their friendly support. I am also thankful to all members of the Genetics and Plant Breeding Division and Environmental Studies Division for their friendly support and encouragement during my work. My sincere appreciation and heartfelt gratitude to all research scholars in the Department of Botany, University of Calicut for their support and care. I express my heartfelt gratitude to **Mr. K. Rajesh** & co-workers of Bina Photostat, Villuniyal for their tireless support in finalising the thesis.*

*Always remain deep in my heart, the support given by my family members throughout the work and for their love and continuous spiritual and material support and being with me in all my pains and gains. I can barely find words to express my profound sense of gratitude to my beloved uppa (**Mr. Abbas V.P.**), ichappa (**Mr. Zakariya K.**), aunty (**Mrs. Risana K.**), uncle (**Mr. Ashik K.**) and lailatha (**Mrs. Laila**) and ichappa (**Mr. Latheef**) for their unconditional love, fidelity, endurance and encouragement throughout my research work and lifting me uphill to this phase of life. I would like to pay high regards to my siblings, **Mrs. Thahani, Mr. Farhan, Mrs. Rehaba Sona, Mrs. Nidha Shekha and Mr. Zafan**, Sister in law (**Mrs. Nasiyath**) and all other family members and relatives for their sincere unconditional love, support, motivation and inspiration. A special word of thanks to **Mummy, Amma, Netaji and Chettan** for their love, support, encouragement and prayers.*

*This thesis would not have been possible without the sacrifice and immense support of my husband, **Mr. Noushad P.K.** He was always by my side at times I needed him most, inspired me to follow my dreams and I am deeply obliged to him. You are my role model to carry out my research to the best of my abilities. My words are not sufficient to thank you for the love and care you showered upon me and being an amazing person and a pure blessing to my life. My affectionate thanks and hugs to my lovely boy, **Adam Ronaq Noushad** who served as my inspiration to pursue this undertaking. The innocent smile and childish pranks of him enabled me to forget all my mental and physical fatigue and to engage myself in my endeavour with fresh enthusiasm. I can never underestimate the irrevocable support, unconditional love, encouragement and prayers I received from my **Umma** (mother in law-God's gift to me). I know how you struggled to take care of Aadhu during my research period. I don't know how to express my sincere thanks to you. Thank you so much umma.*

*I acknowledge with highest sense of regards, my mom **Mrs. Asma K.** who had taught me the value of education from my childhood. You are gone but your belief in me has made this journey possible. I dedicate this thesis for you mom.*

I am extremely obliged and thankful to all people who have contributed, directly or indirectly to the successful completion of the present research work.

My greatest regards to the Almighty for bestowing upon me the courage to for the complexities of life and complete thesis successfully.

Farhad V. P.

ABBREVIATIONS

%	:	Percent
~	:	Approximately
°C	:	Degree Celsius
16S rRNA	:	16S ribosomal ribonucleic acid
AHCA	:	Agglomerative Hierarchical Clustering Analysis
AIC	:	Akaike Information Criterion
ANOVA	:	Analysis of variance
BG-11 medium	:	Blue-Green 11 medium
BI	:	Bayesian Inference
BIC	:	Bayesian Information Criterion
BLAST	:	Basic local alignment search tool
bp	:	Base Pair
BPB	:	Bromophenol blue
Chl	:	Chlorophyll
CTAB	:	Cetyltrimethylammonium bromide
CuSO ₄	:	Copper Sulphate
ddNTPs	:	Di-deoxy nucleoside tri phosphate
ddH ₂ O	:	Double distilled water
DNA	:	Deoxyribonucleic acid
DNA	:	Deoxyribonucleic acid
dsDNA	:	Double stranded DNA
DTT	:	Dithiothreitol
EDTA	:	Ethylenediamine-tetra-acetic acid
et al.	:	et alia (and others)
EtBr	:	Ethidium bromide

etc.	:	et cetera (and other things)
E-value	:	Expect Value
FASTA	:	FAST Alignment
Fig.	:	Figure
g	:	Gram
GC-MS	:	Gas Chromatography Mass Spectrometry
GP	:	Germination Percentage
h	:	Hour
H ₂ O	:	Water
HCl	:	Hydrochloric acid
HPLC	:	High Performance Liquid Chromatography
hrs	:	Hours
K ₂ HPO ₄	:	Dipotassium hydrogen phosphate
Kb	:	Kilobyte
KNO ₃	:	Potassium nitrate
l	:	Litre
M	:	Mole/litre
MEGA7.0	:	Molecular evolutionary genetics analysis 7.0
mg	:	Milligram
mg/g	:	Milligram per gram
mg/ml	:	Milligram per millilitre
MgCl ₂	:	Magnesium chloride
min	:	Minute (s)
min	:	minutes
µg	:	Microgram
µl	:	Microlitre
µg/µl	:	Microgram per millilitre

ML	:	Maximum Likelihood
mm	:	Millimetre
MULTALIN	:	Multiple Alignment
Na ₂ CO ₃	:	Sodium carbonate
NaCl	:	Sodium Chloride
NaOH	:	Sodium hydroxide
NCBI	:	National Center for Biotechnology Information
ng	:	Nanogram
NJ	:	Neighbour Joining
O.D	:	Optical density
PAUP	:	Phylogenetic Analysis Using Parsimony
PCA	:	Principal component analysis
PCR	:	Polymerase Chain Reaction
PMSF	:	Phenylmethylsulfonyl fluoride
pp.	:	Page
PVP	:	Polyvinylpyrrolidone
RNA	:	Ribonucleic acid
RNase	:	Ribonuclease
rpm	:	Rotations per minute
SDS	:	Sodium dodecyl sulphate
SE	:	Standard Error
sec	:	Second
sp.	:	Species (singular)
spp.	:	Species (plural)
sq km	:	Square kilometre
TAE	:	Tris acetate-EDTA
TCD	:	Thermal Conductivity Detector

TE	:	Tris EDTA
Tm	:	Melting temperature
UPGMA	:	Unweighted Pair Group Method with Arithmetic Mean
v/v	:	Volume/volume
viz.	:	Namely
vol	:	Volume
w/v	:	Weight per volume

CONTENTS

<i>Chapter</i>	<i>Title</i>	<i>Page No.</i>
1	Introduction	
2	Review of Literature	
3	Materials and Methods	
4	Morphological characterization of <i>Nostoc</i> species from Kerala	
5	Molecular characterization and phylogenetic analysis of selected <i>Nostoc</i> species.	
6	Biochemical characterization and phylogenetic analysis of <i>Nostoc</i> spp. using Gas Chromatography-Mass Spectrometry analysis	
7	Assessment of <i>Nostoc</i> spp. as a biofertilizer for rice varieties Jyothi and Annapurna	
8	Summary and Conclusions	
9	Recommendations	
	References	
	Appendices	

CHAPTER 1 INTRODUCTION

The increase in the World's population and concerns related to food security, disease outbreaks, global warming, and other environmental issues necessitate a sustainable solution for high productivity of food grains. Cyanobacteria are one of the most successful and sustained organisms during evolution. They are photosynthetic prokaryotes taxonomically classified as members of the Kingdom Bacteria. Historically, cyanobacteria were considered as plants or plant-like organisms and were termed "Cyanophyta", "Cyanophyceae" or "Blue-green algae". The detailed cytological and biochemical studies performed by bacteriologists on axenic cultures led to the proposal to change the common name of "Cyanophyceae" into "Cyanobacteria" and classified as bacteria. Cyanobacteria display some of the most sophisticated morphological differentiation among the bacteria, and many species are truly multicellular organisms. During their evolution, through early symbiotic associations, they gave rise to the plastids of algae and higher plants.

Cyanobacteria are the first organisms to evolve elemental oxygen as a by-product of photosynthesis and thus make the Earth's environment habitable for life. They are the most abundant and potent primary producers on Earth and significant contributors to the sudden increase in atmospheric oxygen during the Great Oxidation Event (Kauff and Budel, 2011). Apart from being the primary oxygen producers, most of them are also efficient in atmospheric nitrogen fixation, which makes them extremely important components of nutrient-deprived ecosystems and extreme environments all over the Earth. Cyanobacteria significantly contribute to the global primary production of the oceans and become dominant primary producers in many extreme environments (Garcia-Pichel, 2009).

1.1 Morphological and ecological diversity of cyanobacteria

Cyanobacteria represents a phylogenetic old photosynthetic group with several structural features including, cell wall made of muramic acid and

glycopeptides, single thylakoid lamellae, light-harvesting antennae, polyphosphate bodies, cyanophycin granules, polyhydroxyalkanoate granules, carboxysomes, lipid bodies, the presence of water-soluble phycobilin pigments as accessory pigments, gas vesicles, DNA-containing areas, and ribosomes (Chittora *et al.*, 2020). ‘Cyan’ means blue-green colour due to combining blue pigment (phycocyanin) and chlorophyll-a pigment. However, not all cyanoprokaryotes are blue-green. Yellow-green, grey-green, black-grey, green, and red-colour are various pigments present in cyanobacteria. Their structural complexity prompted their taxonomic distinction mostly based on the morphological characters.

Morphologically, they vary from unicellular to multicellular filamentous branched or unbranched forms. Cyanobacteria possess intercellular connections or microplasmodesmata, considered a sign of multicellularity. Cells of cyanobacteria contain chlorophyll-a, which participates in oxygenic photosynthesis and carotenoids act as photoprotectants to protect chlorophyll-a from oxidative degradation. Additionally, some genera exhibit morphological and functional cell differentiation such as heterocytes (nitrogen fixing cells) and akinetes (dormant cells). Their distribution, position and number are significant morphological characters used to identify the cyanobacterial genera and species.

Cyanobacteria are a diverse group of organisms that thrive in almost all habitable ecosystems on Earth. They can adapt to various soils and environments. They are distributed ubiquitously and found in lakes, ponds, springs, wetlands, streams, rivers and many terrestrial environments and play a major role in the nitrogen, carbon and oxygen dynamics of many aquatic environments. Cyanobacteria also occur in extreme physical conditions like temperature, light, pH, salinity and desiccation, and nutritional availability (Gaysina *et al.*, 2019). Tropical regions such as those in India provide favourable conditions for the abundant growth of cyanobacteria in natural ecosystems such as different soil types, freshwater bodies, saline backwaters, estuaries and hyper saline saltpans (Rajakumar, 2004; Chellappa *et al.*, 2004).

Aquatic cyanobacteria are divided into two ecological groups: planktonic cyanobacteria (float freely on the water surface) and benthic cyanobacteria (adhere to the submerged solid surfaces like sediments, rocks, stones, algae, and aquatic plants). Cyanobacteria grow on exposed rock surfaces of monuments as epiliths, hypoliths and endoliths, and impart a blackish brown colouration to the rock surfaces (Anand *et al.*, 2019). They are capable of colonizing bare areas of rocks and soil in which higher plant vegetation is restricted or absent. Cyanobacteria have established symbioses with a few invertebrates (echinoid worms and marine sponges), protists, bacteria and plants. Among plants, heterocystous cyanobacteria form symbiotic associations with bryophytes (mosses, liverworts, and hornworts), pteridophytes (*Azolla*), gymnosperms (cycads) and angiosperms (*Gunnera*).

1.2 Genus *Nostoc*

Nostoc is a heterocystous, filamentous cyanobacteria, widely distributed in the free-living state. The genus differs from other heterocystous cyanobacteria in that the trichomes with heterocytes and akinetes are embedded in the colonial mucilaginous sheath. Due to their existence as well-known microbial pioneers in harsh terrestrial ecosystems, they share a range of specific characteristics when confronted with extreme conditions (e.g., UV stresses and drought), such as producing large quantities of exopolysaccharides and mycosporine-like amino acids (Sinha *et al.*, 2001). Despite being first reported in the 19th century with type species *Nostoc commune* Vaucher ex Bornet et Flahault, its taxonomy remains complicated and problematic due to its heterogeneity, vast biogeographical distribution and complicated life cycle. *Nostoc* is a polyphyletic genus, with wide genetic diversity and more new taxa are expected in the future. *Halotia*, *Mojavia*, *Desmonostoc*, *Komarekiella*, *Compactonostoc* and *Desikacharia* are the new genus split from the genus *Nostoc* (Jiminez *et al.*, 2023)

More than 250 species of *Nostoc* have been described worldwide to date; however, they have not yet been sequenced or described in the literature. They are taxonomically complicated warranting nomenclature revision (Komarek, 2013). After an extensive study of *Nostoc* strains from numerous culture collections all

across the globe and the newly identified isolates, it is more than well-established that this genus is complex, diverse and highly heterogeneous and thus has many morphotypes (Rehakova *et al.*, 2007). *Nostoc* spp. are associated symbiotically with diverse taxonomic groups of organisms (such as algae, bryophytes, pteridophytes, gymnosperms and angiosperms), benefiting their hosts with nitrogen in the form of nitrates (Rai and Bergman, 2000). In addition to nitrogen fixation (Adams and Duggan, 2012), *Nostoc* spp. can also release phytohormones such as IAA and cytokinins which improve plant growth (Hussain *et al.*, 2010).

1.3 Ecology and diversity of the genus *Nostoc*

Nostoc spp. form macroscopic or microscopic colonies and are common in both terrestrial and aquatic habitats. The success of the genus *Nostoc* in terrestrial habitats is related to its ability to remain desiccated for months or years and fully recover metabolic activity within hours to days after rehydration. *Nostoc* is filamentous, forms trichomes that are embedded in exopolysaccharides mucilaginous sheath generally divides by binary fission in one plane only and does not exhibit true branching. Heterocytes and akinetes are formed, and reproduction occurs by short, motile trichomes called hormogonia. *Nostoc* spp. sometimes forms a mature colony of trichomes entirely within a firm surface pellicle, whereas others form looser colonies and also produce aggregated filaments. The basic characteristics of *Nostoc* spp. are i) well-developed hormogonial phases in the life cycle ii) terminal heterocytes developing from terminal hormogonial cells after settling and cessation of movement and iii) akinetes developing by differentiation of one vegetative cell midway between two heterocytes, followed by “centrifugal” formation of akinetes (Komarek and Anagnostidis, 1989).

Nostoc has been reported in many lentic habitats including benthos of ultra-oligotrophic lakes, swimming pools, high-altitude Kenyan lakes, Icelandic lakes, Antarctic lakes and streams. *Nostoc* spp. are associated primarily with the benthos but can form floating planktonic masses in aquatic environments. *Nostoc parmelioides* and *N. verrucosum* attach to the benthos and can be found over a wide geographic area. Paddy fields, one of the most common types of tropical and

subtropical wetlands in the world, are favourable to the growth of *Nostoc* spp. The ability of *Nostoc* spp. to withstand desiccation probably is crucial for success in a variety of terrestrial habitats. The members of the genus require special adaptations to obtain sufficient light, nutrients and dissolved inorganic carbon (DIC) in water and to survive in the extreme variations in temperature, water supply and irradiance on soils and barren rock surfaces (Yu and Liu, 2013). The extracellular polysaccharide (EPS) sheaths act as a buffer zone between the cell and the environment and contribute to desiccation tolerance.

1.4 Molecular characterization of cyanobacteria

1.4.1 DNA isolation and purification

The invention of molecular biology techniques brought a plethora of tools that have been used to study species diversity and evolution. DNA isolation and polymerase chain reaction are the basic molecular biology techniques. DNA-based taxonomic evaluation of cyanobacteria is the most reliable method. A combination of light microscopy and analysis of 16S rRNA gene sequences provides the most powerful approach for studying the diversity of photosynthetic prokaryotes *in vivo* and *in vitro* (Lopes-Corte's *et al.*, 2001). While DNA isolation is a routine procedure for most organisms including bacteria, viruses, fungi, parasites, insects, mammals and plants, it is, for various reasons, a rather challenging one when performed on cyanobacteria. Even though many DNA isolation procedures for cyanobacteria have already been reported in the literature (Mazur *et al.*, 1980; Kallas *et al.*, 1983), their respective efficiencies can greatly vary from one species to another. For the isolation of DNA from cyanobacteria, specific protocols are needed even for each genus since their cell walls contain large quantities of cellulose, pectins, murein and xylose as well as synthesis and excretion of compounds (particularly complex polysaccharides and proteins) forming the mucous envelope. The protein S layer present in cyanobacteria interferes with cell lysis and DNA isolation, leading to the isolation of a small quantity of DNA that is also contaminated (Fiori *et al.*, 2000; Kaczynska *et al.*, 2013). Such problems are severe in the genomic DNA isolation of filamentous

cyanobacteria. Most of the heterocystous cyanobacteria contain higher quantities of mucopolysaccharides that make DNA extraction very challenging.

DNA isolation procedures in cyanobacteria include CTAB-based method (Ausubel *et al.*, 1995), phenol chloroform-based extraction (Morin *et al.*, 2010; Nubel *et al.*, 2000), modified phenol extraction method for filamentous cyanobacteria (Kaczynska *et al.*, 2013), xanthogenate-SDS nucleic acid isolation (Tillett and Neilan, 2000), etc. However, the CTAB-based method is one of the best methods for the extraction of cyanobacterial DNA. In cyanobacteria, this method helps to remove the proteins and polysaccharides using CTAB in the presence of NaCl. It also improves the efficiency of the extraction procedure, and the final purity of the extracted DNA (Morin *et al.*, 2010).

1.4.2 Quantification of DNA using agarose gel electrophoresis and spectrophotometer

Agarose gel electrophoresis is a technique used to separate DNA fragments of varying sizes ranging from 100 bp to 25 kb. This molecular method is used to determine the quantity and approximate size of DNA molecules. The principle of DNA quantification by agarose gel electrophoresis is based on the movement of DNA molecules through a gel matrix in response to an electric field. The DNA from the samples to be quantified is mixed with a loading buffer containing a tracking dye, which helps in detecting the migration of DNA. The gel is stained with an intercalating dye, ethidium bromide which binds to DNA and allows visualization of the DNA bands under UV. The size of the DNA fragments can be estimated by comparing their migration distance with that of the DNA ladder (Lee, 2012).

The purity and concentration of DNA can be measured using a NanoDrop spectrophotometer. The micro-volume capability of NanoDrop spectrophotometers allows the researchers to fast and easily run quality control checks of nucleic acid. DNA concentration is determined by measuring the absorbance at 260 nm (A₂₆₀). The purity of the DNA sample is also checked using the UV absorbance at 260 nm and 280 nm. A ratio of less than 1.8 indicated that the DNA samples were contaminated either with proteins or with phenol. Similarly, the ratio of A₂₆₀/A₂₃₀

was also determined for the assessment of nucleic acid purity. The expected ratio of 260/230 is commonly in the range of 2.0-2.2.

1.4.3 16S rRNA

The 16S rRNA gene is one of the most informative and highly studied molecular markers for identifying cyanobacteria and inferring phylogenetic relationships. The 16S rRNA is the “gold standard” in the systematics of prokaryotes (Stackebrandt and Ebers, 2006). This gene has a conserved function and is universally present in cyanobacteria (Ludwig and Klenk, 2001) and most of the novel taxa have been identified using the 16S rRNA sequences (Tindall *et al.*, 2010). Among the molecular methods, the analysis of the 16S rRNA gene sequences has proved to be a useful tool for elucidating the phylogenetic relationships among cyanobacteria. According to the cyanobacterial 16S rRNA gene, the nucleotide sequences are approximately 1.49 kb long. Studies of DNA divergence and gene conversion tract confirmed that the 16S rRNA gene holds an important position in deciding the course of evolution in the microbial world and it is the most highly studied gene in phylogenetic analysis and is useful in determining the identification of cyanobacterial species.

1.4.4 PCR primers to amplify 16S rRNA gene sequences

Polymerase chain reaction (PCR) is a robust technique to selectively amplify a specific segment of DNA *in vitro*. It can be extensively modified to perform a wide array of genetic manipulations. PCR primers are single-stranded DNA (usually 18-25 nucleotides long) that match the sequences at the ends or within the target DNA and these are required to initiate DNA synthesis in PCR. Primer design is based on an alignment of all 16S rRNA sequences of cyanobacteria available from the Ribosomal Database Project and GenBank.

Nubel *et al.* (1997) designed the cyanobacteria specific primers CYA359F (5'-GGGGAATTTTCCGCAATGGG-3'), an equimolar mixture of CYA781R(a) (5'-GACTACTGGGGTATCTAATCCATT-3') and CYA781R(b) (5'-GACTACA GGGGTATCTAATCCCTTT-3') for specific amplification of 379 bp 16S rRNA

partial gene sequence. The 16S rRNA gene of heterocystous cyanobacteria was amplified using the primers A2 (5'-AGAGTTTGATCCTGGCTCAG) and S8 (5'-TCTACGCATTTACCGCTAC-3') (Giovannoni *et al.*, 1988), pA (5'-AGAGTTTGATCCTGGCTCAG-3') and B23S (5'-CTTCGCCTCTGTGTGCCTAGGT-3') (Gkelis *et al.*, 2005) and fD1 (5'-AGAGTTTGATCCTGGCTCAG-3') and rD1 (5'-AAGGAGGTGATCCAGCC-3') (Weisburg *et al.*, 1991). The universal primers 27F (5'-AGAGTTTGATCMTGGC-3') and 1492R (5'-TACCTTGTTACGACTT-3') also targeted 16S rRNA genes of cyanobacteria (Sekar *et al.*, 2009).

1.5 Phylogenetic analysis

Phylogenetic analysis is a method used to elucidate the evolutionary history and relationship among a group of organisms (Horiike, 2016). A phylogenetic tree or phylogeny visualizes a graphical representation of the evolutionary relationship between different organisms, showing the path through evolutionary time from a common ancestor to different descendants. A phylogenetic tree may be built either by character-based methods or distance-based methods. The character-based approach derives trees that optimize the distribution of the actual data pattern for each character. The commonly used character-based methods include the Maximum Parsimony (MP) method and Maximum Likelihood (ML) method. UPGMA (Unweighted Paired Group Method with Arithmetic mean), NJ (Neighbor Joining), ME (Minimum Evolution method), and FM (Fitch-Margoliash method) are built by distance-based method (Roy *et al.*, 2014).

Molecular Evolutionary Genetics Analysis (MEGA) is user-friendly bio-computational software designed for comparative analysis of homologous gene sequences either from multigene families or from different species with a special emphasis on inferring evolutionary relationships and patterns of DNA and protein evolution. The software provides many convenient facilities for the assembly of sequence data sets from files or online repositories, and it includes tools for the visual presentation of the results obtained in the form of interactive phylogenetic trees and evolutionary distance matrices (Kumar *et al.*, 2008). MEGA version 7 is one of the versions of MEGA which contains many sophisticated methods and tools

for phylogenomics. This version is optimized for use on 64-bit computing systems for analyzing larger datasets (Kumar *et al.*, 2016).

Phylogenetic Analysis Using Parsimony (PAUP) is a computational program for phylogenetic analysis using maximum likelihood, parsimony and distance-based methods. This phylogenetic program features an extensive selection of analysis options and model choices and accommodates DNA, RNA, protein and general data types. Usually, this software provides a wide range of pairwise distance measures, from simple absolute differences to more complicated model-based corrected distances. Furthermore, PAUP can use the minimum evolution and least-squares functions to evaluate trees under the distance criterion (Inyang *et al.*, 2022).

1.6 Phytochemistry of the genus *Nostoc*

The genus *Nostoc* is a rich source of secondary metabolites with diverse chemical structures and biological activities. Multiple bioactive compounds produced by different *Nostoc* species showed novel and a varied range of bioactivities and chemical structures including novel peptides (e.g. cyclic depsipeptides, cyclic peptides and lipopeptides), fatty acids, polyketides, alkaloids, amides, terpenes, phenolics, sulphates, carbohydrates and other organic chemicals. The genus *Nostoc* produces numerous peptides, including nostophycin, nostosin, nostopeptolides, nostoweipeptins, and banyasin. These peptides are identified only in this genus or sometimes only in one *Nostoc* strain. Nostocyclamide a macrocyclic peptide was first identified in *Nostoc* sp. and nostophycin has been reported from *Nostoc calcicola* (Juttner *et al.*, 2001). These natural bioactive constituents exhibited antimicrobial, antiviral, antifungal, immunosuppressive, anti-inflammatory, proteinase-inhibiting activities and anticancer activities which are notable targets of biomedical research. The ability of *Nostoc* spp. to produce biologically active compounds could play an important role in the survival of the genus in terrestrial and aquatic environments. Cyanotoxin a bioactive compound isolated from *Nostoc* sp. is used as a pesticide, algicide, fungicide, weedicide and herbicide. The genus *Nostoc* is a sole source of antimicrobial compounds such as nostocyclone A, nostofungicidin, nostocin A, ambigol A and B, hapalindoles, tjipanazoles and

scytophycins that exhibit fungicidal activity against plant pathogens (Singh *et al.*, 2016).

Nostoc spp. produced medium and very long chain hydrocarbons. Most of the fatty acids identified from *Nostoc* spp. were n-saturated, and mono-unsaturated but also di-, tri- and oligo-unsaturated. Most of the bioactive compounds are regarded as good candidates for drug discovery, with applications in agriculture. The secondary metabolites of *Nostoc* species supply novel and useful pharmaceuticals that are hard to produce artificially because of their structural complexity (Nowruzi *et al.*, 2018). The bioactive compounds can affect plant gene expression and encourage the accumulation of a wide range of compounds that help in the stimulation of plant growth and protection against biotic and abiotic stress (Han *et al.*, 2018; Pan *et al.*, 2019).

1.6.1 GC-MS analysis in metabolome profiling

Metabolomics deals with the study of high-throughput characterization of metabolites and is the study of the metabolite composition of cell types, tissues, organs, or organisms. Gas Chromatography and Mass Spectrometry (GC-MS) is an important analytical technique used to qualitatively and quantitatively determine an organic compound's purity and stability and to identify components in a mixture. This technique is widely used in metabolomics research. GC-MS is one of the most employed versatile analytical platforms for metabolic profiling due to its robustness, excellent separation capability, selectivity, sensitivity and reproducibility (Mastrangelo *et al.*, 2015). Chemical ionization (CI) and electron ionization (EI) are the two main forms of ionization used in GC-MS. Nowadays, most GC-MS methods in metabolomics use electron ionization. The advantages of EI-based GC-MS are ease of use (in terms of analysis time and operation), its capability to provide insight into compound identification and the availability of several mass spectral databases/libraries (Beale *et al.*, 2018).

GC-MS technique enables comprehensive and standardized metabolite analyses of complex biological samples. This technique is currently applied for the analysis of metabolites of cyanobacteria (Scwartz *et al.*, 2013) and helps in the

detection of volatile compounds. For metabolomics, the coverage of this technique has been extended by the chemical derivatization of polar metabolites, such as sugars, amino acids, organic acids, and phosphates into volatile products. GC-MS technology allows the analysis of a wide range of chemical metabolite classes in a single run, which was found to be especially convenient for the profiling of primary metabolism including uncharged primary metabolites and small secondary products. GC-MS methodology consists of three steps; sample preparation, operation considerations and data acquisition. This technique requires the analyte to have significant vapour pressure between 30 and 300°C. The mass spectrometer acquires a mass spectrum and visualizes the data as a histogram of the abundance of the ions that reach the detector according to their mass-to-charge ratio (m/z).

1.7 Statistical analysis of metabolomics data

1.7.1 Principal component analysis, hierarchical clustering and heat map of metabolites

Principal Component Analysis (PCA) is a multivariate data reducing technique used to describe (highlight the similarity and dissimilarity between the statistical units and the correlations between the variables), summarize (determine a small number of new variables, uncorrelated linear combinations of the originals with maximal variance) and visualize the information contained in a data set. It is an unsupervised pattern recognition method capable of translating experimental data into information about the similarity between samples and the respective variables responsible for it. Through the grouping of the correlated information into a single major component, this procedure reduces the dimensionality of the experimental data (Beebe *et al.*, 1998). This technique is very useful for identifying effective variables in a process involving the treatment of complex spectroscopic data (Anderson *et al.*, 2017). It is a widely used statistical technique for analyzing metabolomics data.

In PCA, a data set of interrelated variables is converted to a new set of variables known as principal components (PCs). The PCs are uncorrelated and the first few of these PCs retain most of the variation present in the entire data set. The

first PC has the greatest level of variation since it is a linear combination of all the actual variables. The second PC is also a linear combination of the actual variables in such a way that it has the most variation in the remaining PCs (Kerner *et al.*, 2022).

Clustering is most common unsupervised learning approach, in which data is segmented based on the similarity of instances (Hamidi *et al.*, 2019). So data within a cluster have high similarity in comparison to one another and are dissimilar from the members of other clusters. Hierarchical Clustering (HC) is a strategy of cluster analysis that seeks to build a tree based on their evolutionary relationship. In statistics and data mining, hierarchical clustering is a mechanism for grouping data at different scales by creating a dendrogram. Agglomerative Hierarchical Clustering (AHC) is one of the most common HC methods in which clusters are created bottom-up. In AHC, each instance is considered a cluster and then the clusters are merged to create larger clusters (Fernandez and Gomez, 2008).

Heat map clustering is an extensively used graphic display that simultaneously reveals row and column hierarchical cluster structure in a data matrix. Heat map consists of a rectangular tiling of a data matrix with cluster trees appended to its margins. Heat maps facilitate the inspection of row, column, and joint cluster structures within a relatively compact display area. Heat map clustering is useful for the two-dimensional representation of mass-spectrometry-based metabolomics experiments. The colour-coded matrix elements and adjacent dendrograms indicate functional relationships among variables and samples (Wilkinson and Friendly, 2009).

1.8 Cyanobacterial nitrogen fixation in paddy ecosystem

Rice (*Oryza sativa* L.) belongs to the family Poaceae, and is one of the most important and extensively grown cereal crops of South East Asia. It is an important staple food crop with high optimum calorie content consumed by more than 60% of the world's population and the speculative demand for five billion rice consumers by the year 2030 is to be met from available land and water resources (Khush, 2005). It requires large amounts of mineral nutrients, including nitrogen and phosphorus, for

their growth, development, and grain production (Sahrawat, 2000). Rice ranks among the highest, in terms of fertilizer usage, utilizing more than 60 million tonnes of nitrogen in India.

The successful production of rice depends upon the efficient and economic supply of nitrogen, an essential element required in higher quantities in comparison with other essential elements. Modern agricultural practices, heavily dependent on the application of synthetic fertilizers and pesticides, have raised environmental and health problems that include deterioration of soil fertility, overuse of land and water resources, environmental pollution, and an increased cost of agricultural production. Therefore, it has become necessary to look for alternative renewable sources to meet at least a part of the nitrogen demand of paddy. Promoting a stable symbiotic association between agriculturally important crop plants and nitrogen-fixing microorganisms is a topic of special interest for reducing current environmental and health problems, derived from the use of chemical nitrogen fertilizers. In rice fields, cyanobacteria are significant nitrogen contributors to the overall nutrient balance and improving the productivity of rice and they also enhance the soil fertility. The genus *Nostoc* is dominant in populations of fertilized and unfertilized rice fields in India.

Biological nitrogen fixation (BNF) is the conversion of inert dinitrogen (N_2) into a combined form that can be used by plants, through the catalytic process of the enzyme nitrogenase complex. The enzyme consists of two proteins such as dinitrogenase reductase encoded by the gene *nifH*, and dinitrogenase, encoded by genes *nifD* and *nifK*. The nitrogen fixation process is an energy costly process, and the activity of nitrogenase is tightly regulated at both transcriptional and post-transcriptional levels (Diez and Ininbergs, 2014). Cyanobacteria are the only microorganisms that fix nitrogen and simultaneously carry out oxygen-evolving photosynthesis under aerobic or microaerobic conditions (Kulasooriya and Magana-Arachchi, 2016). Rice is cultivated in larger wet to lowlands tropical areas that favour the growth of nitrogen-fixing cyanobacteria, by providing suitable temperature, light, humidity, nutrient and water facilities (Singh

et al., 2018). Repeated cropping of paddy followed by harvesting, maintains the level of nitrogen content in the soil, due to the presence of cyanobacterial mats, thus paddy cultivation is a sustainable practice for economic nitrogen conservation in paddy soil (Pabbi, 2015). In the field, nitrogen-fixing cyanobacteria are dominant in the harvesting stage of rice. Cyanobacteria can withstand extremes of temperature and drought and show remarkable variation in growth, nitrogen fixation and stress compatibility (Whitton and Potts, 2012). They produce exopolysaccharides, especially from the genus *Nostoc*, which help them to overcome conditions of water stress and also bind soil particles to increase the structure and fertility of the soil.

1.9 Cyanobacteria as a source of sustainable agriculture

Nowadays, agricultural practices are heavily dependent on the application of synthetic fertilizers and pesticides, intensive tillage, and over-irrigation, which have undoubtedly helped many developing countries to meet food requirements. The “Green Revolution” practices are helping to boost agriculture productivity and reduce the risk of chemical fertilizers on human health as well as the environment. Thus, ‘green technology’ has been used by researchers to produce an eco-friendly environment by the exploitation of microorganisms and also discusses several aspects of the use of cyanobacteria to improve crop productivity and soil fertility. Cyanobacteria are the most promising microbes for sustainable agriculture development (Singh *et al.*, 2016). Cyanobacteria are bestowed with the ability to fix atmospheric N₂, decompose organic wastes and residues, detoxify heavy metals, pesticides, and other xenobiotics, catalyze nutrient cycling, inhibit the growth of pathogenic microorganisms in soil and water, and also produce some bioactive compounds such as hormones, amino acids, sugar, vitamins, and enzymes that promote plant growth and yield (Higa,1991). The inoculation of cyanobacteria influences various metabolic processes in plants since they activate the production of defense proteins and enzymes that lead to a greater immunity of plants against pathogens (Gonçalves, 2021).

Cyanobacteria can offer a suitable alternative to chemical fertilizers which can improve the quality of soil and plant growth, and minimize the crop production

cost by supplementing the good crop management practices. Heterocystous cyanobacteria are special in atmospheric nitrogen fixation and are capable of converting N_2 to ammonia, nitrites and nitrates, which can be utilised by plants for developing the potential to reclaim saline soil. The application of cyanobacteria in paddy fields reduces the usage of chemical fertilizers (Kumar and Rao, 2012). The nitrogen-fixing capacity of cyanobacteria aids their successful growth and survival in habitats where no nitrogen is available. This trait of cyanobacteria makes them agronomically and economically important as biofertilizers (Vaishampayan *et al.*, 2001).

Cyanobacteria can be used as a biofertilizer for monocot and dicot crops to enhance plant growth and crop yield. *Nostoc* is one of the most promising genera for plant growth promotion and they can supply nitrogen to the plant by using nitrogenase enzymes to fix atmospheric nitrogen (Prasanna *et al.*, 2016; Meeks and Elhai, 2002). The capability of biological nitrogen fixation of cyanobacteria helps the plants to grow without nitrogen in deficient soils and eliminates the need to use expensive nitrogen fertilizers, which are frequently a source of pollution. Cyanobacteria are gaining attention as plant-growth-promoting and biocontrol agents in diverse crops, including rice, wheat, cotton, and legumes (Prasanna *et al.*, 2015).

1.9.1 Cyanobacteria as a biofertilizer

In modern agriculture, microbes play a vital role in determining soil fertility and structure. Cyanobacteria have potential applications in agriculture as biofertilizers. Maintaining soil fertility using renewable bioresources is the main requirement of sustainable agriculture to reduce the use of synthetic fertilizers. Application of cyanobacteria as biofertilizers has been reported in barley, oats, tomato, radish, cotton, sugarcane, maize, chilli and lettuce. Cyanobacterial biofertilizers can control the nitrogen deficiency in plants, improve the aeration of soil, and water holding capacity, and add vitamin B12 (Song *et al.*, 2005). Cyanobacteria play a chief role in the maintenance and build-up of soil fertility, consequently yielding a natural biofertilizer. In addition, they also make the soil

porous and produce adhesive substances, secrete phytohormones (auxin, cytokinins, gibberellins), vitamins, and amino acids, improve the water-holding capacity of soil through their characteristic gelatinous structure, increase biomass of soil after their death and decomposition, decrease soil salinity, control weed growth, soil phosphate availability by excretion of organic acids and extracellular phosphatase are the major actions of cyanobacteria (Fig. 1.1). Cyanobacteria based biofertilizers are cost-effective as they cost one-third to that of chemical fertilizers (Prasanna *et al.*, 2013). Cyanobacterial species are known to produce siderophores and therefore can affect the development and productivity of crops (Rastogi and Sinha, 2009). Due to the adaptation of cyanobacteria to most different environmental conditions, it is widely used to enhance soil quality and fertility, so it has become one of the most important biofertilizers (Dhar *et al.*, 2007).

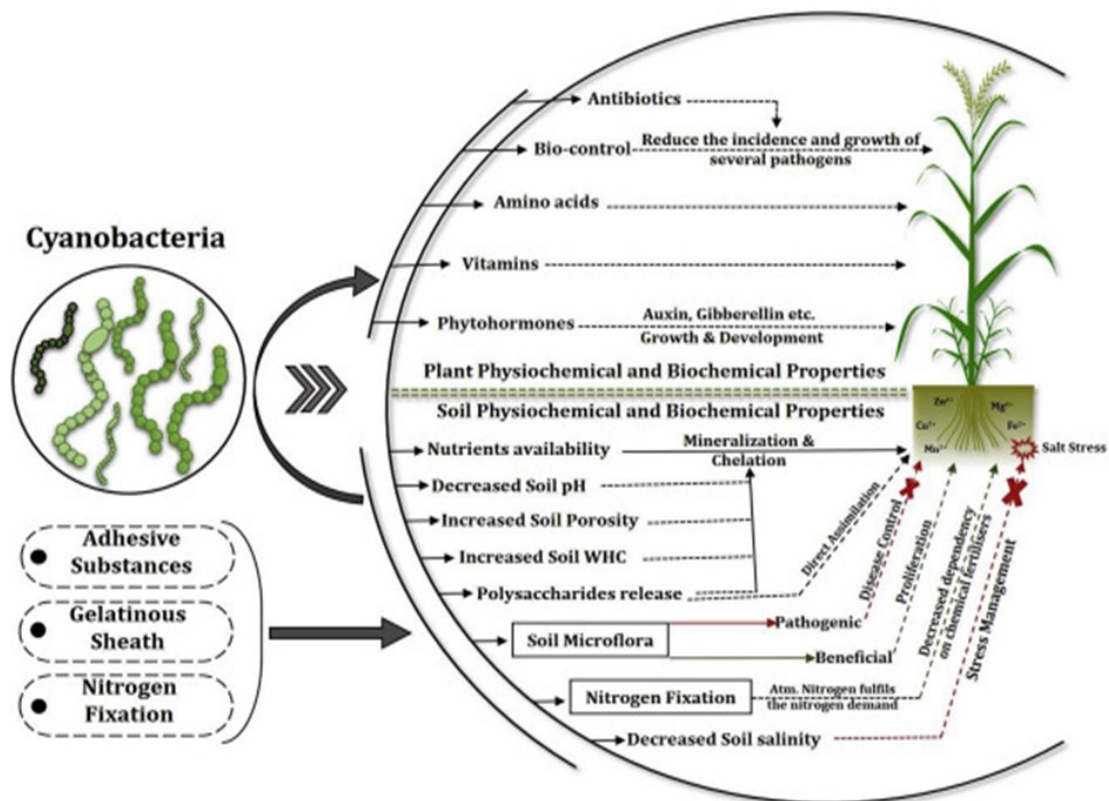


Fig. 1.1 An overview of the beneficial effects of cyanobacteria on the physicochemical and biochemical properties of soil and plant (Singh *et al.*, 2018).

1.9.2 Cyanobacteria as agents of bioremediation

Cyanobacterial species are emerging candidates for their application in developing sustainable agricultural practices where they can be used for removing various contaminants from soil and water. They can accumulate organic or inorganic toxic substances and produce several detoxifying mechanisms, like bioaccumulation, biosorption, biotransformation, and biodegradation (Mondal *et al.*, 2019). Cyanobacteria are capable of degrading a wide range of contaminants such as heavy metals, pesticides, crude oil, phenanthrene, naphthalene, phenol, catechol, and xenobiotics. *Nostoc* sp. can break down lindane residues (El-Bestawy *et al.*, 2007) and help to remove glyphosate from contaminated soils (Forlani *et al.*, 2008). Introduction of culture in soil by spraying and application of *Nostoc* culture immobilized on a neutral carrier are the two ways *Nostoc* biomass can be used for soil bioremediation. *Nostoc linckia* is a potential candidate for bioremediation of soil contaminated with chromium and other metals. *Nostoc* spp. can remove numerous heavy metals such as cadmium, chromium, copper, lead, and zinc (Al-Amin *et al.*, 2021). *Nostoc hatei* and *N. elliposporum* were used to bioremediate toxic pesticides and fungicides (Jha and Mishra, 2005; Barton *et al.*, 2004).

1.9.3 Cyanobacteria against biotic and abiotic stresses

Abiotic stresses, including drought, flood, cold, heat, salinity and nutrient deficiency, along with biotic stresses, including pathogens and pests, represent major limitations on the yield of crops. In rice, an increase in its tolerance to salinity has been analysed by the root inoculation with *Nostoc calcicola* and *N. linckia*, isolated from saline soils increasing root length (El-Sheek *et al.*, 2018) and promoting seedling growth and yield, as with *N. commune* and *N. carneum* (Chittapun *et al.*, 2018). The biostimulants from cyanobacteria can antagonize different pathogens and pests of crops or activate local and systemic resistance in plants. Several plant fungi can be effectively controlled by cyanobacterial extracts, for example, *Fusarium* spp., *Alternaria* spp., *Penicillium* spp., *Botrytis cinerea*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum*. The members of the order Nostocales are very effective against fungal pathogens. *N.*

linckia, and *N. commune* have the antibiosis effect on tomato wilt disease caused by *F. oxysporum* f. sp. *lycopersici*. Additionally, *N. entophytum* and *N. muscorum* decreased the activity of *Rhizoctonia solani* in soybeans by an antibiosis mechanism (Osman *et al.*, 2011). Cyanobacteria offer an alternative and attractive approach, without the negative impact of chemical control that is important for sustainable agriculture.

1.10 Mass cultivation of cyanobacterial fertilizers

Scientific research has increasingly focused on the agricultural and pharmaceutical importance of cyanobacteria. They grow faster than plants and are characterized by a simple genetic system and a high yield of biomass and metabolites (Wijffels *et al.*, 2013). Mass production of cyanobacteria depends on the strain chosen and the effectiveness of the cultivation system (Balasubramaniam *et al.*, 2021). In the future, mass cultivation of cyanobacteria under controlled conditions is the only method to meet the demand. Cyanobacterial vegetative cells have a high potential for regeneration and can form new colonies. Reproduction through trichomes produces a large number of new colonies in a short period and is an effective method in mass cultivation of *Nostoc* spp. (Han *et al.*, 2013).

Commercial production of photosynthetic cyanobacteria can be achieved by three methods such as open-system cultivation using sunlight, closed-system cultivation using sunlight and closed system cultivation using artificial light. In open system cultivation, raceways or shallow open ponds are used. In the closed system cultivation, solar energy is used as the source of energy and plastics or glass trays or pots were used for growing. This method helps in preventing contamination of grazers and competitors. Photobioreactors are used in the closed system of mass cultivation using artificial light. High quality and quantity of the biomass of cyanobacteria can be produced by this method. The cemented tank method, shallow metal troughs method, polythene lined pit method and field method are the other four methods used for the mass cultivation of cyanobacteria. The polythene lined technique is the most suitable for small and marginal farmers for the preparation of

biofertilizer. The polythene lined pit method is most suitable for farmers for the preparation of biofertilizers using *Nostoc inoculum* (Singh *et al.*, 2016).

1.11 Role of cyanobacteria in seed bio-priming

Seed bio-priming is a seed character enhancement technology that refers to the inoculation of seeds with beneficial microorganisms, to increase the seed germination percentage and germination rates in suboptimal environments, suppress disease, control plant pathogens, promote growth, restrain side effects caused by drought, and increase tolerance to high temperatures, salinity and heavy metals (Bisen *et al.*, 2015; Pedrini *et al.*, 2020). Seed bio-priming using microorganisms are useful in almost all crops around the world and are an eco-friendly alternative to chemical fungicides. Microorganisms including cyanobacteria and plant growth-promoting rhizobacteria (PGPR) can help plants by improving, seed germination, seed vigour index and enhancing yield production.

Seed bio-priming with cyanobacteria protects plants against biotic and abiotic stresses, enabling farmers to enhance the production of food with minimal risk and reducing cost. Cyanobacterial species are considered potential bio-control agents and most of them exhibit antagonistic effects against plant pathogens such as bacteria, fungi and nematodes, mainly as a result of the production of a variety of biologically active compounds (Prasanna *et al.*, 2008). Seed bio-priming using cyanobacteria causes an increase in the germination rate, better development of plants, and a higher production yield in a wide variety of cereal, horticultural, and vegetable crops (Singh *et al.*, 2017; Toribio *et al.*, 2021). Abo-Shady *et al.* (2018) reported that priming barley grains in the suspension of *Nostoc muscorum* before cultivation removes the toxic effect of granstar herbicide on the yield parameters of barley plants. Besides, it could stimulate the production of some amino acids which were not present in the control plant.

Objectives

Keeping the above background under consideration, the present investigation was proposed to carry out the following objectives:

- To collect and identify *Nostoc* spp. from different habitats of Kerala State.
- To provide taxonomic key, photomicrographs and morphological descriptions.
- To conduct molecular characterization and phylogenetic analysis of the selected *Nostoc* spp. based on the 16S rRNA gene sequencing.
- To study the biochemical characterization of selected *Nostoc* spp. using Gas Chromatography-Mass Spectrometry (GC-MS) analysis.
- To analyse the effect of selected *Nostoc* spp. as a biofertilizer for rice varieties Jyothi and Annapurna.

2.1 Introduction

Cyanobacteria (Cyanoprokaryotes) are commonly known as blue-green algae occupy an important position in the evolution of eukaryotic algae. They are the primitive life forms of Earth and have been recorded from the microfossils of the Precambrian era (Schopf, 1968). Cyanobacteria have a significant impact on global ecosystems, as their photosynthetic activity provides much of the oxygen necessary for the sustenance of aerobic life forms (Bekker *et al.*, 2004). They have evolved specialized cells known as heterocytes to carry out nitrogen fixation. Cyanobacteria has cosmopolitan distribution and surpassed only by bacteria (Adams, 2000; Govindjee and Shevela, 2011). They can thrive in a wide range of ecological habitats, ranging from marine freshwater, to terrestrial environments (soils, rocks, buildings, tree barks, exposed rock surfaces in highly isolated and high-altitude environments) including extreme habitats such as deserts, hot springs and polar regions. In aquatic habitats, unicellular cyanobacteria are considered as an important group regarding diversity, abundance and ecological character. Cyanobacteria are also commonly encountered in symbiotic relationships with eukaryotic algae, lichens, and plants (Whitton and Potts, 2000).

The effective protective and tolerance mechanisms of cyanobacteria, against various abiotic stresses, such as desiccation (Dadheech, 2010), salinity (Hagemann, 2011), ultraviolet radiation (Zeeshan and Prasad, 2009), high light intensity (Lakatos *et al.*, 2001), extremes of temperature (Singh *et al.*, 2005), oxidative (Hossain and Nakamoto, 2003), acid (Gopaldaswamy *et al.*, 2007) and heavy metals (Turner and Robinson, 1995) were well documented. Cyanobacteria are microscopic ranging from 1-80 μm in diameter and show substantial morphological diversity.

Ultrastructure and morphological diversity of Cyanobacteria

Cyanobacteria exhibit remarkable variability in the morphology and ultrastructure compared to other prokaryotes. Cyanobacteria were identified and

categorized using morphological traits such as cell dimensions, colour and shape, type of branching, cell contents and sheath characteristics. The morphology of cyanobacteria with diverse colours and they occur as unicellular (e.g., Chroococcales) or filamentous (e.g., Oscillatoriales), and as solitary or colonies. Unicellular cyanobacterial colonies have a regular (e.g., *Merismopedia*) or irregular (e.g., *Microcystis*) distribution of cells. In colonies, the numbers of cells vary from two to several thousand. Filamentous cyanobacteria exhibit both false and true branching. False branching is found in all orders of filamentous cyanobacteria, while true branching has been shown only in members of Nostocales (Komarek, 2013). In filamentous cyanobacteria, cells remain adhered to each other after division, forming chains of connected cells termed “trichomes”. Trichomes can be enveloped in exopolysaccharide-based mucilaginous sheaths in some taxa called as filaments. Exopolysaccharide sheaths are often environmentally inducible and provide several putative functions, such as protection from UV radiation, desiccation and anti-herbivory (Ehling-Schulz and Scherer, 1999). In some groups of cyanobacteria specialised cells occur, which are morphologically different from vegetative cells, that is, heterocytes and akinetes.

Heterocytes, are differentiated from vegetative cells by their somewhat larger and rounder shape, diminished pigmentation, thicker cell envelopes, and usually prominent cyanophycin granules. Heterocytes are specialised cells for nitrogen fixation lack oxygenic photosystem, possess extraordinary thick cell walls that lack biliprotein pigments and carboxysomes compared to vegetative cells commonly seen in species adapted to nitrogen-depleted conditions (Anagnostidis and Komarek, 1999). The additional envelope of heterocytes consists of polysaccharides and a glycolipid layer, which help to protect the enzyme nitrogenase from oxygen (Fay, 1992). In unfavourable conditions, cyanobacteria produce akinetes, which are dormant cells helpful in sub-optimal conditions like overwintering. These are spore-like, thick-walled and non-motile cells that germinate when conditions are suitable for growth, and the desiccated akinetes in soil samples can survive up to 70 years.

Hormogonia are a short chain of cyanobacterial filaments with a major role in the relocation and symbiotic colonization of hosts. Hormogonia cells in some cyanobacterial species contain gas vacuoles to regulate buoyancy in the water column (Meeks and Elhai, 2002). These motile filaments associated with dispersal, survival of the species in its natural habitat and reproduction through the creation of necridial cells, can also be formed by filamentous species (Hernandez-Muniz and Stevens, 1986; Damerval *et al.*, 1991). Kumar *et al.* (2010) reported that the formation of hormogonia can be induced by the exchange of metabolites.

The ultrastructure of the cell is visualized by transmission electron microscopy (TEM) and in most cases exhibits significant variability among different cyanobacterial species. Cyanobacterial cells are enclosed by a complex multi-layered structure that protects the bacteria from harsh environmental conditions. The cell wall composition of cyanobacteria is made up of a thin layer of peptidoglycan and lipopolysaccharide outer membrane (Durai *et al.*, 2015). The cyanobacterial peptidoglycan layer is thicker (10-700 nm) compared to most gram-negative bacteria. In filamentous species such as *Oscillatoria princeps* the thickness of this layer was reported more than 700 nm. Apart from protection and sensing environmental stress, the transport of metabolites and nutrients into and out of the cell is one of the major roles of cyanobacterial cellular membranes (Hoiczky, 2000).

According to Chittora *et al.* (2020), the components of the ultrastructure of cyanobacteria are light-harvesting antennae, phycobilisomes, polyphosphate bodies, cyanophycin granules, polyhydroxyalkanoate granules, carboxysomes, lipid bodies, thylakoids, DNA-containing areas, and ribosomes. They are photosynthetic prokaryotes possessing the ability to synthesize chlorophyll a and chlorophyll b. Colour of cyanobacterial cell varies from chartreuse to blue-green to violet red, depending on the ratio between phycocyanin, phycoerythrin, chlorophyll and carotenoids. In a few taxa, chlorophyll b and d are observed (Miyashita *et al.*, 1996; Castenholz, 2001). Chlorophyll f is recently discovered from cyanobacteria grown in wet environments (Behrendt *et al.*, 2015). Cyanobacteria are unique since they are the only prokaryotes that have both the photosystems (PSI and PSII) to perform

oxygenic photosynthesis like vascular plants (Stal, 1995). In addition to photosynthetic pigments, cyanobacteria produce pigments that supposedly protect the cells from intense irradiation, in particular the UV-wavelength range. These pigments can mask the colour of chlorophyll and phycocyanin, for example, scytonemin, a black pigment produced by *Scytonema* spp. (Dillon and Castenholz, 1999). Moreover, cyanobacteria have various storage bodies for carbon, nitrogen, phosphate and the enzyme ribulose 1,5-bisphosphate carboxylase/oxygenase (RubisCO) (Castenholz, 2001).

2.2 Genus *Nostoc*

Nostoc is a genus of heterocystous cyanobacteria that represents one of the earliest described cyanobacterial genera (Bornet and Flahault, 1888) belonging to the family Nostocaceae in the order Nostocales (Castenholz and Waterbury, 1989) that can form macroscopic or microscopic colonies that thrive in both terrestrial and aquatic habitats (Liu and Chen, 2003). Cells of the order Nostocales are characterized by division in a single plane, perpendicular to the filament axis (type species *N. commune*) (Hensyl, 1989). Approximately 250-300 *Nostoc* species have been described to date, indicating the enormous diversity and heterogeneity of the genus *Nostoc*. It has also been reported that the majority of *Nostoc* species require revisions (Komárek, 2013). *Nostoc* is a polyphyletic genus, with wide genetic diversity and more new taxa are expected soon.

Nostoc spp. differs from other heterocyst-forming cyanobacteria in that the trichomes with heterocytes and akinetes are embedded in colonial common exopolysaccharide mucilage. The mucilaginous sheath that excretes EPS, plays a major role in the protection of cells from physical or chemical stress and for anchorage purposes (Garlapati *et al.*, 2019). Heterocytes are specialized cells in response to the lack of combined nitrogen in the environment and are the sites of nitrogen fixation. It may be terminal or intercalary and arranged singly or in series. Hormogonia, which are relatively short, motile filaments produced by *Nostoc* species, and these characteristics in part, distinguish them from the members of the closely related genus *Anabaena* (Sarma *et al.*, 2004). Additionally, in response to

nutritional limitations other than nitrogen, *Nostoc* species produced spore-like structures known as akinetes. All cells in a trichome other than heterocytes in some *Nostoc* species can form akinetes whereas in *N. commune*, an extremely desiccation-resistant terrestrial species, akinetes are never formed (Whitton, 1987).

Colonies of *Nostoc* spp. develop characteristic shapes, dimensions, aromas, textures, and hues. These colonies can grow profusely if there is enough available water (Mollenhauer *et al.*, 1999). For instance, *Nostoc pruniforme* colonies as large as 25 cm in diameter have been seen. Likewise, *N. commune* can produce discoid and flattened crusts as well as spheres ("pearls") with a diameter ranging from tens of microns to three centimeters. In these colonies, the *Nostoc* trichomes are embedded in a thick layer of exopolysaccharide belonging to biopolymer, which also contains protein to protect the cells from the external environment. Colonies are pigmented with a hue from yellow-green to red-brown, dark green to black. Scytonemin, a blackish brown pigment, and nostocine A, a violet pigment, are extracellular pigments identified from this genus. These pigments, along with mycosporine-like amino acids, protect cells from high irradiance and UV radiation (Ferroni *et al.*, 2010; Castenholz and Garcia-Pichel, 2012). *Nostoc* reproduces in different ways, including (1) single cells of *N. commune* fragmented from filaments that can form new colonies; (2) akinetes that disperse and germinate; (3) hormogonia (short free filaments) that disperse and form new colonies; and (4) large colonies that can grow small attached colonies that break off and become separate colonies.

Nostoc is an ecologically important genus that inhabits a wide range of biotopes, from the tropics to the Arctic. It is a common inhabitant of freshwater habitats, humid soils and seasonally inundated habitats (wetlands or estuaries) almost absent in marine habitats (Dodds *et al.*, 1995). Potts *et al.* (1987) identified the colonies of *Nostoc* from extremely cold and dry habitats of Antarctic valleys. Species of *Nostoc* mostly grow in benthonic communities of lakes or aerophytic habitats (Komarek *et al.*, 2003). In aquatic environments, species of *Nostoc* are primarily associated with the benthos but can form floating planktonic masses (Joshi

et al., 1981). Huber-Pestalozzi (1938) reported the formation of water bloom by *Nostoc* spp. Moreover, *Nostoc* spp. occupy in a variety of terrestrial environments. *Nostoc muscorum* and *N. commune* are common species reported from terrestrial habitats. Soil is one of the most potential substrate for their growth, particularly in moist or waterlogged conditions. Devi *et al.* (2010) suggested that water-logged rice fields are ideal for the growth of *Nostoc* spp. *Nostoc* spp. could endure desiccation for very long periods (years), a feature that is largely responsible for the success of this genus in many terrestrial habitats (Dodds *et al.*, 1995). Physiological processes gradually cease as the *Nostoc* colony dries out (Kviderova *et al.*, 2011) and they recover, quickly after being rehydrated (Gao, 1998).

Nostoc spp. participates in a wide range of symbiotic associations with hosts from different groups of organisms. In such associations, motile hormogonia serves as the infection unit, whose differentiation is triggered by the host at the initial stages of the infection process. *Nostoc* spp. developed endophytic symbiotic associations with bryophytes (mosses, hornworts and liverworts), pteridophytes (the water fern *Azolla*), gymnosperms (cycads), and angiosperms (*Gunnera*) (Rai *et al.*, 2000). The cyanobionts are located either outside the cells, in specialized cavities in the thallus as in liverworts and hornworts, or roots as in cycads, or intracellular in symbiotic organs as in the angiosperms *Gunnera* sp. (Johansson and Bergman, 1992) or fungi of the genus *Geosyphon* (Kluge and Kape, 1992).

Symbiotic strains of *Nostoc* are the most common cyanobionts in lichens. About 10% of lichens contain cyanobacteria (like *Nostoc*) as the primary or only photosynthetic partner, and it is the most prevalent phycobiont in N₂-fixing lichens and the N₂-fixing symbiont in a small and diverse group of green plants (Rai *et al.*, 2002). *Nostoc*-containing lichens can be the dominant primary producers in some landscapes (Seaward, 1988). *N. sphaeroides* and *N. commune*, have strong stress resistance, and are usually free-living, other species can develop a symbiotic association with their host plants (Liaimer *et al.*, 2016). The symbiosis between plants and *Nostoc* species constructs a comparatively stable microhabitat for the exchange of metabolites (Rikkinen, 2017).

The genus *Nostoc* is a rich source of low and high-molecular weight organic compounds (e.g. fatty acids, toxins, lectins, peptides, pigments, proteins, polyhydroxyalkanoates, polysaccharides, etc.) having numerous biotechnological applications such as biofertilizers (Chittora *et al.*, 2020; Sholkamy *et al.*, 2015), biofuels (Nagappan *et al.*, 2020; Singh *et al.*, 2016), cosmetics (Morone *et al.*, 2019), nutritional and food additives (Gantar and Svirčev, 2008; Rosales-Loaiza *et al.*, 2017), and pollution prevention (Dixit and Singh, 2014; Kumar *et al.*, 2015; Raghavan *et al.*, 2020; Subash Chandra Bose *et al.*, 2013; Tarawat *et al.*, 2020).

2.3 Systematics and taxonomy of cyanobacteria with emphasis on genus *Nostoc*

Botanical and bacteriological classification systems used the morphology of cyanobacteria for their classification. According to the botanical system of classification, cyanobacteria have been traditionally grouped into five orders, Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales and Stigonematales, which generally correspond to the five subsections proposed in Bergey's Manual of Systematic Bacteriology (Knoll, 2008; Whitton, 2008).

Komárek and Anagnostidis (1986 and 1989) proposed the most comprehensive modern taxonomy revision of cyanobacteria in a series of monographs. According to the modern classification system, cyanobacteria were divided into four orders Chroococcales, Oscillatoriales, Nostocales and Stigonematales. All the filamentous heterocystous cyanobacteria divisible in a single plane were grouped under the order Nostocales (Komarek and Anagnostidis, 1989). The highest degree of morphological diversity has been reported in the order Nostocales (Komarek, 2013; Komarek *et al.*, 2014). This order was divided into two major groups: those taxa which undergo cell division in multiple planes (the Stigonematales *sensu stricto*, e.g., *Hapalosiphon*, *Mastigocladus* and *Stigonema*) and those taxa that never show reproduction in multiple planes (Nostocales *sensu stricto*, e.g., *Nostoc*, *Anabaena* and *Calothrix*).

Since the late 18th century, descriptions of cyanobacteria have begun to appear in botanical literature. Myxophyceae (Stitzenberger, 1860), Cyanophyceae (Sachs, 1874), Cyanophyta (Smith, 1938; Desikachary, 1959), Blue-green algae

(Bourrelly, 1970; Golubic, 1979; Anand, 1981), Cyanochloronta (Bold and Wynne, 1978) and Cyanoprokaryotes (Komarek and Anagnostidis, 1998) are different nomenclatures of this heterogeneous group. The first comprehensive taxonomic monographs on blue-green algae were written by Thuret (1875), Bornet and Flahaut (1888a and 1888b), and Gomont (1892) used as starting points for taxonomic referencing. Geitler (1932) provided an updated taxonomic review and this system of classification marked the beginning of the modern era of cyanobacterial systematics. Since then, many revised systems have been proposed including those of Elenkin (1938), Desikachary (1959), Fritsch (1959), Starmach (1966), Kondrateva (1968), Bourelly (1970) and Golubic (1976). Two volumes of monographic series on Cyanoprokaryota were compiled by Komarek and Anagnostidis (1998, 2005), with Part 1 consisting of the order Chroococcales and Part 2 consisting of the order Oscillatoriales. A manual of Cyanoprokaryota as Part 3, which consists of 'Heterocystous Genera' was published by Komarek (2013), recently which is used for the identification of genus *Nostoc*, *Anabaena*, etc.

Mollenhauer *et al.* (1999) investigated macroscopic forms of cyanobacteria from the genus *Nostoc* from Europe and reported *Nostoc caeruleum*, *N. commune*, *N. microscopicum*, *N. parmelioides*, *N. pruniforme*, *N. verrucosum* and *N. zetterstedtii* can form visible colonies. Three aerophytic morphospecies of *Nostoc* from different habitats in Brazil was studied by Sant'Anna *et al.* (2007). Based on their morphology, especially on the shape and formation of the akinetes, life cycles and ecology, these species were considered as new taxa of the genus *Nostoc*. *N. interbryum*, *N. viride*, and *N. alatosporum* were the identified new species. McGregor (2018) contributed the first detailed account of the Nostocales of north-eastern Australia and described six species of *Nostoc* such as *N. commune*, *N. linckia*, *N. verrucosum*, *N. sphaericum*, *Nostoc* sp. A and *Nostoc* sp. B.

The taxonomic account of the cyanobacterial community in India began in the early 90s. Ghose (1919) studied the blue-green algae of Burma and Punjab, considered to be the earliest contribution regarding cyanobacterial flora of India. Cyanobacteria from different regions of India were investigated by different

researchers. Bharaddwaja (1933) studied the myxophyceae of India. Banerje *et al.* (1938) studied the Myxophyceae of Bengal. Freshwater algae of Manipur were surveyed by Bharaddwaja (1963). Grover and Pandol (1975) investigated the algal flora of rice fields in Ludhiana and its surrounding areas, meanwhile, Bendre and Kumar (1975) surveyed the cyanophyceae of Meerut. Asthekar and Kamat (1980) explored the Nostocales of Maratwada, Maharashtra. Prasad and Mehrotra (1980) examined the blue-green algae of the paddy fields of Uttar Pradesh and Goyal *et al.* (1984) analysed the algal flora of the rice fields of Jammu and Kashmir.

Tripathy *et al.* (1997) conducted a survey of epilithic blue-green algae (cyanobacteria) from temples in India. The study reported thirty-three species of blue-green algae belonging to the genera *Gloeothece*, *Xenococcus*, *Chroococcidiopsis*, *Plectonema*, *Nostoc*, *Calothrix*, *Chlorogloeopsis*, *Hapalosiphon* and *Fischerella*. Tiwari *et al.* (2005) investigated the distribution of cyanobacteria from arid zones of Rajasthan and found that *Nostoc* species was predominant. *Nostoc linckia*, *N. elliposporum*, *N. piscinale*, *N. muscorum* and *N. calcicola* were the identified *Nostoc* spp.

Muthukumar *et al.* (2007) reported the cyanobacterial diversity from different freshwater ponds of Thanjavur, Tamil Nadu which includes thirty-nine species belonging to twenty genera. Prasanna *et al.* (2009) studied the cyanobacterial diversity in the rhizosphere of rice and its ecological significance. The cyanobacterial strains belonging to the genera *Anabaena* and *Nostoc* comprised 80% of the rhizosphere isolates. A total of twenty-five species of *Nostoc* were observed and the study concluded that *Nostoc* is known to be one of the most versatile diazotrophic cyanobacterial genera, observed as free-living and symbiotic associations. Dey, (2010) gave an account of the cyanobacterial diversity in some local rice fields of Orissa and observed fifty-eight taxa of which the majority were non heterocystous and also highest abundance of cyanobacteria belonged to the order Nostocales.

Samad and Adhikary, (2008) recorded *N. commune*, *N. linckia*, *N. microscopicum* and *N. punctiforme* from the building walls and rock surfaces of

monuments. Syiem *et al.* (2010) studied the significance of cyanobacterial diversity in different ecological conditions of Meghalaya and identified sixty-five strains belonging to eleven genera including *Nostoc*, *Cylindrospermum*, *Anabaena*, *Calothrix*, *Fischerella*, *Gleocapsa*, *Tolypothrix*, *Westiellopsis*, *Plectonema*, *Stigonema* and *Loriella*. Cyanobacterial diversity in the Western Ghats of Maharashtra was conducted by Nikam *et al.* (2013). A total of ninety-four cyanobacterial spp. belonged to thirty-eight genera, fourteen families and five orders were reported and the most densely populated genera were *Nostoc* (Nostocaceae), *Chroococcus* (Chroococcaceae) and *Anabaena* (Nostocaceae). *Westiellopsis prolifica* was abundant in the region followed by *N. calcicola*.

Singh *et al.* (2014) identified twenty-nine cyanobacterial species from different unexplored sites in Chhattisgarh, among them two were unicellular, four colonial, nine unbranched non-heterocystous, twelve unbranched heterocystous, and two pseudobranched cyanobacteria. *N. punctiforme*, *N. paludosum*, *N. linckia*, *N. spongiaeforme*, *N. carneum*, *N. ellipso sporum* and *N. commune* were identified from the region. Barman *et al.* (2015), surveyed thirty islands of Indian Sundarbans, a total of thirty-seven cyanobacterial species were collected and heterocystous genera like *Nostoc* (3), *Anabaena* (3) and *Calothrix* (1). The diversity of cyanobacteria in the cultivated fields of Ahmednagar districts, Maharashtra was carried out by Wagh *et al.* (2019). Twenty-nine species belonging to twelve genera including *Nostoc linckia* and *N. muscorum* were identified and catalogued, mostly from sugar cane fields.

The pioneers who worked on the cyanobacterial diversity present in Kerala include, Parukutty, (1940) who studied the blue-green algae of Travancore. Amma *et al.* (1966) studied the occurrence of blue-green algae in acidic soils of Kerala. Anand *et al.* (1987) were the first to survey the blue-green algae occurring in the rice fields of Kerala and reported species of *Nostoc* from the rice fields. Dominic and Madhusoodanan, (1999) explored the blue-green algae from extreme acidic environments of Kerala and identified forty-two species. Among them, nine *Nostoc* species were observed and nineteen cyanobacterial species were recorded for the

first time in India. Umamaheswari, (2005) isolated and characterised the Nostocales of paddy fields of Kerala and observed one hundred and eleven cyanobacterial species, among them *Nostoc* was one of the dominant genera with eleven species. Jose and Francis, (2013) investigated the algal flora of the Idukki District of Kerala, India, and discovered fifty cyanobacterial species under six families. Among these, the unicellular forms of cyanobacteria dominated the cyanobacterial flora compared to the filamentous forms. Sebastian and Joseph, (2013) studied the filamentous cyanobacteria in a hill stream of Kerala and reported *N. commune* from that region.

A field study on the ecology and diversity of cyanobacteria from paddy fields of Kuttanadu in Kerala State was conducted by Dhanya and Ray, (2015). They discovered sixty four species of cyanobacteria belonging to twenty genera and six orders, exhibiting a well-established diversity. Twelve species of *Oscillatoria* predominated followed by *Anabaena* (9 species) and *Nostoc* (7 species). Geethu and Shamina, (2020) investigated the filamentous cyanobacteria from the Western Ghats of North Kerala, India and identified eighteen cyanobacterial taxa. Three *Nostoc* spp. were recorded, among them, *N. nylstromicum* was a new record for India.

Thilak *et al.* (2020) conducted the isolation and taxonomy of blue-green algae (Cyanobacteria) *Nostoc* and *Anabaena*. *Nostoc muscorum*, *N. paludosum*, *N. entophyllum*, *N. humifusum*, *N. linckia*, *N. ellipsoforum*, *N. punctiforme* and *N. spongiaeforme* were the identified *Nostoc* spp. from paddy fields. Ram and Paul, (2022) investigated mangrove-associated cyanobacteria of *Nostoc* spp. from Kerala and reported nine *Nostoc* spp. including, *N. bornetii*, *N. calcicola*, *N. carneum*, *N. commune*, *N. ellipsoforum*, *N. linckia*, *N. sphaericum*, *N. spongiaeforme* var. *varians* and *N. undulatum*. *N. bornetii* was first recorded in India.

2.4 Factors influencing the growth of cyanobacteria

Cyanobacteria exhibit a wide adaptability to environmental conditions, light, temperature, pH and nutrient composition of the suspending medium are the major factors that influence the growth of cyanobacteria. They can survive a wide range of temperatures; however, most of them prefer warm temperatures for optimum growth. According to Pearl (2009), higher temperatures (up to 25°C) due to climate

change may cause increased cyanobacterial growth rates and thus higher cyanobacterial dominance in temperate water bodies. Rai *et al.* (2016) studied the effect of pH, salinity and temperature on the growth of cyanobacteria. All the collected strains showed best growth at 20°C and 30°C and the growth ceased at 40°C and 50°C. Spencer *et al.* (2014) analysed the effect of a wide range of temperatures (10°C, 15°C, 20°C, 25°C, 30°C, and 35°C) on the growth of *N. spongiaeforme*. The optimal growth of *N. spongiaeforme* occurred at 26°C.

Several ecological and environmental factors influence the cyanobacterial colonization in the rhizosphere of paddy plants, as well as their subsequent growth, abundance, physiological and biochemical activity. Environmental factors such as the pH of the soil, nature (acidic or alkaline), temperature, light, moisture availability, crop canopy and nutritional status of the soil may have a significant impact on colonization. pH is one of the most critical soil parameters for cyanobacterial abundance and growth and they prefer a neutral to slightly alkaline pH for optimal growth. Normally cyanobacteria flourish on alkaline soil (Karthikeyan *et al.*, 2007; Singh, 1961). Bano and Siddiqui, (2004) reported the optimum pH for the growth of cyanobacteria between pH 7.4 and 8. Wangwibulkit *et al.* (2008) suggested that a pH higher than 9 or lower than 6 could inhibit the photosynthetic efficiency and adversely affect the morphology of cyanobacteria.

Light intensity is another important factor in the growth of cyanobacteria. Normally, species present in optimal illuminance are associated with their photosynthetic apparatus. An excess or reduction of light can severely alter the morphology and growth pattern of cyanobacteria (Tonk *et al.*, 2005). Poza-Carrión *et al.* (2001) studied the high light intensity significantly decreased the content of phycobiliproteins (PBP) in *Nostoc* sp. Khajepour *et al.* (2010) investigated the effect of light intensity and photoperiod on the growth and biochemical composition of *N. calcicola*. The results concluded that, the optimum light regime for *N. calcicola* is 21 μmol photons and 12/12 h light/dark photoperiod for high biomass production.

2.5 Phytochemistry and Chemotaxonomy of cyanobacteria

Phytochemistry is the study of the plant secondary metabolites, which are synthesized for self-defense against insects, pests, pathogens, herbivores, ultraviolet exposure and environmental hazards. Phytochemistry takes into account the structural compositions of these metabolites, the biosynthetic pathways, functions and mechanisms of action in the living systems as well as their importance in various industries such as pharmaceuticals, nutraceuticals, and dietary supplements. It is an important part of taxonomy, systematic botany, evolutionary sciences, conservation biology, genetics and metabolomics (Egbuna *et al.*, 2018). Natural products, also known as secondary metabolites, are typically defined as substances that are not directly required for an organism's primary metabolism. These secondary metabolites are generally peculiar to specific organisms and are not present during all environmental conditions. Natural products produced by cyanobacteria enable them to survive in a wide range of environments those which are extreme. These compounds from cyanobacteria are used to treat diseases and are useful sources of bioactive compounds for drug discovery (Kultschar and Llewellyn, 2018).

Cyanobacteria are a significant source of potentially useful natural products, as evidenced by the extremely high occurrence of unique, biologically active chemicals that have been identified so far (Moore, 1996; Li and Liu, 2003; Han *et al.*, 2004). These secondary metabolites are often difficult to synthesize chemically due to their complex structure. According to Ordog (2004), intracellular and extracellular cyanobacterial extracts contain various biochemical substances such as carbohydrates, proteins and amino acids, lipids, glycosides, alkaloids, flavonoids, saponins, terpenoids and steroids. Cyanobacterial secondary metabolites are a rich source of novel bioactive compounds applicable for the production of medicines and agriculturally important chemicals.

The polyunsaturated fatty acids (PUFA) from cyanobacteria, which include linoleic acid, α -linolenic acid, γ -linolenic acid, octadecatetraenoic acid, and eicosapolyenoic acids, are particularly fascinating (Vargas *et al.*, 1998; Wang *et al.*,

2000). Lipids, fatty acids, as well as other substances, provide intriguing information for assigning the taxonomic position and some correlation with morphological characteristics of cyanobacteria (Vasyurenko and Frolov, 1986; Kaneda, 1991; Romano *et al.*, 2000; Li and Watanabe, 2001; Dembitsky and Rezanka, 2005). Bioactive compounds from cyanobacteria develop responses to biotic or abiotic stress in the surrounding environment by offering protection and assisting in their survival giving an advantage over other species.

Deyab *et al.* (2021) determined the phytochemical components of *Anabaena variabilis* and *Spirulina platensis* using the GC-MS technique. Pentadecanoic acid (29%), 12, 15-octadecadienoic acid methyl ester (10.26%), hexadecenoic acid methyl ester (Z) (8.3%) and thieno [3,4-C] pyridine,1,3,4,7-tetraphenyl (5.86%) identified as major compounds in *A. variabilis*. Seven compounds were identified from *S. platensis* and the major compounds were hexadecanoic acid (29%), 9, 12, 15-octadecadienoic acid methyl ester (24%) and 9, 12-octadecatrienoic acid (z,z) methyl ester (24.36%).

Devi *et al.* (2020) investigated the GC-MS profile of halophilic cyanobacterial species *Cylindrospermum muscicola* HPUSD12 and *Phormidium* sp. HPUSD13. Nine compounds were identified from the methanolic extract of *C. muscicola* whereas twenty-four compounds were identified from the methanolic extract of *Phormidium* sp. The study concluded that various pharmaceutically important compounds can be identified through GC-MS analysis. A wide range of secondary metabolites are produced by cyanobacteria such as *Microcystis*, *Anabaena*, *Nostoc*, and *Oscillatoria* which include 40% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides and 9% amides. Cyanobacterial lipopeptides contain various cytotoxic compounds (41%), antitumor (13%), antiviral (4%), antibiotics (12%), and the remaining 18% activities include antimalarial, antimycotics, multi-drug resistance reversers, antifeedant, herbicides and immunosuppressive agents (Burja *et al.*, 2001).

Chemotaxonomy aims at the correlation of chemical data with systematic classification based on morphological and phylogenetic characters. Chemotaxonomy

identifies the chemical variation in microbial cells and the use of chemical characteristics in the classification and identification of cyanobacteria. Chemotaxonomic classification of cyanobacteria by analysing the composition of whole-cell fatty acids. Kenyon (1972a) and Kenyon *et al.* (1972b) initiated the analysis of fatty acid profiles of cyanobacteria. Caudales and Wells, (1992) demonstrated that the filamentous heterocystous *Anabaena* and *Nostoc* could be distinguished based on their cellular fatty acids. Vandamme *et al.* (1996) suggested cellular fatty acid as a chemotaxonomic marker. Gugger *et al.* (2002) confirmed that cellular fatty acids are chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix*. Borah *et al.* (2016) studied the chemotaxonomy of the selected species of *Nostoc* and *Cylindrospermum* based on fatty acid profile and Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy (ATR-FTIR). The study proved that fatty acid profiling can be used as a chemotaxonomic marker at least for the generic classification while ATR-FTIR analysis of the strains was not suitable for chemotaxonomic studies.

Krüger *et al.* (1995) evaluated the taxonomic importance of fatty acid composition at the genus and subgenus level of cyanobacteria. Lipid and fatty acid analysis provide useful chemotaxonomic information that can be used for the classification and identification of cyanobacteria (Sato and Murata, 1988). Some types only contain saturated and monounsaturated fatty acids, while others have di and tri -unsaturated fatty acids (Kenyon, 1972; Kenyon *et al.*, 1972). Hydrocarbon and fatty acid composition can be used in the chemotaxonomy of cyanobacteria even at the species level based on examination of fifty-five strains of cyanobacteria belonging to the genera *Nostoc*, *Scytonema* and *Cerococcids* (Dembitsky and Srebnik, 2002; Rezanka *et al.*, 2003).

2.5.1 *Nostoc* GC-MS

Gas Chromatography and Mass Spectrometry is a useful tool to identify the diversity of bioactive compounds from cyanobacteria and used to separate the volatile and thermally stable substances present in the sample. It regularly and reliably offers semi-quantitative information about different chemical compounds,

depending on the biological material. *Nostoc* spp. is a promising source of biologically active secondary metabolites. Secondary metabolites produced by *Nostoc* spp. have significant applications in various industries like medical, pharmaceutical, agriculture, etc. Hydrocarbons, lipids, pigments, mono and polysaccharides, terpenoids and aromatic compounds are the chemical classes reported from *Nostoc* spp. (Dembitsky and Rezanka, 2005; Dembitsky *et al.*, 1999; Temina *et al.*, 2007; Liu *et al.*, 2013). Temina *et al.* (2007) studied the uses of extract of *Nostoc* for fistula treatment, cancer therapy, anti-inflammatory, immune-boosting, and blood pressure control. Cyanovirin, a potential protein molecule produced by *Nostoc* sp., has been shown to have a significant impact on the treatment of HIV and influenza A virus (Boyd *et al.*, 1997). Terpenoids, such as noscomin and comnostins have been reported to have antibacterial activity against different bacteria. *N. flagelliforme*, an edible species, produced a saccharide-rich extract that exhibited anticancer activity.

The genus *Nostoc* is known for the production of structurally different types of peptides and amino acid derivatives (cryptophycins, nostopeptolides, nostoginins, nostocyclin, nostopeptins, nostofungicin, nostocyclopeptides, nostocyclamides ABPs, MCYs, etc.) with a wide range of pharmacological activities (antimicrobial, cytotoxic and enzyme inhibitor) (Dembitsky and Rezanka, 2005). GC-MS analysis of the methanolic extract of *Nostoc* sp. identified thirty-two active compounds. Some of the major compounds were 2,4,4-trimethyl-1-hexene, octadecane, 2,6,10-trimethyl-14- ethylene-14-pentadecane, n-hexadecanoic acid, 9,12-octadecadienoic acid, 9,12-octadecadienoyl chloride, phytol, 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester and vitamin E (Devi and Mehta, 2016). These compounds showed antialgal, antibacterial and antifungal activities. Salem *et al.* (2014) studied the GC-MS analysis of *Nostoc* sp. from the river Nile in the Helwan and Menoufia regions of Egypt. The presence of phenols, plasticizer compounds, phytol (acyclic diterpene alcohol), and flavonoids was identified from in the GC/MS spectrum of *Nostoc* sp.

El Sheek *et al.* (2022) reported the bioactive compounds from *N. calcicola* and *N. linckia* using GC-MS analysis. *N. calcicola* and *N. linckia* were found to

contain numerous bioactive compounds that belong to different classes as fatty acids, phenolics, antioxidants, steroids, alkaloids and flavonoids. *N. calcicola* scored forty-nine bioactive compounds comprising 9-octadecenoic acid and oleic acid (antimicrobial, antibacterial, antioxidant, anti-arthritic, hypocholesterolemic, and anti-cancer activities), 12, 15-octadecadienoic acid methyl ester, p-xylene benzene, 1,4-dimethyl, and hexadecenoic acid methyl ester shows antimicrobial activities against different plant pathogens (Liu and Huang, 2012; Johannes and Litaay, 2015). Cyclohexane, cyclopentane, normal saturated hydrocarbons (C–C), fatty acids and benzene derivatives were identified using GC-MS from *Nostoc* sp. (Dembitsky *et al.*, 1999). Dembitsky and Rezanka, (2005) isolated more than 200 secondary metabolites from *Nostoc* and *Anabaena*. The GC-MS chromatogram of the methanolic extract of *N. fuscescence* showed the presence of twenty-one bioactive compounds. Among them, hexadecanoic acid, methyl ester (19.65%), ethyl oleate (16.45%), heptadecane (9.74%) and benzenepropanoic acid, 3,5-bis (1,1-dimethyl ethyl) 4-hydroxy, methyl ester (6.41%) were the major compounds and these compounds exhibit pesticidal, anti-inflammatory, antioxidant and antifungal activity (Gaikwad and Nalawade, 2020).

Yasin *et al.* (2018) studied the bio-efficacies of methanolic extracts of *Nostoc muscorum* and *Calothrix brevissima* using GC-MS. Forty-two bioactive compounds were identified, and the major compounds were cis vaccenic acid (17.97%), 9-methylnonadecane (16.22%) and heptadecane (15.11%). The methanolic extract of *N. muscorum* had branched alcohols (4.76%), alkane (33%), phenolics (7.14%), fatty acids (23.8%), olefins (4.76%), phthalates (4.76%), terpenes (7.14%), fatty acid esters (4.76%), alkaloids (2.3%) and others (4.76 %). Whereas *C. brevissima* had fifty detectable compounds comprising alkanes (16%), alkaloids (20%), fatty acid (14%), olefins (10%), branched alcohol (2%), phthalates (2%), fatty acid esters (6%), fatty acid amides (2%), terpenes (2%), phenolics (6%) and others (20%). The methanolic extract of *N. muscorum* has significant antioxidant activity as confirmed by DPPH, ABTS, FRAP assay and SOR scavenging assays.

Ananya and Kamal, (2016) identified twenty-nine compounds in the total polar lipid of *N. muscorum* using GC-MS analysis. Docosane (23.11%), phthalic acid (9.65%), tetracontane (8.53%), hexacosane (8.06%), hexatriacontane (7.25%), pentacosane (6.09%), heptacosane (4.87%), tetracontane (3.77%), tetracosane (3.53%), tetra-triacontane (2.51%), 9-octadecanoic acid (2.40%), and hexadecane (2.16%) were the prevailing compounds. 2-pentadecyl-1,3-dioxolane (0.74%), phenol, 2,4-bis (1,1-dimethylethyl (1.40%), hexadecanoic acid methyl ester (0.71%), hexadecanoic acid ethyl ester (1.18%), tetradecanoic acid ethyl ester (0.09%), phthalic acids (9.65%) and 9-octadecenoic acid (Z)-ethyl ester (2.40%) were the potent bioactive compounds with various bioactive properties like anti-inflammatory, anticancer, hypocholesterolemic, 5-Alpha reductase inhibitor, anti-androgenic activity, and antimicrobial activity.

Mundt *et al.* (2003) isolated meroterpenoid, noscomin and comnostins A-E from *N. commune* displayed antibacterial activity against *Bacillus cereus*, biofilm-forming *Staphylococcus epidermidis* and *Escherichia coli*. Volk and Furkert (2006) isolated norharman from *N. insulare* and showed antibacterial activity. Bhateja *et al.* (2006) isolated carbamidocyclophanes from *Nostoc* sp. CAVN 10 showed moderate antibacterial activity against *S. aureus*. Nostocyclamide, an antifungal compound obtained from *Nostoc* sp. Nostodione from *N. commune* showed antifungal activity (Bhadury and Wright, 2004). Cryptophycin, the depsipeptides isolated from *Nostoc* sp. showed antifungal and antiproliferative activity (Schwartz *et al.*, 1990; Chaganty *et al.*, 2004). Nostofungicidine, is an antifungal lipopeptide from the field-grown terrestrial blue-green algae *N. commune* (Kajiyama *et al.*, 1998). Tolypodiol (diterpenoids), noscomin (meroterpenoids) and comnostin A-E (meroterpenoids) from *N. commune* showed antibacterial activity against *S. epidermis* (Prinsep *et al.*, 1996; Jaki *et al.*, 1999; Jaki *et al.*, 2000).

Fatty acid profiling of *N. commune*, *N. paludosum*, *N. pruniforme*, *N. verrucosum* and *Nostoc* sp. analysed using GC-MS (Temina *et al.*, 2007). All selected *Nostoc* spp. synthesized short (C3–C10) carboxylic acids, whose content is usually smaller than that of long-chain fatty acids (C14–C18). Separation of

hydrocarbons, volatile compounds and fatty acid methyl esters from *Nostoc* sp. was carried out using GC-MS (Dembitsky *et al.*, 1999). A total of 130 metabolites including, 41 cyclohexane, 11 cyclopentane, normal saturated hydrocarbons (C7–C30), fatty acids and benzene derivatives were identified from the extract of *Nostoc* sp.

Abdel-Hafez *et al.* (2015) characterized the extracellular metabolites of *N. muscorum* using GC-MS analysis. Twenty-two compounds were identified and beta ionone was reported as the most prevalent component. Piperazine derivatives, isocyclocitral, α -trans-seequicyclocitral, phytol, oleic acid, fatty acids esters (methyl palmitate and linoleic acid methyl ester), myristic alcohol and palmityl chloride were the major components. Extracellular metabolites of *N. muscorum* have the potential as effective fungicides to control the purple blotch disease of onion. Peramuna (2013) studied the synthesis of neutral lipid in *N. punctiforme*. GC-MS analysis results suggest that neutral lipids of *N. punctiforme* are mainly composed of C16, C16:1, C18, C18:2 and C18:3 fatty acid chains.

2.6 Phylogenetics analysis

Phylogenetic analysis help to determine the evolutionary history and relationship among a group of organisms by using molecular data such as DNA sequence and amino acid sequence. It is very common in evolutionary biology and molecular biology (Horiike, 2016). Nowadays, the molecular phylogenetic method is very popular due to several reasons (Nei and Kumar, 2000). These include (1) the popularity of the DNA sequencing method, (2) the establishment of methods for phylogenetic tree constructions using gene or protein sequences, (3) the results of a phylogenetic analysis being treated in a quantitative pattern, (4) availability of many programs for the construction of phylogenetic tree.

Two methods are used for inferring the phylogenetic trees. The first method is the distance-based method, which employs an evolutionary distance matrix. The representative methods are UPGMA (Unweighted Pair Group Method with Arithmetic) and NJ (Neighbor-Joining). The second method is the character-based method, which directly uses the aligned sequences during tree inference. The

representative methods are maximum parsimony, maximum likelihood, and the Bayesian method.

2.6.1 Neighbor-Joining Method (NJ)

Neighbor-Joining method (NJ) is a widely used distance-based method for inferring phylogenetic trees, which overcomes the disadvantage of UPGMA (Saitou and Nei, 1987). The principal NJ method is to identify pairs of operational taxonomic units (OTUs [= neighbors]) that minimize the total branch length at each stage of clustering of OTUs starting with a star-like tree. Analysing a large dataset (hundreds or thousands of taxa) is practical (Tamura, 2004; Niimura and Nei, 2005). If the number of taxa is less than 100, other methods that are not based on evolutionary distance take precedence over the NJ method. MEGA 7, PHYLIP (Felsenstein, 1989), Clustal W and Clustal X are widely used for inferring phylogenetic trees by this method.

2.6.2 Maximum Likelihood Method (ML)

According to Felsenstein (1981), the maximum likelihood method is a character-based (statistical) method for inferring phylogenetic trees. It assigns probabilities to specific possible phylogenetic trees using statistical techniques for inferring probability distributions. As a result, the calculation time is longer than that of the other methods. To assess the probability of particular mutations, this method requires a substitution model. Thus, identifying the best substitution model must be done in advance. The most well-known maximum likelihood programs are PHYLIP and PhyML (Guindon *et al.*, 2010). Currently, MEGA 7, RAxML (Stamatakis *et al.*, 2005) and TOPALi V2 (Milne *et al.*, 2009) are widely used because they can determine the best substitution model and use it to infer the phylogenetic tree using the maximum likelihood method. This method is considered as a standard technique in rRNA-based phylogeny reconstruction.

2.7 Molecular and phylogenetic analysis in Cyanobacteria.

Molecular studies are the most successful methods for inferring the cyanobacterial phylogenetic relationships. The taxonomy of cyanobacteria has

undergone radical changes as a result of the application of advanced molecular biology techniques. Due to their universal prevalence and conserved function, the 16S rRNA genes are the most often employed genetic marker for identifying bacteria and cyanobacteria. The conservative nature of the 16S rRNA gene, its universal distribution and the availability of information in public databases (GenBank, EMBL, DDBJ and RDP) make the gene very useful for phylogenetic studies and taxonomy. Molecular information provides the basic criteria for cyanobacterial taxonomy, but knowledge of their morphology, physiology, and biochemistry is also necessary to construct a comprehensive phylogenetic system of cyanobacteria (Komarek, 2006).

Molecular characterization including PCR-based methods, such as the 16S rRNA sequences, *nif* gene(s), phycocyanin encoding locus and RNA polymerase gene (Giovannoni *et al.*, 1988; Ludwig and Schleifer, 1994; Nelissen *et al.*, 1996; Lee *et al.*, 1996; Zehr *et al.*, 1997; Honda *et al.*, 1999; Neilan *et al.*, 2002; Hartmann and Barnum, 2010; Mishra *et al.*, 2013; Singh *et al.*, 2013), RuBisCO large subunit gene sequence (*rbcLX*), *hetR* (Han *et al.*, 2009; Schleifer, 2009), nitrogenase gene *nifD* (Henson *et al.*, 2004), the gene *ndaF* (Lyra *et al.*, 2005), *RNase P* (*rnpB*) (Schon *et al.*, 2002), etc., have been used as genetic markers for assessing cyanobacterial taxonomy and phylogenetic-relations.

16S rRNA gene-based phylogenetic studies have been published on the genera *Microcystis* (Neilan *et al.*, 1997; Lyra *et al.*, 2001), *Anabaena*, *Aphanizomenon*, *Planktothrix* (Lyra *et al.*, 2001), *Nodularia* (Lehtimäki *et al.*, 2000; Moffitt *et al.*, 2001) and *Nostoc* (Huo *et al.*, 2021). *Nostoc* is a complex and difficult genus whose identification and taxonomy rely strongly on thorough morphological characters, proper ecological evaluation, detailed molecular analyses, and highly supportive and exhaustive phylogeny (Singh *et al.*, 2020).

Arima *et al.* (2012) studied the phylogeny of the terrestrial cyanobacterium *N. commune* and related *Nostoc* species using molecular genetics and chemotaxonomic approaches. Eight genotypes of *N. commune* were characterized by the differences among 16S rRNA gene sequences and the *petH* gene encoding FNR

by random amplified polymorphic DNA analysis. The phylogenetic tree was constructed based on 16S rRNA sequences using the NJ algorithm showed that *N. commune* was clustered in a monophyletic group with *N. flagelliforme* and *N. punctiforme*. Singh *et al.* (2020) examined the phylogeny of the genus *Nostoc* and provided taxonomic descriptions of *N. neudorfense* sp. nov., from the Czech Republic.

Kumar *et al.* (2018) studied the molecular characterization and phylogeny of some cyanobacterial strains isolated from soil and freshwater ecosystems. 16S rRNA gene was selected for the study of molecular characterization. *Nostoc* sp., *Limnothrix* sp. and *Phormidium* sp. were identified using molecular characterization. The constructed phylogenetic trees revealed their evolutionary relatedness with three different OTUs. Makra *et al.* (2019) investigated the taxonomic relationships among the species of *Anabaena* and *Nostoc* using 16S rRNA and *rbcLX* gene sequences. The study concluded that polyphasic taxonomic approaches are required for the reliable identification of cyanobacterial species.

Molecular phylogenetic characterization of two species of the genus *Nostoc* based on the *cpcB*-IGS-*cpcA* locus of the phycocyanin operon studied by Teneva *et al.* (2012). The study indicated that the genus *Nostoc* is heterogeneous and the analysis of the IGS region between *cpcB* and *cpcA* showed that *Nostoc* and *Anabaena* are distinct genera. Reheková *et al.* (2007) conducted a study on the molecular characterization of selected desert soil cyanobacteria. Molecular characterization of *N. lichenoides*, *N. indistinguendum* sp. nov. and *N. desertorum* sp. nov. was done based on the amplification of partial 16S rRNA sequences and 16S-23S internal transcribed spacer (ITS) regions of the strains. Maximum parsimony, distance, and maximum likelihood methods were used to analyse all aligned sequences.

Molecular characterization of *Anabaena*, *Aphanizomenon* and *Nostoc* strains from Portuguese freshwater habitats was done using sequencing of 16S rRNA, *nifH* and *hetR* fragments (Galhano *et al.*, 2011). The strains of *Nostoc* showed a 98% similarity with a 16S rDNA sequence of *N. muscorum*. The *nifH* partial sequence of

strain also showed high similarity (99%) with *N. muscorum*. Singh *et al.* (2016) investigated a new species of *Nostoc* isolated from Pune, using morphological, ecological and molecular attributes. 16S rRNA, nifD, psbA, PC-IGS and rbcL gene were amplified for molecular identification of the species. The observations of phenotypic, genetic and phylogenetic data proved that the strain is a new species to the genus *Nostoc* with the nomenclature *N. punensis* sp. nov. Mesfin *et al.* (2020) described a novel species, *N. oromo* sp. nov. from Ethiopia based on the morphology, ecology, phylogeny, and characterization of the 16S-23S ITS region of the ribosomal operon. This species is phylogenetically in a clade of mostly microscopic *Nostoc* isolated from desert soils in North and South America.

2.7.1 16S ribosomal RNA

16S ribosomal RNA (16S rRNA) gene is highly conserved in different species of archaea and bacteria and has become an important molecular marker for microbial phylogenetic analysis (Woese, 1987; Woese *et al.*, 1990; Case *et al.*, 2007). The comparison of the 16S rRNA gene sequence of an isolate against a type strain of all prokaryotic species is used for the classification and identification of prokaryotes accurately and conveniently. Numerous studies have shown the efficiency of the sequence analysis of genes encoding small-subunit ribosomal RNA (16S rRNA) in the taxonomy of cyanobacteria (Nubel *et al.*, 1997; Komarek, 2006). For the identification of cyanobacteria, a phylogenetic system based on the 16S rRNA gene (rDNA) sequence information retrieved from organisms in pure cultures has been developed (Turner *et al.*, 1999). Nubel *et al.* (1997) designed the most commonly used cyanobacterial PCR primers; CYA359F (forward), an equimolar mixture of CYA781R(a) and CYA781R(b) (reverse) for specific amplification of a 379 bp of 16S rRNA gene sequence. CYA781R(a) and CYA781R(b) differentiate by two polymorphic bases located at positions 7 and 23 (5' to 3') these primers have the benefits of giving a PCR product that corresponds to variable regions V3 and V4, and that contain significant information for phylogenetic analysis (Yu and Morrison, 2004). Based on 16S rRNA gene sequence analysis, the most

comprehensive and widely used phylogenetic scheme of cyanobacteria is currently available (Shiels *et al.*, 2019).

2.8 Richness of cyanobacteria in paddy fields

Rice (*Oryza sativa* L.) is the principal cereal crop belonging to the family Poaceae, it plays a fundamental role as a staple food for more than half of the world's population including Asians and more particularly Indians. Rice cultivation is largely dependent upon climatic conditions and seasons. Rice provides major dietary energy and vocation for more than 40 billion people in Asia (Horie, 2019). Rice growth, production and development depend on the availability of nutrients in the soil. Soil organic matter provides significant quantities of nitrogen which is essential for the growth and optimum yield of rice and is a primary element of amino acid and protein building blocks, nucleic acids and chlorophyll (Shreshta *et al.*, 2020).

Rice fields are agronomically controlled wetland systems, widely known for the vast diversity of cyanobacteria. The majority of paddy soils have a natural population of cyanobacteria, which provides a potential source of nitrogen fixation at no cost. The presence of cyanobacteria in paddy fields is reported in countries such as India, Indonesia, Pakistan, Bangladesh, Sri Lanka, Iraq, Australia, Egypt, Japan, Morocco, Philippines, and South-East Asia (Renaut *et al.*, 1976). The rice field ecosystem offers a favourable environment for cyanobacterial growth concerning their requirements for light, water, high temperature and nutrient availability. Due to this reason, the diversity of cyanobacteria was more abundant in paddy soils than in upland soils. The abundance of N₂-fixing cyanobacteria is positively correlated with soil pH and phosphorous availability (Gupta *et al.*, 2020).

Indian paddy ecosystems harbour mainly N₂-fixing cyanobacterial species belongs to the genus *Nostoc*, *Anabaena*, *Tolypothrix*, *Aulosira*, *Cylindrospermum*, *Scytonema* and *Westiellopsis* (Nayak *et al.*, 2004). Shiozaki *et al.* (2015) observed that cyanobacteria are more abundant in tropical and subtropical regions as compared to the temperate and sub-temperate regions. The most abundant nitrogen-

fixing cyanobacteria include *Nostoc muscorum*, *N. linckia*, *N. calcicola*, *N. commune*, *Anabaena variabilis*, *A. oryzae*, *A. doliolum*, *Calothrix* spp., *Tolypothrix* spp., *Scytonema* spp., *Westielliopsis prolifica*, *Aulosira fertilisima*, *Phormidium fragile*, *Calothrix geitonos*, *Hapalosiphon intricatus*, *T. tenuis*, *Oscillatoria acuta*, *Plectonema boryanumet*, *P. purpurascens*, *O. chlorine*, etc. (Singh *et al.*, 2015; Singh, 1961). Prasanna and Nayak, (2007) reported the dominance of genus *Nostoc* and *Anabaena* in most of the sites whereas Saadania and Riahi, (2009) observed *Anabaena* as an important occupant of paddy field rhizosphere.

2.9 Symbiotic association between plant cells and cyanobacteria

Symbiotic cyanobacteria have unique features that make them particularly significant in acting as N₂-fixers for symbiotic association with plants of commercial interest, such as cereals. They have the mechanism of protecting nitrogenase from inactivation by oxygen (heterocytes). Cyanobacteria are unique in that they can coexist with a wide variety of hosts, including fungi, sponges, protists, and angiosperms. They are not constrained to roots but form symbiosis with various plant parts, and do not need to be located intracellularly within the host plant (Adams *et al.*, 2006; Bergman *et al.*, 2007). In a symbiotic relationship, both partners benefit from each other in different ways. The provision of nitrogen that the cyanobacteria can fix from the air benefits the host organisms (plants).

Symbiotic cyanobacteria are mostly heterocyst-forming, and in particular belong to the genus *Nostoc* (Bergman *et al.*, 1993). Colonization of cyanobacteria in different parts of wheat plant observed by Gantar *et al.* (1999). Cyanobacteria accumulate around the roots in the form of a thick biofilm, they are also found in intercellular spaces in the root epidermis and cortex. The cyanobacteria are enlarged, and the biomass yield increases. The rate of nitrogen fixation and EPS (Exopolysaccharide) production increased at the same time. Nilsson *et al.* (2002) isolated and identified twenty-one symbiotic *Nostoc* spp. from rice roots. Paranodules were formed on the roots and they colonise on the surface and intercellular spaces. The rate of N₂ fixation by associated cyanobacteria was higher

compared with those in free-living cyanobacteria. The symbiosis has diverse effects on the growth and development of cyanobionts.

In *Gunnera-Nostoc* symbiosis an increased heterocyst formation of up to 80% could be observed. This would also demonstrate that only about 12% of the fixed nitrogen remains in the cyanobacteria, while the remaining 88% is supplied to the host organism in the form of NH_3 (Silvester and Mcnamara, 1973). Eily *et al.* (2019) studied the symbiosis of *Azolla-Nostoc*. *N. azollae* cannot exist in nature without the host plant *Azolla*, as the genome of the organism is adapted to the symbiotic way of life. The symbiotic growth not only improves the quantity of many plants but, in some circumstances, also the quality. The positive effects of co-culture on the symbiotic partners and the environment are summarized in Fig. 2.1.

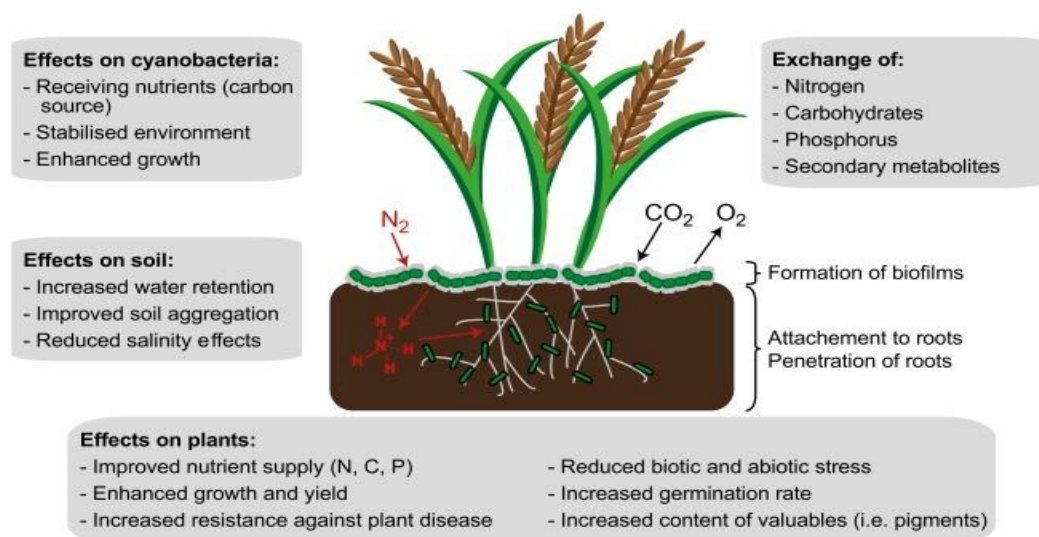
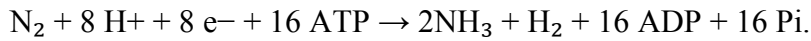


Fig. 2.1 Schematic overview of the effects of cyanobacteria–plant symbiosis on both partners and their surroundings (Kollmen and Strieth, 2022).

2.10 Biological Nitrogen Fixation

Nitrogen (N), one of the most vital elements, plays an important role in plant growth and various metabolic activities. It is often the limiting nutrient in both natural and agricultural systems. Although nitrogen exists abundantly in the earth's atmosphere, dinitrogen (N_2) is relatively inert and accounts for 78% of total atmospheric gases, but this form are not available to plants. Conversion of nitrogen to ammonia (NH_3) by bacteria and archaea that contain an enzyme complex called

nitrogenase (termed ‘diazotrophs’) using energy from ATP and reductant (electrons) supplied by respiration (which can be aerobic or anaerobic) (Dixon and Kahn, 2004) is termed biological nitrogen fixation (BNF). The overall reaction formula is,



In nature, various nitrogen fixers are distributed, and the N_2 fixing ability of those microbes can serve as a substitute for commercial fertilizers (Ladha *et al.*, 1997; Raymond *et al.*, 2004). According to Ali *et al.* (2017), nitrogen-fixing microbes can be divided into two categories (i) symbiotic (mutualistic relationship between bacteria and leguminous plants and non-leguminous trees) and (ii) non-symbiotic (free-living and endophytic organisms). Widely reported symbiotic N_2 fixers in soil are *Frankia* and *Rhizobium* species, whereas diazotrophic PGPR, including Cyanobacteria (e.g. *Nostoc* spp.), *Azospirillum*, *Pseudomonas*, *Azotobacter* and *Acetobacter* are non-symbiotic nitrogen-fixing microbes (Umar *et al.*, 2020). Among them, cyanobacteria are the only prokaryotes that fix nitrogen and simultaneously perform oxygen-evolving photosynthesis (Kulasooriya and Arachchi, 2016).

In cyanobacteria, the specialised cells (heterocysts) procure nitrogen fixation by creating a microoxic environment. In the heterocystous genus *Nostoc* and *Anabaena*, usually, the time required for the formation of mature heterocysts is between 12 and 20h (Adams, 2000b). The enzyme complex nitrogenase is synthesized in heterocysts (Fleming, 1973). Nitrogen cannot diffuse directly into a heterocyst through the cell wall and therefore passes from a vegetative cell into a heterocyst via thin cytoplasmic channels (micro-plasmodesmata) (Fig. 2.2). There, the enzyme nitrogenase reduces nitrogen to ammonia or ammonium. ATP is needed for this reaction and is synthesized in the heterocyst, by PSI which remains active. The reducing agent for nitrogen fixation must be provided by the neighbouring cells. NADPH is formed as a reducing agent via the pentose phosphate pathway. The reduced nitrogen is transferred to glutamate by the enzyme glutamine synthetase, and thus glutamine is formed, which enters the vegetative cells through the micro-plasmodesmata. Here, the enzyme glutamate synthase is present, which catalyzes the reaction of glutamine with ketoglutarate to form two equivalents of glutamate. Glutamate re-enters the heterocyst for further glutamine synthesis or be fed to other

metabolic pathways. However, the fixed nitrogen is not only used by the cyanobacteria to meet their own needs but it can also be released into the medium as ammonium (Andersen and Shanmugam, 1977).

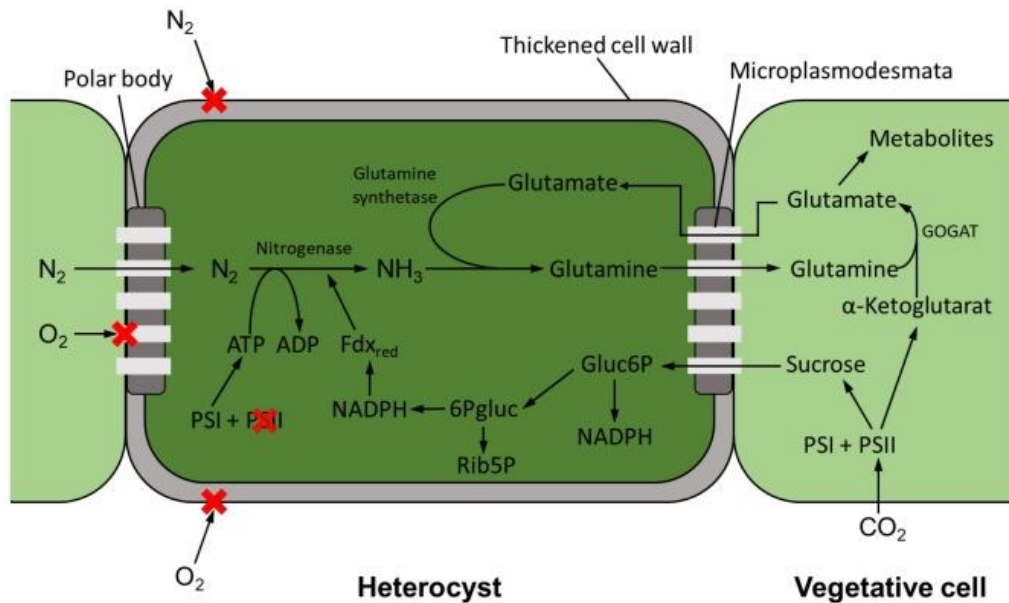


Fig. 2.2 Schematic illustration of nitrogen fixation in heterocysts and metabolic exchange with neighboring vegetative cells. Fdx_{red} = reduced ferredoxin, Gluc6P = glucose-6-phosphate, Rib5P = ribulose-5-phosphate, 6Pgluc = gluconate-6-phosphate, GOGAT = glutamate synthase, PSI = photosystem I, and PSII = photosystem II (Kollmen and Strieth, 2022).

2.11 Cyanobacteria as a biofertilizer

The increase in world population is directly and indirectly dependent on the demand for contamination-free healthy food. By 2029, the World Health Organization expects the production of food to increase by 50%. “Green Revolution” practices are also improving agriculture output and reducing the risk of chemical-based fertilizers on human health as well as the environment. Green technology utilises the use of cyanobacteria to improve crop productivity and soil fertility. Cyanobacteria are crucial for enhancing plant growth and crop production. They are good bio-fertilizers, improve solubilization and mobility of nutrients, stimulate plant growth by their ability to produce bioactive compounds, such as phytohormones and other plant growth regulator substances, like amino acids and polysaccharides, as well as increase essential microelements in soil that are necessary for ion uptake (Fig. 2.3). They are composed of numerous organic

inclusion units capable of carrying out various specialized functions, which give cyanobacteria their unique tasks and applications in sustainable agriculture.

Cyanobacteria can produce several bioactive molecules that enhance the soil nutrient status, boost crop growth, and protect crops from diseases in addition to fixing nitrogen (N_2) to meet their own nitrogen needs. Cyanobacteria are emerging microorganisms for the development of sustainable agriculture (Singh *et al.*, 2016). Since the time immemorial, cyanobacteria have been used as a biofertilizer in agricultural systems. Diazotrophs are cyanobacteria useful for the development of eco-friendly biofertilizers which are easily available and less costly. They are important microbial members in rice fields employed as bioinoculants for improving soil fertility, structure of soil and crop yields (Venkataraman, 1972; Kaushik, 1998; Prasanna *et al.*, 2012). They are significant contributors of carbon, nitrogen, phytohormones, and polysaccharides that enhance rice production and soil fertility (Prasanna *et al.*, 2012). Inoculation with cyanobacteria has also been proposed as a biofortification strategy for staple crops (Rana *et al.*, 2012; Prasanna *et al.*, 2016). Cyanobacteria are used to improve crop productivity and they can degrade a wide range of pollutants and perform different roles in the soil ecosystem to sustain soil fertility (Subramanian and Uma, 1996).

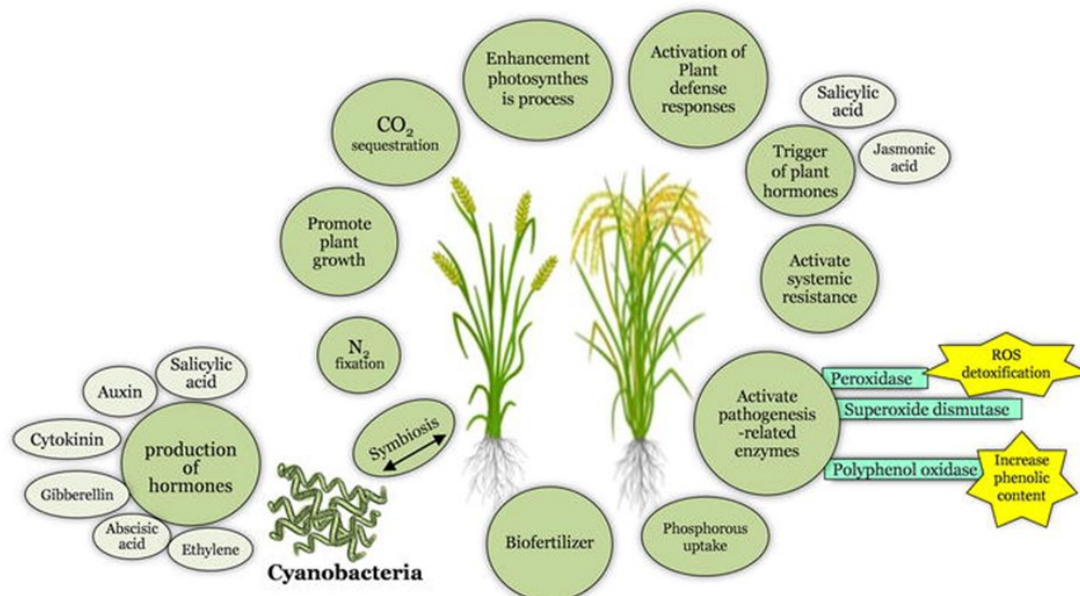


Fig. 2.3 Role of cyanobacteria in enhancing plant growth and stimulating the response of defense systems (Elageamey *et al.*, 2023).

Beneficial effects of cyanobacterial inoculation were reported, not only for rice, but also for other crops such as wheat (*Triticum aestivum* L.), soybean (*Glycine max* L Merr.), oats (*Avena sativa* L.), tomato (*Solanum lycopersicum* L.), bean (*Phaseolus vulgaris* L.), cotton (*Gossypium hirsutum* L.), sugarcane (*Saccharum* sp.), maize (*Zea mays* L.), radish (*Raphanus sativus* L.), muskmelon (*Cucumis melo* L.), chili (*Capsicum annuum* L.), and lettuce (*Lactuca sativa* L.) (Table 2.1) (Venkataraman 1972; Rodgers *et al.* 1979; Singh 1988; Arif *et al.*, 1995; Thajuddin and Subramanian, 2005; Karthikeyan *et al.*, 2007; Maqubela *et al.*, 2008; Saadatnia and Riahi, 2009).

Table 2.1 Application of cyanobacterial inoculum on various plant species and main effects produced.

Cyanobacteria	Positive effects on plant	Plant
<i>Nostoc</i> spp.	Plant growth and pigment content (Takacs <i>et al.</i> , 2019; Mohsen <i>et al.</i> , 2016), seed yield (Toth <i>et al.</i> , 2019; Karthikeyan <i>et al.</i> , 2007), tolerance to cold and drought stress (Setta <i>et al.</i> , 2018), leaf relative water content (Takacs <i>et al.</i> , 2019).	Rapeseed (Toth <i>et al.</i> , 2019), wheat (Takacs <i>et al.</i> , 2019; Karthikeyan <i>et al.</i> , 2007), cantaloupe (Farrag <i>et al.</i> , 2017), lettuce (Mohsen <i>et al.</i> , 2016), bean (Setta <i>et al.</i> , 2018).
<i>Cylindrospermum</i> sp.	Plant growth, pigment content and essential oil content (Shariatmadari <i>et al.</i> , 2015; Haroun and Hussein, 2003)	Peppermint (Shariatmadari <i>et al.</i> , 2015), lupin (Haroun and Hussein, 2003).
<i>Calothrix</i> sp.	Plant growth (Shariatmadari <i>et al.</i> , 2015; Singh <i>et al.</i> , 2011), macro- and micronutrient content (Rana <i>et al.</i> , 2012), germination (Prasanna <i>et al.</i> , 2014), seed yield (Karthikeyan <i>et al.</i> , 2007), essential oil content (Shariatmadari <i>et al.</i> , 2015), phytohormones content (Priya <i>et al.</i> , 2015)	Rice (Singh <i>et al.</i> , 2011), coriander (Kumar <i>et al.</i> , 2013), peppermint (Shariatmadari <i>et al.</i> , 2015), wheat (Rana <i>et al.</i> , 2012; Karthikeyan <i>et al.</i> , 2007), cotton (Prasanna <i>et al.</i> , 2014).
<i>Anabaena</i> sp.	Plant growth (Singh <i>et al.</i> , 2014; Mahmoud <i>et al.</i> , 2019), germination (Kumar <i>et al.</i> , 2013) macro- and micronutrient content and photosynthesis rate (Grzesik <i>et al.</i> , 2017) pigment content (Haroun and	Rice (Singh <i>et al.</i> , 2014), spinach (Mahmoud <i>et al.</i> , 2019), peppermint (Shariatmadari <i>et al.</i> , 2015), lupin (Haroun and

	Hussein, 2003), essential oil content (Shariatmadari <i>et al.</i> , 2015) phytohormones content (Bharti <i>et al.</i> , 2019), tolerance to thermal stress (Piotrowski <i>et al.</i> , 2015).	Hussein, 2003), willow (Grzesik <i>et al.</i> , 2017), wheat (Rana <i>et al.</i> , 2012), cotton (Prasanna <i>et al.</i> , 2014), cumin (Kumar <i>et al.</i> , 2013).
<i>Arthrospira fusiformis</i>	Bulb diameter (Shalaby and El-Ramady, 2014).	Garlic (Shalaby and El-Ramady, 2014).
<i>Scytonema hofmanii</i>	Tolerance to salt stress (Rodríguez <i>et al.</i> , 2006).	Rice (Rodríguez <i>et al.</i> , 2006).
<i>Aphanothece</i> sp.	Plant growth, macro- and micronutrient content (Mutale-joan <i>et al.</i> , 2020).	Tomato (Mutale-joan <i>et al.</i> , 2020).

2.11.1 Cyanobacteria as bio-control agents

The bioactive compounds produced by cyanobacteria belong to the group of polyketides, amides, fatty acids, indoles, alkaloids, and lipopeptides (Abarzua *et al.*, 1999; Burja *et al.*, 2001) which have antifungal, antibacterial and antiviral potential (Teuscher *et al.*, 1992; Dahms *et al.*, 2006). The potency and mode of action of various cyanobacterial secondary metabolites are species as well as stress-specific. Dahms *et al.* (2006) reported the anti-algal potential of cyanobacterial compounds which inhibit the growth of pathogens by disturbing their metabolic and physiological activities.

Research has focused mostly on the order Nostocales and Oscillatoriales since early 2000. Extracts of *Nostoc* spp., *Scytonema* spp., *Lyngbya lutea*, *Oscillatoria* spp., *Microcystis aeruginosa*, *Anabaena* spp., *Fischerella* sp. *Phormidium tenue*, *Trichodesmium hildebrandtii*, *Synechococcus elongates*, and *Synechocystis* sp. inhibited *Aspergillus* spp. mycelial growth in agar disk diffusion assay (Marrez and Sultan, 2016; Shishido *et al.*, 2015; El-Sheek *et al.*, 2006; Pawar and Puranik, 2008). Exo-metabolites from *Nodularia harveyana* and *Nostoc insulare* exhibit antibacterial and antifungal properties (Volk and Furkert, 2006). Extracellular diterpenoids from *N. commune* showed antibacterial effects (Jaki *et al.*, 2000). Additionally, cyanobacteria possess molecules that can act as biopesticides,

thus protecting the plant against pathogens, mainly fungi or bacteria (Table 2.2). In addition, cryptophycin producer *Nostoc* sp. is the natural source of pesticides against insects, fungi, and bacteria (Biondi *et al.*, 2004).

The algicidal activity of cyanobacteria is restricted to the order Stigonematales and Nostocales, and genera, *Nostoc*, *Anabaena*, *Calothrix*, *Fischerella* and *Scytonema*. *Nostoc* sp. produced compounds cyanobacterins LU-1 and LU-2 show algicidal activity (Juttner *et al.*, 2001). Nostocine A, nostocyclamide, and nostocarboline are reported from different strains of *Nostoc* spp. shows antialgal activity whereas *Nostoc spongiaeforme* TISTR 8169 produces nostocine A with herbicidal, antibiotic, and algicidal properties.

Extracts of *Anabaena oryzae*, *Tolypothrix tenuis*, and *Nostoc muscorum* possess a significant decrease in the natural infestation of Indian rice together with the stem borer *Chilo agamemnon* and leaf miner *Hydrellia prosternalis* (Yanni and Abdallah, 1990). Nostocarboline is an alkaloid and protease inhibitor obtained from *Nostoc* sp. active against species of *Trypanosoma*, *Plasmodium* and *Leishmania*.

Table 2.2 Cyanobacterial species exhibiting antagonistic effects against different plant pathogens.

Cyanobacteria	Plant disease/ plant pathogens	References
<i>Nostoc muscorum</i>	Cottony rot of vegetables and flowers (<i>Sclerotinia sclerotiorum</i>), damping off, root and stem rots (<i>Rhizoctonia solani</i>), wood blue stain (<i>Aureobasidium</i> , <i>Alternaria</i> , <i>Cladosporium</i>).	De Caire <i>et al.</i> , 1990 Tassara <i>et al.</i> , 2008 Kulik, 1995 Zulpa <i>et al.</i> , 2003
<i>Nostoc</i> strain ATCC 53789	Root rot (<i>Armillaria</i> spp.), fusarium wilt (<i>Fusarium oxysporum</i>), fruit rot disease (<i>Penicillium expansum</i>), bud-rot of palms, fruit-rot or kole-roga of coconut and areca nut (<i>Phytophthora palmivora</i>).	Biondi <i>et al.</i> , 2004
<i>N. commune</i>	Black mold (<i>Aspergillus niger</i>), ear rot disease in maize (<i>A. flavus</i>).	Pawar and puranik, 2008

<i>N. linckia</i>	Tomato vascular wilts (<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>).	Alwathnani and Perveen, 2012
<i>N. entophytum</i>	Damping off (<i>R. solani</i>).	Osman <i>et al.</i> , 2011
<i>N. piscinale</i>	Rice sheath blight (<i>R. solani</i>).	Zhou <i>et al.</i> , 2020
<i>Fischerella muscicola</i>	Brown rust (<i>Uromyces appendiculatus</i>), powdery mildew (<i>Erysiphe graminis</i>), rice blast (<i>Phytophthora infestans</i> and <i>Pyricularia oryzae</i>).	Hagmann and Juttner, 1996 Papke <i>et al.</i> , 1997
<i>Calothrix elenkenii</i>	Damping off (<i>R. solani</i>).	Manjunath <i>et al.</i> , 2010
<i>Anabaena variabilis</i>	Damping off (<i>R. solani</i>).	Chaudari <i>et al.</i> , 2012

2.11.2 Improvement of physical properties of soil by Cyanobacteria

Cyanobacteria can excrete several extra-cellular compounds like polysaccharides, peptides and lipids during their growth in soil. They can secrete mucilage or slime, which increases the availability of nutrients and enhances soil structure creating an ideal environment for the growth of advantageous microorganisms and plays a role in enhancing soil characteristics. *N. muscorum* excrete exopolysaccharides and enhance saline soil stability. Exopolysaccharides (EPSs) of cyanobacteria, act as gluing agents, binding soil particles, and promoting the formation of soil aggregates, thus increasing soil stability (Mazor *et al.*, 1996). Cyanobacteria can aid in the improvement and recovery of infertile soils by releasing the holding and aggregation of soil particles together, the accumulation of organic content, and an improvement in the water-holding capacity of the upper soil layer. The addition of cyanobacteria to the soil will enhance soil characteristics and texture by adjusting soil stabilization, nutrients, moisture-holding capacity, and crust formation. Excretion of vitamins, amino acids, and phytohormones (auxin, gibberellins, etc.), improves the water-holding capacity of soil through their characteristic jelly structure, makes soil porous and produces adhesive substances, increases in biomass of soil after their death and decomposition, lowers the salinity of the soil, availability of soil phosphate is caused by organic acid excretion, efficient absorption of heavy metals on the microbial surface (bioremediation) are the major action of cyanobacteria (Roger and Reynaud,

1982; Rodríguez *et al.*, 2006; Saadatinia and Riahi, 2009; Wilson, 2006; Ibraheem, 2007).

2.11.3 Cyanobacteria for the protection against abiotic stress

Abiotic stress in plants is caused by a variety of factors, such as temperature, droughts, light, and soil-related factors, including salinity, soil acidity and the presence of heavy metals (Cramer *et al.*, 2011). Response to high soil salinity can be influenced by cyanobacteria that synthesise and accumulate protective substances, maintain low intracellular ion concentrations, and express the so-called salt stress proteins. Rodríguez *et al.* (2006) demonstrated the effect of the extracellular products of *Scytonema hofmanni* on the growth of rice plants under salt stress and these extracellular products made rice plants able to cope with stress caused by high salt concentrations. In addition, rice plants showed an increase in tolerance to salinity after inoculating roots with strains isolated from saline soils, such as *N. calcicola*, *N. linckia*, and *Anabaena variabilis* (El Sheek *et al.*, 2018). Singh *et al.* (2011) reported treatment of rice roots with *Oscillatoria acuta* and *Plectonema boryanum* showed an effective response to abiotic stress on rice by increasing the activity of peroxidase, phenylpropanoid and phenylalanine ammonia-lyase.

Cyanobacteria effectively remove heavy metals from agriculture soil and water. *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima*, and *Tolypothrix tenuis*, may absorb and remove Cr, Cu, Pb, and Zn, whereas *Oscillatoria* sp. and *Synechocystis* sp. can remove Cr, this was linked to increasing the growth of wheat (Faisal *et al.*, 2005).

2.12 *Nostoc* spp. as a biofertilizer for rice and other crops

Nostoc spp. are potential candidates that fix atmospheric nitrogen, so that can be used as a biofertilizer for the cultivation of economically important crops like rice, wheat and beans. This genus is found in both free-living forms and in symbioses, covering a wide range of habitats, and is known to survive under different C and N nutrition (Potts, 2000; Rai *et al.*, 2000). Since *Nostoc* is the most prevalent cyanobacterial genera in natural symbiotic associations (Rai *et al.*, 2000),

symbiotically competent *Nostoc* strains would likely be more prone than other non-symbiotic cyanobacteria to form associations with a 'new' plant, such as rice. Previous studies reported that some strains of *Nostoc* and *Anabaena* can colonize the roots of wheat and carry out associative N₂-fixation (Gantar and Elhai, 1999; Gantar, 2000). The genus *Nostoc* can fix atmospheric nitrogen to ammonia, which may then be able to be utilized or converted to a form that is beneficial for plant growth and development (Esch, 2014). Researches indicate a positive effect of *Nostoc* spp. inoculation on rice cultivation (Nillson *et al.*, 2002; Perez *et al.*, 2020).

Pereira *et al.* (2009) studied the effect of filamentous nitrogen-fixing cyanobacteria as a biofertilizer for rice crops in Chile. *N. commune* showed the highest N₂ fixation and survival rates despite the drastic desiccation system utilized. The study concluded that *Anabaena iyengarii* var. *tenuis*, *Nostoc commune*, *N. linckia* and *Nostoc* sp.1 proved to be good options for the formulation of biofertilizers for rice varieties. They also reported that using local strains of heterocystous cyanobacteria as biofertilizers can reduce the use of synthetic fertilizer by 50%, resulting in the same grain yield as the use of full doses of chemical fertilizer. Hassan, (2020) reported the propagation of rice was tremendously fast when treated with cyanobacteria such as *Nostoc* and *Anabaena*. An increase in 51% of plant height, 68% in root length, 56% in fresh shoot weight, 92% in fresh root weight, 120% in dry shoot weight, 146% in dry root weight, 32% in soil moisture, 30% in soil porosity and 9.3% decrease in soil bulk density were analysed by the application of cyanobacterium *Nostoc*.

Begum *et al.*, (2011) investigated the importance of cyanobacterial biofertilizers on the growth and yield components of two rice varieties. The study reported cyanobacterial inoculum 1 kg ha⁻¹ (*Nostoc spongiaeforme* Dh 164, *N. commune* Dh 169, *Calothrix marchica* Dh 167 and *Stigonema* Dh 168) produced a significantly higher number of tillers/hills, panicles/hill, length of panicle, the weight of 1000 grains, and yields of grain and straw as compared to the control. El-Sheek *et al.* (2018) examined the effect of cyanobacterial isolates on rice seed germination in saline soil and the results of the investigation showed that the

inoculation with *Nostoc calcicola* and *N. linckia* increased root length and shoot length of rice plants. The study also recommended the use of *Nostoc calcicola* and *N. linckia* as biofertilizers for rice in saline soil for increasing growth and decrease soil electrical conductivity.

Chittapun *et al.* (2017) studied the effects of cyanobacteria and chemical fertilizers on the growth and yield of rice (*Oryza sativa* L.) cv. Pathum Thani, Thailand. The results concluded that *N. carneum* TUBT04 and *N. commune* TUBT05 showed promising characteristics as biofertilizers in terms of IAA and exopolysaccharide production and secretion. In addition, the combined biofertilizer with half of the recommended dose of chemical fertilizer significantly enhanced rice production, and the study also recommended using a half dose of chemical fertilizer combined with cyanobacteria to decrease rice production costs for farmers without compromising any effects on the yield of rice.

Rogers *et al.* (1994) demonstrated the polysaccharide secretion by *Nostoc muscorum* increased soil stability by 18% within 300 days. Simultaneously, the carbon content of the soil was increased resulting in a 50% increase in the germination rate of lettuce plants. Toth *et al.* (2019) investigated the influence of *N. entophytum* and *Tetracystis* sp. on winter survival of rapeseed (*Brassica napus* L.). The treatments with *N. entophytum* showed plant growth increase and survival, which contributed to the significant increase of thousand seed weight (18%-25%) and total yield (by 10%-24%). Tackacs *et al.* (2019) studied the biostimulating effects of the cyanobacterium *Nostoc piscinale* on winter wheat in field experiments. The cyanobacterial treatments increased the ear number, ear length, grains per ear, thousand-grain weights and the yield of the winter wheat variety “Bóség.

Rao and Burns, (1990) demonstrated that the inoculation of a flooded brown earth silt loam with a consortium of cyanophyceae (including *N. muscorum*) increased the soil carbon and nitrogen content leading to a subsequent improvement in soil aggregate stability. The effects of *N. muscorum* on soil physical, chemical, and biological properties indicated the possible benefits of cyanobacteria as soil

inoculants, not only for the improvement of soil aggregate stability but also as a means of improving seedling emergence, plant growth, and yield.

Obreht *et al.* (1993) reported stimulation of root growth in the co-cultivation of wheat with *Nostoc* 2S9B, *Nostoc* 2S6B and *Anabaena* C5 for 15 days in both nitrogen-containing and nitrogen-free medium. A large stimulation of root length was observed without any increase in root dry weight and also increased the nitrogen concentration of roots and shoots co-cultivated with cyanobacteria. Depending on the cyanobacterial strain, the cyanobacteria adhered to the roots and led to an increase in the nitrogen content in the roots.

The growth of maize plants in combination with the cyanobacterial strains, *Anabaena* sp., *A. doliolum*, *Nostoc carneum*, and *N. piscinale* was assessed (Prasanna *et al.*, 2016). An increase in the amount of pigment in the cyanobacteria was shown in combination with increased growth of the plants. Also, the amount of carbon, nitrogen, and phosphate in the soil increased by 10-39%, 4-43%, and 13-32% respectively. Karthikeyan *et al.* (2007) observed the gain of yield and total biomass of wheat by co-cultivation with *Nostoc* sp., *Calothrix ghosei* and *Hapalosiphon intricatus*. In addition, the nitrogen and protein contents of the grains also increased. Inoculation of soil with a suspension of *N. entophytum* and *Oscillatoria angustissima* or a combination of two strains significantly increased the germination percentage and accelerated the other measured growth parameters and photosynthetic pigment fractions of pea (Osman *et al.*, 2010). The study also concluded that *N. entophytum* fixed more nitrogen, produced more exopolysaccharides, and contained more auxin and cytokinin than *O. angustissima*. According to Obana *et al.* (2007), heterocystous cyanobacteria have a beneficial impact on plant development and nutrient uptake. They demonstrated that the application of *Nostoc* improved plant growth and ion uptake while also increasing the organic C and N content of the surface soil, and they also reported *Nostoc* spp. provide the microelements required for the growth of plants.

2.13 Seed bio-priming

Seeds are the primary requirements for agriculture. Therefore, it is essential to maintain the quality of the seeds using nature-based solutions having multiple advantages. Bio-priming has proven to be the simple, cost-effective and environmentally friendly delivery system of beneficial microorganisms in the agro-ecosystem among pre-sowing procedures. Seed bio-priming is an innovative skill of biological seed treatment which includes the integration of seed hydration and inoculation of the seeds with beneficial microorganisms. Microbial inoculants such as plant growth-promoting rhizo-microorganisms (bacteria, cyanobacteria or fungi) are used for bio-priming of seeds. It is a sustainable ecological approach using specific microorganisms that promote plant growth by producing plant growth-promoting substances enhancing nutrient uptake or protecting seedlings/plants against soil-/seed-borne plant pathogenic organisms (Prasad *et al.*, 2016).

Recently, bio-priming of seeds has been used as a substitute for synthetic chemical pesticides and fertilizers for being eco-friendly and safer for future agriculture and gaining importance in the seed, plant and soil health improvement programs. Seed bio-priming refers to a standard method for the introduction of disease resistance through bio-control agents (Prabha *et al.*, 2019). It is a promising technique that improves the stress tolerance of seeds before germination (Harman and Taylor 1988; Jenson *et al.*, 2004). It improves crop yield by enhancing seed germination, vigour, viability, plant growth and protection against seed or soil-borne pathogens. Bio-priming of seeds is a suitable substitute for seed treatment, because the microorganisms multiply continuously, occupy the growing root surfaces, form a biofilm around the roots and protect the plants from diseases throughout the crop-growing stage. The elicitation of systemic resistance in plants that can protect the plants from foliar pathogens during the later stages of their growth and development is another benefit of seed bio-priming. This supports the idea of promoting seed bio-priming techniques among farmers, which will not only protect the health of the seeds and crops but also contribute to ensuring ecological sustainability.

Seed bio-priming can also improve the nutritional and physiological qualities of the seed, resulting in better germination and adaptation under various soil conditions. When combined with beneficial microbial agents associated with plant roots, seed bio-priming can augment plant immunity and productivity. Recent research by several groups revealed that these microbes also elicit what is known as induced systemic tolerance (IST) against biotic and abiotic challenges (Yand *et al.*, 2006).

2.13.1 Role of cyanobacteria in seed bio-priming for plant growth promotion

Cyanobacteria have been found to secrete growth-promoting substances e.g. auxins, cytokinins and gibberellins (Phytohormones) in their exopolysaccharides (Chamizo *et al.*, 2018). These chemicals have important functions in plant growth metabolism, regulations and development (Hashtroudi *et al.*, 2013). For example, cytokinins enhance seed germination, shoot length and grain weight, amongst other attributes of plant growth (Mazhar *et al.*, 2013). According to Mahmood *et al.* (2016), seeds soaked in cyanobacterial suspensions allow imbibition into the seeds and favour optimal conditions for their inoculation and colonization in the seed. Moreover, the application of advantageous microbes to seeds, such as cyanobacteria, can be a successful method for delivering the inoculum into the soil after germination (O'Callaghan, 2016). Seed immersion or priming in cyanobacterial inoculum results in an increase in the germination rate, improved plant development, and a higher productive yield in a wide variety of cereal, horticultural and vegetable crops (Rodríguez *et al.*, 2006; Saadatnia and Riahi, 2009).

According to Jacq and Roger (1977), rice seeds pre-soaked with cyanobacterial culture extracts showed enhanced germination, promotion of root and shoot growth and an increase in the weight and protein content of grains (Fig. 2.4). Muñoz-Rojas *et al.* (2018) studied the positive effect of bio-priming seeds with cyanobacteria on germination and seedling growth of *Acacia hilliana*, *Senna notabilis*, *Grevillea wickhamii*, *Triodia epactia* and *Triodia wiseana*. Seeds of plants were soaked in cultures of *Nostoc microscopicum* and *Microcoleus paludosus*. A significant positive effect of seeds bio-primed with cyanobacteria on germination and seedling growth of two species, *Senna notabilis* and *Acacia hilliana*,

respectively was noticed. Chua *et al.* (2019) conducted a study on the bio-priming of seeds with cyanobacteria and analysed the effects on native plant growth and soil properties. *Leptolyngbya* sp., *Microcoleus* sp., *Nostoc* sp. and *Scytonema* sp. were used for the study. Bio-primed seeds using cyanobacteria produced seedlings with longer shoot and radicle lengths for several species, compared with non-primed seeds. Bio-primed seeds of *Eucalyptus gamophylla*, *Grevillea wickhamii* and *Triodia wiseana* produced both longer shoots and radicles, whereas bio-primed seeds of *Acacia hilliana* and *Senna notabilis* produced seedlings with noticeably longer radicles.

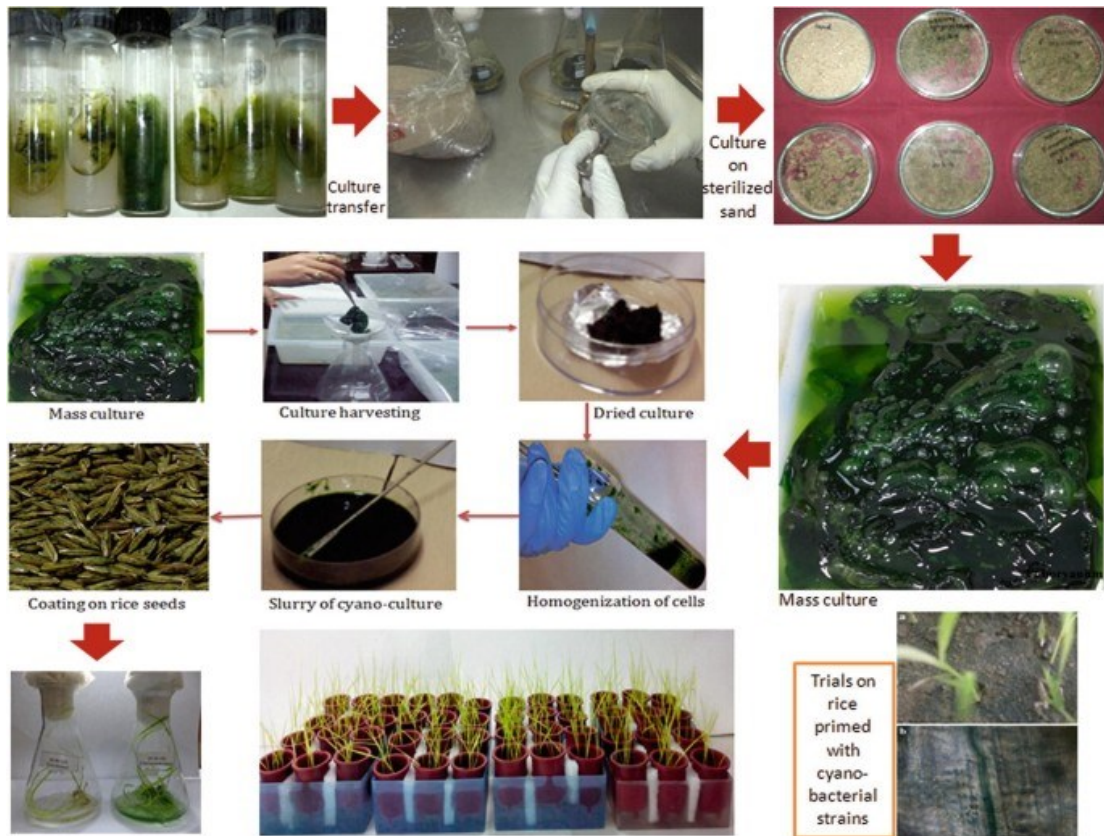


Fig. 2.4 Seed bio-priming in rice plants with cyanobacterial inoculum (Prabha *et al.*, 2019).

Ismail and Abo-Hamad, (2017) investigated the effect of different treatments of *Anabaena variabilis* on the growth and physiological aspects of *Hordeum vulgare* and *Trigonella foenum-graecum*. Three treatments of *A. variabilis* (seeds primed in 1% fresh cyanobacterial extract, seeds sown in soils inoculated with 3 g fresh or dry cyanobacterial cells per 1 kg soil and wet seeds primed with 3 g dry cyanobacterial

powder per 1 kg seeds) were examined. In all treatments, the germination percentage, shoot length, and fresh and dry weights of plants were increased. The physiological parameters such as photosynthetic pigments, proteins, glutamic-oxaloacetic and glutamic-pyruvic transaminases (GOT and GPT) activities were also markedly increased especially in seeds primed with cyanobacterium dried cells.

Fitriati *et al.* (2015) examined the influence of *Nostoc* spp. on germination of paddy seedlings. Rice seeds were soaked in a medium that contained *Nostoc* strains in different quantities. Application of *Nostoc* strains CPG24 and GIA13a on the germination of the seedling of the paddy variety influenced the plant height. The study concluded that effective inoculum for paddy seedlings is between 0.1 g to 0.3 g fresh weight given on the 1st day of germination. Toribio *et al.* (2021) analysed the effect of cyanobacterial extract from *Anabaena* spp., *Tolypothrix* spp., *Nostoc* spp. and *Trichormus* spp. to control the incidence of damping-off caused by *Pythium ultimum* in cucumber seedlings. Seed bio-primed with extracts of cyanobacteria showed a remarkable promoter effect in the early stages of plant development, although the extract is an effective control agent against damping-off caused by *P. ultimum* in cucumber seedbeds.

CHAPTER 3

MATERIALS AND METHODS

To study the morphological, biochemical and molecular characterization of *Nostoc* spp. and its assessment as biofertilizer for rice, the following methodologies were adopted.

3.1 Chemicals and Glasswares

The analytical and molecular grade chemicals used for the study were purchased from HiMedia, SRL and Merck (India). The master mix for PCR amplification was purchased from Takara, Japan. The primers used for PCR were purchased from Eurofins, Genomics India Pvt. Ltd., Bangalore. All the glassware and plastic ware were purchased from Borosil, Riviera and Tarsons (India). Conical flasks (100, 250, 500 and 1000 ml), petri dishes (100×15 cm), measuring cylinders (10, 100, 250, 500 and 1000 ml), beakers (5, 10, 50, 10 and 250 ml) and micropipettes (0.5-10, 10-100 and 100-1000 μ L) were used for the preparations of culture media, buffers and biochemical assays. For the molecular characterizations, Eppendorf tubes were used.

3.2 Morphological characterization of *Nostoc* spp.

3.2.1 Study area

The State of Kerala located in the southwestern fringe of the Indian Peninsula, is rich in flora and fauna including several endemic species. Among the lower plants, algae are one of the least known and less documented groups (Easa, 2004). The geographical area of the State is 38,852 sq km, which is 1.18% of the country and the State lies between 8°17'N to 12°47'N latitude and 74°52'E to 77°24'E longitude. Kerala shares its border with Karnataka in the north & northeast, Tamil Nadu in the east (Western Ghats) & southeast and the Arabian Sea in the west. Based on physiography, Kerala can be divided into three climatically distinct zones, viz. coastal, midland and highland zones.

The climate of the State is humid with an average temperature ranging from 19.8°C to 36.7°C and the average annual rainfall from 1,520 mm to 4,075 mm. Rain in Kerala is mostly the result of seasonal monsoon, which experiences two monsoons *i.e.*, the Southwest monsoon starting in June and the Northeast monsoon starting in mid-October finally ends around mid-November. The soil of Kerala can be broadly grouped into coastal alluvium, mixed alluvium, acid saline, kari, laterite, red, hill, black cotton and forest soils. A warm humid climate with perennial water resources and nutrient-rich soil has contributed to diverse vegetation with enormous species diversity in the State. The present study was carried out in the Western Ghats regions and paddy fields of Kerala. Rice is the staple food for the people of Kerala, and the rice ecosystem is correspondingly varied and strongly conditioned by the regional climate and landforms. Heterocystous cyanobacteria are abundant in the paddy fields and appear as floating masses on the surface.

The Western Ghats is one of the eight hotspots of biodiversity in the world and is an ecologically sensitive area. Western Ghats regions of Kerala refers to the practically unbroken hill chain (except the Palakkad Gap) running roughly in the north-south direction, for about 1500 km parallel to the Arabian Sea coast, from the river Tapi (about 21° 16' N) down to just short of Kanyakumari (about 8° 19' N) at the tip of the Indian peninsula. About 40% of Western Ghats lies in Kerala and all the fourteen districts of Kerala are included in the Western Ghats ecoregion. Due to the great topographic heterogeneity (from sea level to 2695 m at its highest point, the Anamudi peak) and a strong rainfall gradient (annual precipitation of 700 cm along west-facing slopes) contribute to tremendous cyanobacterial diversity in this area. Location details of the study areas (Table 3.1) and a map of the study areas are presented in Fig. 3.1.

Table 3.1 Location of study sites in different districts of Kerala, India

Sl. No.	District	Study areas	Location (Latitude/Longitude)
1	Kasargod	Ranipuram	12.4280°N, 75.3616° E
2	Kozhikode	Kakkayam	11°33'03"N, 75°55'32" E
		Peruvannamuzhi	11°34'37"N, 75°49'22" E
		Koodaranji	11°33'03"N, 76°03'23" E
		Mavoor	11°26'67"N, 75°91'67" E
		Purakkattiri	75°75'89" N, 11°34'41" E
		Mukkam	11°19'66"N, 75°59'54"E
3	Wayanad	Kuruva island	11°49'25"N, 76°05'37" E
		Kalpetta	11°61'03"N, 76°08'28" E
4	Malappuram	Nilambur	11°28'55"N, 76°23'86" E
		Nadukkani	10°05'24"N, 76°40'29" E
		Ramanattukara	11°25'87"N 75°78'03"E
		Calicut University	11°16'99"N 76°10'27"E.
		Chelari	11° 6' 46"N, 75° 53' 18" E
		Tanur	10°98'20"N, 75°87'54" E
5	Thrissur	Sholayar	10°17'29"N, 76°52'03" E
		Mattampuram	10°56'77"N, 76°24'84" E
		Alapad	10°44'79"N, 76.15'59"E
6	Palakkad	Nelliampathy	10°32'09"N, 76°52'03" E
		Attapadi	11°06'81"N, 76°56'62" E
		Pothundi dam	10°54'18"N, 76°62'54"E
		Nenmara	10°59'34"N, 76'60'06" E
7	Idukki	Vagamon	9°40'21"N, 76°52'03" E

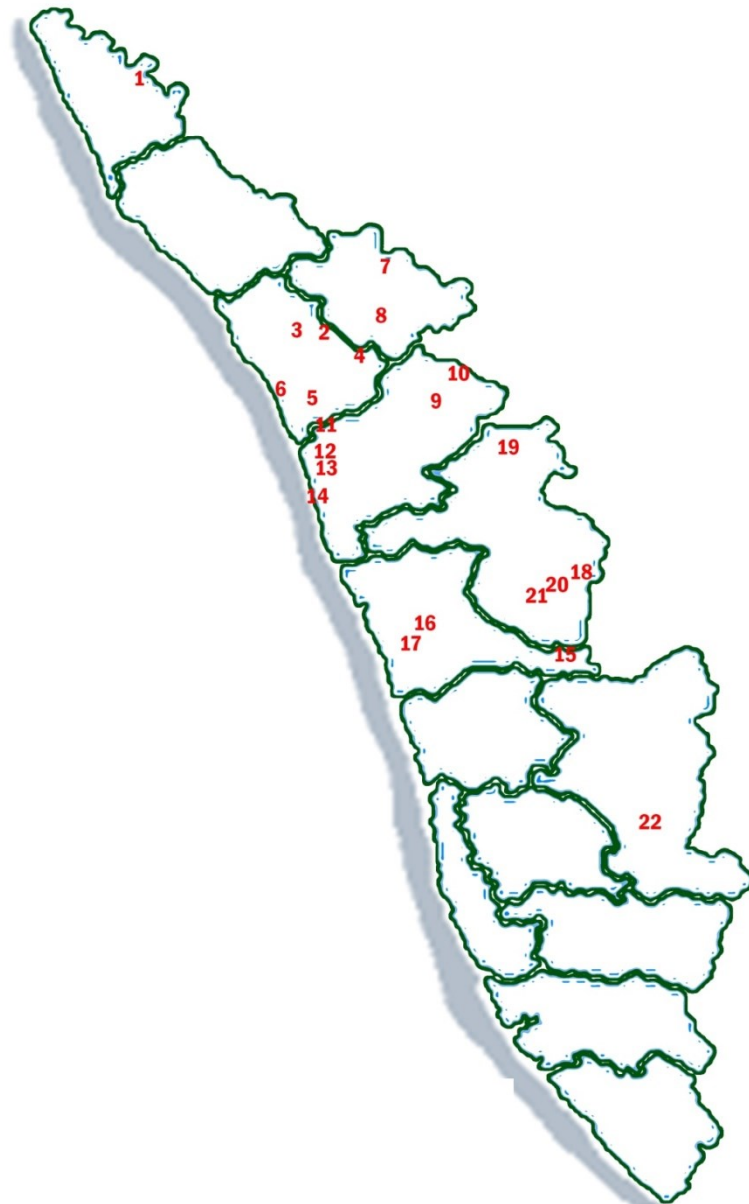


Fig. 3.1 Map of Kerala showing the study area



Fig. 3.2 Habitat of epipsammic *Nostoc* spp. in the paddy fields

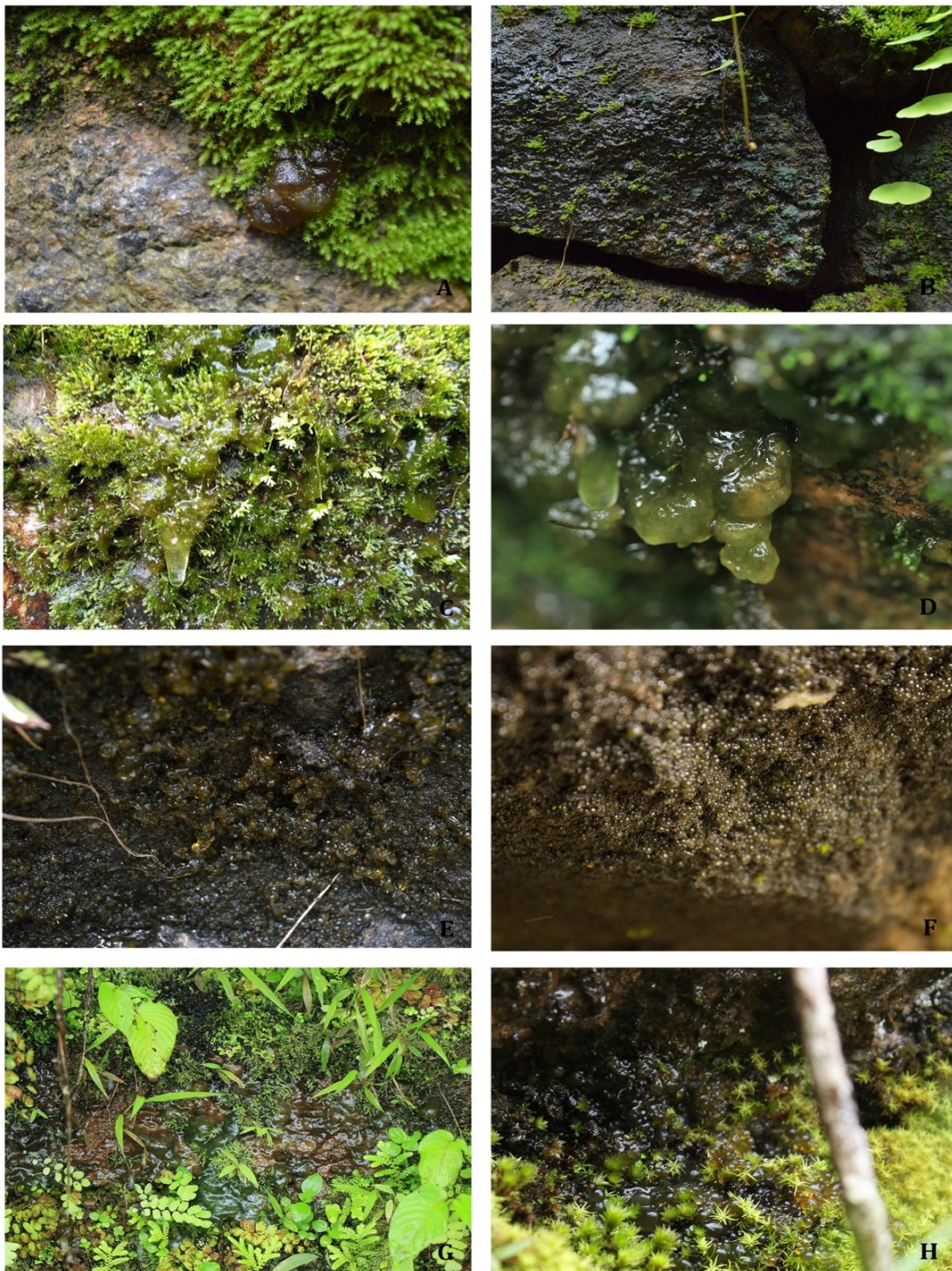


Fig. 3.3 Habitat of epilithic *Nostoc* species

3.2.2 Sampling methods for cyanobacteria

Sampling was done from different habitats including agricultural fields, rock surfaces, soil, fresh water and bark of trees from different districts of Kerala during the southwest monsoon (June to September) and northeast monsoon (October to December) during 2015-2017 (Fig. 3.2 & 3.3). Specimens were collected using spatula, forceps, needles and scalpels in collection bottles. The identification number for each specimen was given according to the collection area and number of collections from each area. Samples were collected and kept in separate bottles based on their morphological appearance. Field observations of habit and habitat were noted in the field book at the time of collection.

3.2.3 Culturing, isolation, purification and maintenance of *Nostoc* spp.

Collected samples were cleaned using distilled water and cultured in BG-11₀ medium (Table 3.2 devoid of nitrogen source, NaNO₃) (Rippka *et al.*, 1979). pH of the medium was adjusted to 7.4 with 1N HCl or NaOH using a digital pH meter and the medium was autoclaved at 121°C for 20 minutes. The cultures were maintained in the culture room under fluorescent light (3000 lux) at 25±2°C and illuminated with 14h light followed by 10h darkness.

Table 3.2 Composition of BG-11₀ medium (Rippka *et al.*, 1979)

Composition	g/l
MgSO ₄ .7H ₂ O	0.075
K ₂ HPO ₄ .3H ₂ O	0.04
CaCl ₂ .2H ₂ O	0.036
Citric Acid	0.006
Ferric Ammonium Citrate	0.006
Disodium EDTA	0.001
Na ₂ CO ₃	0.02
Trace Metal Solution	1 mL
The final pH was adjusted to 7.4	

Table 3.3 Composition of trace metal solution

Chemicals	g/l
H ₃ BO ₄	2.86
MnCl ₂ .4H ₂ O	1.81
ZnSO ₄ .7H ₂ O	0.22
CuSO ₄ .5H ₂ O	0.079
Co(NO ₃).6H ₂ O	0.049
Na ₂ MoO ₄ .2H ₂ O	0.39

To isolate *Nostoc* spp. streak plate, dilution and pour methods were adopted. For preparing semi-solid medium 1.2% w/v (12 g/l) of agar was used. The plates were sealed and kept on culture racks provided with white fluorescent lamps (3000 lux) at 25±2°C and provided light for 14h. Colonies of *Nostoc* spp. started to appear on agar plates within 14-21 days of incubation. Individual colonies were picked up and re-streaked on new agar plates. Alternatively, all the individual colonies were shifted into a 20 ml liquid medium in a conical flask.

3.2.4 Identification of *Nostoc* spp.

Photomicrographs were taken using a system-connected Leica DM 1000 LED microscope and cell measurements were made from the digital images using Leica Application Suite (LAS version 5.0.2). The morphological characteristics such as the nature of the colony and filaments, thickness and colour of the mucilaginous sheath, shape, size and colour of vegetative cells, size and position of heterocysts, the occurrence of akinetes, granules presence or absence were recorded. Identification of *Nostoc* spp. was made using cyanobacterial taxonomic manuals of Desikachary (1959), Prescott (1982), Anand (1989) and Komárek (2013). The validity of the species name was verified as per Algaebase (<http://www.algaebase.org>) (Guiry and Guiry, 2022).

3.3 Biochemical characterization of *Nostoc* spp.

Biochemical characterizations of *Nostoc* spp. were conducted by analysing the chlorophyll content (Mackinney, 1941). Quantitative estimation of chlorophyll-a content is important to study the growth and photosynthetic rates.

Procedure

- Centrifuged 10 ml of culture at 5000 rpm for 5 minutes.
- Washed the culture pellets and suspended them in 4 ml of methanol and vortex thoroughly.
- Solvent evaporation was prevented using aluminium foil covers on the mouth of the tubes.
- Incubated the tubes in a water bath at 60°C for 1h in the dark, with occasional stirring to prevent oxidation.
- Cool the tubes and centrifuge the contents at 5000 rpm for 10 min at room temperature.
- The clear supernatant was transferred to a clean test tube and the pellet was re-extracted as mentioned above until the extraction was completed.
- Pool out the supernatants and makeup to a 10 ml final volume with 80% methanol.
- Read the absorbance at 663 nm in a spectrophotometer against a methanol blank.

Calculate the quantity of chlorophyll using the following equations;

$$\text{Amount of Chl a} = A_{663} \times 12.63$$

(12.63 is the correction factor)

3.4 Molecular characterizations of *Nostoc* spp. using 16S rRNA gene sequence analysis

3.4.1 Isolation of genomic DNA

Genomic DNA was extracted and purified using the CTAB method (Ausubel *et al.*, 1995). The preparation of buffers and stock solution for genomic DNA isolation is presented in Tables 3.4 & 3.5. The procedure adopted for isolating pure DNA is as follows;

Procedure

- Exponentially growing cyanobacterial cultures grown in BG-11₀ medium were harvested (14-21 days old cultures) by centrifugation at 9000 rpm for 5 minutes. Discard the supernatant.
- Added double distilled water again, centrifuged at 9000 rpm for 5 minutes. Discarded the supernatant.
- Re-suspended cells in 564 μ L lysis buffer (10 mM Tris-HCl (pH-8); 1 mM trisodium citrate; 1.5% SDS), spun well.
- Incubated for 1h at 37°C after the addition of 30 μ L of 10% SDS and 6 μ L of 10mg/ml proteinase K and 20 μ L of lysozyme. Spin well.
- Added 100 μ L 5M NaCl. Mixed well and 100 μ L CTAB/NaCl buffer (pre-warmed) was added. Mixed thoroughly and incubated the mixture at 65°C for 10 min.
- Lysate was extracted with an equal volume of chloroform: isoamyl alcohol (24:1). Centrifuged at 14000 rpm for 10 min at room temperature.
- Transfer the aqueous phase to a 1.5 ml clean microcentrifuge tube. Added an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1). Mix well.
- Centrifuged at 14000 rpm for 10 min at room temperature.

- Take the aqueous phase and transfer it to a new 1.5 ml microcentrifuge tube (without interrupting the white bottom layer).
- Added an equal volume of ice-chilled isopropanol. Invert gently and incubate at (-80°C) for 1h.
- Centrifuge at 14000 rpm for 15 min at 4°C.
- Discarded the supernatant and washed the pellet with 200 µL of 70% (v/v) ethanol. Centrifuged at 14000 for 5 min at 4°C.
- Discarded the supernatant and let the pellet air dry for 15-20 min at room temperature.
- Resuspend the dried pellet in a minimum quantity of TE buffer containing *RNase* (10mg/ml).
- Incubated at 37°C for 20 min. The purified DNA was quantified using a nanodrop 2000 spectrometer (UV scanning Thermo scientific).
- Isolated DNA was stored at -20°C until further use.

Table 3.4 Preparation of buffers for cyanobacterial genomic DNA isolation (Sambrook *et al.*, 1989)

Sl. No.	Buffer	Method of preparations	Comments
1	Lysis buffer-100 ml (10 mM Tris- HCl (pH-8); 1 mM trisodium citrate; 1.5% SDS),	Take 1 ml of Tris HCl (1 M), 0.029 g of trisodium citrate (Himedia) and add 1.5 g of SDS adjusted final volume to 100 ml	Store at 4°C. Avoid foaming
2	CTAB/NaCl extraction buffer: 100 ml, 10% CTAB in 0.7 M NaCl	Add 10 g CTAB (Himedia) in 80 ml 0.7 M NaCl (Himedia heat at 60°C and stir. Adjust the final volume to 100 ml with 0.7 M NaCl.	CTAB will take time to dissolve. Avoid foaming.
3	TE (10 mM:1 mM) buffer: 100 ml, 100 mM Tris- HCl (pH-8), 1 mM EDTA (pH-8)	Take 1 ml of Tris HCl (1 M), 0.2 ml of EDTA (0.5 M). Makeup to 100 ml with sterile double distilled water	TE (10 mM) is written since there is TE with 1 mM EDTA.

		taken in a reagent bottle, mixed thoroughly and autoclaved.	
4	TAE buffer 50X ; 1 litre	Measure 242 g of Tris base; added 100 ml of EDTA (0.5 M); 57.1 ml of glacial acetic acid and around 0.50 ml of sterile double distilled water. Dissolved the salt and adjusted volume to 1 litre. Autoclaved.	It will dissolve much more easily in a 500 ml solution
5	Gel loading buffer (6X): 100 mL 0.25% (w/v) bromophenol blue 30% (v/v) glycerol	Dissolved 0.25 gm of bromophenol blue (Himedia) in 99 ml of 30% (v/v) glycerol (Himedia). Kept on a magnetic stirrer for several hours to get the dye completely dissolved. Dispensed to reagent bottles and kept at 4°C.	Strong dyes handle carefully
6	Proteinase K-storage buffer: Glycerol (50 mL), 1 M Tris-HCl (pH 7.5) (1 ml), CaCl ₂ (0.29 g) dd H ₂ O to 100 ml.	Kept 100 ml storage buffer in a screw cap tube, added 100 mg of proteinase K, mix thoroughly and aliquot to 1.5 ml microcentrifuge tubes.	Store at -20°C
7	<i>RNase A</i>	Prepare 10 mg per ml stock solution in 10 mM sodium acetate buffer, pH 5.2. Heat to 100°C for 15 minutes; cool at room temperature, and adjust the pH to 7.4 using 0.1 volume of 1 M Tris-HCl, pH 7.5.	Store at -20°C.
8	Lysozyme buffer; 1 ml	Prepare 10 mg lysozyme per 1 ml of 10 mM Tris-HCl (pH-8) stock solution	Store at -20°C

Table 3.5 Preparation of stock solution for cyanobacterial genomic DNA isolation (Sambrook *et al.*, 1989)

Sl. No.	Reagents	Method of preparations	Comments
1	Tris (pH 8.0), 500 ml	Weighed 60.55 g Tris base (Himedia) and dissolved in 300 ml of double distilled water. Adjusted the pH to 8 by adding conc. HCl. Made the volume to 500 ml. Dispensed to reagent bottles and sterilized by autoclaving.	Tris base will take time to dissolve.
2	0.5M EDTA (pH 8.0), 100 ml	Dissolved 18.61 g of EDTA-disodium salt (Himedia) in 100 ml of water. Adjusted pH to 8.0 by adding NaOH pellets. Made the volume to 100 ml. Dispensed into reagent bottles and autoclaved.	pH of EDTA solution is temperature dependent. EDTA will entirely dissolve only when pH becomes 8.
3	5M NaCl, 500 ml	Added 146.1g NaCl (Himedia) to 200 ml of water and mixed well. When the salts get completely dissolved, adjust the final volume to 500 ml with double distilled water. Dispensed into reagent bottles and autoclaved.	Nacl will take much time to dissolve.
4	3M sodium acetate (pH 5.2), 100 ml	Weighed 24.60 g of anhydrous sodium acetate (Merk) and dissolved in 100 ml of water and mixed well. When dissolved completely adjusted the pH of the solution to 5.2 with glacial acetic acid (99-100%). Dispensed to reagent bottles and autoclaved.	Sodium acetate will take much time to dissolve.
5	Ethidium bromide (100 mg/ml), 100 ml	Weighed 1 g ethidium bromide and added to 100 ml of double distilled water. Kept on magnetic stirrer to ensure that the dye	Ethidium Bromide is a powerful mutagen and is moderately toxic. So handle with

		has dissolved completely. Dispensed to amber coloured reagent bottle and stored at 4°C	caution.
6	Chloroform: Isoamyl alcohol (24:1), 500 ml	Measured 480 ml of chloroform and 20 ml of isoamyl alcohol. Mixed well and stored in amber bottle in room temperature.	Chloroform will evaporate so close the cap tightly and keep in amber coloured bottles.
7	10% SDS, 100 ml	Weighed 10 g SDS and dissolved in distilled water and make up to 100 ml. Mixed well and stored at 4°C.	Avoid foaming
8	Phenol: chloroform: Isoamyl alcohol (25:24:1), 500 ml	Measured 250 ml of phenol, 240 ml of chloroform and 10 ml of isoamyl alcohol. Mixed well and stored at room temperature	Stored in amber coloured bottle.
9	70% (v/v) ethanol, 500 ml	Take 355 ml of ethanol: mix with 145 ml of double distilled water. Dispensed to reagent bottle and stored at 4°C.	Stock ethanol is 99% (v/v) hence 355 ml is taken instead of 350 ml

3.4.2 Quantification of DNA

Genomic DNA was quantified using UV scanning Thermo scientific NanoDrop™ 2000 spectrophotometer. The amount of DNA was calculated based on its OD at 260/280 nm. The DNA quantity was calculated based on 1 OD of DNA=50ng of double-stranded DNA. 1% (w/v) agarose gel was used for DNA visualization for qualitative analysis. 5 µL DNA samples were mixed with 1 µL 6X gel loading dye and loaded in each well. The gel was incorporated with 1 µl of (10 µg/µL) ethidium bromide and the gel were run using a horizontal electrophoretic unit containing 1x TAE buffer until the tracking dye reached the bottom edge of the gel. A 1 kb DNA ladder was used to compare the molecular weight of the DNA sample. The genomic DNA bands were visualized using UV-based E-Gel image analyzer.

3.4.3 PCR amplification of 16S rRNA gene

PCR amplification of the 16S rRNA gene using genomic DNA and cyanobacterial gene-specific primers was performed using a PCR thermal cycler (Eppendorf- Vapoprotect, Germany). Emerald green master mix consisting of (*Taq* DNA polymerase, 10X reaction buffers, dNTPs and magnesium chloride) from Takara, Bangalore, India was used as a PCR reaction mixture. DNA primers were obtained from Sigma Aldrich, Bangalore. The composition of the PCR reaction mixture is shown in Table 3.6 and primer sequences and reaction conditions are shown in Table 3.7. PCR amplification products were examined by 1% agarose gel prepared in 1X TAE buffer containing 100 mg/ml ethidium bromide. 5 µl of PCR products was loaded and electrophoresis was performed at 90 V power supply with 1X TAE as electrophoresis buffer for about 45 minutes. 100 bp DNA ladder was used as the molecular weight standard. After the amplification, the image of agarose gel electrophoresis was captured under UV light using a Gel documentation system (E- gel imager-Life technology). The amplified PCR products were sequenced from Agrigenome Lab Pvt. Ltd. (Cochin, Kerala), on a charge basis.

Table 3.6 PCR primer sequences and their reaction conditions

Gene	Primer Sequences (5' to 3')	Reaction conditions	References
16S rRNA	Forward primer- CYA359F GGG GAA TYT TCC GCA ATG GG 27F-AGA GTT TGA TCC TGG CTC AG Reverse primer- Equimolar mixture of CYA781Ra and CYA781Rb CYA781R(a) GAC TAC TGG GGT ATC TAA TCC CAT T CYA781R(b) GAC TAC AGG GGT ATC TAA TCC CTT T 1492R- TAC GGY TAC CTT GTT ACG ACT T	94°C 3 min, 94°C 45 sec 50°C 1 min and 72°C 1.30 min in 30 cycle, 72°C 10 min	Nubel <i>et al.</i> , 1997 Lane, 1991

Table. 3.7 PCR mix consisted of the following components

Reagents	Reaction Volume (μL)
Master mix	12.5
Forward primer (10 μM)	1
Reverse primer(10 μM)	1
Template DNA (~50ng)	1
Sterile double distilled water to make the final volume up to 25 μL	9.5
Final volume per tube	25 μL

3.4.4 Sequence analysis

16S rRNA PCR amplicons of the isolates were purified and sequenced from Agrigenome Lab Pvt., Ltd. (Cochin, Kerala) using ABI 3730XL DNA Analyzer according to the Sanger dideoxy sequencing method. The reverse and forward DNA sequences were analyzed and cloned using Bio-Edit.v.7.1.3 software (Ibis Biosciences, Carlsbad). Species identification and homology between the sequences were identified from the I6S rRNA sequence using BLAST (<https://www.ncbi.nlm.nih.gov/BLAST/>). The nucleotide sequences (16S rRNA partial sequence) of *Nostoc* spp. were submitted to GenBank, NCBI-database (<http://www.ncbi.nlm.nih.gov>).

3.4.5 Phylogenetic analysis

MEGA 7.0 software (Kumar *et al.*, 2016) based on the neighbor-joining and maximum likelihood method (Felsenstein, 1981) and the branching support of 1000 bootstrap (Felsenstein, 1985) was used for the phylogenetic analysis of *Nostoc* spp. Phylogenetic tree construction based on 16S rRNA sequence was initially performed using the cloned sequence from this study and also with the sequences retrieved from GenBank and aligned with ClustalW. Table 3.8 shows the list of sequences retrieved from GenBank with strain name, accession number and location.

Table 3.8 List of sequences retrieved from NCBI for phylogenetic analysis with strain number, GenBank accession number and location

Sl. No.	Strain name	GenBank accession number	Location
1	<i>N. elliposporum</i> str Lukesova 51	MG596757	Czech Republic
2	<i>N. elliposporum</i> V strain V	AJ630450	Czech Republic
3	<i>N. minutum</i> NIES-26	LC228976	Japan
4	<i>Nostoc</i> sp. CENA67	MN551902	Brazil
5	<i>Nostoc</i> sp. HKAR-6	KF751605	India
6	<i>N. punctiforme</i> HPU NR 08	MK122775	India
8	<i>N. punctiforme</i> SULS E-1	KP792332	India
9	<i>Nostoc</i> sp. MZUC6	MF109994	India
10	<i>N. piscinale</i> AUS	KP052646	India
11	<i>N. punctiforme</i> SAG 60.79	GQ287652	France
12	<i>Nostoc</i> sp. Ru1-3d	LC325254	Japan
13	<i>N. commune</i> BEA	ON032966	Spain
14	<i>Nostoc insulare</i> SAG 54.79	KM019927	Germany
15	<i>Nostoc</i> sp. <i>Collema</i> sp. AR75	KF359719	Finland
16	<i>Nostoc</i> sp. <i>Peltigera aphthosa</i> UK52	KF359692	Finland
17	<i>Nostoc</i> sp. <i>Pannaria Sphinctrina</i>	EF174203	Chile
18	<i>Nostoc</i> sp. CY7	KY421772	India
19	<i>N. punctiforme</i> BHU83	KY129709	India
20	<i>N. punctiforme</i> SAG 60.79	GQ287652	Egypt
21	<i>N. calcicola</i> TSZ 2203	OQ627023	Kazakhstan
22	<i>Desmonostoc muscorum</i> ACSSI 046	MK248032	Russia

3.5 GC-MS analysis of *Nostoc* spp.

3.5.1 Sample preparation for GC-MS

- 15 days old actively growing cyanobacterial cells were harvested by filtration.
- Cyanobacterial cells washed with distilled water (3 times) and kept in a dry place for 5 to 6 days.

- The dried biomass was subjected to organic extraction by crushing using a mortar and pestle and extracted using HPLC gradient methanol.
- The powder was mixed in methanol (1g: 10mL), vortexed for 5 min and sonicated for 2 min.
- Centrifuged at 3000rpm for 10 minutes at 4°C.
- The supernatant was pooled and centrifuged with methanol until the pellet became white in colour.
- Filtered the sample using Whatman No. 1 filter paper.
- The extract was evaporated and concentrated by air drying.
- The air-dried residue was dissolved in HPLC gradient methanol to make the stock solution.
- The extract was then sterilized using a nylon syringe filter (diameter 25 mm and pore size 0.22 µm) and used for GC-MS analysis for bioactivity studies.

3.5.2 Analysis of methanolic extract using GC-MS

Shimadzu GC-MS, QP2010S equipped with Elite-5 MS (0.25 mm×30 m, 0.25 µm film thickness) capillary column was used for GC-MS analysis of the methanolic extract of *Nostoc* spp. The operating conditions were as follows: the GC temperature program consisting of 70°C for 2 min, incremented at 70°C to 200°C and holding for 5 min. The temperature was finally adjusted to 280°C for 15 min. The injector temperature was 260°C; the sample injection volume was 1 µl; and the split ratio was 100:1. Mass spectrometry conditions were: electron impact as the ion source, ionizing voltage 70 eV, source temperature 200°C, electron multiplier at 2000 eV, scan speed 1000 amu/s, and scan range 50-500 m/z. The constituents of the methanolic extracts were identified by comparing the retention indices and matching the recorded mass spectra of each compound with NIST11 and WILEY8 Library. The quantification of all the identified components was investigated using a percent relative peak area.

3.5.3 Phylogenetic analysis using chemical components

The components identified using GC-MS from the methanolic extracts of *Nostoc* spp. were scored for the presence or absence of specific components and the data were analyzed using PAUP software. Cluster analysis was carried out with UPGMA (Unweighted Pair Group Method with Arithmetic averages) using the components from the methanolic extracts of the four samples to determine the presence of identical and distinct chemical compounds in each species.

3.6 Assessment of *Nostoc* spp. as a biofertilizer for rice varieties

3.6.1 Bio-priming of rice seeds using *Nostoc* inoculum

3.6.1.1 Plant material

Rice seeds of two high-yielding varieties Jyothi and Annapurna were collected from the Regional Agricultural Research Station (RARS), Pattambi, Kerala, India. Table 3.9 shows the duration of variety, bran colour, grain type and major characteristics of the rice varieties.

Table 3.9 Major characteristics of the rice varieties

Rice variety	Duration (Days)	Bran colour and grain type	Important characteristics
Jyothi (PTB 39)	110-125	Red long and bold	Susceptible to sheath blight disease. Tolerant to brown plant hopper.
Annapurna (PTB 35)	95-100	Red short and bold	Susceptible to blast and sheath blight diseases. Plant hopper-resistant variety.

3.6.1.2 Surface sterilization of rice seeds

Uniform-sized rice seeds were selected for the experiments and were pre-washed for 1 min in teepol solution to remove dirt. The washed seeds were surface sterilized using 0.1% (w/v) HgCl₂ solution for 5 min and further washed thoroughly with distilled water for 4-5 times.

3.6.1.3 Experimental design for bio-priming of rice seeds

Nostoc spp. biomass was harvested by centrifugation after growing for 15 days (exponential phase). The cells were washed in distilled water thoroughly. Cyanobacterial biomass was homogenized using a sterilized mortar and pestle. 1% of the extract was prepared by suspending 1 g of the cell in 100 ml of distilled water (Shariatmadari *et al.*, 2011). Equal quantity of seeds were soaked in different *Nostoc* inoculum. Bio-primed seeds and control were kept in paper glass for germination.

Experimental design for seed bioprimering

Treatment 1: Seeds primed in water (control)

Treatment 2: Seeds primed in 1% extract of *Nostoc ellipsosporum* FY150125

Treatment 3: Seeds primed in 1% extract of *Nostoc* sp. CU150162

Treatment 4: Seeds primed in 1% extract of *Nostoc punctiforme* FY150188

Treatment 5: Seeds primed in 1% extract of *Nostoc* sp. CU150191

3.6.1.4 Growth parameter analysis of rice seedlings

3.6.1.5 Germination Percentage (GP)

The germination percentage of seeds of all the treatments was calculated using the following formula described by Abdul-Baki and Anderson, (1973) and expressed as in percentage.

$$GP = \frac{\text{Seed germinated}}{\text{total seeds}} \times 100$$

3.6.1.6 Analysis of morphological characters

Root length, shoot length and leaf number of rice seedlings of each treatment and control were analysed on the 7th day of treatment to find out the effect of seed bio-priming.

3.6.2 Assessment of rice growth parameters using biofertilizer

3.6.2.1 Sterilization of soil

The soil used for the experiment was collected from the paddy fields of Chovva, University of Calicut, Kerala. The soil was packed in autoclavable polythene bags and sterilized for 40 min in an autoclave at 121°C at 15 psi pressure.

3.6.2.2 Experimental design

Seeds primed with different *Nostoc* species and water were separately grown in grow bags (38 cm × 35 cm). The primed seeds were transplanted on the 14th day to the grow bags which contain sterilized soil. Added dried *Nostoc* inoculum to the soil on the 7th day after planting in the ratio 1 g: 1 kg (cyanobacteria inoculation: soil) (Chittapun *et al.*, 2017). All the grow bags were maintained at an equal distance and each treatment had an experimental unit of n=3. Observations were recorded on the 7th, 14th and 21st days after the multiplication of inoculum in the grow bags and also analyzed during the harvesting time.

Treatment 1: Hydroprimed (control) and without *Nostoc* inoculum

Treatment 2: Seeds primed in 1% extract of *N. elliposporum* and 1g/ 1kg inoculum

Treatment 3: Seeds primed in 1% extract of *Nostoc* sp. CU150162 and 1g/ 1kg inoculum

Treatment 4: Seeds primed in 1% extract of *N. punctiforme* and 1g/ 1kg inoculum

Treatment 5: Seeds primed in 1% extract of *Nostoc* sp. CU150191 and 1g/ 1kg inoculum

3.6.2.3 Effect of *Nostoc* inoculum on the growth and yields of rice plant

The effect of different *Nostoc* inoculums on rice growth and yield was quantitatively analyzed by examining the morphological and biochemical parameters.

3.6.2.4 Quantification of chlorophyll content

Total chlorophyll content from rice plant tissues treated with different *Nostoc* spp. inoculum was determined using Arnon's (1949) protocol.

Procedure

- 0.05 g of fresh leaves of rice from all the treatments were homogenized in a pre-cooled mortar and pestle in 10 ml of 80% chilled acetone.
- Homogenate was centrifuged at 5000 rpm at 4°C for 10 min and the supernatant was collected in a 100 ml conical flask.
- The procedure was repeated until the pellet became white in colour.
- The final volume of the homogenate was made up to 20 ml using 80% (v/v) acetone.
- The absorbance of the solution was measured at 645 nm and 663 nm using UV-Visible spectrophotometer (Shimadzu, Japan) against the blank solvent (80% acetone).

The total chlorophyll was calculated using the equations;

$$\text{Total chl (mg/g) of tissue} = (20.2 (A_{645}) + 8.02 (A_{663}) / 1000 \times W) V$$

Where, A= Absorption at a specific wavelength

V= Final volume of chlorophyll extract in 80% acetone

W=Fresh weight of tissue extracted

3.6.2.5 Quantification of total protein

Total protein content from plant tissues treated with different *Nostoc* spp. inoculum was determined using Lowry *et al.* (1951) protocol.

Procedure

- 100 mg leaf tissues from all the treatments were separately homogenized on a prechilled mortar and pestle using 2 ml of extraction buffer.
- The extract was collected in a 2 ml microcentrifuge tube and centrifuged at 10,000 rpm for 10 min at 4°C.
- The supernatant was used for total protein quantification.
- 50 µl of the supernatant from each sample was taken in a test tube and the volume was made up to 10 ml with double distilled water.
- To it, 5 ml of solution C was added and kept at room temperature for 10 min.
- 0.5 ml of solution D was added to the mixture and the contents were mixed well, then incubated in the dark for 30 min.
- The absorbance was measured using a UV spectrophotometer (Shimadzu, Japan) at 660nm.
- A standard curve was prepared using a gradient concentration of BSA (1 mg/ml) and the total protein content was measured.

Table 3.10 Reagents used for the estimation of total protein

Reagents	Preparations
Extraction buffer	100 mM Tris-HCl, 10 mM DTT, 1 mM PMSF and 1% PVP dissolved in 80 ml double distilled water and the final volume made up to 100 ml using double distilled water.
Solution A	2.0 g of Na ₂ CO ₃ dissolved in 0.1 N NaOH solution and final volume made up to 100 ml.
Solution B	0.1 g of sodium potassium tartrate and 0.005 g CuSO ₄ .5H ₂ O dissolved separately in 5 ml distilled water and both solutions were mixed well and the final volume was adjusted to 10 ml.
Solution C	100 ml of solution A and 2 ml of solution B were mixed well to obtain solution C.
Solution D	Folin-Ciocalteau Phenol was prepared by diluting with distilled water in the ratio 1:1 and kept at 4°C until use.

3.6.2.6 Quantification of total sugar

Total sugar content from plant tissues treated with different *Nostoc* spp. inoculum was determined according to the Anthrone method (Hedge and Hofreiter, 1962).

Procedure

- Leaf samples (10 mg) from each treatment were incubated with 0.5 ml of 2.5 N HCl for three hours in a boiling water bath.
- After cooling to room temperature, the samples were neutralized with sodium carbonate powder until the effervescence stopped.
- Diluted the neutralized samples to a final volume of 10 ml using double distilled water and centrifuged at 5000 rpm for 10 min.
- 0.5 ml of the supernatant from each sample was taken in separate test tubes and the final volume was made up to 1 ml with double distilled water.
- To each tube, 4 ml of anthrone reagent was added and kept in a boiling water bath for 8 min and was rapidly cooled by keeping it on ice.
- Absorbance at 630 nm was measured using UV-Visible spectrophotometer (Shimadzu, Japan).
- The standard curve was prepared using gradient concentrations of glucose (10 mg/100 ml). The total sugar content of the samples was quantified from the standard graph.

Table 3.11 Reagents used for total sugar and soluble sugar quantification

Reagents	Preparation
Anthrone reagent	200 mg of anthrone powder dissolved in 100 ml of ice-chilled 98% (v/v) H ₂ SO ₄ and kept at 4°C until use
2.5 N HCl	20.83 ml of concentrated HCl was diluted to 100 ml by adding double distilled water

3.6.2.7 Morphological parameters

Sl. No.	Characters
Growth characters	
1	Plant height
2	Leaf Numbers
3	Leaf length
4	Leaf breadth
5	Number of tillers
6	Panicle length
Yield characters	
1	Number of panicle
2	Number of spikelets per panicle
3	Number of grains
4	Number of chaffed grains
5	Number of filled grains
6	Weight of 100 grains

3.6.2.8 CHNS analysis of rice seeds

Elemental analysis of dried rice seeds (5 mg) from different treatments was done using a CHNS analyzer (Thermo Scientific FLASH 2000 HT). Total nitrogen, carbon, hydrogen and sulfur content were determined using a CHNS analyzer. For the CHNS analysis, freeze-dried and crushed samples are weighed (5 mg) and mixed with an oxidizer in a tin capsule, which is then combusted in a reactor at 950°C. The combustion products CO₂, SO₂, and NO₂ are carried by a constant flow of carrier gas (helium) that passes through a glass column. The integrated TCD (thermal conductivity detector) and dedicated Eager 300 software allow the operation of the FLASH 2000 HT analyzer as a stand-alone instrument to determine elemental weight percentages of N, C, H, and S.

3.7 Statistical analysis

PCA (Principal Component Analysis), HCA (Hierarchical Cluster Analysis), and Clustered heatmap of phytoconstituents were executed with XLSTAT 2023

software (Addinsoft, USA). A Venn diagram of chemical compounds was generated using BioVinci software 2.0. version (BioTuring Inc., San Diego, CA, USA). Analysis of Variance (ANOVA) and heterogeneity test were conducted using SPSS 23.0 software for the study of the effect of different *Nostoc* inoculum on the growth of rice varieties Jyothi and Annapurna. All the results were given as mean \pm SE for three replicates of each sample.

CHAPTER 4

MORPHOLOGICAL CHARACTERIZATION OF *NOSTOC* SPECIES FROM KERALA

4.1 Abstract

Nostoc is a cyanobacterial genus most commonly found in a wide range of terrestrial and aquatic ecosystems where their growth becomes conspicuous and abundant. The diversity of *Nostoc* species from Kerala has not been completely explored. Hence, this study investigated the diversity of *Nostoc* species from different locations from paddy fields, rock surfaces, bark of trees and freshwater bodies of different districts of Kerala. The vegetative and reproductive characteristics such as shape, colour and size of the colonies; width and length of trichome; entangled or not, the form of trichome; shape, colour and thickness of the sheath; shape, size and colour of vegetative cells, heterocysts and akinetes as well as colour and ornamentation of cell walls of the akinetes, were used for the identification. Collected samples were subjected to species-level identification based on the cyanobacterial taxonomy manuals. A total of thirty-nine *Nostoc* spp. were identified, among them thirty-four species were identified up to species level and the remaining five species were identified up to genus level. Among them, most of the species were collected from rock surfaces of the Western Ghats. Twenty-five *Nostoc* spp. were collected as epilithic. *Nostoc aureum* f. *variabilis*, *N. bornetii*, *N. caeruleum*, *N. calcicola*, *N. carneum*, *N. citrisporum*, *N. comminutum*, *N. commune*, *N. edaphicum*, *N. elliposporum*, *N. elliposporum* var. *violaceum*, *N. flagelliforme*, *N. fuscescens*, *N. gelatinosum*, *N. hatei*, *N. humifusum*, *N. indistinguendum*, *N. interbryum*, *N. kihlmanii*, *N. linckia*, *N. locularis*, *N. microscopicum*, *N. minutum*, *N. muscorum*, *N. nylstromicum*, *N. oryzae*, *N. paludosum*, *N. passerinianum*, *N. pruniforme*, *N. punctiforme*, *N. sphaericum*, *N. sphaeroides*, *N. tibeticum*, *N. undulatum*, *Nostoc* sp. CU150162, *Nostoc* sp. CU150191, *Nostoc* sp. CU167213, *Nostoc* sp. CU150122 and *Nostoc* sp. CU150172 were the identified *Nostoc* species. Among the collected *Nostoc* spp. *N. indistinguendum*, *N. locularis* and *N. tibeticum* are new records of the cyanobacterial species of India.

4.2 Results

4.2.1 Systematic account

Phylum Cyanobacteria Stanier 1973

Synonyms: Blue-green algae, Cyanophyceae, Myxophyceae, Cyanophytes, Cyanoprokaryotes

Cyanobacteria are photosynthetic prokaryotes that lack a cell nucleus and other cell organelles. They occur as unicellular, colonial or multicellular filamentous forms. Cells (vegetative cells, heterocysts and akinetes) can be spherical, ellipsoid, barrel-shaped, cylindrical, conical or disc-shaped. Some cyanobacterial taxa can produce aerotopes. The position, number and distribution of the heterocysts and akinetes are considered important morphological characteristics of species and genera. Cyanobacteria can be identified based on cell dimensions, shape, colour, presence or absence of heterocysts and akinetes, type of branching, sheath characteristics and cell contents.

Order Nostocales

The order Nostocales represents a large monophyletic cluster of filamentous cyanobacteria, comprising both branched and unbranched forms. The species has diversified thallus and specialised cells (heterocysts and akinetes). The order contains false and true branched forms, unbranched and isopolar and the filaments of a few families have heteropolar structures. The order consists of 19 families, including almost 132 genera.

Family Nostocaceae C.A. Agardh 1824 ex Kirchner 1898

The family is characterised by filamentous, heterocystous, isopolar cyanobacteria with uniseriate trichomes, surrounded by fine or firm mucilaginous sheaths, filaments straight, flexuous, contorted or screw-like coiled, without false or true branching. They grow in solitary filaments or form microscopic or macroscopic colonies. Trichomes isopolar, moniliform or cylindrical, constricted or not constricted at the cross-walls, sometimes attenuated terminal cells. Cells barrel,

spherical, cylindrical, terminal cells rounded, conical, acutely pointed or cylindrical. Heterocysts present (absent only in genera *Isocystis* and *Raphidiopsis*), terminal or intercalary. Akinetes are sometimes larger than vegetative cells.

Genus *Nostoc* Vaucher ex Bornet et Flahault 1888

Thallus micro-macroscopic, gelatinous, amorphous or spherical, later irregularly spherical, lobate, smooth or warty on the surface. Filaments aggregated and loosely or densely entangled in gelatinous colonies, usually macroscopic, sometimes gathered in peripheral parts enveloped by periderm, which is flattened, lobate, irregular mass, or forming an amorphous mucilaginous mat. The mucilaginous envelope surrounds the trichome colourless or yellow-brownish, rarely slightly violet. Trichomes are uniseriate, unbranched, moniliform, isopolar (same width along the whole length), apical cells morphologically not different from other cells, flexuous, cylindrical and always constricted at cross-walls. Cells barrel to cylindrical, terminal mostly rounded. Heterocysts solitary, rarely adjacent, intercalary or terminal (their frequency or absence is dependent on nitrogen metabolism), sometimes in the apical position of initial colonies, spherical to oval. Akinetes arise in rows, maximally 2 times larger than vegetative cells, usually oval, cylindrical or spherical. Reproduction occurs by motile hormogonia of different type, by disintegration of colonies and less frequently by germination of akinetes.

Among 228 species (infraspecific) reported up to date, 108 are currently accepted as taxonomically valid taxa. A total of 39 *Nostoc* species of epilithic, epipsammic, epiphytic, benthic and corticolous forms were collected from different regions of Kerala. Among them, 34 species were identified up to the species level and the remaining 5 species were identified up to the genus level. Taxonomic descriptions of characters of each taxon along with their photomicrographs and taxonomic key for identification are described here.

1a. Trichomes enveloped by clear and distinct sheath.....	24
1b. Trichomes not enveloped by clear and distinct sheath	2
2a. Trichomes with indistinct envelope	3

2b. Trichomes without indistinct envelope	6
3a. Heterocysts terminal	<i>N. sphaericum</i>
3b. Heterocysts terminal or intercalary	4
4a. Trichomes loosely entangled	5
4b. Trichomes densely entangled.....	<i>N. linckia</i>
5a. Cells greyish, 3.7-4.3 μm broad.....	<i>N. carneum</i>
5b. Cells pale green, 2.5- 3 μm broad.....	<i>N. calcicola</i>
6a. Akinetes present.....	7
6b. Akinetes absent or unknown.....	13
7a. Cells usually more than 5 μm in length	8
7b. Cells usually less than 5 μm in length.	9
8a. Cells elongated.....	<i>N. borneyi</i>
8b. Cells quadrate or barrel shaped.....	12
9a. Cells barrel shaped.....	10
9b. Cells cylindrical to quadrate	<i>Nostoc</i> sp. CU150162
10a. Heterocysts single	11
10b. Heterocysts more than 2 in a row	<i>N. undulatum</i>
11a. Cells barrel shaped, yellowish brown	<i>N. sphaeroides</i>
11b. Cells barrel shaped, blackish brown	<i>N. humifusum</i>
12a. Akinete with sculptured exposure.....	<i>N. gelatinosum</i>
12b. Akinetes in row, without sculptured exposure	<i>N. muscorum</i>
13a. Cells spherical to compressed spherical	14
13b. Cells other than spherical.....	18
14a. Filaments mostly straight.....	<i>Nostoc</i> sp. CU150172
14b. Filaments mostly not straight.....	15
15a. Cells less than 4 μm in diameter	<i>N. aureum</i> f. <i>variabilis</i>
15b. Cells more than 4 μm in diameter	16

16a. Heterocysts long attenuated	<i>Nostoc</i> sp. CU150122
16b. Heterocysts not attenuated	17
17a. Cells dark green, granules not prominent	<i>N. comminutum</i>
17b. Cells yellowish green, granules prominent	<i>Nostoc</i> sp. 167213
18a. Cells rectangular or ellipsoidal	19
18b. Cells barrel or quadrate shaped	20
19a. Cells less than 5 μm in length	<i>N. hatei</i>
19b. Cells more than 5 μm in length	21
20a. Cells less than 5 μm broad and more than 3 μm long	22
20b. Cells less more than 5 μm broad and less than 3 μm long	<i>N. interbryum</i>
21a. Cells less than 4 μm broad	<i>N. nylstromicum</i>
21b. Cells more than 4 μm broad	<i>N. passerinianum</i>
22a. Cells quadrate, constricted at cross wall	23
22b. Cells quadrate, not constricted at cross wall	<i>N. caeruleum</i>
23a. Cells violet, granules prominent	<i>N. elliposporum</i> var <i>violaceum</i>
23b. Cells green, granules prominent	<i>N. oryzae</i>
24a. With envelope, akinetes absent	28
24b. With envelope, akinetes present	25
25a. Sheath thick and lamellated	<i>N. tibeticum</i>
25b. Sheath thin and unlamellated	26
26a. Cells elongated or cylindrical, akinetes more than 6 μm broad	27
26b. Cells barrel shape, akinetes less than 6 μm broad	<i>N. punctiforme</i>
27a. Cells cylindrical more than 5 μm broad, akinetes spherical to ellipsoidal more than 12 μm broad	<i>N. citrisporum</i>
27b. Cells cylindrical less than 5 μm broad, akinetes elongated more than 12 μm broad	<i>N. elliposporum</i>
28a. Heterocysts usually terminal in position	29
28b. Heterocysts terminal or intercalary	31

29a. Cells barrel shaped.....	30
29b. Cells other than barrel shaped.....	<i>N. indistinguendum</i>
30a. Colonies macroscopic, olive green.....	<i>N. commune</i>
30b. Colonies microscopic, yellow brown.....	<i>N. edaphicum</i>
31a. Heterocysts spherical.....	32
31b. Heterocysts elongated.....	<i>N. fuscescens</i>
32a. Cells globose.....	33
32b. Cells compressed spherical.....	<i>Nostoc</i> sp. CU 150191
33a. Aerotopes present.....	<i>N. locularis</i>
33b. Aerotopes absent.....	<i>N. pruniforme</i>
34a. Cells spherical or barrel shaped, more than 3.5 µm long.....	35
34b. Cells spherical or barrel shaped, less than 3.5 µm long.....	<i>N. minutum</i>
35a. Colonies free, microscopic.....	36
35b. Colonies attached together, macroscopic.....	<i>N. kihlmanii</i>
36a. Colonies olive green, cells spherical.....	37
36b. Colonies yellow, cells barrel shaped.....	<i>N. paludosum</i>
37a. Heterocysts terminal spherical.....	<i>N. microscopicum</i>
37b. Heterocysts terminal intercalary.....	<i>N. flagelliforme</i>

1. *Nostoc aureum* f. *variabilis* B.N.Prasad, R.K.Mehrotra & Y.Singh 1977

Komárek, 2013, vol. 19, pp. 998–999, fig. 1295.

Description: Colonies small, attached to a substrate, light green, soft, mucilaginous. Sheath not observed. Trichomes are short, flexuous, and loosely entangled. Cells globose to short barrel, 3.5 µm wide, blue-green. Heterocysts terminal and intercalary, solitary, olive, spherical to oblong, 4.5 µm in diameter. Akinetes not observed.

Distribution in India: West Bengal (Gupta, 2012).

General Environment: Freshwater species.

Habitat: Appeared as a green-coloured gelatinous mass on wet soil of paddy field.

Comment: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Malappuram, Calicut University, 30.08.2016, CU No: 150121 (Fig. 4.1A & B).

2. *Nostoc bornetii* Gain 1911

Komárek, 2013, vol. 19, pp. 998–999, fig. 1298.

Description: Colonies gelatinous brown, spherical red mass. Sheaths are not visible, hyaline. Trichomes are loosely entangled. Cells elongated or cylindrical, greenish-brown, 6.0–10.2 μm long, 4 μm broad, granules prominent. Heterocysts spherical or oblong, 8.3–9.8 μm long, 5–6 μm broad. Akinetes in rows, oval, up to 7.3 μm long, 4.9 μm broad.

Distribution in India: Kerala (Ram and Paul, 2022).

General environment: Terrestrial species.

Habitat: Appeared as brown-coloured mucilaginous balls on rock surfaces.

Specimens examined: INDIA: Kerala; Palakkad, Pothundi dam, 27.08.2015, CU No. 144445; Thrissur, Sholayar, 02.07.2016, CU No: 144490 (Fig. 4.1 C&D)

3. *Nostoc caeruleum* Lyngbye ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Komárek, 2013, vol. 19, pp. 963–964, fig. 1256.

Description: Colonies yellow brown gelatinous spherical mass. Sheaths around the trichome were lacking or indistinct. Trichomes are densely and irregularly arranged, flexuous, yellowish brown, barrel to quadrate, sometimes slightly longer or shorter than wide, 5.0–8.4 μm long, 4.6 μm broad, granules prominent. Heterocysts are intercalary, olive green, spherical, 6.4–7.8 μm in diameter. Akinetes unknown.

Distribution in India: Kerala (Philip, 2020); West Bengal (Gupta, 2012)

General environment: Freshwater species.

Habitat: Appeared as a yellowish-brown coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Kozhikode, Kakkayam, 08.06.2017, CU No: 167207 (Fig. 4.1E & F)

4. *Nostoc calcicola* Brébisson ex Bornet Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 384; Komárek, 2013, vol. 19, pp. 984–986, fig. 1280.

Description: Colonies are irregular, flat, gelatinous, blue-green, and rarely yellowish-green. Sheaths are usually indistinct, more distinct at colonial margins, and colourless. Trichomes are loosely entangled, long, flexuous, and irregularly curved. Cells barrel-shaped, isodiametric or almost spherical, green, pale green or blue-green, 2.5–4.5 µm long, 2.5–3.0 µm broad, granular content greenish, prominent. Heterocysts are spherical, terminal or intercalary, slightly larger than vegetative cells, 4–6 µm in diameter. Akinetes are unknown.

Distribution in India: Kerala (Ram and Shamina, 2016); Assam (Basumatary *et al.*, 2022); Goa (Gomes, 2012); Maharashtra (Nikam *et al.*, 2013); Tamil Nadu (Kumar and Sridhar, 2012); Uttar Pradesh (Yadav *et al.*, 2021); Tripura (Kant *et al.*, 2020). West Bengal (Gupta, 2012); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019); Khandesh (Jaiswal, 2017); Ladakh (Kant and Gupta, 1998).

General environment: Terrestrial species

Habitat: Appeared as a green mucilaginous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Palakkad, Nelliampathy, 15.06.2017, CU No: 150178 (Fig. 4.1G & H).

5. *Nostoc carneum* [Lyngbye] Agardh ex Bornet et Flahault 1888

Synonyms: *Nostoc rufescens* C.Agardh 1824; *Anabaena rufescens* (C.Agardh)

Kirchner 1878; *Nostoc spongiiforme* C.Agardh ex Bornet & Flahault 1886.

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 381, pl. 69, fig. 6; Komárek, 2013, vol. 19, pp. 968–970, fig. 1261.

Description: Colonies yellow gelatinous mass, macroscopic, fine, lobate, and irregularly clustered. Trichomes are loosely contorted and flexuous. Sheaths are indistinct and unclear. Cells are almost isodiametrical, or cylindrical, greyish, 4.5–8 µm long, 3.7–4.3 µm broad, with granules present. Heterocysts are intercalary, oblong to spherical, light yellow, 5.6–7.1 µm long, 5.5 µm broad. Akinetes not observed.

Distribution in India: Kerala (Ram and Shamina, 2016); Manipur (Silvia *et al.*, 2015), Tamil Nadu (Muthukumar *et al.*, 2007); Goa (Gomes, 2012); Telengana (Srinivas and Aruna, 2016); Punjab (Singh *et al.*, 2022); Kolkata (Gupta, 2012); Karnataka (Gupta and Das, 2019); Khandesh (Jaiswal, 2017); Punjab (Singh *et al.*, 2022); Rajasthan (Jain, 2018).

General environment: Freshwater species

Habitat: Appeared as a pale yellow-coloured gelatinous mass on rock surfaces and paddy fields.

Specimens examined: INDIA: Kerala; Malappuram, Tanur, 13.08.2015, CU No: 144440; Malappuram, Nadukkani, 13.07.2016, CU No: 150108; Malappuram, Nilambur, 03.06.2016, CU No: 144479 (Fig. 4.2A & B).

6. *Nostoc citrisporum* Prasad et Mehrotra 1976

Komárek, 2013, vol. 19, pp. 1000, fig. 1300.

Description: Colonies mucilaginous, small, soft. Filaments are flexuous and loosely entangled. Sheath distinct only in the periphery, wide, hyaline. Cells cylindrical, green, 5.4–6.8 µm long, 5.5 µm broad, granules prominent. Heterocysts intercalary, spherical, 6.1–7.8 µm in diameter. Akinetes are spherical to ellipsoidal, 12–14 µm in diameter.

Distribution in India: Rajasthan (Jain, 2018); West Bengal (Gupta, 2012).

General environment: Terrestrial species

Habitat: Appeared as pale green-coloured gelatinous balls on rock surfaces.

Comment: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Idukki, Vagamon, 08.06.2017, CU No: 167283 (Fig. 4.2C & D).

7. *Nostoc comminutum* Kützing 1850

Kützing, 1850, vol. 2, pp. 1–8; Komárek, 2013, vol. 19, pp. 1000–1001, fig. 1301.

Description: Colonies green, irregularly lobed, gelatinous, membranous softballs. Trichomes are flexuously curved and densely entangled. Cells blue-green or dark green, spherical to compressed spherical, 4.3-5.0 μm in diameter. Heterocysts are terminal, green, spherical, and distinctly larger than vegetative cells, 5.8 μm in diameter. Akinetes are unknown.

Distribution in India: Kerala (Philip, 2020); West Bengal (Gupta, 2012).

General environment: Freshwater species

Habitat: Appeared as a dark green-coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Kozhikode, Kakkayam, 08.06.2017, CU No: 150174 (Fig. 4.2E & F).

8. *Nostoc commune* Vaucher ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 387; Komárek, 2013, vol. 19, pp. 979–980, fig. 1273.

Description: Colonies are macroscopic, olive-green gelatinous masses, initially spherical, later irregularly flattened, crispy or wavy, sometimes like mats. Sheaths are present, visible mostly at the margin of colonies. Trichomes are flexuous, densely entangled, and later freely entangled. Cells spherical or barrel, olive green, 3.9-6.4 μm in diameter, granules prominent. Heterocysts are mostly terminal,

sometimes intercalary, spherical or hemispherical, olive green, 5.6–6.5 µm in diameter. Akinetes are unknown.

Distribution in India: Kerala (Geethu and Shamina, 2021; Vijayan and Ray, 2015); Manipur (Silvia *et al.*, 2015); Goa (Gomes, 2012); Orissa (Samad and Adhikary, 2008); Tamil Nadu (Anand and Subramanian, 1994); Sikkim (Bhakta *et al.*, 2010); Assam (Dasgupta and Ahmed, 2013); Punjab (Singh *et al.*, 2022); Uttarakhand (Verma and Asthana, 2005); Chattisgarh (Singh *et al.*, 2014); Tripura (Kant *et al.*, 2020); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019).

General environment: Freshwater species

Habitat: Green-coloured gelatinous balls collected as epilithic and epiphytic forms.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 27.08.2016, CU No: 150126; Thrissur, Mattampuram, 02.06.2016, CU No. 144476 (Fig. 4.2G & H).

9. *Nostoc edaphicum* Kondratyeva 1962

Kondratyeva, 1962, vol. 19, pp. 58–65; Komárek, 2013, vol. 19, pp. 975–978, fig. 1268.

Description: Colonies are microscopic, globose, gelatinous, enveloped by periderm, intact, oval and yellowish brown. Trichomes are densely coiled together, not attenuated towards ends, and flexuous. Cells shortly barrel or spherical, yellowish-brown or green, granules prominent, 4.5 µm in diameter. Heterocysts are mainly terminal, rarely intercalary, spherical, yellow, 4.9-7.0 µm in diameter. Akinetes are unknown.

General environment: Terrestrial species

Habitat: Appeared as green-coloured jelly-like balls on rock surfaces.

Comments: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar dam sites, 09.09.2016, CU No: 150128 (Fig. 4.3A & B).

10. *Nostoc elliposporum* [Desmazieres] Rabenhorst ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 383, pl. 69, fig. 5; Komárek, 2013, vol. 19, pp. 989–990, fig. 1285.

Description: Colonies are brown, gelatinous, flattened, and irregular. Trichomes are loosely entangled, and flexuous. Sheath present, distinct at the periphery, hyaline. Cells elongated or cylindrical, brown, 5.2–10.0 µm long, 4.1–4.3 µm broad, granules prominent. Heterocysts are oblong to cylindrical, olive green, 8.1–15.2 µm long, 5.9–7.02 µm broad. Akinetes in a series, olive green, cylindrical to elongated, 11–16 µm long, 6.5 µm broad.

Distribution in India: Kerala (Vijayan and Ray, 2015; Thilak *et al.*, 2020; Ram and Paul, 2022); Goa (Gomes, 2012); Manipur (Silvia *et al.*, 2015); Maharashtra (Nikam *et al.*, 2013); West Bengal (Gupta, 2012); Tripura (Kant *et al.*, 2020); Uttar Pradesh (Yadav *et al.*, 2021); Punjab (Singh *et al.*, 2022); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019); Rajasthan (Jain, 2018).

General environment: Terrestrial species

Habitat: Appeared as a yellowish brown-coloured gelatinous mass on rock surfaces and soil.

Specimens examined: INDIA: Kerala; Wayanad, Kuruva Island, 07.07.2015, CU No: 144414; Malappuram, Calicut University, 30.08.2016, CU No: 150125; Thrissur, Alappad, 25.09.2016, CU No. 150164 (Fig. 4.3C & D).

11. *Nostoc elliposporum* var. *violaceum* Rao 1937

Rao, 1937, vol. 6, pp. 339–375; Desikachary, 1959, pp. 383, pl. 68, fig. 4.

Description: Colonies gelatinous, irregularly expanded, light violet. Trichome flexuous, loosely entangled. Sheath absent. Cells quadrate, constricted at cross-walls, 4–8 µm long, 4 µm broad. Heterocysts are oblong or cylindrical with rounded ends, 4–8 µm long, 4–6 µm broad. Akinetes not observed.

Distribution in India: Kerala (Ram and Paul, 2022); West Bengal (Gupta, 2012); Karnataka (Gupta and Das 2019); Ladakh (Kant and Gupta, 1998).

General environment: Freshwater species

Habitat: Appeared as a light violet gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Kozhikode, Kakkayam, 12.10.2016, CU No: 150165 (Fig. 4.3E).

12. *Nostoc flagelliforme* Berkeley et Curtis ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Komárek, 2013, vol. 19, pp. 975, fig. 1264.

Description: Colonies are macroscopic, gelatinous, olive green, and on the surface slightly verrucose. Sheaths are distinct, and hyaline. Trichomes are flexuous. Cells spherical, olive green, 5.6–6.0 µm wide. Heterocysts spherical or hemispherical, 5 µm diameter. Akinetes are unknown.

General environment: Terrestrial species

Habitat: Appeared as green-coloured mucilaginous balls on rock surfaces.

Comments: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Kozhikode, Kakkayam, 02.08.2016, CU No: 150120 (Fig. 4.3F-H).

13. *Nostoc fuscescens* Fritsch 1912

Fritsch, 1912, pp. 1–66; Komárek, 2013, vol. 19, pp. 1003–1004, fig. 1307.

Description: Colonies spherical, yellow-brown, with thin periderm. Sheaths are prominent, recognizable only near the laminated colonial periphery, with an inner layer of yellow-brown. Cells are barrel-shaped, yellow-brown, 4.0–5.1 µm long, 3.5–4.9 µm broad. Heterocysts are terminal and intercalary, mostly not enveloped by

sheaths, spherical or slightly elongated with pointed ends, 6.0–6.7 μm long, 5.2 μm broad. Akinetes are unknown.

Distribution in India: Maharashtra (Gaikwad and Nalawade, 2020).

General environment: Freshwater species

Habitat: Appeared as brown-red coloured mucilaginous balls on rock surfaces.

Comments: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 10.09.2016, CU No: 150145 (Fig. 4.4A-C).

14. *Nostoc gelatinosum* Schousboe ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Komárek, 2013, vol. 19, pp. 988–989, fig. 1284.

Description: Colonies olive green gelatinous mat, irregular, flat. Sheaths are not clear. Trichomes are loosely entangled. Cells long cylindrical, longer than wide, constricted at the cross wall, yellow-green, 5–8 μm long, 4 μm broad. Heterocysts are intercalary and terminal, olive green, oval to ellipsoidal, 6–11 μm long, 5–6 μm broad. Akinetes are long elongated oval, with sculptured exospores (small warts or short spines) and dark green cell walls.

Distribution in India: West Bengal (Gupta, 2012); Karnataka (Gupta and Das, 2019); Kerala (Philip, 2020); Tripura (Kant *et al.*, 2020).

General environment: Freshwater species

Habitat: Appeared as green-coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Idukki, Vagamon, 26.08.2017, CU No: 167229 (Fig. 4.4D-H).

15. *Nostoc hatei* Dixit 1936

Dixit, 1936, pp. 93–106, fig. 4; Desikachary, 1959, pp. 389, pl. 67, fig. 2; Komárek, 2013, vol. 19, pp. 1004, fig. 1305.

Description: Colonies mucilaginous, blackish brown, up to 2 cm in diameter. Sheaths absent. Trichomes are irregularly curved or twisted, densely entangled. Cells are ellipsoidal, 4.4–5.6 μm broad, longer than wide. Heterocysts are single, almost spherical, pale yellow, 5–6 μm broad. Akinetes not observed.

Distribution in India: Assam (Basumatry *et al.*, 2022); Goa (Gomes, 2012); Maharashtra (Nikam *et al.*, 2013); Punjab (Singh *et al.*, 2022); West Bengal (Gupta, 2012); Karnataka (Gupta and Das, 2019); Khandesh (Jaiswal, 2017), Punjab (Singh *et al.*, 2022); Kerala (Philip *et al.*, 2020).

General environment: Freshwater species

Habitat: Appeared as a brown-coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Malappuram, Nadukkani, 11.09.2015, CU No: 144461; Sholayar, Thrissur, CU No: 144488 (Fig. 4.5A & B).

16. *Nostoc humifusum* Carmichael ex Bornet et Flahault 1888

Desikachary, 1959, pp. 384; Komárek, 2013, vol. 19, pp. 989, fig. 1283.

Description: Colonies mucilaginous, irregular, sometimes small, olive green-blackish green. Filaments densely entangled, flexuous. Sheath absent. Cells barrel shaped blackish green. 4 μm broad, 4.5 μm long. Heterocysts are oblong, elongated, olive yellow, 4.5 μm wide, 6 μm long. Akinetes in a series, oval, elongated, sometimes dumbbell shaped, 8.2 μm long, 4.5 μm wide.

Distribution in India: West Bengal (Gupta, 2012); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019).

Specimens examined: INDIA: Kerala; Kozhikode, Purakkattiri, 24.08. 2016, CU No: 150116 (Fig. 4.5C & D).

General environment: Terrestrial species

Habitat: Appeared as black-coloured gelatinous balls on the wet soil of paddy fields.

17. *Nostoc indistinguendum* Rehakova et Johansen 2007

Rehakova *et al.*, 2007, vol. 46, pp. 481–502; Komárek, 2013, vol. 19, pp. 1004–1005, fig. 1308.

Description: Colonies microscopic, blackish-green gelatinous mass. Microcolonies spherical to oval later completely confluent with trichomes, at first compact and indiscernible, then loosely arranged. Sheaths thick. Trichomes are curved and bent, irregularly arranged. Cells globose or compressed barrel, 5.6 µm long, 4.8–6.7 µm broad, granules prominent. Heterocysts are spherical or hemispherical, usually apical, rarely intercalary, olive green, 4.5–7.0 µm in diameter.

General environment: Terrestrial species

Habitat: Appeared as a dark green-coloured gelatinous mass on rock surfaces.

Comments: The species has been reported as a first record from India.

Specimens examined: INDIA: Kerala; Kozhikode, Peruvannamuzhi, 25.08.2017, CU No: 167242 (Fig. 4.5E & F).

18. *Nostoc interbryum* Sant'Anna *et al.*, 2007

Sant'Anna *et al.*, 2007, vol. 34, pp. 96, fig. 1; Komárek, 2013, vol. 19, pp. 1004–1005, fig. 1309.

Description: Colonies green mucilaginous mass, microscopic, with firm colourless periderm. Filaments are short (up to 30 cells), moniliform, slightly coiled or curved, and embedded in a yellow gelatinous mass. Cells shortly barrel-shaped, almost spherical, smaller than wide, green, 3 µm long, 5.8 µm broad, granules prominent. Heterocysts are mostly terminal, rarely intercalary, spherical, olive green, 6 µm in diameter. Akinetes are unknown.

Distribution in India: Kerala (Geethu and Shamina, 2021).

General environment: Terrestrial species

Habitat: Appeared as a yellowish-coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Idukki, Vagamon, 29.08.2017, CU No: 167296 (Fig. 4.5G & H).

19. *Nostoc kihlmanii* Lemmermann 1900

Synonym: *Nostoc planctonicum* Poreckij et Cernov 1929

Lemmermann, 1900, vol. 18, pp. 24–32; Komárek, 2013, vol. 19, pp. 960–961, fig. 1252.

Description: Colonies attached, firm, gelatinous, irregularly torn, pale blue-green, composed of two or a few colonies together, periderm firm, and smooth at the surface. Trichomes are irregularly entangled, without individual sheaths. Cells are barrel-shaped or spherical, isodiametric, shorter than wide, green, with aerotopes, 3.7–4.6 µm long, 3.9–5.3 µm broad. Heterocysts are spherical, terminal intercalary, solitary, and olive green; 4.9–6.6 µm in diameter. Akinetes are unknown.

Distribution in India: Tripura (Kant *et al.*, 2020).

General environment: Freshwater species

Habitat: Pale green-coloured gelatinous balls collected as epilithic and corticolous forms.

Comments: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Kozhikode, Koodaranji, 01.10.2016, CU No: 150171; Kozhikode, Koodaranji, 01.10.2016, CU No. 150168 (Fig. 4.6A-E).

20. *Nostoc linckia* [Roth] Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 377; Komárek, 2013, vol. 19, pp. 968–969, fig. 1260.

Description: Colonies are mucilaginous, attached to plants or rock substrates, later irregularly clustered, amorphous, usually brownish green or dirty olive green, disintegrating, gelatinous clusters. Filaments are flexuous, densely entangled, and

variously curved. Sheaths may or may not be present, colourless, and visible occasionally in the marginal parts of the colonies. Cells are almost spherical or shortly barrel-shaped, pale blue-green, 5.6 μm in diameter, granules present. Heterocysts are spherical larger than vegetative cells, 4.5–6.6 μm in diameter. Akinetes are unknown.

Distribution in India: Kerala (Thilak *et al.*, 2020); Telangana (Srinivas and Aruna, 2016); Goa (Gomes, 2012), Maharashtra (Nikam *et al.*, 2013); Orissa (Samad and Adhikary, 2008); West Bengal (Gupta, 2018); Uttar Pradesh (Yadav *et al.*, 2021); Punjab (Singh *et al.*, 2022); Tripura (Kant *et al.*, 2020); Khandesh (Jaiswal, 2017).

General environment: Freshwater species

Habitat: Green-coloured gelatinous mass collected as epilithic, epipsammic, and benthic forms.

Specimens examined: INDIA: Kerala; Idukki, Vagamon, 26.09.2016, CU No: 150163; Palakkad, Nenmara, 25.08.2015, CU No: 144443; Wayanad, Kuruva Island, 07.07.2015, CU No: 144416 (Fig. 4.6F & H).

21. *Nostoc locularis* An 1992

Komárek, 2013, vol. 19, pp. 1006, fig. 1313.

Description: Colonies are soft, spherical, small gelatinous, pale yellow, like beads. Colonies are divided into numerical ellipsoidal loculi, mucilaginous, wide, soft, transparent, pale yellow, 75–88 μm broad. Trichomes are loosely arranged within loculi. Cells spherical, 4.4–4.9 μm in diameter, aerotopes present. Heterocysts terminal, spherical, 5.8 μm in diameter. Akinetes not observed.

General environment: Freshwater species

Habitat: Appeared as yellowish spherical balls on rock surfaces.

Comments: The species has been reported as a first record from India.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 17.09.2016, CU No: 150150 (Fig. 4.7A & B).

22. *Nostoc microscopicum* Carmichael ex Bornet et Flahault 1888

Synonym: *Nostoc macrosporum* Meneghini ex Bornet et Flahault 1888 incl.

Komárek, 2013, vol. 19, pp. 978–979, fig. 1269–1270.

Description: Colonies irregular spherical, mucilaginous, olive green, enveloped by periderm. Filaments flexuous, densely and later loosely entangled, with yellow sheath at the colonial margin. Cells barrel or almost spherical shaped, olive-green to dark green, 4.47–5.8 μm in diameter. Heterocysts terminal, spherical-oblong, olive green, 5.7–8.9 μm long, 6.04–6.8 μm broad. Akinetes not observed.

Distribution in India: West Bengal (Gupta 2012); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019); Rajasthan (Jain, 2018); Kerala (Ram and Shamina, 2017).

General environment: Terrestrial species

Habitat: Appeared as a dark green-coloured gelatinous mass on the wet soil of paddy fields.

Specimens examined: INDIA: Kerala; Mavoor, Kozhikode. 09.07.2015, CU No: 144433 (Fig. 4.7C & D).

23. *Nostoc minutum* Desmazieres ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Komárek, 2013, vol. 19, pp. 984–985, fig. 1278.

Description: Colonies are green gelatinous in appearance, small, at first spherical, later flattened. Filaments are flexuous, densely entangled, and often enveloped by individual sheaths. Sheaths are yellowish. Trichomes with sheath 12.0–13.7 μm broad. Cells shortly barrel-shaped to spherical, yellowish green, 3.5 μm in diameter, granules prominent. Heterocysts barrel to spherical, pale yellow, 3.5–4.3 μm in diameter. Akinetes are unknown.

Distribution in India: Kerala (Philip, 2020); Tripura (Kant *et al.*, 2020); West Bengal (Gupta, 2012); Khandesh (Jaiswal, 2017).

General environment: Freshwater species

Habitat: Appeared as yellow-coloured gelatinous balls on rock surfaces.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 02.08.2016, CU No: 150131 (Fig. 4.7E & F).

24. *Nostoc muscorum* Agardh ex Bornet et Flahault 1888

Synonym: *Desmonostoc muscorum* (Agardh ex Bornet et Flahault 1888) Hrouzek et Ventura 2013.

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Hrouzek *et al.*, 2013, vol. 13, pp. 201–213; Komárek, 2013, vol. 19, pp. 1016, fig. 1281, 1327.

Description: Colonies are brown gelatinous, flattened masses up to 2–5 cm in diameter, attached by the lower surface. Filaments are densely entangled. Cells quadrate to cylindrical, longer than wide, 5.6–10.4 μm long, 3.7–5.0 μm broad, brown, granules prominent. Heterocysts are terminal or intercalary, spherical to oblong, olive green, 6.4–8.3 μm long, 5.4–7.3 μm broad. Akinetes present in a row, oblong, olive green, 7.6–9.2 μm long, 5.5–6.6 μm broad.

Distribution in India: Kerala (Thilak *et al.*, 2020); Meghalaya (Kharkongor and Ramanujam, 2014); Assam (Dasgupta and Ahmed, 2013); Punjab (Singh *et al.*, 2022); Telengana (Srinivas and Aruna, 2016); Tripura (Kant *et al.*, 2020); Maharashtra (Nikam *et al.*, 2013); Goa (Gomes, 2012); Karnataka (Gupta and Das, 2019); Khandesh (Jaiswal, 2017); Rajasthan (Jain, 2018); West Bengal (Gupta, 2012).

General environment: Freshwater species

Habitat: Brown-coloured gelatinous balls collected as epilithic, epiphytic and epipsammic forms.

Specimens examined: INDIA: Kerala; Kozhikode, Peruvannamuzhi, 24.08.2015, CU No: 144441; Kozhikode, Mukkam, 26.08.2015, CU No. 144444; Malappuram, Chelari, 03.09.2016, CU No: 150133 (Fig. 4.7G & H).

25. *Nostoc nylstromicum* Claassen 1961

Komárek, 2013, vol. 19, pp. 1008–1009, fig. 1315.

Description: Colonies gelatinous, dark brown. Trichomes are loosely entangled, and flexuous. Cells are cylindrical, greenish-brown, 4.5–8.4 µm long, 3.3 µm broad. Granules present. Heterocysts are mostly terminal, spherical, 5 µm in diameter, intercalary, cylindrical, 5.1–7.5 µm long, 3.9–4.7 µm broad. Akinetes not observed.

Distribution in India: Kerala (Geethu and Shamina, 2021)

General environment: Freshwater species

Habitat: Yellowish brown-coloured gelatinous mass collected as epilithic and epiphytic forms.

Specimens examined: INDIA: Kerala; Kasargod, Ranipuram, CU No: 150192, 15.08.2016; Kozhikode, Peruvannamuzhi, 24.08.2015, CU No: 144441(Fig. 4.8 A-B).

26. *Nostoc oryzae* (Fritsch) Komarek et Anagnostidis 1989

Synonym: *Anabaena oryzae* Fritsch 1949

Komárek, 2013, vol. 19, pp. 1008–1009, fig. 1316

Description: Colonies green gelatinous mass. Trichomes are straight, densely aggregated. Cells barrel shaped, green, 5.5–6 µm long, 4.5 µm broad, and granules prominent. Heterocysts terminal oblong, intercalary spherical, olive yellow, larger than vegetative cells, 6–7 µm in diameter. Akinetes not observed.

Distribution in India: West Bengal (Gupta, 2012); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019); Khandesh (Jaiswal, 2017); Ladakh (Kant and Gupta, 1998); Punjab (Singh *et al.*, 2022); Rajasthan (Jain, 2018).

General environment: Terrestrial species

Habitat: Appeared as a yellowish-green-coloured gelatinous mass on paddy soil.

Specimens examined: INDIA: Kerala; Malappuram, Ramanattukara, 03.07.2015, CU No: 144413 (Fig. 4.8C & D).

27. *Nostoc paludosum* Kützing ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 375, pl. 69, fig. 2; Komárek, 213 vol. 19, pp. 980–981, fig. 1274.

Description: The colony is microscopic in clusters, gelatinous mass, pale green, without periderm. Filaments loosely entangled, with yellowish gelatinous sheaths. Trichomes are flexuous and irregularly coiled. Cells barrel to quadrate, pale green, in some parts of trichome sometimes cylindrical, 4.0–6.3 μm long, 4.5 μm broad. Heterocysts are spherical or oblong, sometimes slightly elongated, a little larger than vegetative cells, 3.9–6.7 μm in diameter. Akinetes not observed.

Distribution in India: Kerala (Ram and Shamina, 2016); Assam (Dasgupta and Ahmed, 2013); Maharashtra (Nikam *et al.*, 2013); Goa (Gomes, 2012); Punjab (Singh *et al.*, 2022); Tripura (Kant *et al.*, 2020); West Bengal (Gupta, 2012); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019); Ladakh (Kant and Gupta, 1998); Punjab (Singh *et al.*, 2022); Rajasthan (Jain, 2018).

General environment: Freshwater species

Habitat: Appeared as green -coloured gelatinous balls on rock surfaces.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 27.08.2016, CU No: 150129 (Fig. 4.8E & F).

28. *Nostoc passerinianum* [DeNotaris] Bornet et Thuret ex Bornet et Flahault 1888.

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Desikachary, 1959, pp. 385; Komárek, 2013, vol. 19, pp. 988–989, fig. 1282.

Description: Colonies gelatinous attached by the lower surface, yellowish brown. Trichomes are mostly parallel, slightly flexuous, and loosely entangled. Cells rectangular, ellipsoidal, constricted at cross walls, yellowish green, 5.0–8.7 μm long,

4.5 µm broad. Heterocysts are intercalary, rarely terminal, oblong or elongated, olive green, 7–8 µm long, 7 µm broad. Akinetes are unknown.

Distribution in India: Goa (Gomes, 2012); Tripura (Kant *et al.*, 2020); West Bengal (Gupta, 2012); Karnataka (Gupta and Das, 2019); Kerala (Ram and Shamina, 2017).

General environment: Terrestrial species

Habitat: Appeared as yellowish green coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Malappuram, Nadukkani, 24.06.2016, CU No: 150106 (Fig. 4.8G & H).

29. *Nostoc pruniforme* [Linnaeus] Agardh ex Bornet et Flahault 1888

Komárek, 2013, vol. 19, pp. 962–963, fig. 1253–1254.

Description: Thallus globose, soft, olivaceous. Colonies are mucilaginous, green, and spherical. Trichomes are irregularly entangled. Cells globose, olive green, 4.5 µm broad, 4.5–6.4 µm long, Heterocysts spherical, 5.5–6 µm in diameter. Akinetes not observed.

Distribution in India: Tripura (Kant *et al.*, 2020); West Bengal (Gupta, 2012; Sen and Naskar, 2003).

General environment: Freshwater marine species

Habitat: Appeared as green-coloured gelatinous balls on rock surfaces.

Comments: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Ranipuram, Kasargod, 23.06.2016, CU No: 150104 (Fig. 4.9A).

30. *Nostoc punctiforme* (Kützing ex Hariot) Hariot 1891

Synonym: *Nostoc hederulae* [Kützing] Meneghini ex Bornet et Flahault 1888.

Hariot, 1891, vol. 5, pp. 29–32; Komárek, 2013, vol. 19, pp. 973–974, fig. 1267.

Description: Colonies are small, spherical, dark green or blackish mucilaginous masses, attached to a substrate, less than 1.5 mm, later confluent into a blackish gelatinous mass, several cm large. Sheaths are thin and firm. Trichomes are flexuous, in the young stage agglomerated together. Cells shortly barrel to ellipsoidal, blackish green, 4.4–6.1 μm long, 5.1–5.6 μm broad, granules prominent. Heterocysts are intercalary, spherical or barrel-shaped, olive green, 4.4–5.7 μm long, 4.4–6.2 μm broad. Akinetes in a row, ellipsoidal to elongated, green, 7.1–8.8 μm long, 4.3–5.6 μm broad with a smooth colourless cell wall.

Distribution in India: Kerala (Thilak *et al.*, 2020); Assam (Saha *et al.*, 2007); Goa (Gomes, 2012); Maharashtra (Nikam *et al.*, 2013); Orissa (Samad and Adhikary, 2008); Meghalaya (Kharkongor and Ramanujam, 2014); Uttar Pradesh (Yadav *et al.*, 2021); Punjab (Singh *et al.*, 2022); Tripura (Kant *et al.*, 2020); West Bengal (Gupta, 2012); Jharkhand (Gupta, 2021); Karnataka (Gupta and Das, 2019); Khandesh (Jaiswal, 2017); Ladakh (Kant and Gupta, 1998); Punjab (Singh *et al.*, 2022); Rajasthan (Jain, 2018).

General environment: Terrestrial species

Habitat: Appeared as a greenish brown-coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Idukki, Vagamon, 19.07.2016, CU No: 150188 (Fig. 4.9B & C).

31. *Nostoc sphaericum* Vaucher ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–186; Desikachary, 1959, pp. 390; Komárek, 2013, vol. 19, pp. 965–966, fig. 1257.

Description: Colonies spherical or oval with firm periderm, smooth at surface, olive green, gelatinous, attached on stony substrate, upto 2–3 cm in diameter. Individual colony is spherical, 270.2 μm long, 144 μm broad. Sheaths are visible only at the periphery, hyaline. Trichomes are green and densely entangled. Cells barrel to compressed spherical, 5.0–5.6 μm long, 4.0–4.3 μm broad, granules prominent. Heterocysts terminal, spherical, olive green, 4.4–5.6 μm in diameter. Akinetes not observed.

Distribution in India: Kerala (Ram and Paul, 2022); Goa (Gomes, 2012); Meghalaya (Kharkongor and Ramanujam, 2014); Punjab (Singh *et al.*, 2022).

General environment: Freshwater species

Habitat: Green-coloured gelatinous balls collected as epilithic and corticolous forms.

Specimens examined: INDIA: Kerala; Kozhikode, Peruvannamuzhi, 24.08.2015, CU No: 144442 (Fig. 4.9D-F).

32. *Nostoc sphaeroides* Kützing ex Bornet et Flahault 1888

Bornet & Flahault, 1888, vol. 7, pp. 177–262; Komárek, 2013, vol. 19, pp. 967–968, fig. 1259.

Description: Colonies gathered together in agglomerations, attached firmly to the stony surface, usually upto 1–2 cm in diameter, mucilaginous, and brown. Trichomes are flexuous, irregularly aggregated, usually without visible individual envelopes or sheaths. Cells barrel shaped or almost spherical, yellowish brown, 4.6–5.02 μm long, 5.16–5.40 μm broad, granules prominent. Heterocysts are terminal and intercalary, spherical, slightly compressed globose or slightly oval, olive green, 5.7 μm broad. Akinetes in series, spherical to oval, yellow to olive green, 5.4 μm long.

Distribution in India: Uttar Pradesh (Prasad and Mehrotra, 1978); West Bengal (Gupta, 2012).

General environment: Freshwater species

Habitat: Appeared as reddish brown-coloured gelatinous balls on rock surfaces.

Comments: The species has been reported as a first record from Kerala.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 10.09.2016, CU No: 150146 (Fig. 4.9G & H).

33. *Nostoc tibeticum* Jao et Lee 1974

Jao *et al.*, 1974, pp. 92–126; Komárek, 2013, vol. 19, pp. 1012, fig. 1321.

Description: Colonies spherical, yellow mucilaginous mass. Filaments are flexuous, not densely arranged. Sheaths are distinct in marginal parts of the colonies, 27 µm broad, lamellated, slightly yellowish layer and with inner blackish brown. Cells are ellipsoidal, at the ends pointed, pale green, sometimes each cell has several concentric continuous sheaths in layers in addition to a broad envelope, 4–8 µm long, 4.1 µm broad, granules prominent. Heterocysts terminal and intercalary, sometimes not enveloped by a sheath, spherical or oblong, 4.0–6.7 µm in diameter. Akinetes are oval, granular, and elongated up to 13 µm long.

General environment: Freshwater species

Habitat: Appeared as a brown-coloured gelatinous mass on the wet soil of paddy field.

Comments: The species has been reported as a first record from India.

Specimens examined: INDIA: Kerala; Kozhikode, Kakkayam, 10.09.2016, CU No: 167205 (Fig. 4.10A & B).

34. *Nostoc undulatum* Archibald 1967

Komárek, 2013, vol. 19, pp. 1012, fig. 1323.

Description: Reddish gelatinous mass. Sheath absent. Filaments are flexuous. Cells barrel, sometimes isodiametric, densely entangled reddish, 4-5 µm broad, 4.5 µm long. Heterocysts are mostly spherical sometimes compressed spherical, 2-3 in a row, 4.5-6 µm in diameter. Akinetes not observed.

Distribution in India: Kerala (Ram and Paul, 2022).

General environment: Terrestrial species

Habitat: Appeared as red-coloured gelatinous balls on rock surfaces.

Specimens examined: INDIA: Kerala; Palakkad, Attapadi, 07.06.2016, CU No: 144481 (Fig. 4.10C & D).

35. *Nostoc* sp. CU150162

Description: Thallus gelatinous brown balls, mucilaginous. Filaments are flexuous and densely entangled. Sheath not observed. Cell greenish brown to blackish green,

cylindrical to quadrate, 4–5.6 μm broad, 5.3–10 μm long, granules present. Heterocysts terminal mostly intercalary, oblong, light green, 7.1–10 μm broad, 6.7–12.6 μm long, terminal heterocysts 8.9 μm broad, 10.42 μm long. Akinetes elongated, 13 μm long, 8–10 μm broad.

General environment: Terrestrial species

Habitat: Appeared as brown-coloured gelatinous balls on the wet soil of paddy field.

Specimens examined: INDIA: Kerala; Malappuram, Calicut University, 24.09.2016, CU No: 150162 (Fig. 4.10E-H).

36. *Nostoc* sp. CU150122

Description: Colonies microscopic, aggregation of small green gelatinous mass. Sheath absent. Filaments densely entangled. Cells barrel to quadrate, green, 3.5–5 μm long, 4.5–6.5 μm broad, granules prominent. Heterocysts intercalary or terminal, oblong sometimes attenuated, 5–6 μm broad, 5–7.5 μm long. Akinetes not observed.

General environment: Terrestrial species

Habitat: Appeared as green-coloured gelatinous balls (like beads) on rock surfaces.

Specimens examined: INDIA: Kerala; Wayanad, Kuruva Island, 22.08.2016, CU No: 150122 (Fig. 4.11A-C).

37. *Nostoc* sp. CU150191

Description: Colonies are microscopic, aggregates of small spherical colonies that look like beads, later changing to wrinkled (on agar), which are dark green, later yellow brown. Sheaths are colourless. Cells spherical to barrel-shaped, longer than broad, 3–5.5 μm in diameter, olive green. Heterocysts spherical, pale green, 3–6 μm in diameter. Akinetes not observed.

General environment: Terrestrial species

Habitat: Appeared as olive green-coloured gelatinous mass on rock surfaces.

Specimens examined: INDIA: Kerala; Thrissur, Sholayar, 08.08.2017, CU No: 150191 (Fig. 4.11D).

38. *Nostoc* sp. CU167213

Description: Thallus small brown gelatinous balls, adherent to stones, mucilaginous. Trichome yellowish green, loosely entangled. Cells quadrate, 4–4.2 μm broad, 4.5–6.8 μm long, granules prominent. Heterocysts intercalary and terminal, spherical to oblong, pale green to olive, 6–6.8 μm long, 5.9–6.6 μm broad. Akinetes not observed.

General environment: Terrestrial species

Habitat: Appeared as brown-coloured gelatinous balls on rock surfaces.

Specimens examined: INDIA: Kerala; Idukki, Vagamon, 08.06.2017, CU No: 167213 (Fig. 4.11E & F).

39. *Nostoc* sp. CU150172

Description: Thallus green gelatinous mass. Sheath absent. Filaments mostly straight, sometimes flexuous. Cell barrel-isodiametric, olive yellow, 3.7–4.2 μm broad, 4.6–5.7 μm long, granules prominent. Heterocysts subspherical to spherical, 4.4–4.9 μm broad, 4.3–5.3 μm long. Akinetes not observed.

General environment: Terrestrial species

Habitat: Appeared as green-coloured gelatinous mat on wet paddy fields.

Specimens examined: INDIA: Kerala; Kalpetta, Wayanad, 19.10.2017, CU No: 150172 (Fig. 4.11G & H).

4.2.2 Habitat

Nostoc spp. can be found in a wide range of habitats including rock surfaces (epilithic), soil of paddy fields (epipsammic), attached to plants (epiphytic), freshwater bodies (benthic) and bark of trees (corticolous). *Nostoc* spp. formed in a variety of colours in nature, including blackish green, brown, pale green, dark green, reddish brown and violet. Different types and quantity of pigments present in the cells determined the colour of the thallus of *Nostoc* species on the substratum. Mostly *Nostoc* thallus forms gelatinous balls and the size and shape of the ball are different. In paddy field soil, *Nostoc* spp. formed as a gelatinous mass on the soil

surface and in dry conditions it was observed as a thin papery crust on the soil surface. The majority of *Nostoc* spp. were collected as epilithic in nature. They appear in different colours and shapes on moist rock surfaces. In the sampling locations of Kerala, the highest number of *Nostoc* spp. was observed in Sholayar (Thrissur-10 species) followed by Vagamon (Idukki-6 species) and Kakkayam (Kozhikode-5 species). Among the identified *Nostoc* spp., 25 species are epilithic, 6 species are epipsammic, 2 species co-occur in epilithic and epiphytic, 2 species co-occur in epilithic and epipsammic, 2 species co-occur in epilithic and corticolous, 1 species co-occur in epilithic, epipsammic and benthic and 1 species co-occur in epilithic, epiphytic and epipsammic habitat. Table 4.1 shows the distribution of *Nostoc* spp. in different habitats.

Table 4.1. Distribution of *Nostoc* spp. in different habitats.

No.	Name of species	EL	ES	CO	EP	BE
1	<i>Nostoc aureum</i> f. <i>variabilis</i>	-	+	-	-	-
2	<i>N. bornetii</i>	+	-	-	-	-
3	<i>N. caeruleum</i>	+	-	-	-	-
4	<i>N. calcicola</i>	+	-	-	-	-
5	<i>N. carneum</i>	+	-	-	-	-
6	<i>N. citrisporum</i>	+	-	-	-	-
7	<i>N. comminutum</i>	+	-	-	-	-
8	<i>N. commune</i>	+	-	-	+	-
9	<i>N. edaphicum</i>	+	-	-	-	-
10	<i>N. ellipso sporum</i>	+	+	-	-	-
11	<i>N. ellipso sporum</i> var. <i>violaceum</i>	+	-	-	-	-
12	<i>N. flagelliforme</i>	+	-	-	-	-
13	<i>N. fuscescens</i>	+	-	-	-	-
14	<i>N. gelatinosum</i>	+	-	-	-	-
15	<i>N. hatei</i>	+	-	-	-	-
16	<i>N. humifusum</i>	+	-	-	-	-
17	<i>N. indistinguendum</i>	+	-	-	-	-
18	<i>N. interbryum</i>	+	-	-	-	-
19	<i>N. kihlmanii</i>	+	-	+	-	-
20	<i>N. linckia</i>	+	+	-	-	+

Morphological characterization of Nostoc species from Kerala

21	<i>N. locularis</i>		+	-	-	-	-
22	<i>N. microscopicum</i>		-	+	-	-	-
23	<i>N. minutum</i>		+	-	-	-	-
24	<i>N. muscorum</i>		+	+	-	+	-
25	<i>N. nylstromicum</i>		+	-	-	+	-
26	<i>N. oryzae</i>		-	+	-	-	-
27	<i>N. paludosum</i>		+	-	-	-	-
28	<i>N. passerinianum</i>		+	-	-	-	-
29	<i>N. pruniforme</i>		-	+	-	-	-
30	<i>N. punctiforme</i>		+	+	-	-	-
31	<i>N. sphaericum</i>		+	-	-	-	-
32	<i>N. sphaeroides</i>		+	-	-	-	-
33	<i>N. tibeticum</i>		+	-	-	-	-
34	<i>N. undulatum</i>		+	-	-	-	-
35	<i>Nostoc</i> CU150162	sp.	-	+	-	-	-
36	<i>Nostoc</i> CU150191	sp.	+	-	-	-	-
37	<i>Nostoc</i> CU167213	sp.	+	-	-	-	-
38	<i>Nostoc</i> CU150122	sp.	+	-	-	-	-
39	<i>Nostoc</i> CU150172	sp.	-	+	-	-	-

EL: Epilithic; **ES:** Epipsammic; **EP:** Epiphytic; **BE:** Benthic; **CO:** Corticolous

4.2.3 New records of cyanobacterial flora.

Out of 39 *Nostoc* spp. identified from various regions of Kerala, 3 species are considered new records of Indian cyanobacterial flora. *N. indistinguendum*, *N. locularis* and *N. tibeticum* are new records from India. A total of 11 *Nostoc* spp. were reported as new to cyanobacterial flora of Kerala. *N. aureum* f. *variabilis*, *N. citrisporum*, *N. edaphicum*, *N. flagelliforme*, *N. fuscescens*, *N. indistinguendum*, *N. kihlmanii*, *N. locularis*, *N. pruniforme*, *N. sphaericum* and *N. tibeticum* are new addition to cyanobacterial flora of Kerala.

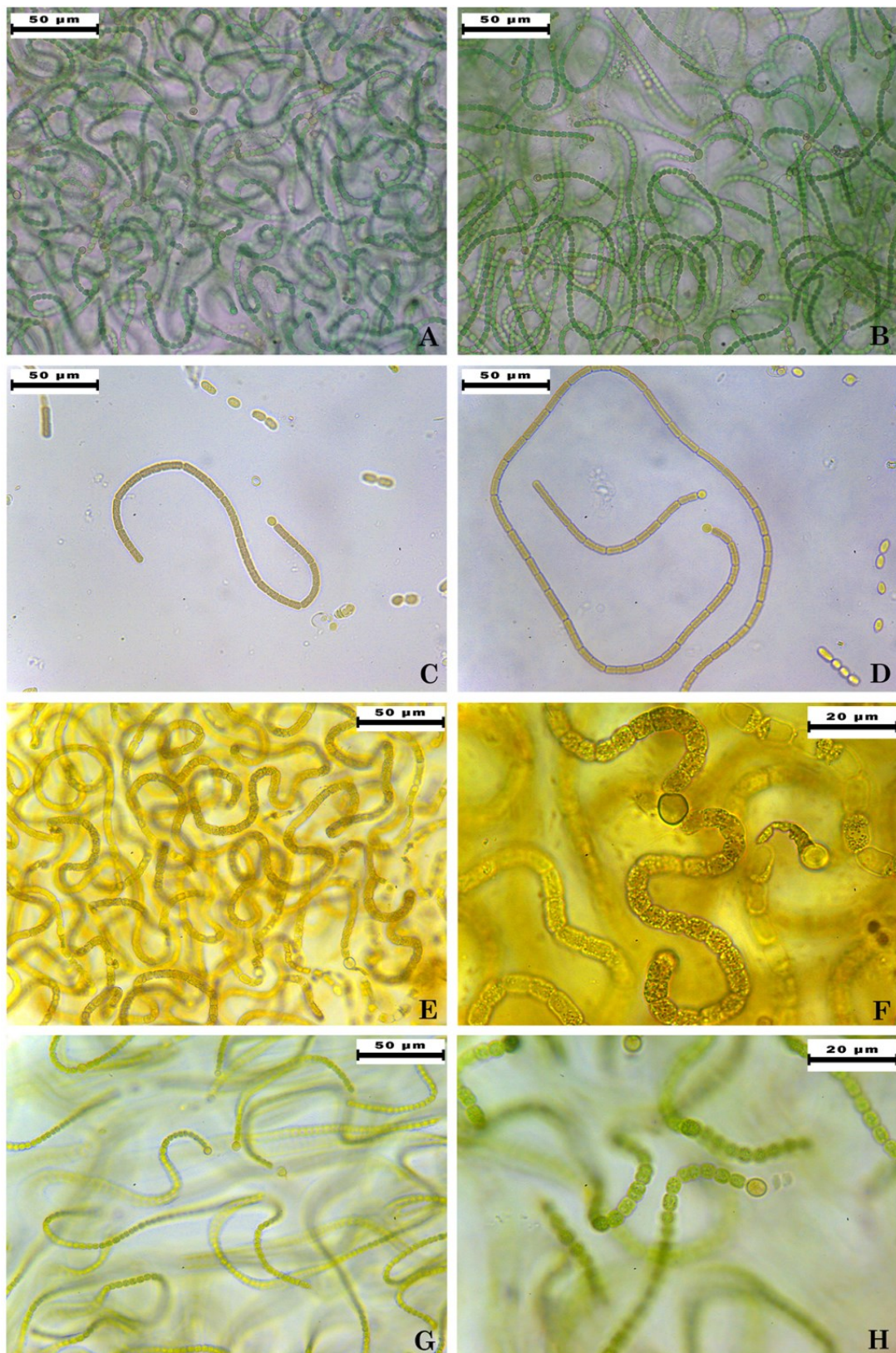


Fig. 4.1 A & B – *Nostoc aureum* f. *variabilis*; C & D – *N. bornetti*; E & F – *N. caeruleum*; G & H – *N. calcicola*. Scale bars: A–E, G = 50 µm, F & H = 20 µm

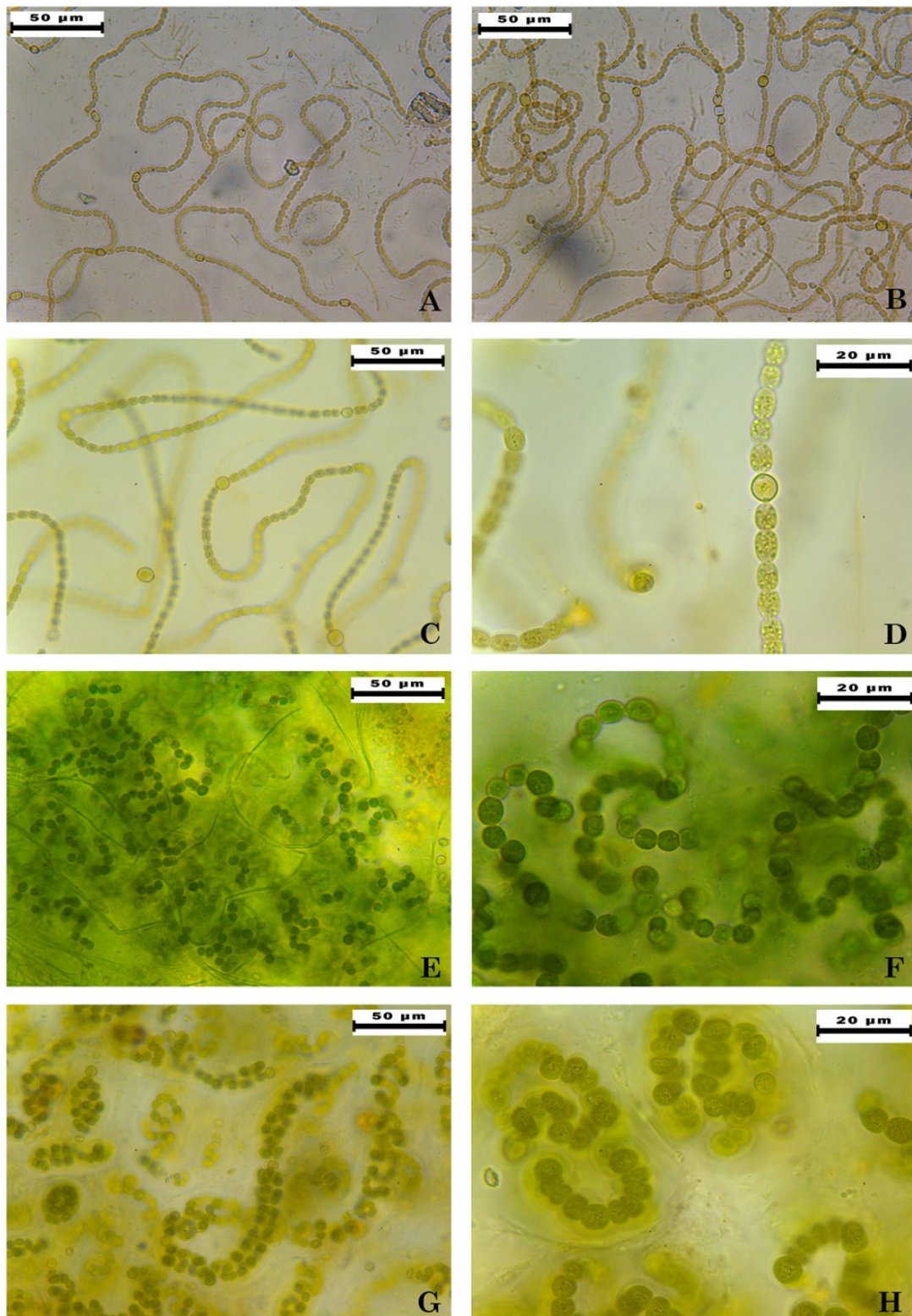


Fig. 4.2 A & B – *Nostoc carneum*; C & D – *N. citrisporum*; E & F – *N. comminutum*; G & H – *N. commune*. Scale bars: A-C, E & G = 50 µm, D, F & H = 20 µm

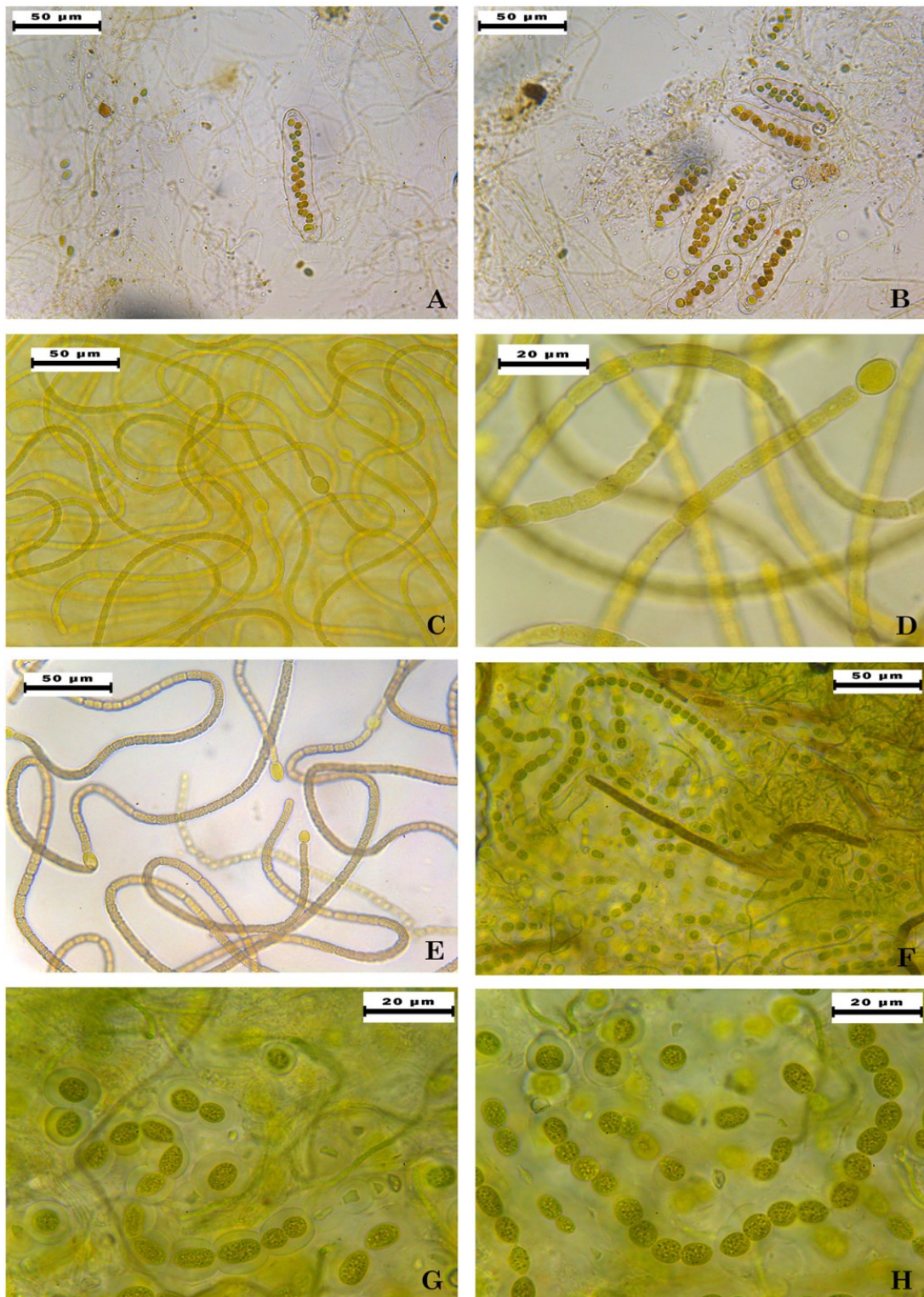


Fig. 4.3 A & B – *Nostoc edaphicum*; C&D – *N. elliposporum*; E – *N. elliposporum* var. *violaceum*; F-H – *N. flagelliforme*. Scale bars: A-C, E & F = 50 µm, D, G & H = 20 µm

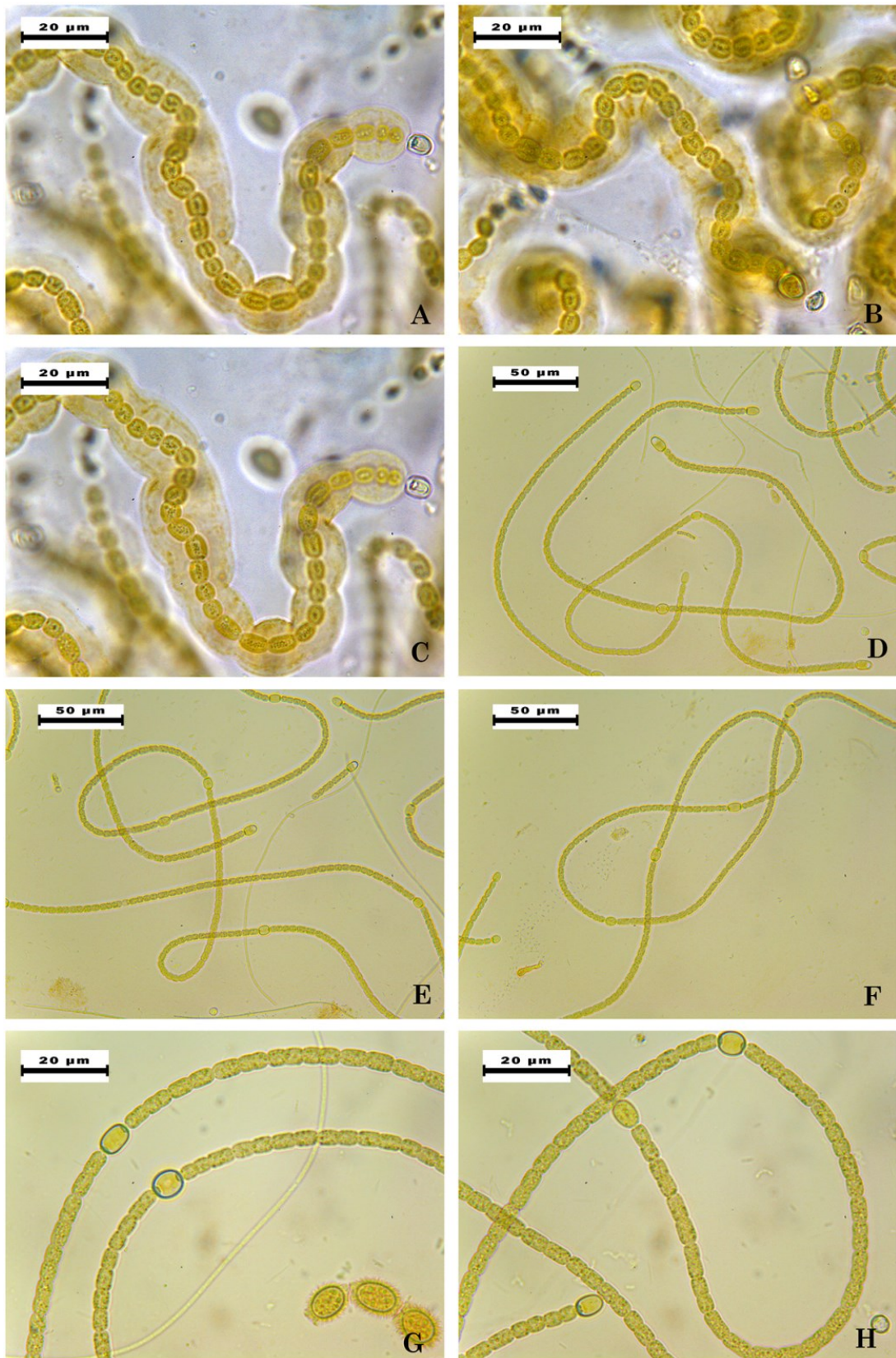


Fig. 4.4 A-C – *Nostoc fuscescens*; D-H – *N. gelatinosum*. Scale bars: A-C, G & H = 50 µm, D-F = 20 µm

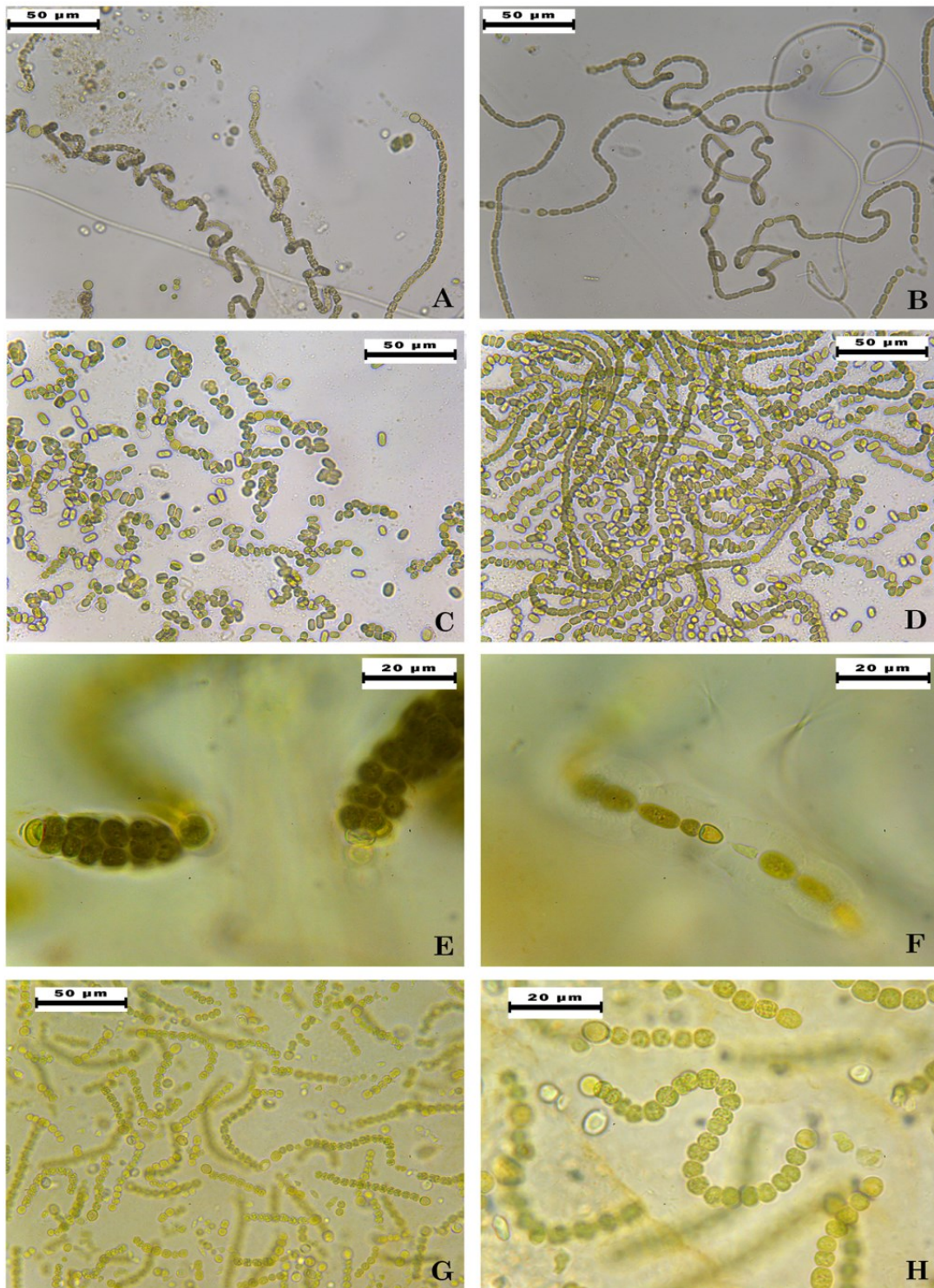


Fig. 4.5 A & B – *Nostoc hatei*; C & D – *N. humifusum*; E & F – *N. i. indistinguendum*; G & H – *N. interbryum*. Scale bars: A–D & G = 50 μm, E, F & H = 20 μm

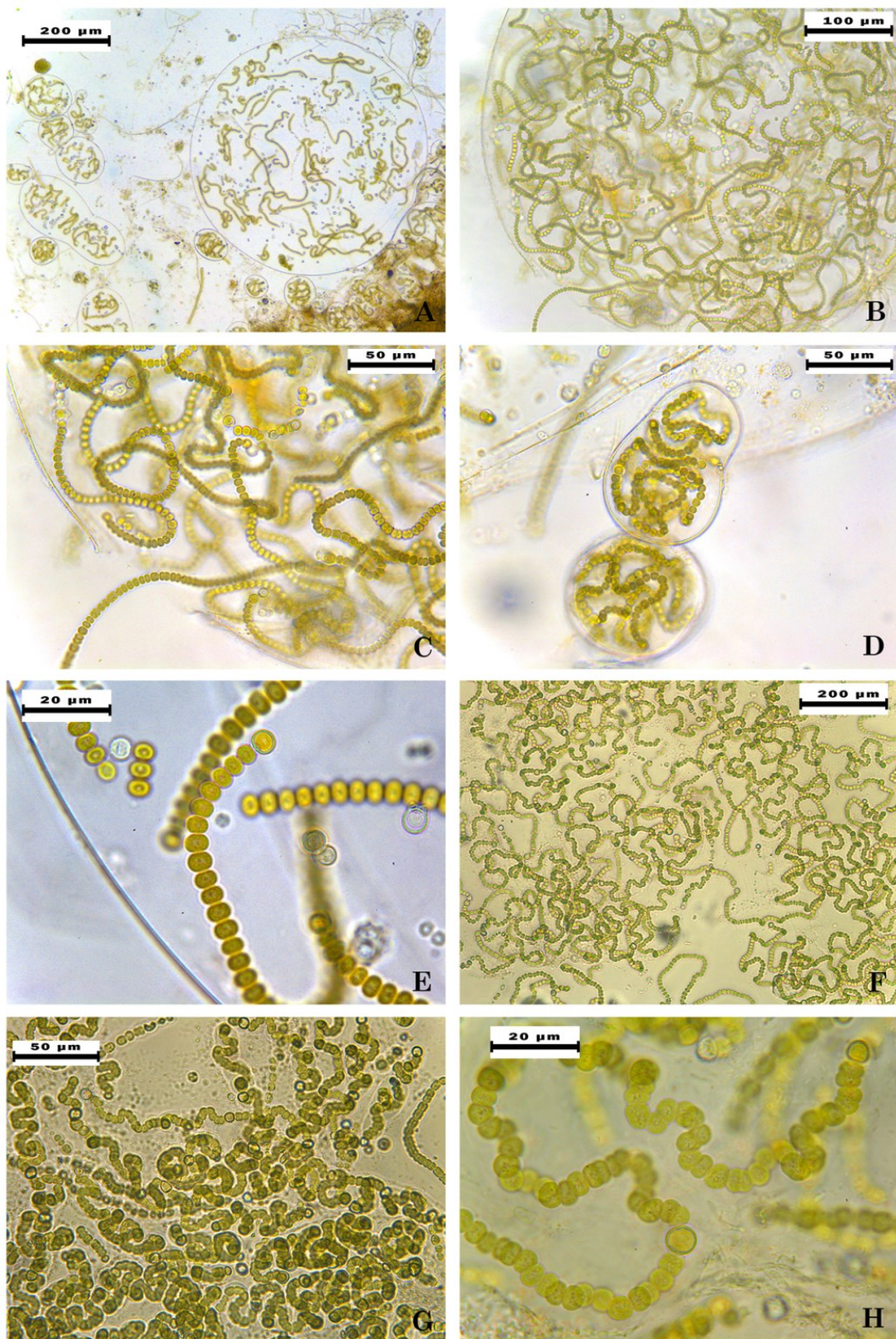


Fig. 4.6 A-E – *Nostoc kihlmanii*; F-H – *N. linckia*. Scale bars: A, B & F = 100 µm; C, D & G = 50 µm, E & H = 20 µm

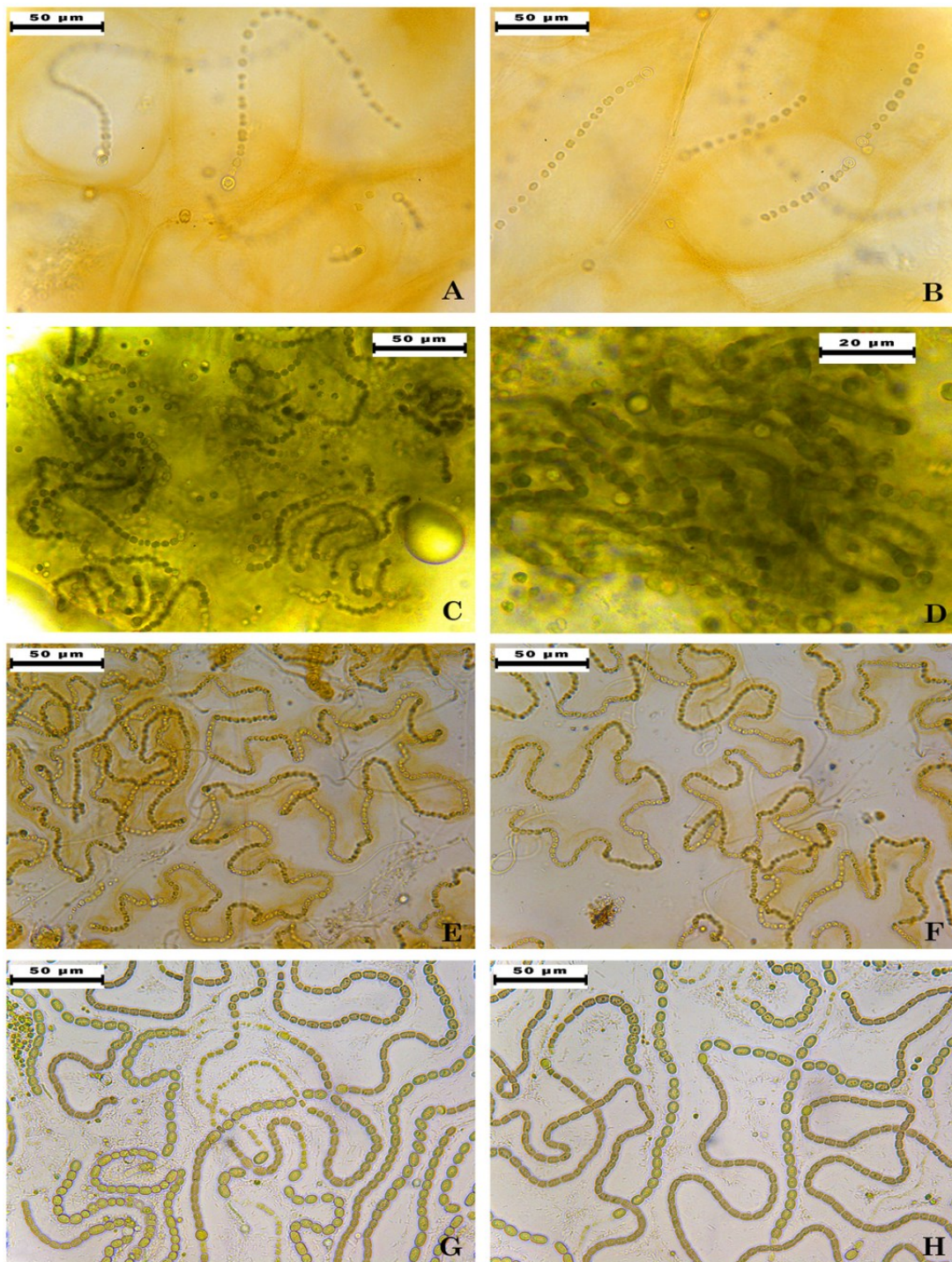


Fig. 4.7 A & B – *Nostoc locularis*; C & D – *N. microscopicum*; E & F – *N. minutum*; G & H – *N. muscorum*. Scale bars: A, B, C, E, F, G & H = 50 µm; C, D & G = 50 µm, D = 20 µm

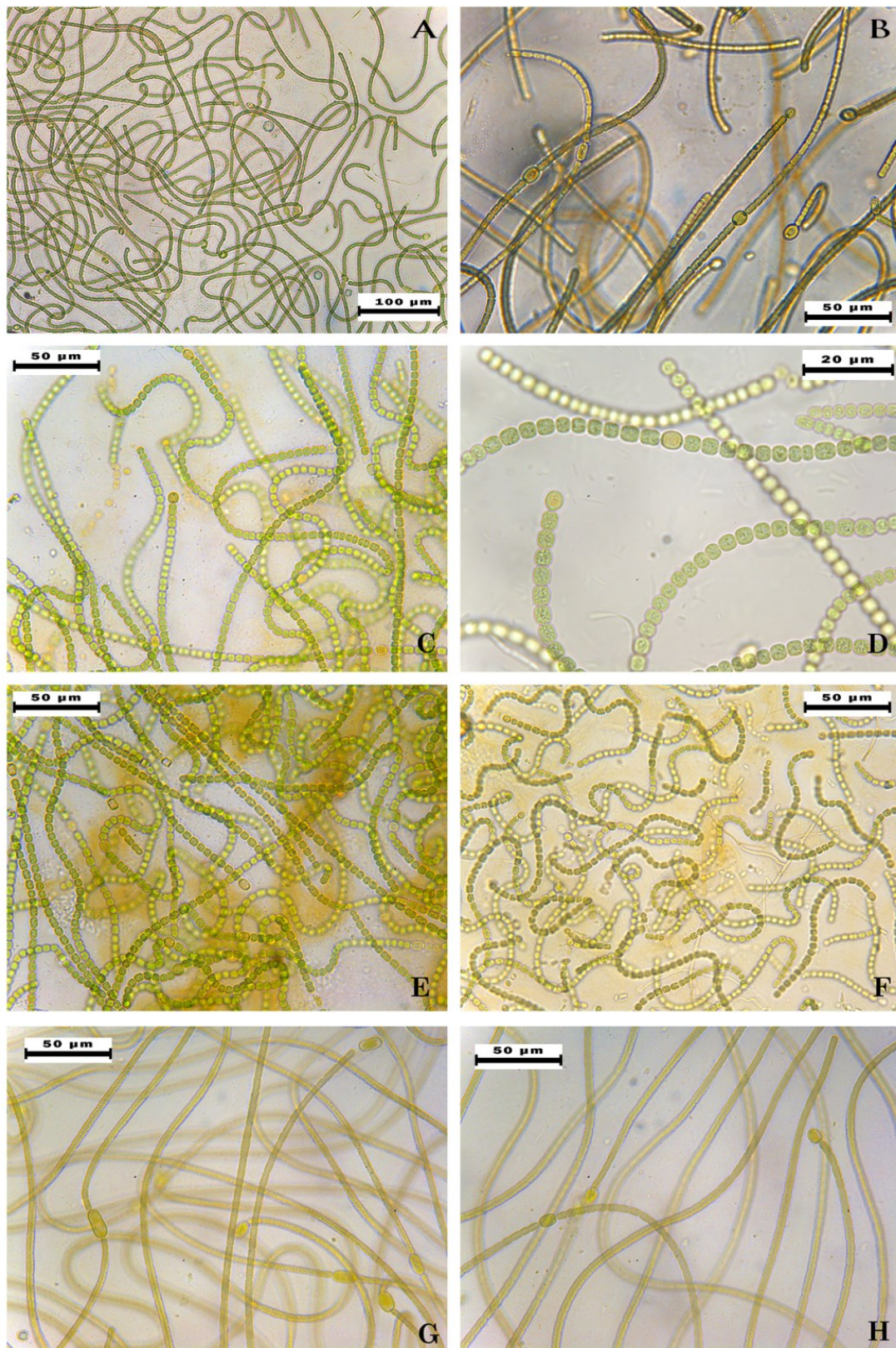


Fig. 4.8 A & B – *Nostoc nylstromicum*; C & D – *N. oryzae*; E & F – *N. paludosum*; G & H – *N. passerinianum*. Scale bars: A = 100 μm; B, C, E, F, G & H = 50 μm

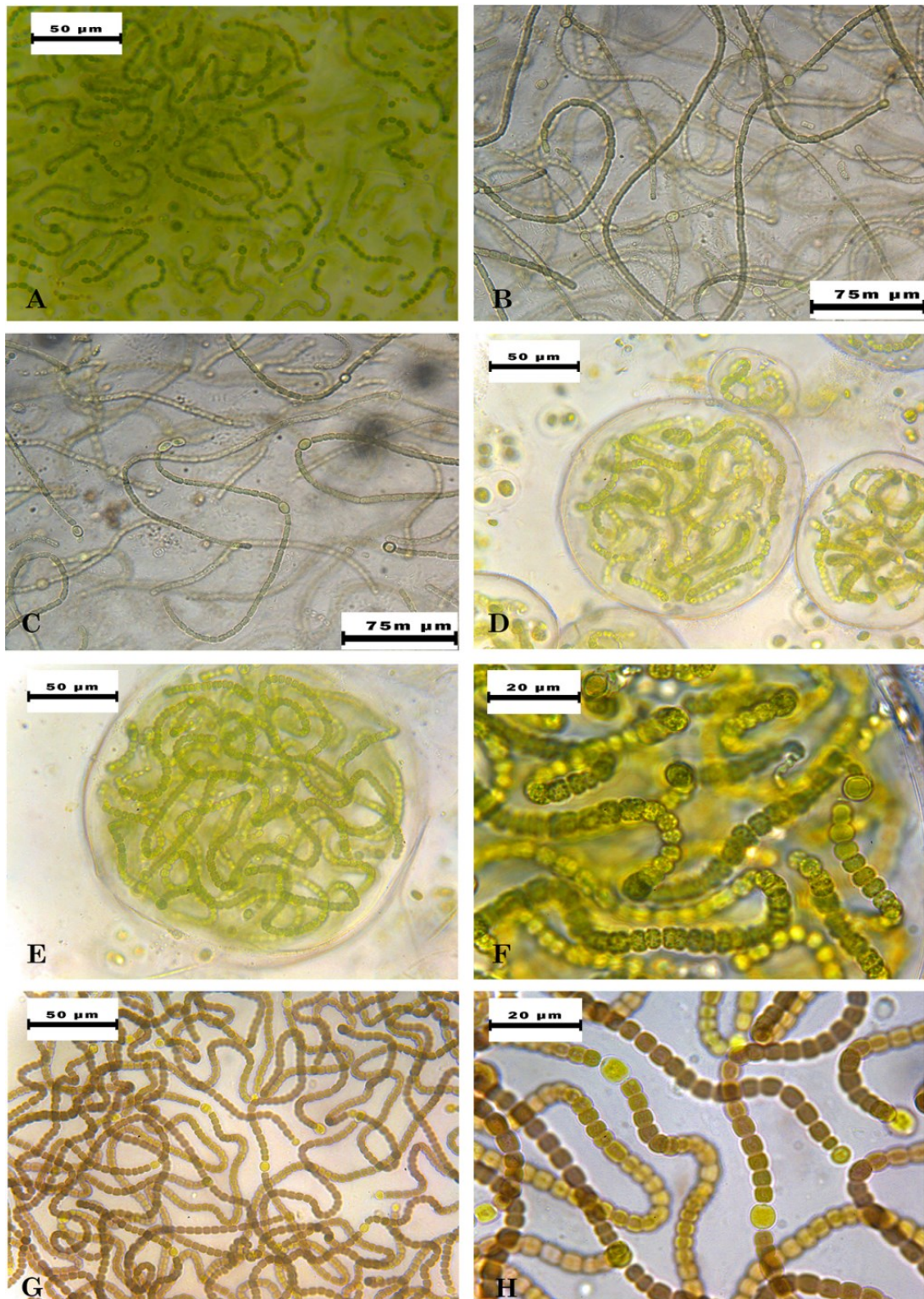


Fig. 4.9 A – *Nostoc pruniforme*; B & C – *N. punctiforme*; D & E – *N. shaericum*; G & H – *N. sphaeroides*. Scale bars: A, D, E & G = 50 µm; B & C = 75m µm; F & H = 20 µm.

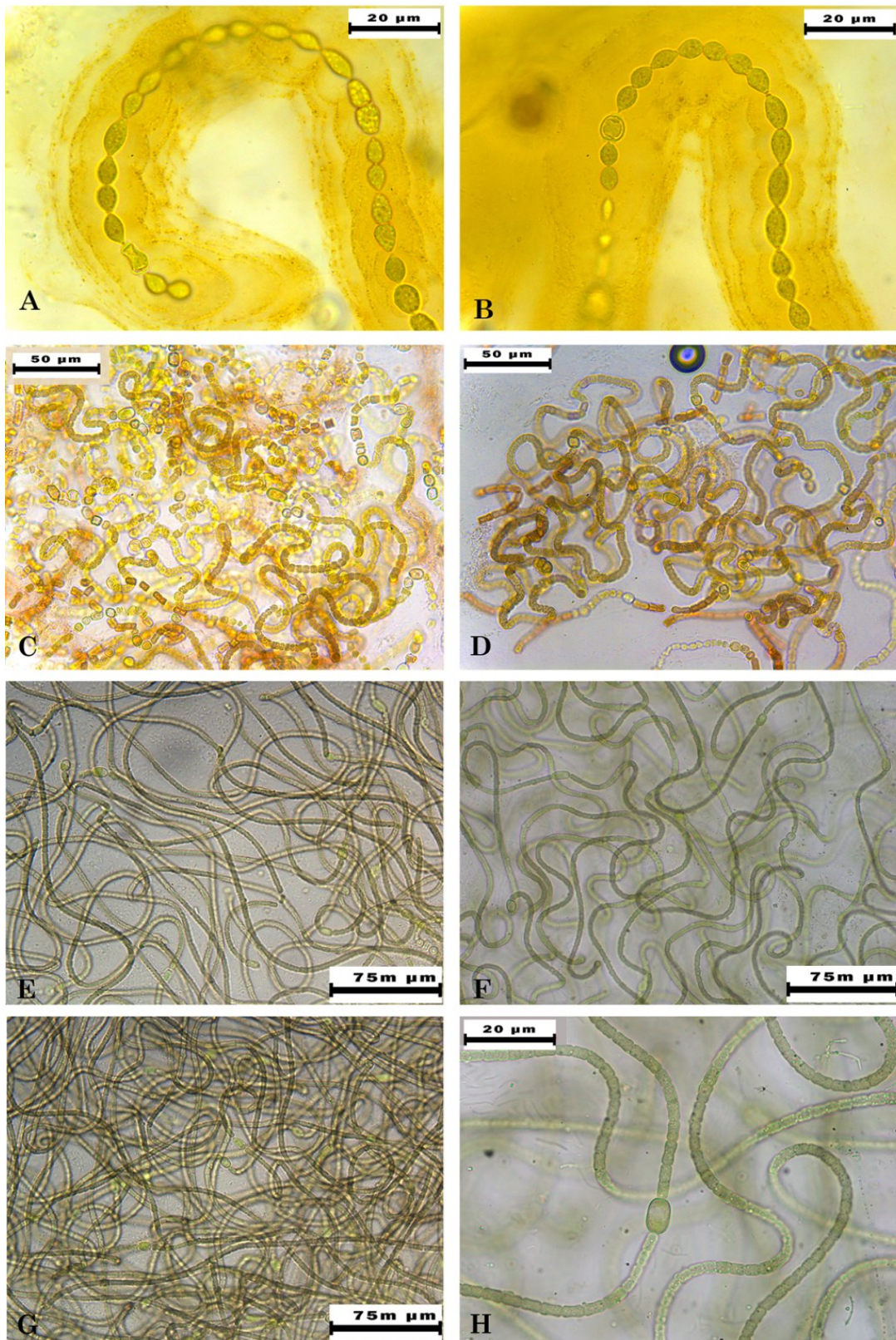


Fig. 4.10 A & B – *Nostoc tibeticum*; C & D – *N. undulatum*; E-H – *Nostoc* sp. CU150162. Scale bars: A, B & H = 20 µm; C & D = 50 µm; E, F & G = 75 µm

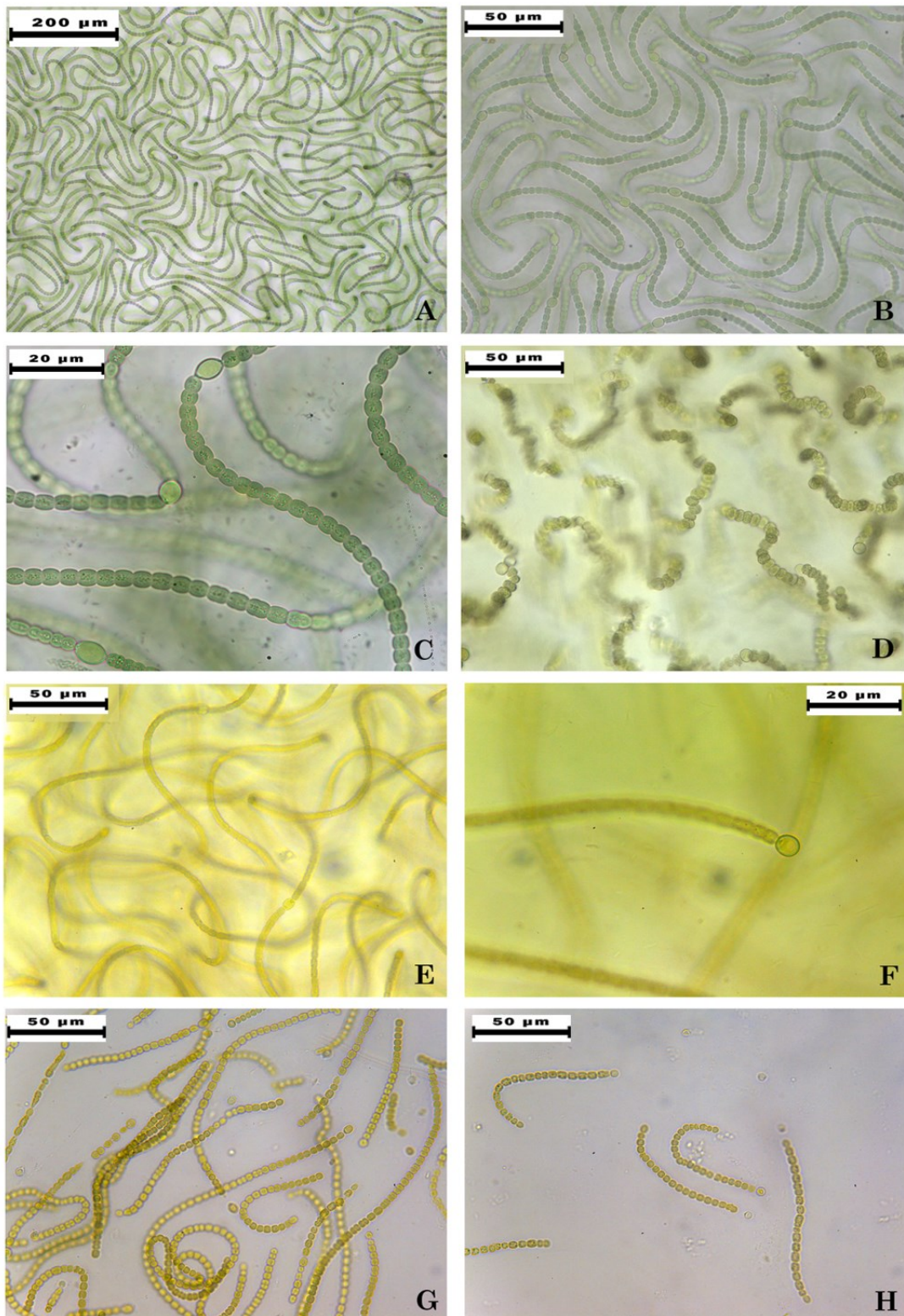


Fig. 4.11 A-C – *Nostoc* sp. CU150122; D – *Nostoc* sp. CU150191; E & F – *Nostoc* sp. CU167212; G & H – *Nostoc* sp. CU150172. Scale bars: A = 200 µm; B, D, E, G & H = 50 µm; C & F = 20 µm

Discussion

Nostoc species are universal in distribution over a wide range of terrestrial and aquatic ecosystems, which helps the colonies to become both conspicuous and abundant (Mollenhauer *et al.*, 1999; Potts, 2002). All the species of primarily soil-dwelling genus *Nostoc* is characterized by a complex life cycle that includes differentiation into heterocysts (nitrogen-fixing cells), akinetes (resting spores) and hormogonia (motile filaments, serving for dispersion and as infective units in symbiosis) (Mollenhauer, 1970; Rippka *et al.*, 1979). They showed wide diversity based on morphology and approximately 228 species are currently listed in Algaebase (Guiry and Guiry, 2023) and out of these, 108 species were taxonomically accepted. In India, Desikachary (1959) reported 23 *Nostoc* spp. in the monograph Cyanophyta. Among the identified species, most were recorded from crop fields, rest were observed on rock surfaces. Paddy fields of Kerala have recorded the highest degree of diversity of cyanobacteria so far reported. The cultivated soil from the paddy fields supports the growth of heterocystous cyanobacteria, and the water-logged conditions of the paddy fields provide a favourable environment (optimum levels of light, water, temperature, humidity and nutrient availability) for the growth and dominance of heterocystous cyanobacteria. *Nostoc* can prevail in terrestrial habitats on a variety of substrates such as rocks, soils and tree bark (Desikachary, 1959). In this study, *Nostoc* species were collected from epilithic, epipsammic, epiphytic, benthic and corticolous habitats.

Previous studies on the cyanobacterial diversity in India revealed that *Nostoc* spp. is dominant in the paddy fields of Kerala (Thilak *et al.*, 2020), Assam and Haryana (Venkataraman, 1972), Manipur (Devi *et al.*, 1999), West Bengal (Saha and Mandal, 1978), Orissa (Sahu *et al.*, 1996), Tamil Nadu (Anand, 1989) and Uttar Pradesh (Yadav *et al.*, 2021). In the paddy fields, cyanobacteria make significant contributions to soil fertility in terms of the physico-chemical, biological and soil-water relations (Nayak and Prasanna, 2007). Thilak *et al.* (2020) reported twelve *Nostoc* spp. from the paddy fields of Kerala. *N. paludosum*, *N. entophyllum*, *N. piscinale*, *N. linckia*, *N. carneum*, *N. muscorum*, *N. ellipso sporum*, *N. punctiforme*,

N. spongiaeforme and *Nostoc* sp. were the identified species. Similarly, *N. carneum*, *N. linckia*, *N. elliposporum*, *N. punctiforme* and *N. muscorum* were collected from paddy fields of Kerala during this study. In another study, Prasanna and Nayak (2007) observed *Nostoc* and *Anabaena* as the most dominant genera in most of the sites. Singh *et al.* (2015) investigated the distribution of cyanobacteria in rice fields of Varanasi, UP. Among thirty-two taxa identified only three species of *Nostoc* were recorded. *N. muscorum*, *N. calcicola* and *N. commune* reported from rice fields. Likewise, in our study, *N. muscorum* was reported from paddy fields of Mukkam, Kozhikode. *Nostoc* spp. richness in the paddy fields of Kerala, Tamil Nadu, West Bengal, Assam and Haryana were studied by Venkataraman (1972). Singh *et al.* (2001) have also demonstrated the abundance of *Nostoc* spp. in the paddy fields of West Bengal, India. Previous researchers argued that the genus *Nostoc* is common in paddy fields of Bangladesh, Spain, Australia, Egypt, Indonesia, Iraq, Japan, Morocco, Philippines and Uruguay (Song *et al.*, 2005; Sinha and Hader, 1996).

In the present study, *Nostoc elliposporum* and *N. linckia* were recorded from paddy fields of Malappuram and Palakkad respectively. The result is corroborated with the previous study regarding the diversity of *Nostoc* spp. from the paddy fields of Bangladesh (Begum *et al.*, 2008). Vijayan and Ray (2015) identified seven *Nostoc* spp. from the paddy fields of Kuttanadu, Kerala. *N. punctiforme*, *N. paludosum*, *N. elliposporum*, *N. muscorum*, *N. carneum*, *N. commune*, *N. linckia* and *N. spongiaeforme* were the identified species. In this study, *N. carneum*, *N. linckia*, *N. elliposporum*, *N. punctiforme* and *N. muscorum* were reported from paddy fields. Anand and Hopper (1987) identified *N. commune* from Ernakulam, *N. calcicola* and *N. paludosum* from Thrissur paddy fields.

The diversity of epilithic cyanobacteria has been studied in several regions around the world and some of the studies reported *Nostoc* spp. as epilithic. Budel (1999) studied the ecology and diversity of rock-inhabiting cyanobacteria in tropical regions and reported *N. muscorum* as epilithic from Ivory Coast, Africa. Another investigation of Hauer (2008), identified *N. commune* and *N. microscopicum* as epiliths from South Bohemia. Likewise, in the present study *N. muscorum* and *N.*

commune are reported from epilithic habitats of Kerala. According to Tamaselli *et al.* (2000), photosynthetic microorganism living in epilithic communities establish close relationships with lithotypes and they concluded that *Nostoc* spp. were most frequently associated with artificial substrates.

Rock surfaces are suitable substratum for the luxuriant growth of most *Nostoc* spp. and it occurs as a slimy mucilaginous mass on rock surfaces. They can easily adhere to the surface of rocks due to the mucilage on the trichomes. In the present investigation, the majority of *Nostoc* spp. were collected from rock surfaces of the Western Ghats regions of Kerala. Western Ghats regions are one of the hotspots of biodiversity with tremendous species richness (Myers *et al.*, 2000). The diversity of microalgae in high ranges of Western Ghats regions is influenced by a combination of environmental factors (Nasser and Suresh Kumar, 2013). Gelatinous colonies of *Nostoc* spp. on rock surfaces are brown, yellowish brown, olive green, pale green and dark green in colour. Colony colour depends on the presence of pigments and environmental conditions. Dark brown or brown coloured colonies of *Nostoc* spp. may be due to extracellular pigments the brownish scytonemin, the violet nostocine A and intracellular mycosporine-like amino acids. The colonies also exhibit various morphological forms including spherical balls, spherical balls with granulated surfaces and mat forms. Cells of *Nostoc* spp. are embedded in an extensive gelatinous matrix of polysaccharides and many other organic substances like exopolysaccharides (EPS), which influence the texture of colonies and provide biological and environmental protection (Jensen, 2014). Excretion of EPS is a key step in the adhesion of *Nostoc* spp. to rock surfaces (Rossi and Phillips, 2015).

Nostoc spp. in terrestrial habitats is subjected to a variety of stresses, such as high light, ultraviolet radiation, high and low temperatures, osmotic stress, drought, and desiccation (Allakhverdiev *et al.*, 2000; Singh *et al.*, 2002; Lin *et al.*, 2004; Potts *et al.*, 2005; Budel *et al.*, 2008). Compared to freshwater and marine environments, aero-terrestrial cyanobacteria in the tropics are exposed to more adverse environmental conditions, especially desiccation and high solar irradiation and UV. The resistance of the cyanobacteria to extreme environmental conditions

provides them with a distinct advantage for survival on exposed surfaces. These organisms are inactive when desiccated, even though they can recover quickly with the availability of water, indicating desiccation tolerance (Scherer and Potts, 1989; Higo *et al.*, 2007). *Nostoc commune* retains viability for over 100 years when desiccated (Lipman, 1941; Cameron, 1962). Mycosporine-like amino acids in *N. commune* form strong interactions with water-stress proteins (Scherer and Potts, 1989), which are also present in substantial amounts in desiccated colonies and are released from colonies upon rehydration (Hill *et al.*, 1994).

N. muscorum, *N. linckia*, *N. elliposporum* and *N. punctiforme* recorded in the study from the rice fields also occur as epilithic on rock surfaces. The distribution of these species might indicate the lower nitrogen status in both environments (rice fields and rock surfaces). Previous studies by Saha *et al.* (2007) in agreement with our results. *N. punctiforme* recorded from different environments. Gaysina *et al.* (2018) studied the biodiversity of terrestrial cyanobacteria in the South Ural region and recorded *N. calcicola*, *N. commune*, *N. elliposporum*, *N. microscopicum* and *N. punctiforme* from the regions. Additionally, *N. microscopicum*, *N. punctiforme* and *N. commune* were recorded in the flora of steppe and forest-steppe zones of Bashkiria (Bakieva *et al.*, 2012). Similarly, *N. commune*, *N. elliposporum* and *N. microscopicum* were also identified during the present study. The biodiversity of epilithic cyanobacteria from freshwater streams of Kakoijana Reserve forest of Assam, India was estimated by Saha *et al.* (2007) and identified *N. humifusum*, *N. oryzae* and *N. punctiforme* as epiliths.

Nikam *et al.* (2013) reported 12 *Nostoc* spp. from the Western Ghats regions of Maharashtra and concluded that *Nostoc* is one of the most dominant genera in that area and *N. calcicola* is the most prominent and common cyanobacteria in Western Ghats regions of Maharashtra. Kant *et al.* (2020) studied the diversity of *Nostoc* spp. from the Indo-Bangla regions of Tripura and reported seventeen species of *Nostoc*. About 23.5% of them grow in rice fields and the rest 76.5% flourish in other habitats (rock surface, building walls, subaerial, etc.). The study concluded that the species richness in other habitats is due to the chemical nature of the soil and availability of

various pesticides in rice fields. Excessive use of chemical fertilizer on the field is causing to a shift in the nutrient status of the ecosystem, ultimately generating nutrient stress for the heterocystous cyanobacteria (Choudary and Bimal, 2010).

Ram and Paul (2022) reported nine *Nostoc* spp. from the mangrove environments of Kerala. Among these, *N. carneum*, *N. commune*, *N. elliposporum* and *N. linckia* were considered as new species to the mangrove cyanobacterial flora of Kerala. Ram and Shamina (2016) studied the biodiversity of freshwater epilithic cyanobacteria of Kerala and reported five *Nostoc* spp. as epiliths. Geethu and Shamina (2021) studied filamentous cyanobacteria from the Western Ghats of north Kerala and reported three *Nostoc* species namely *N. commune*, *N. interbryum* and *N. nylstromicum*. Among these, *N. interbryum* and *N. nylstromicum* are two new reports from India. In accordance with this study, *N. interbryum*, *N. nylstromicum* and *N. commune* were reported from Western Ghats regions of Kerala. Furthermore, Sant' Anna *et al.* (2007) recorded three new aerophytic morphospecies of *Nostoc* (*N. interbryum*, *N. viride*, and *N. alatosporum*) from São Paulo State, Brazil based on their morphology, especially by the formation and shape of the akinetes, life cycles and ecology. John and Francis (2013) identified epilithic species such as *N. linckia* and *N. commune* from the Idukki district of Kerala. Correspondingly, *N. linckia* and *N. commune* were identified from the epilithic habitats of Kerala.

Corticolous cyanobacteria inhabit the bark of trees and the information on the distribution of cyanobacteria in this habitat is extremely scarce. According to Bakta *et al.* (2014), corticolous cyanobacteria are observed as gelatinous, greenish or dark green, red dark or brown patches, streaks or velvet masses on the tree bark. They are exposed to air and absorb water, minerals and other nutrients directly from the atmosphere. Corticolous cyanobacteria including *Nostoc*, *Phormidium*, *Scytonema* and *Stigonema* are responsible for the variety of colour and textures of the bark surface. *N. commune* was reported as corticolous cyanobacteria from Similipal Biosphere Reserve, Mayurbhanj, Odisha, India. Silva *et al.* (2012) reported corticolous *Nostoc* sp. from tropical forest remnants in northwestern Sao Paulo State, Brazil. Kant *et al.* (2020) identified *N. pruniforme* as corticolous

cyanobacterial species from Tripura, India. In addition, Neustupa and Škaloud (2008) identified *Aphanothece*, *Phormidium*, *Nostoc*, and *Scytonema* species from tree bark. A total of four *Nostoc* spp. such as *Nostoc* sp. 1, *Nostoc* sp. 2, *N. cf punctiforme*, and *N. cf entophytum* were reported as corticolous cyanobacteria from a South-East Asian mountain rainforest locality in Cibodas, West Java.

Benthic cyanobacteria are those living in the bottom or stagnant and flowing water. Ram (2022) reported *N. bornetii* from the benthic habitat of southern Kerala. According to Joshi *et al.* (1981), in aquatic environments, *Nostoc* is associated primarily with the benthos but can form floating planktonic masses. In addition, Gupta and Das (2019) identified *N. linckia* from freshwater habitats of Karnataka. Similarly, *N. linckia* was reported from freshwater bodies of the Wayanad district. According to Hoagland *et al.* (1982), epiphytic cyanobacteria are attached to or growing on plant surfaces. They derive moisture and nutrients from the environment and may colonize submerged or emergent plants. Ram (2022) recorded *N. commune* as epiphytic from the mangrove environments of Kerala. In the current study, *N. nylstromicum*, *N. muscorum* and *N. commune* reported from epiphytic habitat.

N. indistinguendum from Kozhikode is a new report on the flora of Indian cyanobacteria. Recently, Afify *et al.* (2023) studied the biodiversity, morphology and taxonomy of cyanobacteria from soils of Egypt. The study concluded that *Nostoc lichenoides*, *N. indistinguendum* and *N. favosum* were the most predominant cyanobacterial species in the soil sample. Furthermore, Davydov (2021) demonstrated cyanobacterial species from the Northern Polar Ural Mountains. A total of ninety-three cyanobacterial species were identified, among them five species were belongs to the genus *Nostoc*. *Nostoc commune* was the most frequently encountered taxon from this region, which typically had soil and rock inhabitants. In the current study, *N. commune* was reported from epilithic and epiphytic habitats.

CHAPTER 5

MOLECULAR CHARACTERIZATION AND PHYLOGENETIC ANALYSIS OF SELECTED *NOSTOC* SPECIES

5.1 Abstract

There is an increased interest in using molecular techniques to resolve the taxonomic problems of cyanobacteria as the systematics of *Nostoc* spp. is complicated. This study was undertaken to identify the selected species using a molecular approach. Among the species identified using morphological characters, four were selected for further studies based on their easiness of isolation, culture and convenience for mass cultivation. The 16S rRNA gene is a prominent tool for phylogenetic analysis and is useful in identifying cyanobacterial species. 16S rRNA gene sequencing is used for the correct identification of species and the evaluation of relationships between them. Mega 7.0 software was used to construct a phylogenetic tree using neighbour-joining and maximum likelihood methods to study the evolutionary distance and interrelationship. Molecular characterization using 16S rRNA gene sequences showed that the species belonged to the genus *Nostoc*, and the identified species were *N. elliposporum* FY 150125, *N. punctiforme* FY 150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191. The phylogenetic analysis revealed the evolutionary distance and genetic relatedness of the *Nostoc* spp.

5.2 Results

5.2.1 Isolation and maintenance of axenic cultures of *Nostoc* spp.

Nostoc species collected from epilithic and epipsammic habitats were isolated by streak plate method on BG-11₀ (1.2% agar) medium. After 21 days of culture, the desired colonies were identified and the individual filaments or colonies were re-inoculated in an agar medium to isolate single species. The cultures were heterogeneous at the initial stages and the colony homogenization was achieved by continuous subculturing. The pure colonies of *Nostoc* spp. were transferred to BG-11₀ liquid medium for multiplication.

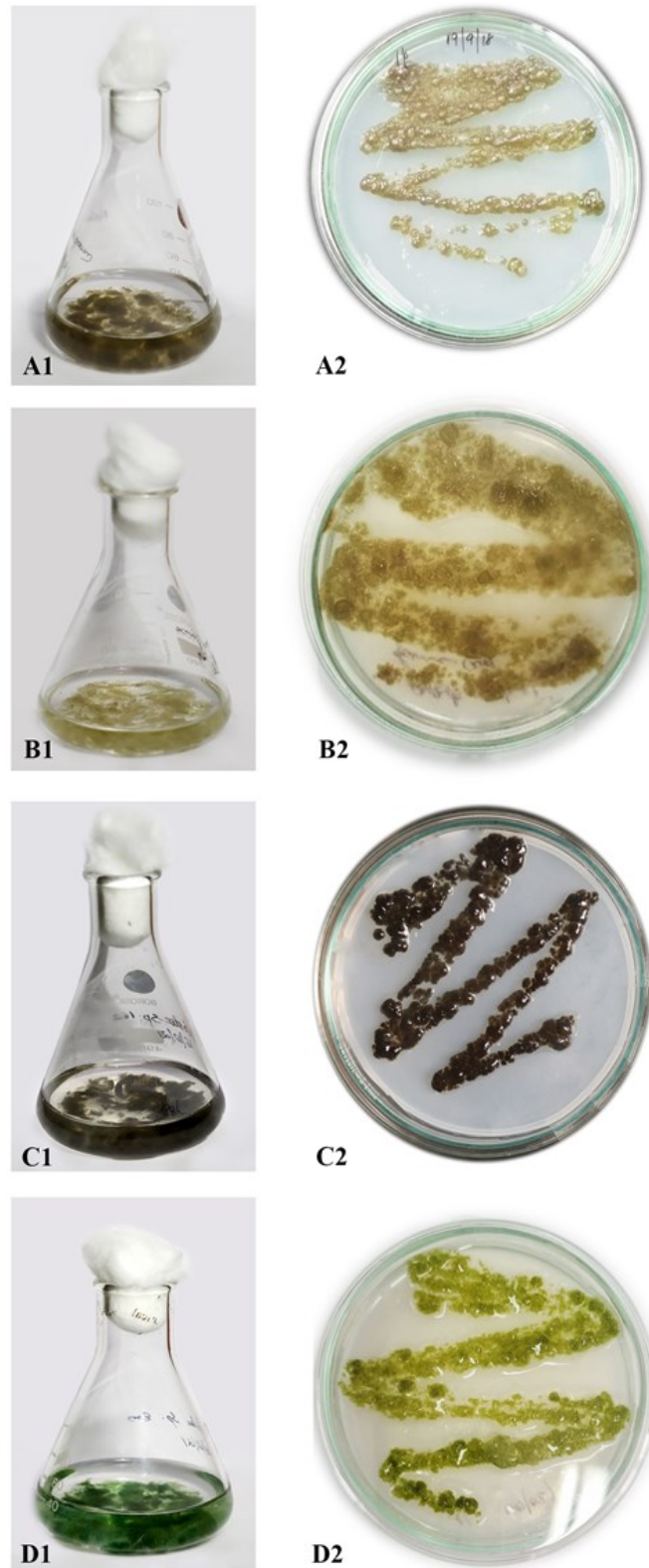


Fig. 5.1 Liquid and solid cultures of *Nostoc* spp. showing different growth patterns. A1 & A2 – *Nostoc ellipsosporum* FY150125; B1 & B2 – *N. punctiforme* FY150188; C1 & C2 – *Nostoc* sp. CU150162; D1 & D2 – *Nostoc* sp. CU150191

The cultures were maintained in the culture room under fluorescent light (3000 lux) at 25±2°C and illuminated with 14 hrs light followed by 10 hrs darkness. They multiply as gelatinous balls on agar plates in different colours. The colour varied from pale blue-green to brownish. *N. elliposporum* FY 150125 formed as brown gelatinous balls on medium, *N. punctiforme* FY 150188 formed a pale green gelatinous mass and spread on the agar plate unevenly, *Nostoc* sp. CU150162 formed blackish brown small gelatinous balls without spreading giving a jelly appearance on an agar plate, subsequent transfer to liquid medium changed to a lobed mass and *Nostoc* sp. CU150191 formed small blue-green to olive green balls on agar. When it is transferred to medium it is changed to blue or blue-green mucilaginous sheath. Continuous subculturing was done to maintain pure cultures after 14-21 days.

5.2.2 Molecular characterization of *Nostoc* spp. using 16S rRNA gene sequences

5.2.2.1 DNA extraction

The selected *Nostoc* spp. were preliminarily identified based on morphological characters and 16S rRNA typing was done. Twenty-one days (exponential stage) old pure cultures were used for DNA isolation to ensure the quality of the isolated DNA.

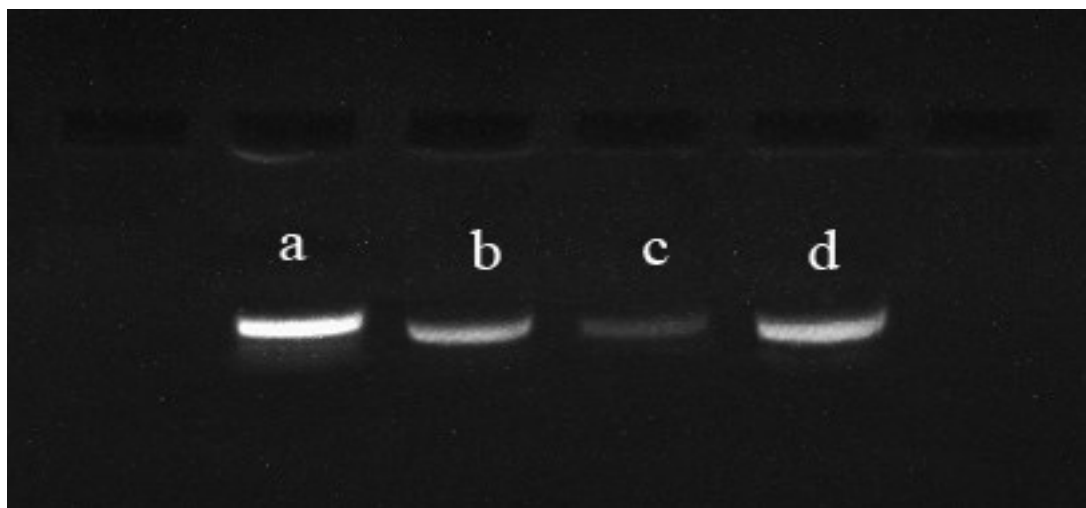


Fig. 5.2 Genomic DNA of *Nostoc* spp. on agarose gel. **a)** *N. elliposporum* FY 150125 **b)** *N. punctiforme* FY 150188 **c)** *Nostoc* sp. CU150162 **d)** *Nostoc* sp. CU150191

The genomic DNA from *Nostoc* species was isolated by the modified CTAB method. All the DNA samples showed the 260/280 ratio ranging from 1.9-2.0 indicating the purity of the isolated DNA (Fig. 5.2) and further used for PCR amplification.

5.2.2.2 Cloning of 16S rRNA gene from *Nostoc* spp.

The 16S rRNA gene was amplified from the isolated DNA using cyanobacterial universal primers. Agarose gel electrophoresis of the PCR products showed a 1500 bp (primer pair 8-27F and 1496R) band for *N. elliposporum* and 450 bp for all the other species (primer pair CYA 359F and equimolar mixture of CYA781Ra and CYA 781Rb) (Fig. 5.3).

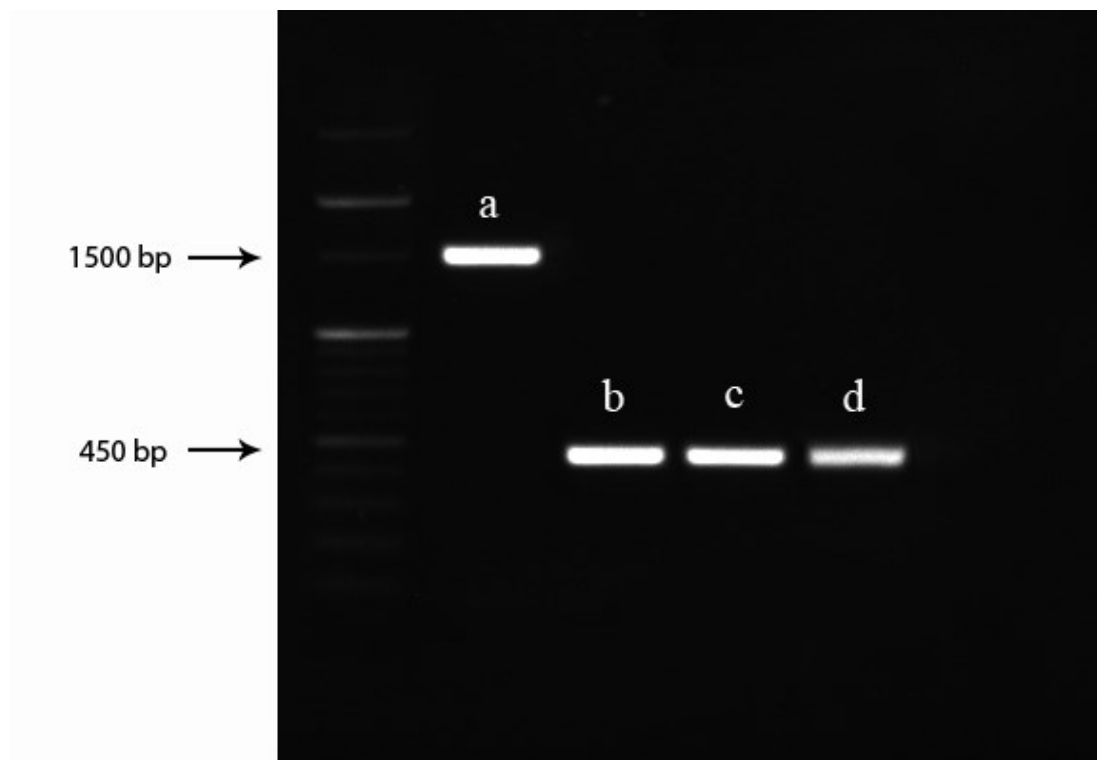


Fig. 5.3 Amplified 16S rRNA fragment of *Nostoc* spp. on agarose gel. **a)** *N. elliposporum* FY150125 **b)** *N. punctiforme* FY150188 **c)** *Nostoc* sp. CU150162 **d)** *Nostoc* sp. CU150191

The cloned PCR products encoding the 16S rRNA gene were sequenced by automated DNA sequencing and the obtained sequence was analyzed using Bio-Edit.v.7.2.5 software (Ibis Biosciences, Carlsbad, CA, USA). The obtained forward

and reverse sequences were aligned and edited. BLAST homology search tools were used to detect the sequence homology. The sequences were deposited in the NCBI GenBank database (<http://www.ncbi.nlm.nih.gov>) and accession numbers were provided. Molecular characterization based on 16S rRNA gene sequencing confirmed that the selected species were *Nostoc ellipsosporum* FY 150125, *N. punctiforme* FY 150188, *Nostoc* sp. CU150191 and *Nostoc* sp. CU150162. The sequences of the 16S rRNA gene of *Nostoc* spp. in FASTA format are listed below.

>*Nostoc ellipsosporum* FY 150125 16S ribosomal RNA gene, partial sequence
(Accession No: OM230033)

```
GGGCAATGGCGGATGCTACCATGCAGTCGACGGTGTCTTCGGACACAGTGGCGGAC
GGGTGAGTAACCGGTGAGAATCTAGCTTCAGGTCGGGGACACAGCCACATGGAAAC
GAGTGGCTAATGCCGGATTTGCCGAGAGGTACCAGGCTTGCTGCCTGAAGCTGAGC
TCGGCGCTCTGATTAGCTAGTAGGTGTGGTCAAGAGCGCACCTAGGCGACGATCAG
TAGCTCGGTCTGAGTTGATGATCAGTCCACACTGGGACTGAGACACGGCCAGCCT
CCTACGGGAGGCAGCAGTGGGGCATTTCGCCCATGGGCGAAAGCCTGACGGAGCA
ATCCCGCGTGAGGGAGGAATGCTCTTGGGTTGTAAACCTCTTTTCTCAGTGAAAGA
GAGCAATGACGGTACGTGAGGAGTCAGCATCGGCTAACTCCGTGCCAGCAGCCGCG
GTAATACGGAGGATGCGAGCGTTATCTGGAATGATTGGGCGTAAAGCGTCCGCAGG
TGGTGATGTAAGTCTGTTGTCAAAGCGTCTAGCTCAACTCAGATAAAGGCAGTGGA
AACTACATGACTAGAGTGCCTTCGGGGCAGAGGGAATTCCTGGTGTAGCGGTGAAA
TGCGTATATATCAGGAAGAACACCGGTGGCGAGAGCGCTCTGCTAGGCCGCAACTG
ACATTGAGGGACGAACGCTAGGGGAGCGAATGGGATTAGATACCTCAGTAGTCCTA
GCCGTAAACGATGGATACTAGGCGTGGCTTGTATCGACCCGAGCCGTGCCGTATCT
AACGCGTTAAGTATCCCGCCTGGGGAGTACGCACGCAAGTGTGAAACTCAAAGGAA
TTGACGGGGGCCCGCACCAAGCGGTGGAGTATGTGGTTTTATTTCGATGCAACGGGA
AGAGCCTTACCAAGACTTGACATGTCGCGAATTGGAGTGAGAGCTTCACGTGCCTT
AGGGAGCGCGAACACAGGTGGTGCATGGGCTGTCGTCAGCTCGTGTGCTGAGATGT
TGGGTTAAGTCCCGCAACGAGCGCAATCCTCGTTTTTTAGTTGCCAGCATTATGTTG
GGCACTCTAGAGAGACTGCCGGTGACAGACCTGAGGTAGGTGGGGATGACGTCAAG
TCAGCATGCCCCTTACGTCTTGGGGTACACACGTACTACAATGCTCCGGACAGAGT
GCAGCTAGCATGCGAATGCAAGTCAAATCCCGTAAACCTGAGCTCAGTTCAGATTG
CAGGTCTGCCACTCGCTGTGACGTGAAGGAGGAATCGCTAGTAATTGCAGGTCAGC
TATACTGCGAGTGAATATGGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATG
GAAGCTGGCAACGCCCGAAGTCATTACTCCAACCGTCAAAGAGGAGGATACCAAG
TCAGTCCCGACGTT
```

>*Nostoc punctiforme* FY 150188 16S ribosomal RNA gene, partial sequence (Accession No: OM230032)

```
GTGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATACCGCGTGAGGGAG
GAAGGCTCTTGGGTGTAAACCTCTTTTCTCAGGGAAGAAAAAATGACGGTACCT
GAGGAATCAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCA
AGCGTTATCCGGAATGATTGGGCGTAAAGCGTCCGCAGGTGGCTATGTAAGTCTGC
TGTTAAAGAGTCTAGCTCAACTAGATAAAGGCAGTGGAACTACACAGCTAGAGTG
CGTTCGGGGCAGAGGGAAATTCCTGGTGTAGCGGTGAAATGCGTAGATATCAGGAAG
AACACCGGTGGCGAAAGCGCTCTGCTAGGCCGCAACTGACACTGAGGACGAATGCT
AAGCTGGGAGCGAAAGGGATTAGATACCCCTGTAGTCA
```

>*Nostoc* sp. CU150162 16S ribosomal RNA gene, partial sequence

```
TCCGCAATGGGCGAAAGCCTGACGGAGCAATACCGCGTGAGGGAGGAAGGCTCTTG
GGTTGTAAACCTCTTTTCTCAGGGAAGAACACAATGACGGTACCTGAGGAATCAGC
ATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCAAGCGTTATCCG
GAATGATTGGGCGTAAAGCGTCCGCAGGTGGCGATGTAAGTCTGCTGTTAAAGAGC
AAAGCTCAACTTTGTAAAAGCAGTGGAACTACATAGCTAGAGTGCGGTAGGGGCA
GAGGGAATTCCTGGTGTAGCGGTGAAATGCGTAGAGATCAGGAAGAACACCGGTGG
CGAAAGCGCTCTGCTAGGCCGCAACTGACACTGAGGGACGAAAGCTAGGGGAGCGA
AAGGATTAGATACCCCTGTAGTCA
```

>*Nostoc* sp. CU150191 16S ribosomal RNA gene, partial sequence (Accession No: OR635663)

```
GGGGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATACCGCGTGAGGGAGG
AAGGCTCTTGGGTCGTAAACCTCTTTTCTCAGGGAAGAAAGAAATGACGGTACCTG
AGGAATAAGCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCAA
GCGTTATCCGGAATGATTGGGCGTAAAGCGTCCGCAGGTGGCGATGTAAGTCTGCT
GTTAAAGCGTCTAGCTCAACTAGATAAGAGCAGTGGAACTACATGGCTAGAGTGC
GTTTCGGGGCAGAGGGAATTCCTGGTGTAGCGGTGAAATGCGTAGAGATCAGGAAGA
ACACCGGTGGCGAAGGCGCTCTGCTAGGCCGCAACTGACACTGAGACGACTGAAAG
CTTCAAGGGGAGCGAATGGGATTAGATACCCCTGTAGTCA
```

Pairwise similarity alignment was performed using BLASTN of the above sequences to identify the species by comparing with the sequences available in NCBI GenBank database, *N. elliposporum* FY 150125 showed 96.47% identity with *N. elliposporum* V (accession no. AJ630450), *N. punctiforme* FY 150188 showed 98.36% identity with *N. punctiforme* SAG60.79 (accession no. GQ287652), *Nostoc* sp. CU150162 showed 98.32% identity with *Nostoc* sp. HKAR (accession no. KF751605) and *Nostoc* sp. CU150191 showed 99.27% identity with *Nostoc* sp. *Collema* sp. AR75 (accession no. KF359719).

5.2.2.3 Nucleotide substitution model selection

Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) are the best-fit nucleotide substitution models determined using Molecular Evolutionary Genetics Analysis (MEGA) 7.0 software. K2+G+I model with the lowest BIC scores (2730.118) and lowest AIC score (2353.021) were considered to describe the best-fit nucleotide substitution pattern for 16S rRNA gene sequence of *Nostoc* spp. and the homologous gene sequences retrieved from NCBI (Fig. 5.4). A discrete Gamma distribution (+G) with rate categories for the modelling of non-uniformity of evolutionary rates among sites by assuming that certain fraction of sites is evolutionary invariable (+I).

Model	#Param	BIC	AICc	lnL	Invariant	Gamma	R	Freq A	Freq T	Freq C	Freq G	A=>T	A=>C	A=>G	T=>A	T=>C	T=>G	C=>A	C=>T	C=>G	G=>A	G=>T	G=>C	
K2+G+I	52	2730.118257	2353.021214	-1124.247555	0.72688116	0.485531203	1.20640567	0.25	0.25	0.25	0.25	0.25	0.06	0.06	0.14	0.06	0.14	0.06	0.06	0.14	0.06	0.14	0.06	0.06
HKY+G+I	55	2735.350353	2336.528378	-1112.970627	0.723479332	0.469091797	1.232783753	0.276163343	0.186603989	0.211206078	0.324026591	0.04	0.05	0.17	0.06	0.11	0.08	0.06	0.1	0.08	0.15	0.04	0.05	0.05
JC+G+I	51	2735.576109	2365.721148	-1131.617472	0.721635043	0.489463803	0.5	0.25	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
TN92+G+I	53	2736.939493	2352.597754	-1123.025681	0.725345719	0.499177917	1.189629838	0.232383666	0.232383666	0.267616334	0.267616334	0.05	0.06	0.15	0.05	0.15	0.06	0.05	0.13	0.06	0.13	0.05	0.05	0.06
K2+G	51	2739.680763	2369.825802	-1133.659799	na	0.05	1.16080937	0.25	0.25	0.25	0.25	0.25	0.06	0.06	0.13	0.06	0.13	0.06	0.06	0.13	0.06	0.13	0.06	0.06
TN93+G+I	56	2744.557351	2338.493637	-1112.943135	0.722511073	0.497104511	1.221822223	0.276163343	0.186603989	0.211206078	0.324026591	0.04	0.05	0.17	0.06	0.12	0.08	0.06	0.11	0.08	0.14	0.04	0.05	0.05
JC+G	50	2745.130504	2382.518011	-1141.015882	na	0.05	0.5	0.25	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
HKY+G	54	2746.080551	2354.503501	-1122.956718	na	0.05	1.178103753	0.276163343	0.186603989	0.211206078	0.324026591	0.05	0.05	0.17	0.07	0.11	0.08	0.07	0.1	0.08	0.14	0.05	0.05	0.05
TN92+G	52	2747.367403	2370.27036	-1132.872128	na	0.05	1.149742975	0.232383666	0.232383666	0.267616334	0.267616334	0.05	0.06	0.14	0.05	0.14	0.06	0.05	0.12	0.06	0.12	0.05	0.06	0.06
GTR+G	58	2751.825475	2331.23404	-1107.315213	na	0.066656763	1.227871136	0.276163343	0.186603989	0.211206078	0.324026591	0.04	0.12	0.17	0.05	0.12	0.07	0.15	0.11	0	0.14	0.04	0	0
GTR+G+I	59	2751.825715	2324.0449	-1102.684341	0.708718039	0.468058907	1.070134377	0.276163343	0.186603989	0.211206078	0.324026591	0.03	0.12	0.16	0.05	0.09	0.06	0.15	0.08	0.03	0.15	0.04	0.02	0.02
TN93+G	55	2755.280515	2356.435539	-1122.935708	na	0.05	1.163944955	0.276163343	0.186603989	0.211206078	0.324026591	0.05	0.05	0.16	0.07	0.12	0.08	0.07	0.1	0.08	0.14	0.05	0.05	0.05
K2+I	51	2819.686329	2449.831369	-1173.662583	0.438271635	na	1.103917133	0.25	0.25	0.25	0.25	0.25	0.06	0.06	0.13	0.06	0.13	0.06	0.06	0.13	0.06	0.13	0.06	0.06
HKY+I	54	2824.010259	2432.430208	-1161.931572	0.438271635	na	1.110412051	0.276163343	0.186603989	0.211206078	0.324026591	0.05	0.05	0.16	0.07	0.11	0.08	0.07	0.1	0.08	0.14	0.05	0.05	0.05
JC+I	50	2824.830274	2482.217782	-1180.865547	0.438271635	na	0.5	0.25	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GTR+I	58	2825.809501	2405.268067	-1144.307226	0.438271635	na	1.130194946	0.276163343	0.186603989	0.211206078	0.324026591	0.04	0.13	0.15	0.05	0.12	0.06	0.17	0.11	0	0.13	0.04	0	0
TN92+I	52	2826.241725	2449.144682	-1172.308289	0.438271635	na	1.102234175	0.232383666	0.232383666	0.267616334	0.267616334	0.06	0.06	0.14	0.06	0.14	0.06	0.06	0.12	0.06	0.12	0.06	0.06	0.06
TN93+I	55	2832.712531	2453.891556	-1161.651717	0.438271635	na	1.103807786	0.276163343	0.186603989	0.211206078	0.324026591	0.05	0.05	0.15	0.07	0.12	0.08	0.07	0.11	0.08	0.13	0.05	0.05	0.05
K2	50	2867.333199	2504.720706	-1202.117009	na	na	1.094414715	0.25	0.25	0.25	0.25	0.25	0.06	0.06	0.13	0.06	0.13	0.06	0.06	0.13	0.06	0.13	0.06	0.06
HKY	53	2872.841697	2488.502858	-1190.970283	na	na	1.095554269	0.276163343	0.186603989	0.211206078	0.324026591	0.05	0.05	0.16	0.07	0.11	0.08	0.07	0.1	0.08	0.14	0.05	0.05	0.05
JC	49	2872.930447	2517.560808	-1209.546825	na	na	0.5	0.25	0.25	0.25	0.25	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
GTR	57	2873.438183	2460.136515	-1172.752559	na	na	1.116304086	0.276163343	0.186603989	0.211206078	0.324026591	0.04	0.13	0.15	0.05	0.12	0.06	0.17	0.11	0	0.13	0.04	0	0
TN92	51	2874.187457	2504.332497	-1200.913147	na	na	1.098895598	0.232383666	0.232383666	0.267616334	0.267616334	0.06	0.06	0.14	0.06	0.14	0.06	0.06	0.12	0.06	0.12	0.06	0.06	0.06
TN93	54	2881.428706	2489.848656	-1190.640796	na	na	1.094694177	0.276163343	0.186603989	0.211206078	0.324026591	0.05	0.05	0.15	0.07	0.12	0.08	0.07	0.11	0.08	0.13	0.05	0.05	0.05

Fig. 5.4. Maximum likelihood fits of 24 different nucleotide substitution models for the 16S rRNA sequences of *Nostoc* spp. and homologous sequences retrieved from NCBI; K2: Kimura 2 parameter; JC: Jukes-Cantor; TN93: Tamura-Nei; HKY: Hasegawa-Kishino-Yano; GTR: General Time Reversible)

Estimated values of transition/transversion bias (R) were shown for each model they were followed by nucleotide frequencies (f) and rates of base substitutions (r) for each nucleotide pair. Relative values of instantaneous r were considered and for simplicity, the sum of the r values is made equal to 1 for each model. The nucleotide frequencies are A=25.0%, T/U=25.0%, C=25.0% and G=25.0%. For estimating ML values, a tree topology was automatically computed. The analysis involved 26 nucleotide sequences. The evolutionary distances are expressed in terms of the number of transitional substitutions per site and were computed using the Tamura-Nei technique. Codon positions included were 1st +2nd +3rd +Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 417 positions in the final dataset.

5.2.3 16S rRNA-based phylogenetic analysis of *Nostoc* spp. using neighbour-joining and maximum likelihood methods.

Phylogenetic tree of *Nostoc* spp. were constructed using the cloned sequences and homologous sequences retrieved from GenBank, NCBI. Maximum likelihood and neighbour-joining methods with 1000 bootstraps were used to draw the phylogenetic tree. Models with the lowest BIC scores were considered to represent the best nucleotide substitution pattern. Multiple sequence alignments using MEGA 7.0 software and K2+G+1 model based on bootstrap resampling with 1000 replicates were performed to estimate the confidence of the tree topologies. The K2+G+1 model with the lowest BIC scores (2730.118) and lowest AIC score (2353.021) was considered to describe the best-fit nucleotide substitution pattern to construct consensus neighbour-joining and maximum likelihood tree of the sequences. The evolutionary distances are expressed in terms of the number of transitional substitutions per site and were computed using the Tamura-Nei technique. The analysis involved 26 nucleotide sequences. Codon positions included were 1st +2nd +3rd +Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 417 positions in the final dataset.

In the neighbour-joining tree, group I consists of *Nostoc* sp. CU150191, *Nostoc punctiforme* FY150188 and *N. ellipsosporum* FY 150125 and group II

consists of *Nostoc* sp. CU150162 (Fig. 5.5). The majority of the native strains show affinities with their corresponding strains retrieved from the GenBank except for *N. punctiforme*, which form the group I whereas all other *N. punctiforme* are grouped in group II and III. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown beside the branches. In NJ tree, *Nostoc* sp. CU150191 showed high relatedness with *Desmonostoc muscorum* ACSSI 046. *Desmonostoc muscorum* is the synonym of *Nostoc muscorum*. *N. elliposporum* FY 150125 showed the closest relatedness with *N. elliposporum* V and *Nostoc* sp. CU150162 showed the highest similarity to *Nostoc* sp. HKAR-6. *Nostoc punctiforme* FY150188 existed as a single clade.

The phylogenetic tree constructed using the maximum likelihood method with 1000 bootstraps (Fig. 5.6). The evolutionary relationship was inferred by using the maximum likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-1171.90) is observed. The percentage of replicate trees in which the associated taxa clustered together in bootstrap tests (1000 replicates) is shown next to the branches. Initial trees for the heuristic search were obtained automatically by applying Neighbour-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The analysis involved 26 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 405 positions in the final dataset. The maximum likelihood tree is also grouped into three. Group I consists of *Nostoc* sp. CU150191, group II consists of *N. punctiforme* FY 150188 and *Nostoc* sp. CU150162 and group III consists of *N. elliposporum* FY 150125. In group I, *Nostoc* sp. CU150191 clustered with *Nostoc* sp. *Peltigera aphthosa* UK 52. The sequence of *Nostoc punctiforme* FY 150188 in this study was observed as a single clade within group II. *Nostoc* sp. CU150162 and *Nostoc* sp. HKAR-6 was observed as a single cluster within the group II and both of them are tightly clustered with a 90 bootstrap value. In group III, *N. elliposporum* FY 150125 and *N. elliposporum* V were observed as a cluster.

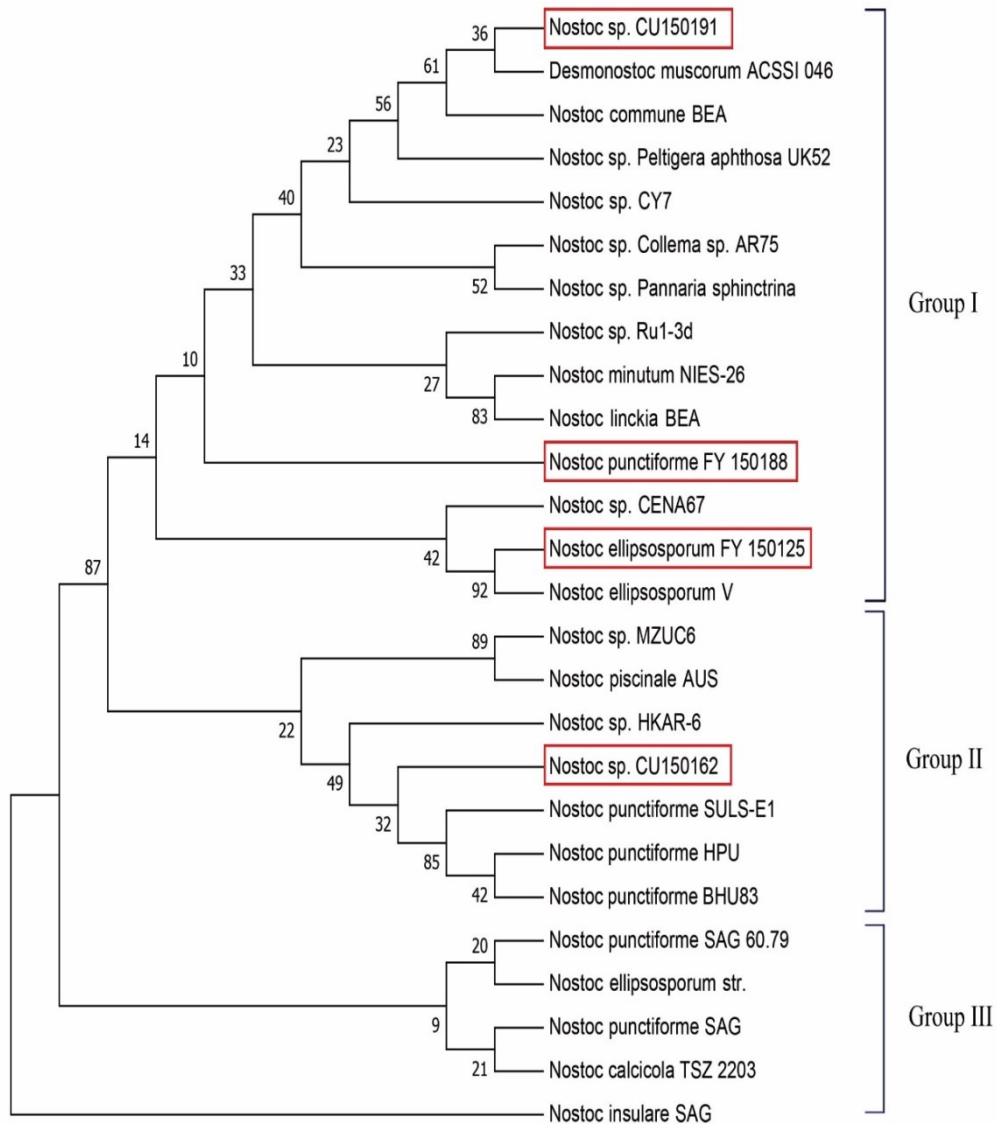


Fig. 5.5 Neighbour-Joining tree constructed using 16S rRNA gene sequences of *Nostoc* spp. and the homologous gene sequences retrieved from NCBI. The selected species in this study are represented in boxes.

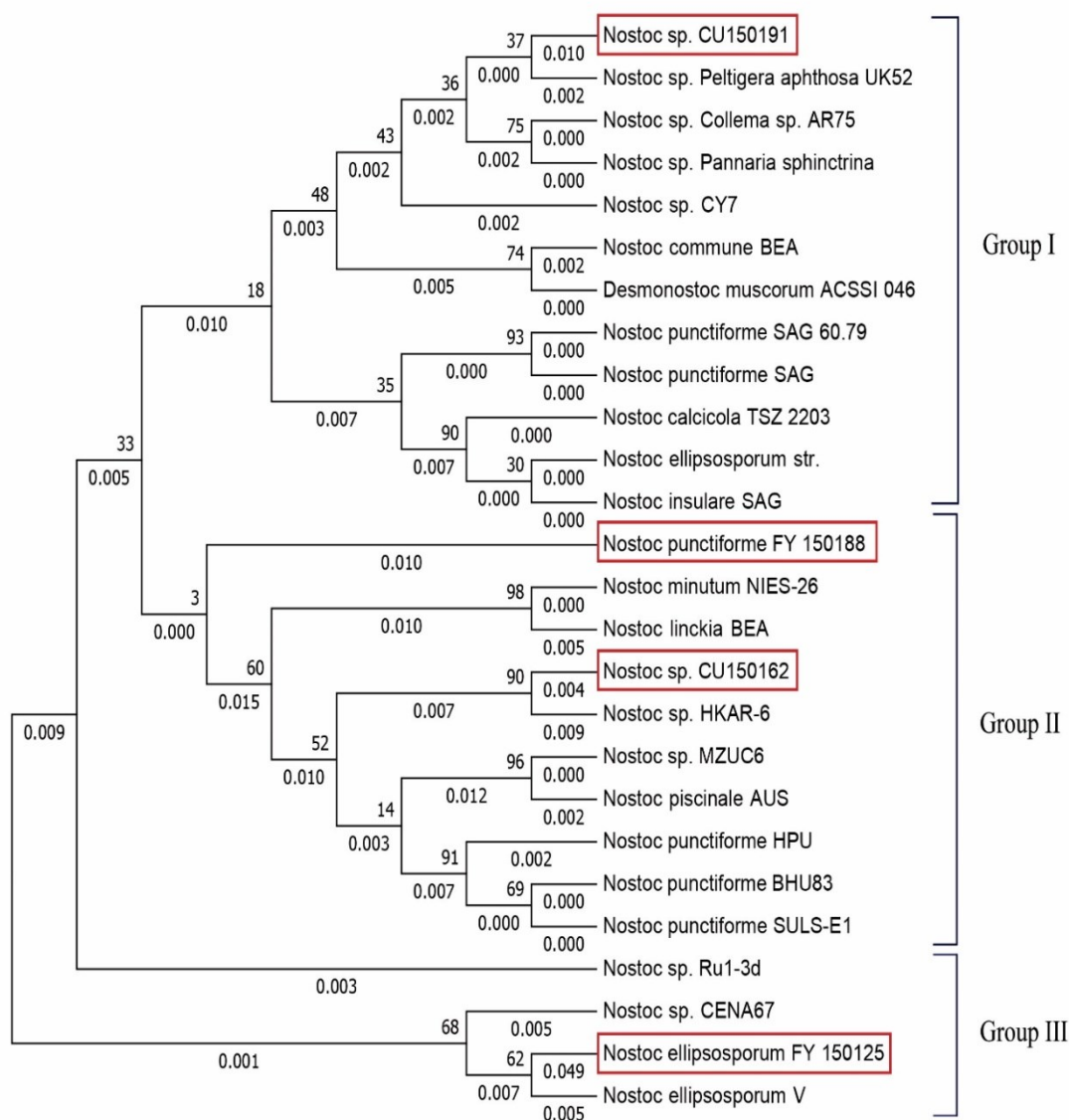


Fig. 5.6 Maximum likelihood tree constructed using 16S rRNA gene sequences of *Nostoc* spp. and the homologous gene sequences retrieved from NCBI. The selected species from the study are represented in boxes.

Discussion

Molecular and biochemical approaches have been used to identify cyanobacteria, instead of morphological and ecological characters (Gugger *et al.*, 2002; Rajaniemi *et al.*, 2005; Hayes *et al.*, 2007; Nayak *et al.*, 2007; Li *et al.*, 2008; Zapomelova *et al.*, 2010). Isolation and purification of cyanobacterial species are time-consuming and the procedure is very difficult. Similarly, the isolation of

cyanobacteria in pure form is also difficult (Nowruzi *et al.*, 2012). According to Choi *et al.* (2008), axenic cultures of microscopic and macroscopic cyanobacteria are typically made by single-cell isolation using a variety of techniques, such as streak plate method, serial dilution technique, UV irradiation, filtration, and treatment with different antibiotics. In the current study, the streak plate method was used for the isolation of *Nostoc* spp. After 21 days of inoculation cyanobacteria colonies were grown well and filled most of the area of agar plates (Mendhekar, 2023).

The establishment of modern cyanobacterial taxonomy and the identification of phylogenetic relationships among cyanobacteria have been made possible by molecular methods based on PCR amplification targeting conserved areas inside the 16S rRNA gene (Komarek, 2006). All cyanobacteria contain the 16S rRNA gene, which has a conserved function. According to Ludwig and Klenk (2001), for the phylogenetic analyses of bacteria and cyanobacteria, one of the most informative genes is the 16S rRNA gene. The most often used gene marker for cyanobacterial identification and evolutionary relationship analysis is the 16S rRNA. Despite the 16S rRNA gene contains numerous evolutionarily conserved sequences, many species-specific variable sequences are also present in the 16S rRNA gene. It has been previously supported that, among the molecular techniques, the analysis of the 16S rRNA gene sequences has proved to be an effective tool for understanding phylogenetic relationships among cyanobacteria (Gugger *et al.*, 2002; Pan *et al.*, 2008; Han *et al.*, 2009; Zapomelova *et al.*, 2010; Svenning *et al.*, 2005; Rehakova *et al.*, 2007). Moreover, Ozturk *et al.* (2018) and Kumar *et al.* (2018) concluded that 16S rRNA will be useful in the classification and identification of cyanobacterial strains. In this study, amplification of 16S rRNA gene sequences is used for the molecular identification and phylogenetic analysis of the selected *Nostoc* spp.

There are various methods proposed for the DNA isolation of cyanobacteria. For many reasons, DNA isolation from cyanobacteria is difficult (Wu *et al.*, 2000). The most common difficulties encountered in cyanobacterial DNA isolation mainly range from cell lysis efficiency (Fiore *et al.*, 2000), to purification issues (Porter,

1988). Even though several methods are available for cyanobacterial DNA extraction, the efficiencies vary from species to species (Fiore *et al.*, 2000). This may be due to the morphological, ecological and metabolic diversities found in different cyanobacterial species (Garcia-Pichel, 2009). DNA isolation adopted in this study is a modification of the DNA isolation method proposed by Ausubel *et al.* (1995). The major difficulty during DNA isolation was the effective breakdown of the cell wall of *Nostoc* spp. made up of exopolysaccharides. Magana-Arachchi and Wanigatunge (2011) isolated RNA-free DNA by adopting a pure enzymatic method. Kumar *et al.* (2018) recommended the use of enzymes like proteinase K and lysozyme for effective DNA isolation whereas in this study we used these enzymes in modified quantities for DNA isolation. CTAB, SDS and β -mercaptoethanol were used for the cell lysis of cyanobacteria for DNA isolation (Tillet and Neilan, 2000; Ma *et al.*, 2016). In this study, chemicals such as SDS, EDTA, proteinase K and CTAB also have a significant effect on cell disruption. The same protocol when used for the cells of *Nostoc*, and it was observed that DNA was extracted rapidly. In addition, Chakraborty *et al.* (2008) employed proteinase K to hydrolyze membrane proteins and they discovered that proteinase K, EDTA, and SDS block the action of nucleases degrading DNA. Furthermore, Morin *et al.* (2010) also suggested that the enzymatic cell disruption method can be used for an efficient DNA isolation protocol for filamentous cyanobacteria.

Most of the scientists recommended the 16S rRNA gene sequences and a database can be prepared for cyanobacterial phylogeny and taxonomy (Wanigatunge *et al.*, 2014). PCR amplification of 16S rRNA with the cyanobacteria-specific oligonucleotide primers CYA359F and equimolar mixture of CYA781Ra and CYA781Rb yielded a unique fragment of ~450bp (Nubel *et al.*, 1997). These universal primers are employed for the successful study of the diversity of cyanobacteria from different ecosystems (Garcia-Pichel *et al.*, 2001; Hagemann *et al.*, 2017; Magana-Arachchi *et al.*, 2008). In our study, the primer CYA359F and equimolar mixture of CYA781Ra and CYA781Rb were used for the PCR amplification of 16S rRNA gene.

The molecular identification of *N. elliposporum* FY 150125 and *N. punctiforme* FY 150188 also supported the morphological character-based identification using the morphological characters. *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191 was identified up to the genus level using the taxonomical manuals (Komarek, 2013). The percentage identity of all the selected *Nostoc* spp. with the sequences deposited in NCBI showed >96% identity. According to Yarza *et al.* (2014), a percentage identity greater than 95% serves as good evidence for species belonging to the same genus. The genus *Nostoc* was found to be heterogenic and thus their species-level identification was challenging.

In this study, the selected species of *Nostoc* were clustered with the corresponding sequences of *Nostoc* species retrieved from the NCBI database and the neighbour-joining and maximum likelihood phylogenetic tree constructed. The evolutionary relationship of the *N. punctiforme* FY 150188 showed a divergence between the same species of *N. punctiforme* retrieved from GenBank, which could be caused by environmental influences on their genes. Likewise, native *N. punctiforme* did not show any affinity to the sequences of *N. punctiforme* retrieved from GenBank, and it exists as a single clade (Thilak and Madusoodanan, 2020). *Nostoc* sp. CU150191 and *Nostoc* sp. CU150162 whose species identity is not clear, in the NJ tree demarcated *Nostoc* sp. CU150191 appeared as a sister to *Desmonostoc muscorum*, but their morphology does not have any coherence. *Desmonostoc* is a recently established genus, primarily based on phylogenetic evidence (Hrouzek *et al.*, 2013). Species belonging to *Desmonostoc* form long vegetative filaments embedded in diffluent mucilaginous envelopes, except for primordial stages they never form a firm periderm and the filaments are never densely coiled with compact trichomes as found in *Nostoc*. These morphological characteristics of the genus *Desmonostoc* are entirely different from the species *Nostoc* sp. CU150191. In addition, the phylogenetic tree constructed based on 16S rDNA showed *N. elliposporum* V and *Desmonostoc muscorum* clustered as a single group (Cia *et al.*, 2021).

Whole genome analyses of *Nostoc ellipsosporum* strain NOK showed its close relationship with *N. punctiforme* strain PCC73102 (Ghosh and Bhadury, 2021). Similarly, *N. ellipsosporum* FY 150125 and *N. punctiforme* FY 150188 clustered in a single group in the NJ tree. In the phylogenetic analyses of the partially sequenced genes of 16S rRNA, nifH and hetR were used for the discrimination of the three studied strains in distinct clusters belonging to the genera *Anabaena*, *Aphanizomenon* and *Nostoc*. However, at the species level, there are hurdles for the proper identification due to the lack of sequences deposited (Galhano *et al.*, 2011). The findings are corroborated by our study; two species are only identified up to the genus level using molecular technique.

CHAPTER 6

**BIOCHEMICAL CHARACTERIZATION AND PHYLOGENETIC
ANALYSIS OF *NOSTOC* SPECIES USING GAS
CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS**

6.1 Abstract

Cyanobacteria are one of the most sustained prokaryotic organisms on Earth that are rich sources of metabolites of great importance. The complex photosynthetic mechanism of cyanobacteria channels the assimilated solar energy into other forms of energy to produce food and metabolites. Moreover, they are promising biocatalysts and can be used in “white biotechnology” to enhance the sustainable production of food, metabolites, and green energy sources such as biodiesel. The genus *Nostoc* is a filamentous nitrogen-fixing cyanobacterium and is known to produce dozens of pharmacologically active compounds, including antiviral, antitumor, antibacterial, anti-fungal, anti-HIV and enzyme-inhibiting agents. Moreover, the compounds have a potential role in non-biomedical applications, including herbicides, algicides, nematocides, and insecticides. This study is focused on the biochemical characterization of *Nostoc* spp. by analysing the chlorophyll-a content and GC-MS analysis to develop a chemotaxonomic profile of *Nostoc* spp. Maximum chlorophyll-a quantity was observed in *Nostoc* sp. CU150162 on the 21st day and the minimum quantity in *Nostoc* sp. CU150191. The major compounds identified from the methanolic extracts of *Nostoc* spp. using the Gas Chromatography-Mass Spectrometry (GC-MS) technique are phytol (diterpene alcohol), heptadecane (alkane hydrocarbon), lupeol (pentacyclic triterpene), methyl palmitate (fatty acid), methyl stearate (fatty acid), neophytadiene (sesquiterpenoids), propyl hexanoate, isobutyl caproate (hexanoate esters), 9,12-octadecadienoic acid, methyl ester, 10-octadecenoic acid, methyl ester (fatty acid methyl esters) and guanosine (nitrogen-rich alkaloid). Among the identified bioactive compounds, lupeol, propyl hexanoate, and isobutyl caproate are reported for the first time in *N. elliposporum*. Cluster analysis using PAUP was performed on *Nostoc* spp. based on the presence of major chemical compounds. Principal component analysis,

agglomerative hierarchical clustering and heatmap clustering of the compounds are done based on the percentage quantity of identified compounds from *Nostoc* spp. using XLSTAT statistical software. A Venn diagram of phytoconstituents of *Nostoc* spp. generated using BioVinci 2.0 software. *Nostoc* spp. constitutes an important source of potentially bioactive compounds, which can be used to improve cultivation practices and biomedical applications.

6.2 Results

6.2.1 Biochemical characterization of *Nostoc* spp.

6.2.1.1 Quantification of chlorophyll-a

The samples containing the *Nostoc* species were collected from different localities and biochemically characterized. The identified species were *N. elliposporum* FY150125, *N. punctiforme* FY150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191. *N. elliposporum* CU150125 and *Nostoc* sp. CU150162 were collected from epipsammic habitats whereas *N. punctiforme* FY150188 and *Nostoc* sp. CU150191 collected as epilithic. Chlorophyll-a content in the selected *Nostoc* spp. was analysed using the Mackinney method on the 7th, 14th, 21st and 28th day of inoculation (Table 6.1). The results showed maximum chlorophyll-a content in *Nostoc* sp. CU150162 with 3.71 ± 0.08 $\mu\text{g/ml}$ on the 21st day followed by *N. elliposporum* FY150125 (2.82 ± 0.07 $\mu\text{g/ml}$), *N. punctiforme* FY150188 (2.26 ± 0.06 $\mu\text{g/ml}$) and *Nostoc* sp. CU150191 (1.50 ± 0.01 $\mu\text{g/ml}$). A significant difference in chlorophyll-a content was observed from 7 to 28 days from inoculation. Chlorophyll content was increased up to the 21st day of inoculation thereafter, it gradually decreased (Fig. 6.1). The results indicated that the maximum chlorophyll-a content was estimated on the 21st day of inoculation.

Table 6.1. Variations in the chlorophyll-a content in different *Nostoc* species cultured in BG-11₀ medium.

Treatment days	<i>N. elliposporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191
7 th	0.782±0.02	0.563±0.02	0.961±0.01	0.348±0.04
14 th	1.713±0.01	1.002±0.02	1.995±0.05	0.903±0.02
21 st	2.822±0.07	2.263±0.06	3.712±0.08	1.503±0.1
28 th	2.160±0.06	1.910±0.05	2.701±0.04	1.08±0.05

Values are given as mean±SE for each sample.

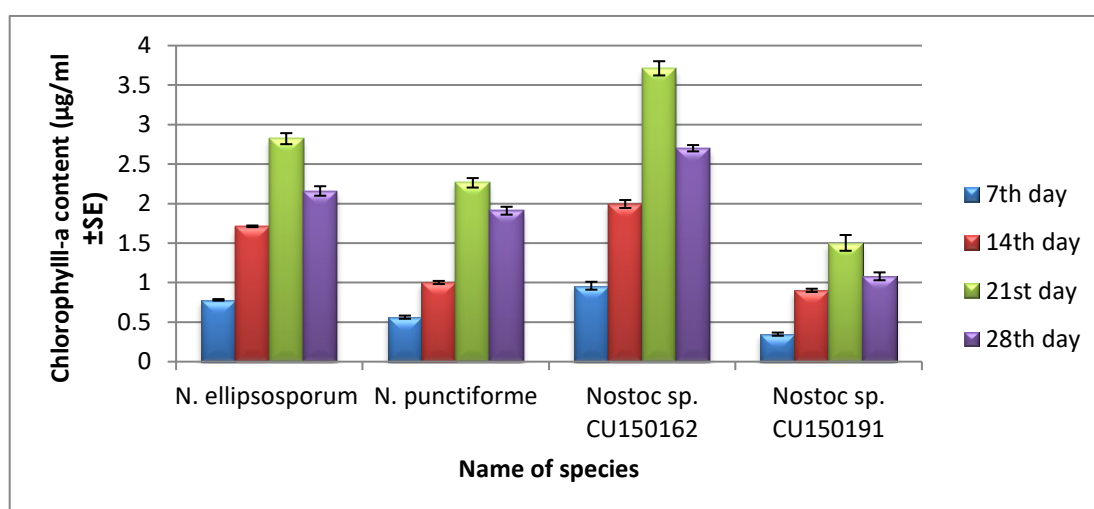


Fig. 6.1 Chlorophyll-a content in different *Nostoc* species cultured in BG-11₀ medium. Values are given as mean±SE of the three replicates (n=3).

6.2.2 GC-MS analysis of *Nostoc* spp.

GC-MS chromatogram of methanolic extract of *Nostoc* spp. showed the presence of several compounds belonging to different chemical groups, including methyl esters of fatty acids, steroids/triterpenes, alkanes, diterpenes, and acyclic diterpene alcohols (Table 6.2). The identified major components of the methanolic extract of *Nostoc* spp. are given below:

N. elliposporum FY150125

The GC-MS analysis of a methanolic extract of *N. elliposporum* is represented in Fig. 6.2. A total of eight major compounds were identified viz, 3-

deoxy-d-mannitol (26.64%), lupeol (20.28%), isobutyl caproate (19.65%), heptadecane (10.58%), propyl hexanoate (7.82%), lupan-3yl acetate (7.39%), phytol (5.09%) and neophytadiene (2.55%). Lupeol, isobutyl caproate and propyl hexanoate were first reported in this species.

***N. punctiforme* FY150188**

The major compounds detected in *N. punctiforme* represented in Fig. 6.3. Guanosine (75.86%), heptadecane (5.82%), heptadecane, 3- methyl ester (2.28%), neophytadiene (5.64%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (0.86%), phytol acetate (1.81%), methyl palmitate (3.44%) , 10-octadecenoic acid, methyl ester (2.12%), phytol (1.04%) and methyl stearate (1.12%). A total of ten compounds were identified from *N. punctiforme*.

***Nostoc* sp. CU150162**

Fourteen major compounds were detected from *Nostoc* sp. CU150162 using GC-MS analysis (Fig. 6.4). The major components were heptadecane, 4-methyl- (10.71%), hexadecane, 4-methyl ester (5.97%), octadecane, 4-methyl ester (6.59%), neophytadiene (20.61%), 8-heptylpentadecane (1.70%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (6.66%), methyl palmitoleinate (3.07%), methyl palmitate (11.57%), 9,12-octadecadienoic acid, methyl ester (5.04%), 8,11,14-docosatrienoic acid, methyl ester (5.78%), 10-octadecenoic acid, methyl ester (3.63%), phytol (14.85%), methyl stearate (2.16%) and methyl lignocerate (1.66%). The majority of compounds identified from *Nostoc* sp. CU150162 were fatty acid methyl esters.

***Nostoc* sp. CU150191**

GC-MS profiling of methanolic extract of *Nostoc* sp. CU150191 detected five major compounds as represented in the chromatogram (Fig. 6.5). Phytol (24.64%), tetradecane, 5 -methyl (22.56%), neophytadiene (19.93%), guanosine (18.97%) and heptadecane (13.90%).

Table 6.2: Phytochemical constituents identified from *Nostoc* spp. using GC-MS analysis.

Species	Compound name	Retention time	Peak area percentage	Chemical compound
<i>N. elliposporum</i> FY150125	3-Deoxy-d-mannitol	11.567	26.64	Aliphatic alcohol
	Isobutyl caproate	11.671	19.65	Hexanoate ester
	Propyl hexanoate	11.867	7.82	Hexanoate ester
	Heptadecane	14.864	10.58	Alkane
	Neophytadiene	16.64	2.55	Diterpene
	Phytol	22.328	5.09	Acyclic diterpene alcohol
	Lupan-3-yl acetate	33.760	7.39	Pentacyclic triterpenoid
	Lupeol	34.409	20.28	Pentacyclic triterpenoid
			100	
<i>N. punctiforme</i> FY150188	Guanosine	19.493	75.86	Purine nucleoside (Alkaloid)
	Heptadecane	23.579	5.82	Alkane
	Heptadecane, 3-methyl	25.113	2.28	Alkane
	Neophytadiene	26.556	5.64	Diterpene
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.068	0.86	Acyclic diterpene alcohol
	Phytol, acetate	27.432	1.81	Diterpenoid
	Methyl palmitate	28.334	3.44	Fatty acid methyl ester
	10-Octadecenoic acid, methyl ester	31.654	2.12	Fatty acid methyl ester
	Phytol	31.900	1.04	Acyclic diterpene alcohol
	Methyl stearate	32.111	1.12	Fatty acid methyl ester
				100

<i>Nostoc</i> sp. CU150162	Heptadecane, 4-methyl-	24.816	10.71	Alkane
	Hexadecane, 4-methyl-	26.024	5.97	Alkane
	Octadecane, 4-methyl-	26.303	6.59	Alkane
	Neophytadiene	26.546	20.61	Diterpene
	8-heptylpentadecane	26.793	1.70	Alkane
	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	27.421	6.66	Acyclic Diterpene alcohol
	Methyl palmitoleinate	27.924	3.07	Fatty acid methyl ester
	Methyl palmitate	28.323	11.57	Fatty acid
	9,12-Octadecadienoic acid, methyl ester	31.543	5.04	Fatty acid methyl ester
	8,11,14-docosatrienoic acid, methyl ester	31.664	5.78	Fatty acid methyl ester
	10-Octadecenoic acid, methyl ester	31.747	3.63	Fatty acid methyl ester
	Phytol	31.894	14.85	Acyclic diterpene alcohol
	Methyl stearate	32.101	2.16	Fatty acid methyl ester
	Methyl lignocerate	41.747	1.66	Fatty acid methyl ester.
		100		
<i>Nostoc</i> sp. CU150191	Guanosine	12.517	18.97	Purine nucleoside
	Heptadecane	14.875	13.90	Alkane
	Tetradecane, 5-methyl ester	15.462	22.56	Alkane
	Neophytadiene	16.654	19.93	Diterpene
	Phytol	22.334	24.64	Acyclic diterpene alcohol
		100		

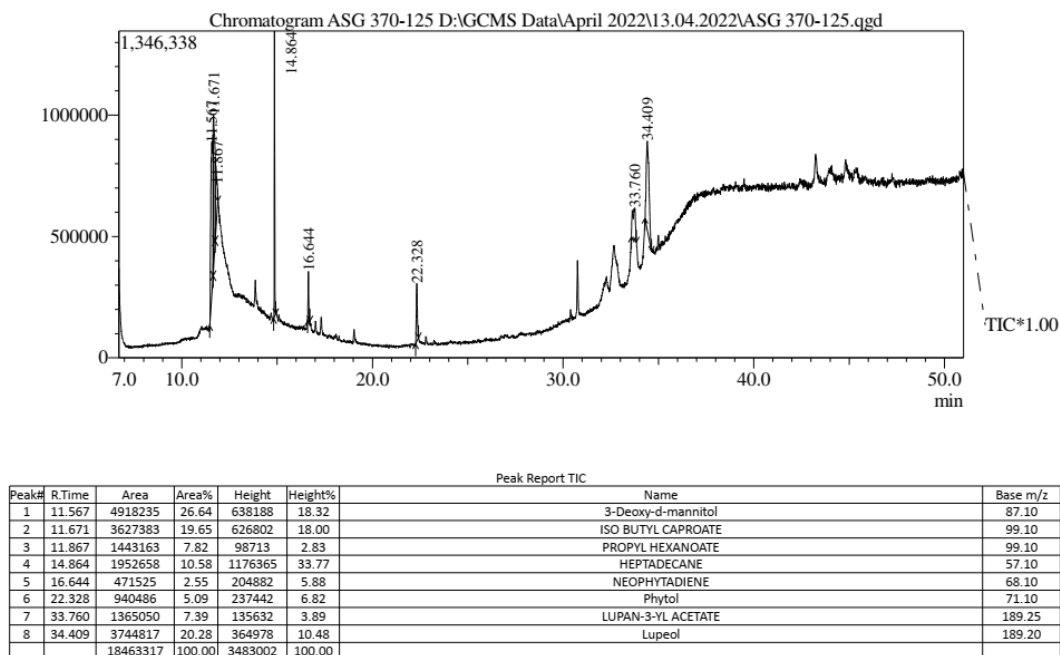


Fig. 6.2 GC-MS Chromatogram of methanolic extract of *N. elliposporum* FY150125

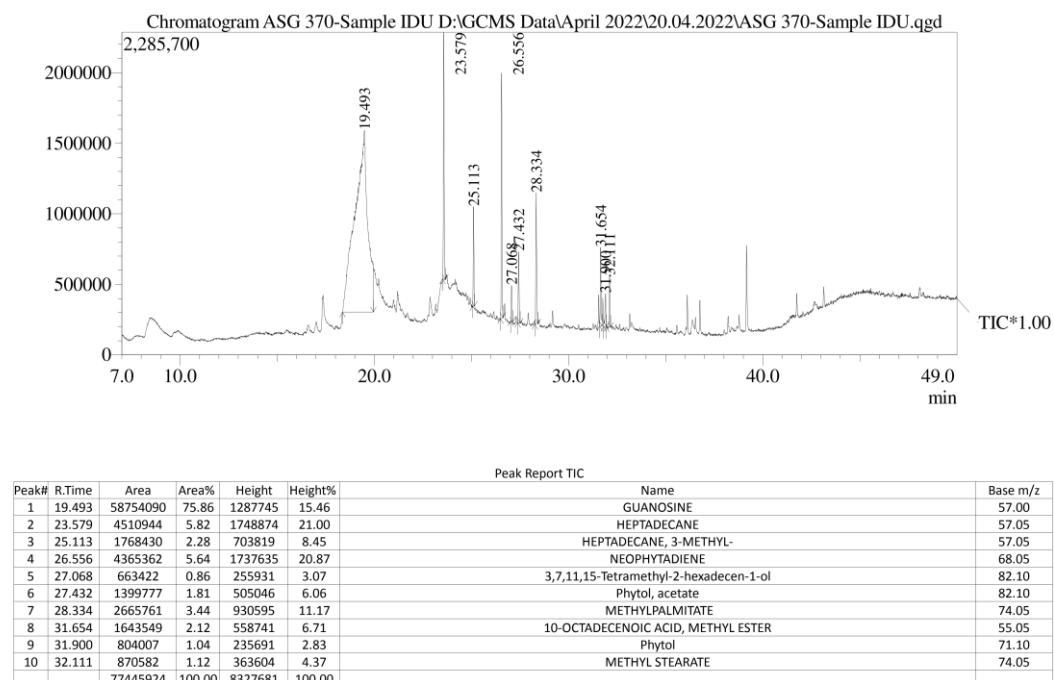


Fig. 6.3 GC-MS Chromatogram of methanolic extract of *N. punctiforme* FY150188

Biochemical characterization and phylogenetic analysis of *Nostoc* species using Gas Chromatography-Mass Spectrometry analysis

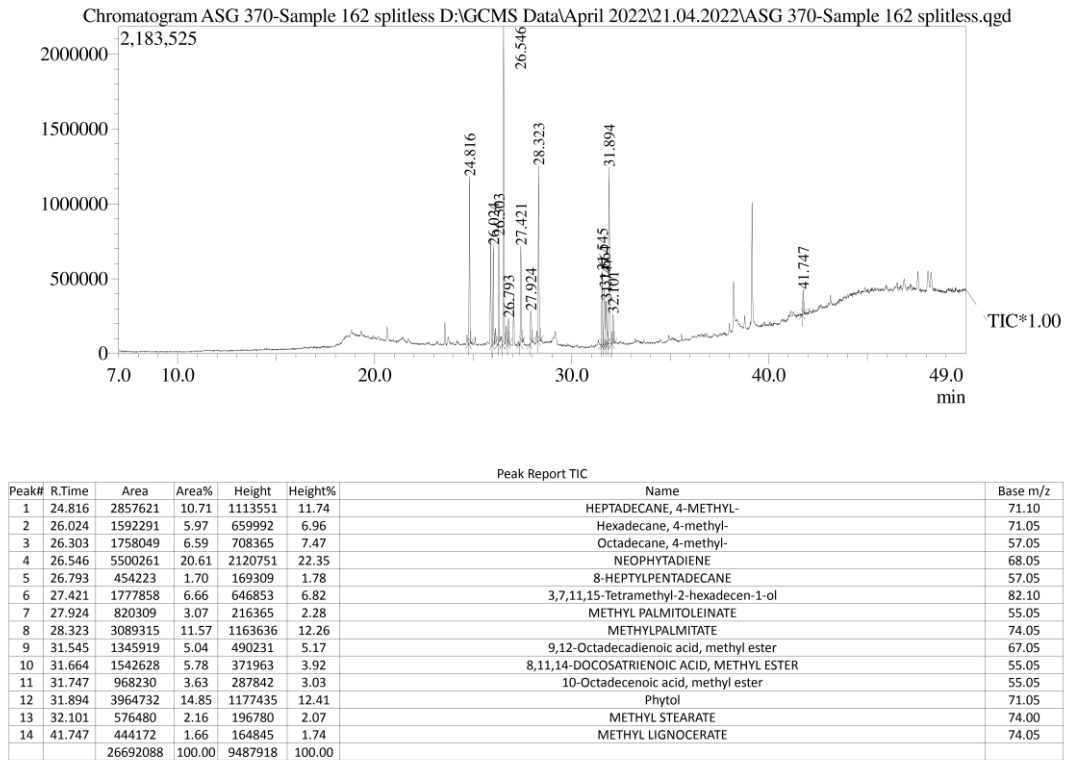


Fig. 6.4 GC-MS Chromatogram of methanolic extract of *Nostoc* sp. CU150162

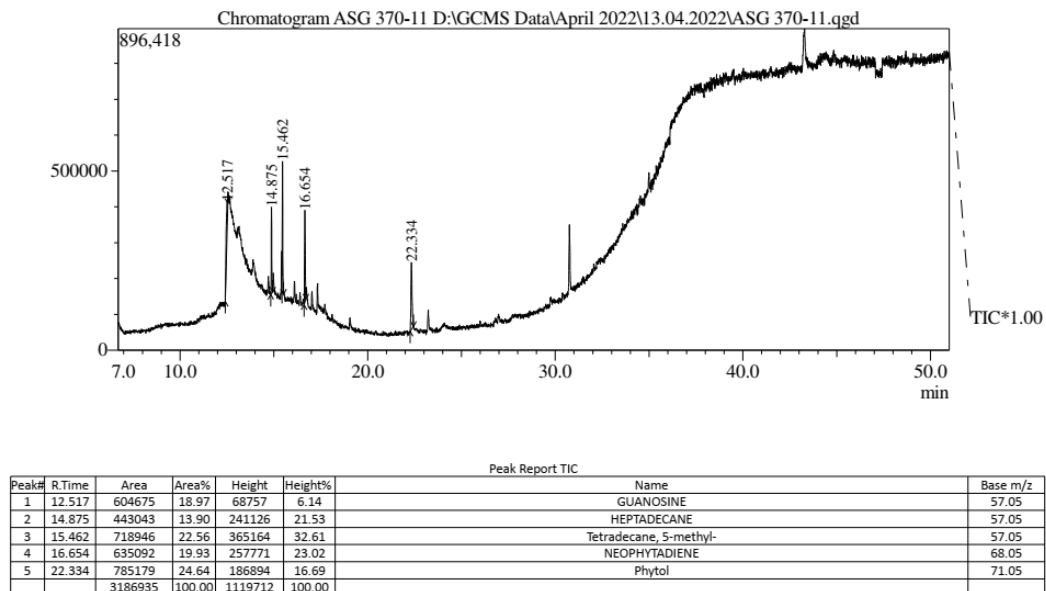


Fig. 6.5 GC-MS Chromatogram of methanolic extract of *Nostoc* sp. CU150191

Neophytadiene, phytol and heptadecane are the major bioactive compounds detected in four *Nostoc* spp. In terms of peak area, the major component identified in *N. elliposporum* FY150125 is 3-deoxy-d-mannitol (26.64%), *N. punctiforme* FY150188 is guanosine (75.86%), *Nostoc* sp. CU150162 is neophytadiene (20.61%) and *Nostoc* sp. CU150191 is phytol (24.64%). Phytol is one of the major bioactive compounds identified in all selected *Nostoc* spp. The percentage of phytol content in each *Nostoc* spp. was different. *Nostoc* sp. CU150191 contained the highest quantity of phytol (24.64%) followed by *Nostoc* sp. CU150162 with 14.85%, *N. elliposporum* FY150125 with 5.09%, and *N. punctiforme* FY150188 with 1.04%. Neophytadiene is another common compound detected in all *Nostoc* spp. *Nostoc* sp. CU150162 contains a significant quantity (20.61%) followed by *Nostoc* sp. CU150191 with 19.93%, *N. punctiforme* FY150188 with 5.64% and *N. elliposporum* FY150125 with 2.55%.

Methyl palmitate (palmitic acid, methyl ester), 10-octadecenoic acid methyl ester (methyl cis-10-octadecenoate), methyl stearate (stearic acid, methyl ester), methyl palmitoleinate (palmitoleic acid, methyl ester), 9,12-octadecenoic acid methyl ester, 10-octadecadienoic acid methyl ester, 8,11,14-docosatrienoic acid methyl ester and methyl lignocerate (tetracosanoic acid, methyl ester), are the fatty acid methyl esters present in selected *Nostoc* spp. Lupeol is a pentacyclic triterpenoid (also known as fagarsterol) observed only in *N. elliposporum* FY150125. This is the first report of *N. elliposporum* having 20.28% of lupeol. In addition, a higher quantity of isobutyl caproate (isobutyl hexanoate) and propyl hexanoate (19.65% and 7.82%), was first reported in *N. elliposporum* FY150125 respectively.

The phytochemical profiling of *Nostoc* spp. showed the presence of varying quantities of alkanes. Octadecane, heptadecane, hexadecane, heptylpentadecane and tetradecane are the different alkanes identified from *Nostoc* spp. The most dominant alkane percentage is tetradecane (22.5%) followed by heptadecane (13.90%) in *Nostoc* sp. CU150191 and the most common alkane in all the species is heptadecane. Guanosine is an alkaloid constructed with a guanine base, which is

present in *N. punctiforme* with 75.86% of quantity, and also in *Nostoc* sp. CU150191 (18.97%). 3-deoxy mannitol is an aliphatic alcohol identified with 26.64% in *N. elliposporum* FY150125.

6.2.3 Phylogenetic analysis based on chemical profile

The methanolic extracts of *Nostoc* spp. showed similarity in the chemical composition of alkanes, diterpenes and fatty acid methyl esters. The constructed dendrogram showed the phylogenetic relation of the identified *Nostoc* spp. Heptadecane, phytol and neophytadiene are the major common bioactive compounds identified in all the *Nostoc* species. The dendrogram derived from the active compounds generated two main clusters (Fig. 6.6). Cluster 1 is subdivided into sub-clusters as *N. elliposporum* FY150125, *Nostoc* sp. CU150191 and *Nostoc punctiforme* FY150188. In cluster 1 *N. elliposporum* FY150125 and *Nostoc* sp. CU150191 showed more similarity in compounds and was considered a sister group. Cluster 2 consists of *Nostoc* sp. CU150162, which contains more bioactive compounds compared to other selected *Nostoc* spp.

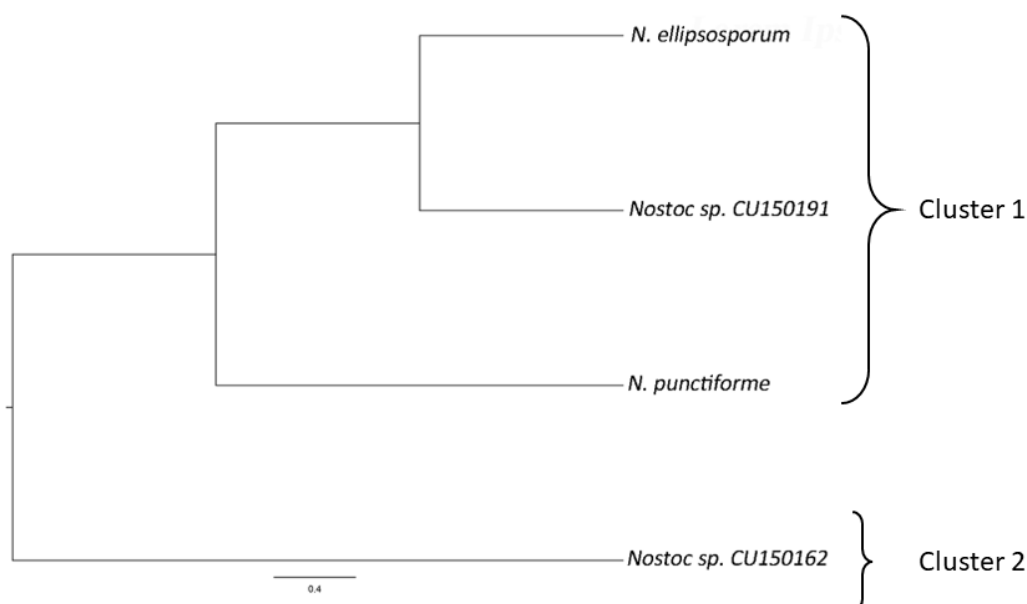


Fig. 6.6 UPGMA (Unweighted Pair Group Method with Arithmetic averages) based dendrogram of *Nostoc* spp. based on GC-MS analysis of methanolic extracts. Data analyzed using PAUP software.

6.2.4 Principle component analysis (PCA) of the chemical compounds from different *Nostoc* species

A comparison of the percentage of phytoconstituents present in different *Nostoc* spp. was carried out using Principal Component Analysis (PCA). PCA is a multivariate explanatory method used for data dimensionality reduction and is used to compress the data based on their similarities and dissimilarities. In this study, the correlation circles and biplot of PCA were constructed using XLSTAT statistical software. The data matrix consists of 26 rows (phytoconstituents) and 4 columns (percentage of compounds in different *Nostoc* spp.) subjected to PCA analysis. The first two factors of PCA represent 71.85% of the initial variability of the data and are used for the map construction. The first map of correlation circles of *Nostoc* spp. based on the percentage of phytoconstituents (Fig. 6.7) showed a projection of the initial variables in the factor space. The result of correlation circles of PCA showed *N. punctiforme* and *Nostoc* CU150191 are significantly positively correlated (close to 1) in the phytoconstituent content and *N. elliposporum* and *Nostoc* CU150162 are negatively correlated (close to -1). The horizontal axes are linked with *N. punctiforme* and *Nostoc* sp. CU150191. *N. elliposporum* and *Nostoc* sp. CU150162 is linked with the vertical axis. The results are interpreted as the positively correlated variables (*N. punctiforme* and *Nostoc* sp. CU150191) are grouped together and negatively correlated species are positioned on opposite sides of the plot origin (*N. elliposporum* and *Nostoc* sp. CU150162)

The Biplot of PCA is a representation of the interrelationship and congruence of variables and observations in a PCA space. The longer lines of the biplot denote high variance and the angles between the lines indicate the correlation between the variables. The *Nostoc* spp. are considered as variables and the relative quantity of phytoconstituents represented as observations (Fig. 6.8). The F1 component contributes 40.20% of the variability, while F2 contributes 31.65%. The lines and dots in the biplot indicate the *Nostoc* spp. and phytoconstituents respectively. The result of the biplot demonstrates that the variables, *N. punctiforme* and *Nostoc* sp. CU150191 are positively correlated (angles between them are small)

based on compound quantity and *N. elliposporum* is negatively correlated to *Nostoc* sp. CU150162 (angle between them is 180°). The distance between two points in the biplot indicates the Euclidean distance between the two compounds in the multivariate space. Compounds far away from the center have high Euclidean distance, and vice versa. The distance of a variable from the biplot center positively correlates to relative importance. The distance obtained for each of the compounds from center is less, which indicates the compound contained in *Nostoc* spp. with a low percentage quantity. Guanosine from *N. punctiforme* has a high percentage quantity (75.86%) when compared to all other compounds and it is clear from the biplot, that the dot denoting guanosine is positioned far away from the center of the biplot. Most of the fatty acid methyl esters and alkanes from *Nostoc* spp. are present in moderate quantity percentages, so in the biplot, the fatty acids and alkanes are positioned near the center.

6.2.5 Agglomerative hierarchical clustering analysis (AHCA)

The agglomerative hierarchical cluster analysis (AHCA) is a multivariate explanatory method of cluster analysis that builds a hierarchy of clusters using agglomerative functions. The agglomerative hierarchical cluster analyses according to Ward's method (ANOVA based approach) with squared Euclidean distances are subjected to detect multivariate homogeneity and multivariate heterogeneity concerning the percentage of compounds identified from different *Nostoc* spp. The dendrogram constructed shows how the algorithm works to group the observations, and then the subgroups of observations. In the present study, algorithm has successfully grouped all the observations. The use of agglomerative hierarchical cluster analysis for studying the quantity of compounds from *Nostoc* spp. yielded a dendrogram (agglomerative bottom-up tree-like structure), with four statistically significant clusters (Fig. 6.9). Cluster 1 consists of 3-deoxy-d-mannitol, isobutyl caproate, heptadecane and lupeol, cluster 2 consists of propyl hexanoate, lupan-3 yl-acetate, heptadecane, 3-methyl, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol acetate, methyl palmitate, 10-octadecenoic acid, methyl ester, methyl stearate, heptadecane,4-methyl, hexadecane, 4-methyl, octadecane, 4-methyl, 8-

heptylpentadecane, methyl palmitoleinate, 9,12-octadecadienoic acid, methyl ester, 8,11,14-docosatrienoic acid, methyl ester, methyl lignocerate, cluster 3 consists of neophytadiene, phytol, tetradecane, 5-methyl and cluster 4 consist guanosine. All the fatty acids methyl esters identified from different species are grouped in cluster 2.

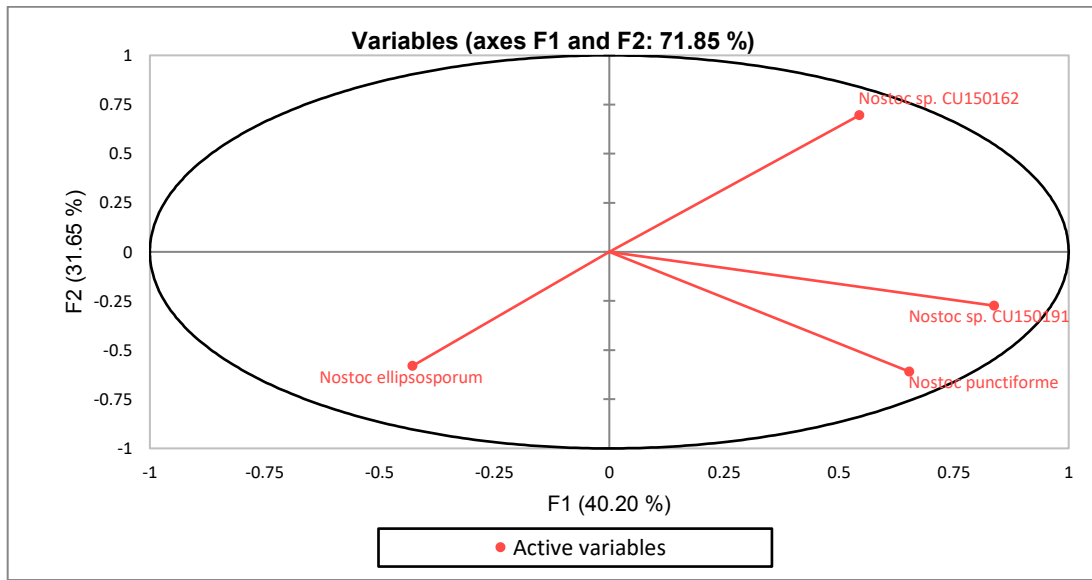


Fig. 6.7 PCA: Projection of the variables on the factor plane (Correlation circles of *Nostoc* spp.)

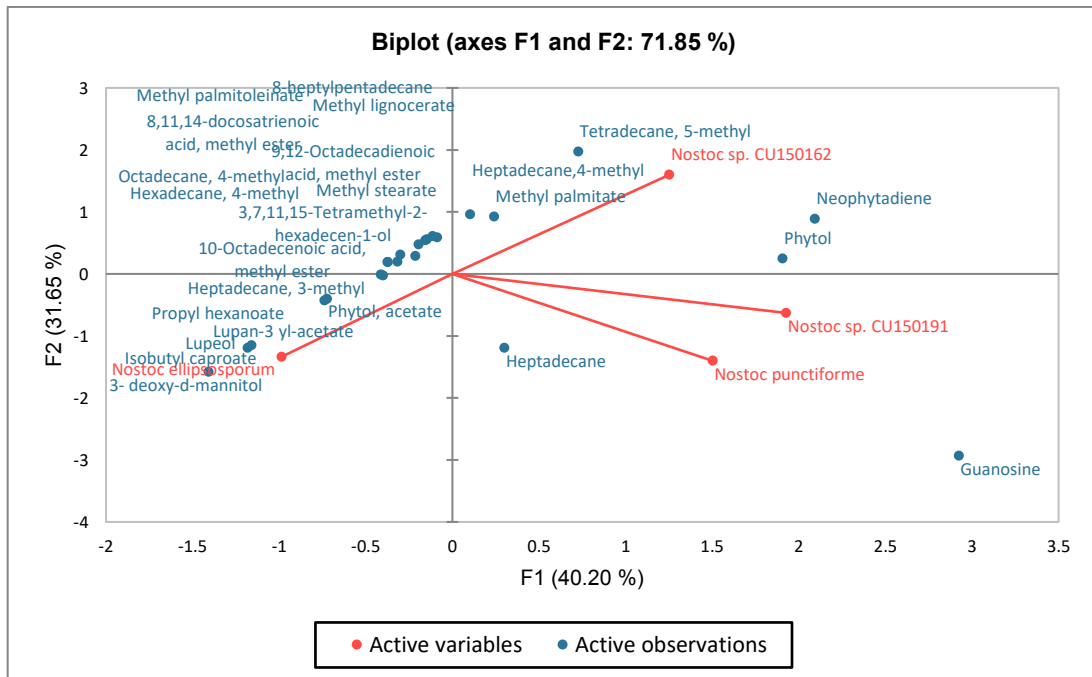


Fig. 6.8 PCA: Biplot of the principal component analysis of the different *Nostoc* spp.

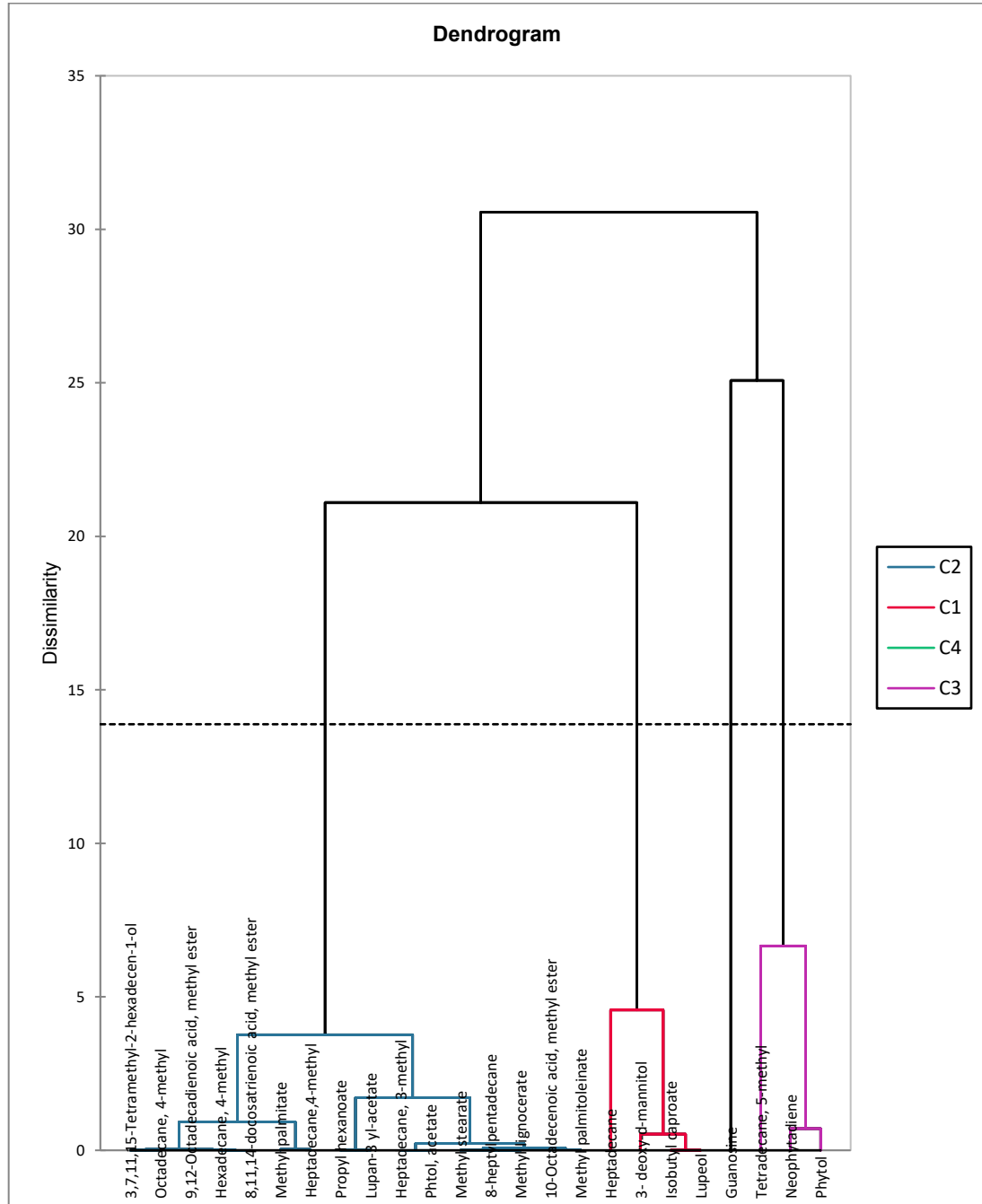


Fig. 6.9 Dendrogram for the agglomerative hierarchical clustering analysis (AHCA) using Ward's clustering algorithm.

6.2.6 Heat map clustering phytoconstituents of *Nostoc* spp.

A clustered heat map is a coloured visualization tool that is widely used in biological sciences as it allows the synthesis of large sets of information compactly and with coherence. In this study, a clustered heat map was constructed based on the quantity of compounds present in *Nostoc* spp. using the statistical software XLSTAT and the colours red to green through black colour were the options of the heat map (Fig. 6.10). In the heat map, the default colour gradient sets the lowest value in bright red, the highest value to bright green, and mid-range values to black, with a corresponding transition (or gradient) between these extremes. Bright red stands for a lower quantity of compounds, bright green for a high quantity and black for intermediate values. The heat map chart clustered the compounds in rows and *Nostoc* spp. in columns. The heat map grouped 24 compounds into two main clusters and four sub-clusters (left dendrogram) corresponding to the percentage of compounds. In the main cluster I, nine compounds, methyl stearate, 10-octadecenoic acid, methyl palmitate, heptadecane 4-methyl, tetradecane 5-methyl, octadecane 4-methyl, hexadecane 4-methyl, docosatrienoic acid, 9,12-octadecadienoic acid, methyl palmitoleinate, 8-heptylpentadecane, methyl lignocerate, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, phytol and neophytadiene were grouped. All the fatty acid methyl esters are grouped in cluster 1. The remaining 9 compounds such as guanosine, lupeol, deoxy mannitol, isobutyl caproate, propyl hexanoate, lupan-3-yl acetate, heptadecane, heptadecane 3-methyl and phytol acetate are grouped in cluster 2. *Nostoc* spp. based on the percentage of compounds is grouped into 2 clusters (upper dendrogram) and cluster 1 on the left consists of spp. *N. elliposporum*, *N. punctiforme* and *Nostoc* sp. CU150191 and cluster 2 consist of *Nostoc* sp. CU150162. Red large rectangle on the left side of the heat map shows that the identified percentage of compounds in that area is low for the species viz, *N. elliposporum*, *N. punctiforme* and *Nostoc* sp. CU150191. In other words, the compounds from *Nostoc* sp. CU150162 are relatively high percentage for the bottom cluster of compounds and a relatively low concentration for the top cluster of compounds.

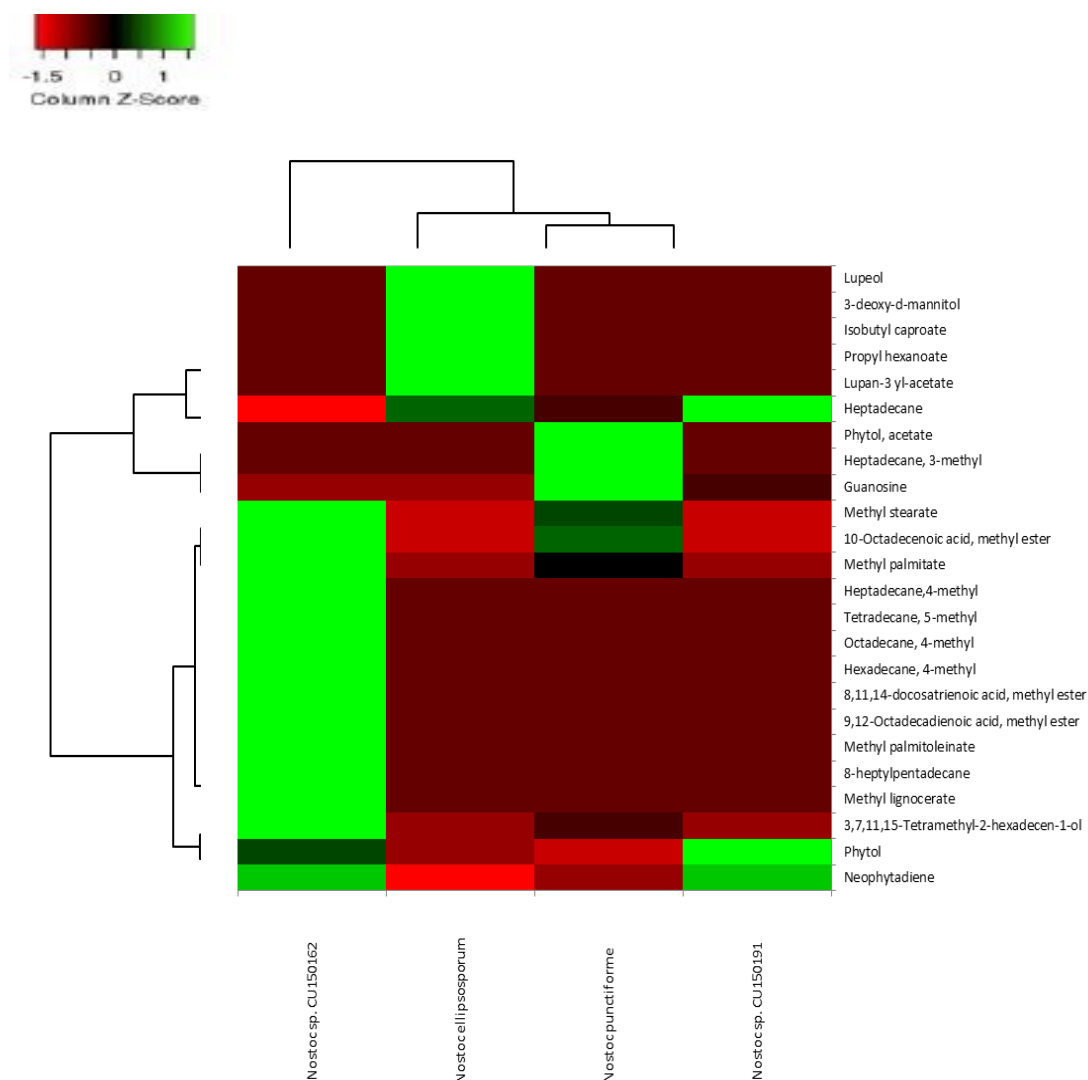


Fig. 6.10 Heat map visualization showing correlation of metabolites peaks identified from *Nostoc* spp. using GC-MS. Hierarchical clustering was performed on both *Nostoc* spp. and compound quantities. Green colour indicates Z-scores > 1, black scores indicate Z-scores = 0.1 and red indicates Z-scores < -1.

6.2.7 Venn diagram of the phytoconstituents of *Nostoc* spp.

A Venn diagram (Fig. 6.11) depicts the graphical representation of similar and dissimilar compounds in *Nostoc* spp. generated using the software Biovnici 2.0. Circles that overlap have a commonality (inner circles) while circles that don't overlap do not share the compounds. Among the identified compounds, phytol, neophytadiene and heptadecane are the common compounds in all *Nostoc* spp. In the Venn diagram, it is clear that three compounds were identified in all selected

species. In the case of *Nostoc* sp. CU150162, fourteen compounds are identified using GC-MS and among them, 7 compounds are only present in that species.

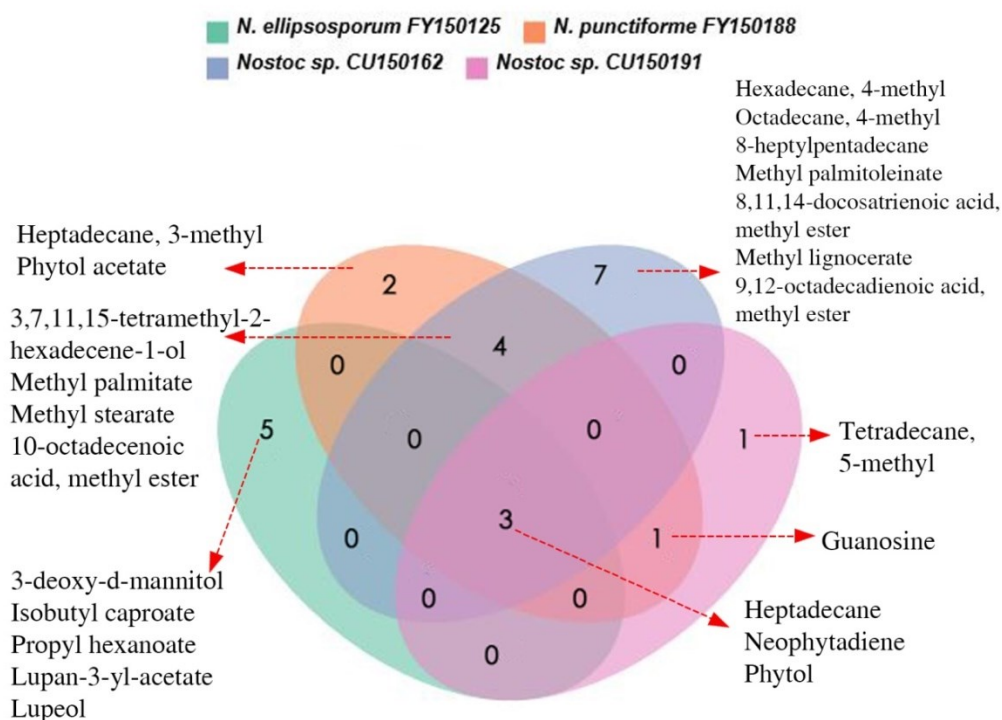


Fig. 6.11 Venn diagram comparing the similar and dissimilar compounds in different species. The numbers indicate the number of shared compounds between *Nostoc* species.

Discussion

Cyanobacteria have chlorophyll-a as a major pigment for harvesting light and conducting photosynthesis. Quantification of chlorophyll-a can be used as a standard for assessing the growth and photosynthesis in filamentous cyanobacteria, where the number of cells cannot be counted directly (Li *et al.*, 2014). Serpa and Calderon (2006) also supported that the amount of chlorophyll is positively correlated with cell density or biomass. Chlorophyll-a content of *N. ellipsosporum*, *N. punctiforme* and *Nostoc* sp. CU150162 was higher than *N. spongiaeforme* ($1.76 \pm 0.00 \mu\text{g/ml}$) (Ojit *et al.*, 2012). The results of chlorophyll-a content of *Nostoc* spp. analysed in our study are very much supported by the earlier report of Silvia *et al.* (2015). In the present study, among the selected species least chlorophyll content

was reported in *Nostoc* sp. CU150191 (1.50 ± 0.01 $\mu\text{g/ml}$) incongruence with Silvia *et al.*, (2015), who reported the chlorophyll content of *Nostoc* sp. BTA972 from Manipur possesses the least chlorophyll content (1.22 ± 0.01 $\mu\text{g/ml}$). The chlorophyll-a content of *N. elliposporum* isolated from Loktak Lake in Manipur was reported to be higher than in the present study, which was 2.82 ± 0.07 $\mu\text{g/ml}$ on the exponential period. In this study, the chlorophyll content was found to be maximum on the 21st day of incubation. The present observations are in corroboration of an earlier work (El-Hameed *et al.*, 2021) which proved that the chlorophyll content of *N. muscorum* was maximum on the 21st day of incubation. Similarly, Bortoli *et al.* (2014) also reported that, the growth of cyanobacteria was maximum on the 21st day of incubation.

Cyanobacteria are utilised as a source of biologically active compounds. The cyanobacterial genus *Nostoc* produces a wide range of secondary metabolites with antibacterial, antifungal, anti-tumor, enzyme-inhibiting, immunostimulant, nematocidal, insecticidal and herbicidal activities (Rawat and Bhargava, 2011). In the present study, GC-MS analysis was done for the biochemical characterization of *Nostoc* spp., to separate, identify and quantify the bioactive constituents of long-chain, branched-chain hydrocarbons, alcohols, acids, esters, etc. The GC-MS analysis of *Nostoc* spp. revealed the presence of 24 bioactive compounds. From the GC-MS analysis, it is obvious that the methanolic extract of *Nostoc* spp. is rich in biologically active constituents. Among the identified phytochemicals produced by *Nostoc* spp., phytol is present in all four *Nostoc* spp. in varying concentrations and *Nostoc* sp. CU150191 produced a higher percentage of phytol. Phytol is an acyclic diterpene alcohol and is a product of chlorophyll metabolism that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K (Daines, 2003; Netscher, 2007) used in treating arthritis (Ogunlesi *et al.*, 2009). Phytol also has an important role in cosmetics and detergent production as a natural compound. In addition, phytol is used as cytotoxic, anti-inflammatory, anti-diabetic, anti-hyperalgesic, antibiotic, chemotherapeutic, antimicrobial, anti-tumour, antifungal, antimutagenic, anti-teratogenic, anticonvulsant, anti-schistosomal, lipid

restriction, antiplasmodic, antidepressant and as an immune adjuvant (Ghaneian *et al.*, 2015; Taj *et al.*, 2021). Moreover, this compound is a safe food additive, also observed in *Spirulina platensis*, which is a well-known cyanobacterium used commercially as a dietary supplement. *N. muscorum* and *N. punctiforme* also showed the presence of phytol (Abdel-Hafez *et al.*, 2015; Gupta *et al.*, 2015).

Neophytadiene (diterpenoid) is another essential oil found in all *Nostoc* spp., with the highest quantity in *Nostoc* sp. CU150162. It has multiple functional properties including antioxidant, anti-inflammatory, analgesic, antipyretic, and antimicrobial activities (Raman *et al.*, 2012; Palic *et al.*, 2002) hence used in the treatment of headache, rheumatism and some skin diseases (Suresh *et al.*, 2010). Dikhoba *et al.*, (2019) reported that natural plant products such as neophytadiene and palmitic acid are potential agents used as synthetic fungicide substitutes. GC-MS analysis of *N. linckia* showed the presence of neophytadiene with 0.83% and the study concluded that the antioxidant and antitumour activity of the species (Ramadan *et al.*, 2021).

Heptadecane, long-chain normal paraffin derived from octadecanoic acid (C₁₈H₃₆O₂), present in the crude extracts of cyanobacteria and plants exhibited antimicrobial and antifungal activities (Amudha *et al.*, 2018). Zhou *et al.* (2020), first reported the inhibitory effects of heptadecane, octadecanoic acid, and palmitoleic acid against *Rhizoctonia solani*. Ozdemir *et al.* (2004) documented that *Spirulina platensis* consisted of heptadecane (39.70%) and tetradecane (34.61%) which can inhibit bacterial and fungal growth. All the *Nostoc* spp. studied are excellent sources of heptadecane though with variations. Among them, *Nostoc* sp. CU150191 showed the highest quantity, followed by *Nostoc ellipsosporum*, *Nostoc punctiforme*, and *Nostoc* sp. CU150162.

Mannitol is a polyol used in food and pharmaceutical industries as a sweetener and antioxidant respectively, and is of high commercial value (Saha and Racine, 2011) and cyanobacteria produced mannitol via direct conversion of CO₂. 3-deoxy-d-mannitol is an aliphatic alcohol identified in *N. ellipsosporum* with a high quantity (26.64%). Torrez-Maza *et al.* (2020), also reported the presence of mannitol in

Nostoc species. In the present study, lupeol was detected in *N. elliposporum*, and it is a therapeutic and chemopreventive agent for the treatment of inflammation and cancer. Various studies showed that the anti-inflammatory activity of lupeol through the modulation of p-38 pathways inhibits neuroinflammation in the cerebellum and induces neuroprotection. It has the potential to act against different types of cancers such as human prostate, breast cancer, skin, liver, lung and blood cancer (Sharma *et al.*, 2020). Mannitol is also essential for root nodule formation (Delis *et al.*, 2011).

Fatty acids and lipids can be used as an important tool to determine the taxonomic position and are also useful in deciphering the relationship with morphological properties of cyanobacteria (Li and Watanabe, 2004; Dembitsky and Rezenka, 2005). The fatty acid profile of cyanobacteria is quite varied with marked differences occurring even within the same genus (Murata *et al.*, 1992). Cyanobacteria contain many important fatty acids such as linolenic, linoleic, palmitic acid, stearic acid, oleic acid and arachidonic acids, which are prerequisites for healthy growth and insecticidal activities (Lopez *et al.*, 2012). Fatty acids and their derivatives directly inhibit the growth of plant pathogens within the rhizosphere, and improve the environment of the plant rhizosphere aiding in reducing the occurrence of crop diseases and promoting growth (Davis *et al.*, 1997; Liu *et al.*, 2008; Liu *et al.*, 2012). Methyl palmitate, has pesticidal activity and insect repellent capacity (Ananya *et al.*, 2016). This compound is safe for vertebrates, as it is widely used in food, pharmaceuticals, cosmetic and industrial applications (Pearson, 2010). Wang *et al.*, (2009) isolated methyl palmitate from the green walnut husk and suggested methyl palmitate as a promising botanical miticide. Methyl palmitate identified from the three *Nostoc* spp. is consistently present in higher levels (Borah *et al.*, 2016) in *N. carneum*, *N. hatei*, and *N. muscorum*. In our study, high quantity methyl palmitate was present in *N. punctiforme* and *Nostoc* sp. CU150162. Linoleic acid (9, 12-Octadecadienoic acid, methyl ester) and stearic acid (Methyl stearate) have insecticidal activity against *Spodoptera frugiperda*, an insect that infests cotton, corn, and many grass crops (Lopez *et al.*, 2012). The present study identified these two compounds in *Nostoc* sp. CU150162 and *N. punctiforme*.

Polyunsaturated fatty acids (PUFAs) production is a characteristic of eukaryotic organisms (algae and higher plants), whereas SAFAs (saturated fatty

acids) and MUFAs (monounsaturated fatty acids) are the only constituents of the bacterial fatty acid profile. Cyanobacteria share characteristics of both bacteria and algae, as, some of the species of cyanobacteria do not produce PUFAs resembling the eubacteria and others synthesize PUFAs as algae (Caudales *et al.*, 2000). The analysis of chemical components of the *Nostoc* spp. revealed the presence of monounsaturated and polyunsaturated fatty acids. Among the various fatty acids identified from the *Nostoc* sp. CU150162, octadecadienoic acid and docosatrienoic acid are the polyunsaturated fatty acids (PUFA) known to exhibit anticancer, anti-inflammatory, vasodilator and antimicrobial properties (Bano and Deora, 2020).

The GC-MS analysis of *N. punctiforme* and *Nostoc* sp. CU150162 showed the presence of 10-octadecanoic acid, methyl ester used as a plant defense substance against pathogens (Bihana *et al.*, 2018). The present finding is in congruence with the report of Shukla *et al.* (2015), in which octadecanoic acid was identified in *Nostoc* spp. Furthermore, this compound exhibited potent cytotoxicity in all human gastric tumors, hepatocellular carcinoma and leukemia cell strains (Yu *et al.*, 2008). Generally, the major FAMES in cyanobacteria are palmitoleic, hexadecadienoic, palmitic, stearic and oleic acid. Based on the differences in their composition cyanobacteria are classified into four groups (Kenyon *et al.*, 1972; Murata *et al.*, 1992); the first group organisms are devoid of PUFA and contain SAFA and MUFA; the second and third groups contain either α -linolenic acid or γ -linolenic acid, respectively and the fourth group contains octadecatetraenoic acid.

Cyanobacteria are used as a promising feedstock for carbon-neutral biofuels due to their ability to accumulate large quantities of neutral lipid (20–50%) stored in cytosolic lipid bodies (Halim *et al.*, 2013; Mutanda *et al.*, 2011). Therefore, these photosynthetic microorganisms can potentially be employed for the economically effective and environmentally sustainable production of biofuels. Cyanobacteria are regarded as a more viable solution for biofuel production in comparison to other microorganisms (Ruffing, 2014). The GC-MS analysis of *Nostoc* spp. revealed the presence of a wide spectrum of fatty acid methyl esters (FAME) such as methyl palmitate, methyl palmitoleinate, methyl stearate, 10-octadecenoic acid methyl ester,

9,12-octadecenoic acid methyl ester, 8,11,14-docosatrienoic acid methyl ester, 10-octadecadienoic acid methyl ester and methyl lignocerate. In addition, FAME profiling of *Nostoc* sp. grown in pulp and paper wastewater showed the presence of various fatty acids like myristic acid, palmitic acid, palmitoleic acid, hexadecadienoic acid, stearic acid, oleic acid, linoleic acid and linolenic acid (Kimani, 2016). Cyanobacterial strains with these constituents are more suitable for biofuel applications. The GC-MS chromatogram of *Nostoc* sp. CU150162 showed the presence of FAME components including palmitic acid, palmitoleic acid and stearic acid. Peramuna and Summer (2014) analyzed the fatty acid composition of *N. punctiforme* and investigated the major fatty acids such as hexadecanoic acid and octadecanoic (stearic) acid, with a trace amount of phytol acetate also detected using GC-MS. Similarly, hexadecanoic acid, octadecanoic acid and phytol were also isolated from *N. punctiforme*.

Alkane hydrocarbon, heptadecane is one of the major components of petrodiesel and this cyanobacterium-derived alkane can be used directly as a biodiesel component, without converting triglycerides into liquid hydrocarbons (Knothe, 2010). Cyanobacteria can synthesize hydrocarbons such as long-chain C15–C19 alkanes and alkenes, mainly C15 and C17 and these compounds have been detected in the cyanobacterial thylakoid and cytoplasmic membranes, and their functions are related to membrane flexibility (Lea-Smith *et al.*, 2016). GC-MS analysis of *Nostoc* sp. KNUA003 showed the presence of heptadecane (C₁₇H₃₆), along with other common microalgal fatty acids such as palmitic acid, palmitoleic acid, linoleic acid and linolenic acid, which are major components of biodiesel (Hong *et al.*, 2018). Previously, this hydrocarbon was observed in the extracts of *Nostoc* sp. CENA175 (5.1%) and *Nostoc punctiforme* PCC73102 (Armstrong, 2019). In our study, heptadecane is detected in all *Nostoc* spp. with *Nostoc* sp. CU150191 and *N. elliposporum* produced a high quantity of heptadecane.

According to Thacker and Paul (2004), secondary metabolite profiles have recently been utilised to classify cyanobacteria with traditional morphological taxonomy. The present study showed that the species *N. elliposporum* FY150125,

N. punctiforme FY150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191, collected from Kerala, can be recognized as a group that represents an analogous chemotaxonomic basis which is characteristic of the family Nostocaceae. Dembitzky and Rezanka (2005) demonstrated the importance of fatty acids, hydrocarbons and other compounds in the chemotaxonomy of cyanobacteria at the genus and subgenus levels. Likewise, Shukla *et al.* (2012) constructed a dendrogram based on FAME profiling and seven comprehensive parameters (percentage of SAFA, MUFA, PUFA, the ratio between MUFAs/ PUFAs, SAFAs/MUFAs, and the degree of unsaturation) of the cyanobacterial strains. The cluster analysis indicated that the FAME profiling of cyanobacterial strains might be used to study the cyanobacterial diversity at the genus and species level. According to the present study, the phylogenetic relationship using the chemical composition of *Nostoc* spp. revealed the close relationship of *N. elliposporum* and *N. punctiforme*, both of them considered as a single cluster. This observation is corroboration with the earlier report by Ghosh and Badury (2021), as the whole genome analysis of *N. elliposporum* strain NOK showed a close relationship with *N. punctiforme* strain PCC73102.

Principal component analysis (PCA) and hierarchical cluster analysis (HCA) are the most popular multivariate explanatory techniques. It is used to reduce the number of parameters and retain only those which are the most significant for the explanation of some phenomena (Milovanovic *et al.*, 2015). Most often these two techniques are applied in solving classification problems (Ragno *et al.*, 2007). In PCA, the smaller angle between the arrows indicates a high positive correlation between the variables (Celis-Pla *et al.*, 2021). In our study, the angle between *N. punctiforme* and *Nostoc* sp. CU150191 was less than 90°. The results also showed that the two species are positively correlated, and the results of the heat map also supported this. In PCA, factor 1 and factor 2 accounted for 40.20% and 31.65% of the variation, respectively, also suggesting the validity and reliability of the method. Previous comparative analysis also demonstrated the first two factors are valid for PCA analysis of volatile compound concentration in selected strains of

cyanobacteria (Milovanovic *et al.*, 2015) and the study also recommended that both techniques are best for the grouping of strains using the type and concentration of volatile compounds.

Ward's method and the Euclidean distance metrics are the most suitable and efficient strategies for hierarchical cluster analysis (Punj and Stewart, 1983; Rencher, 2002; Tamasauskas *et al.*, 2012). In this study, AHCA with Ward's method and Euclidean distance was employed for cluster analysis. HCA uses an appropriate metric as a measure of the distance between pairs of observations (Erisoglu *et al.*, 2010). The dendrogram of HCA serves as a summary of the distance matrix and reveals the similarities and differences between individuals in each cluster, it is corroborated with the present study in which all fatty acids formed a single cluster. The Euclidean distance of HCA was used as a measure for the similarity and dissimilarity between the compounds. Yerel (2010) also reported that the selection of Euclidean distance as a distance measure and the Wards method as a linkage rule produced the most distinctive groups. Large Euclidean distances indicated heterogeneous clusters whereas short Euclidean distances indicated homogeneous clusters (Ukpatu *et al.*, 2015). The present study suggested cluster 2 (displayed in blue colour) is more homogeneous than other clusters. Cluster heatmaps are mostly used to reveal the hierarchical clusters in data matrices (Engle *et al.*, 2017). The clustered heatmap of *Nostoc* spp. constructed based on the percentage of compounds that showed similarities with the chemotaxonomic dendrogram of *Nostoc* spp. constructed using PAUP software based on the presence of compounds. In both, the four species are grouped in two clusters. *N. elliposporum*, *N. punctiforme* and *Nostoc* sp. CU150191 grouped in one cluster and *Nostoc* sp. CU150162 grouped in another cluster. Likewise, clustered heatmaps have been used by several researchers in cyanobacteria for the hierarchical classification of metabolites (Le-Manach *et al.*, 2019; Le-Moigne *et al.*, 2021).

CHAPTER 7

**ASSESSMENT OF NOSTOC SPECIES AS A BIOFERTILIZER FOR
RICE VARIETIES JYOTHI AND ANNAPURNA**

7.1 Abstract

Modern agriculture relies heavily on applying synthetic fertilizers and pesticides, intensive tillage, and excess water consumption causing environmental problems including the deterioration of soil fertility. Therefore, to increase agricultural production, it is necessary to find an alternative technology that enhances the output by utilizing limited resources without destroying environmental quality. The application of cyanobacterial biofertilizers in paddy fields would be an eco-friendly approach, contributing to the development of sustainable agriculture. In rice fields, nitrogen-fixing cyanobacteria are important microbial members that are employed as bio-inoculants for enhancing fertility, beneficial for growth, and nutrient uptake, and exhibit induced resistance or antagonism towards pathogens, improving the structure of soils and crop yields. The present investigation aimed to analyse the effect of *Nostoc* spp. in rice plant growth and the species that promotes better plant growth. Bio-priming of seeds with different *Nostoc* inoculum enhanced seed germination, shoot and root length of seedlings. The morphological and biochemical characters of rice varieties Jyothi and Annapurna were significantly affected by the inoculation of *N. elliposporum* FY150125, *N. punctiforme* FY150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191. Germination parameters were evaluated in lab conditions and pot experiments were conducted for the assessment of morphological and biochemical characters. Statistical data analysis using SPSS 23.0 software revealed that the morphological and biochemical characters of rice varieties treated with different *Nostoc* inoculums showed a statistically significant ($p < 0.05$) effect.

7.2 Results

7.2.1 Effects of *Nostoc* spp. on the growth of rice varieties Jyothi and Annapurna

7.2.1.1 Effects of bio-priming on seed germination of rice seeds with *Nostoc* spp.

Surface sterilized rice seeds were bio-primed with 1% of *Nostoc* spp. to improve the seed germination of rice varieties. Rice seeds were bio-primed with different *Nostoc* spp. and hydroprimed seeds as control. The rice seeds treated with different *Nostoc* spp. germinated faster than the control (Table 7.1). Compared to the effects of all other species, *Nostoc* sp. CU150162 showed the highest seed germination during 24 hrs of incubation, followed by *N. elliposporum* FY150125 in both the varieties. Jyothi variety bio-primed with *Nostoc* sp. CU150162 showed 97.22% and Annapurna variety with 94.21% of seed germination (Fig. 7.1).

Table 7.1 Effects of bio-priming with *Nostoc* spp. on seed germination of rice varieties Jyothi and Annapurna.

Treatments	Seed germination percentage in Jyothi rice seeds (%)	Seed germination percentage in Annapurna rice seeds (%)
<i>N. elliposporum</i> FY150125	88.88±2.77 ^{ab}	91.11±0.44 ^{ab}
<i>N. punctiforme</i> FY150188	86.11±5.55 ^{ab}	91.11±0.44 ^{ab}
<i>Nostoc</i> sp. CU150162	97.22±2.77 ^a	94.21±1.92 ^a
<i>Nostoc</i> sp. CU150191	86.11±2.77 ^{ab}	89.77±0.44 ^b
Control	75.00±4.81 ^b	87.55±0.44 ^b

Values are given as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

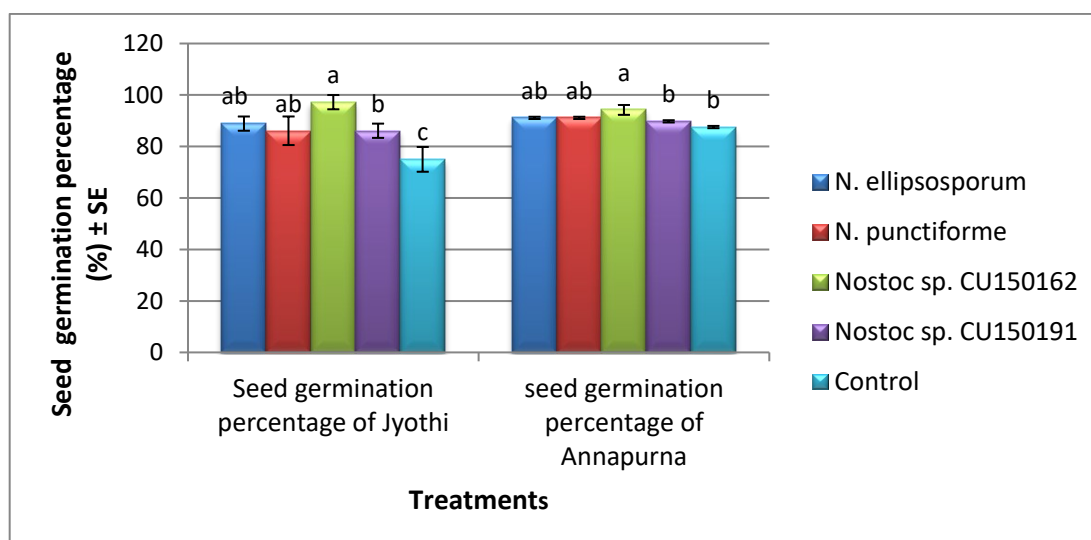


Fig. 7.1. Effects of different bio-priming treatments on the seed germination of rice varieties Jyothi and Annapurna. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

7.2.1.2 Effects of bio-priming on the morphological parameters of rice seedlings with different *Nostoc* spp.

Morphological characters like shoot length and root length were analysed on the 14th day old seedling (Tables 7.2 & 7.3). The best plant height after 14 days was recorded in the plants raised from seeds bio-primed with *Nostoc* sp. CU150162 (10.46 ± 0.17 cm) followed by *N. elliposporum* FY150125 (9.96 ± 0.44 cm) in Jyothi (Fig. 7.2A-7.2B). Compared to all treatments shortest plant height was found in the seedlings of hydro-primed seeds. The effect of bio-priming on root length showed a significant difference within the treatments. The control plants produced (6.166 ± 0.28 cm) shortest roots. In the Annapurna variety, the highest shoot length (11.23 ± 0.14 cm) and root length (6.80 ± 0.88 cm) were observed in seedlings treated with *Nostoc* sp. CU150162 compared to other treatments (Fig. 7.2C-7.2D). In general, rice seeds bio-primed using *Nostoc* spp. had better seedling growth than hydro-primed seeds during the 14th day.

Table 7.2 Effects of bio-priming on the morphological characters of rice variety Jyothi on the 14th day.

Treatments	Shoot length (cm±SE)	Root length (cm±SE)
<i>N. elliposporum</i> FY150125	9.96±0.44 ^{ab}	7.83±1.04 ^{ab}
<i>N. punctiforme</i> FY150188	9.46±0.46 ^{ab}	7.83±0.28 ^{ab}
<i>Nostoc</i> sp. CU150162	10.46±0.17 ^a	9.33±0.76 ^a
<i>Nostoc</i> sp. CU150191	9.20±0.37 ^{ab}	7.50±1.30 ^{ab}
Control	8.43±0.49 ^b	6.16±0.28 ^b

Values are given as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

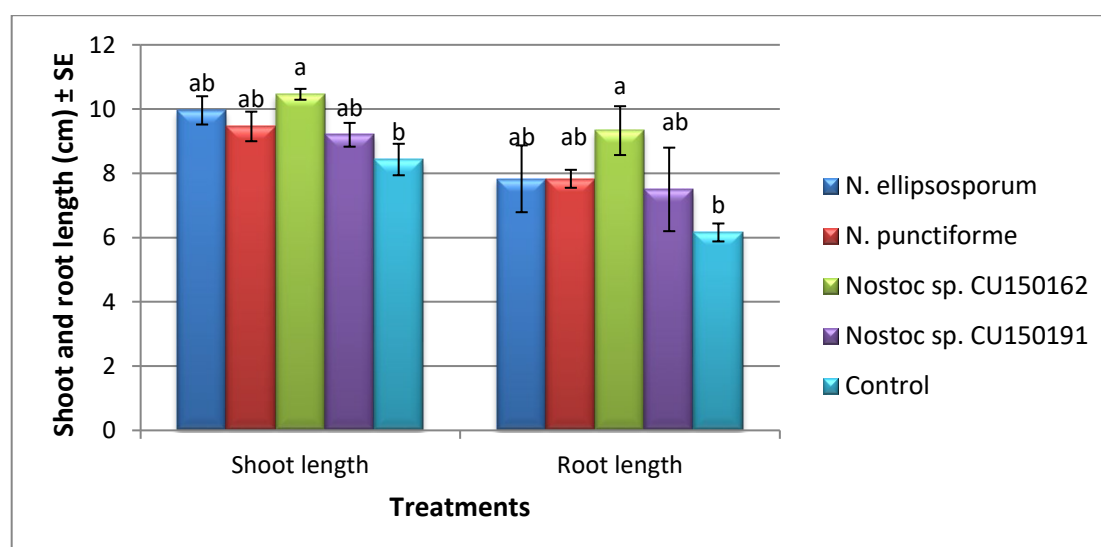


Fig 7.2A Effects of bio-priming on the morphological characters of rice variety Jyothi on the 14th day. Values are given as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

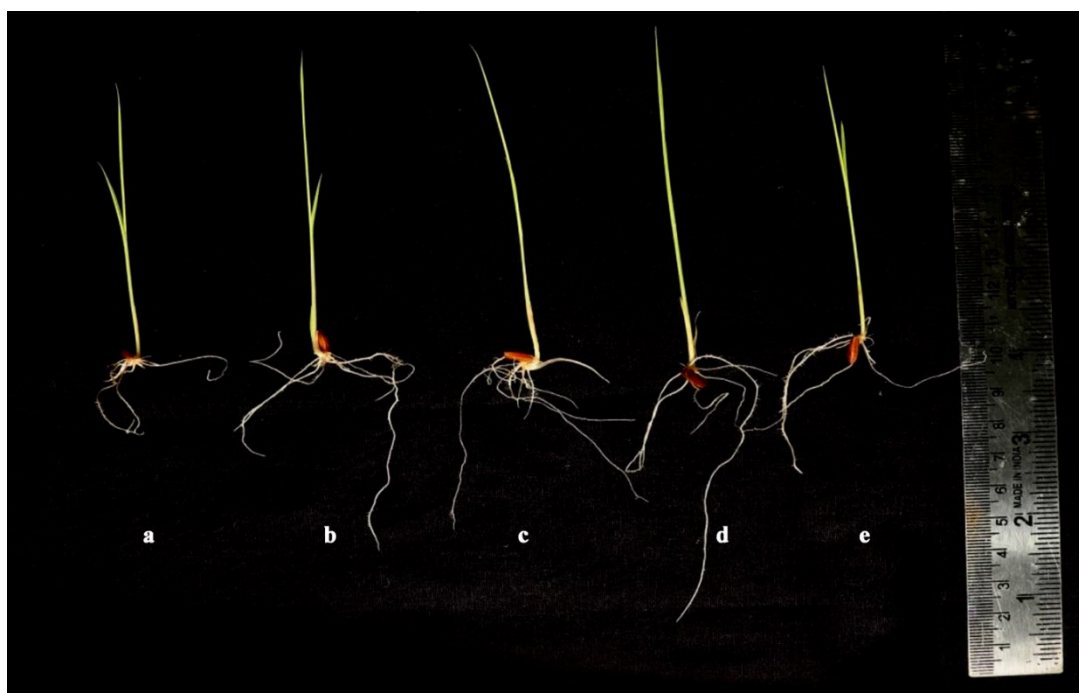


Fig. 7.2B Effects of treatments with a) Control b) *N. punctiforme* FY150188 c) *N. ellipsosporum* FY150125 d) *Nostoc* sp. CU150162 e) *Nostoc* sp. CU150191 on the morphological characters of Jyothi seedlings.

Table 7.3 Effects of bio-priming on the morphological characters of rice variety Annapurna on the 14th day.

Treatments	Shoot length (cm±SE)	Root length (cm±SE)
<i>N. ellipsosporum</i> FY150125	10.8±0.20 ^a	5.50±0 ^{ab}
<i>N. punctiforme</i> FY150188	10.55±0.24 ^a	5.30±0.33 ^{ab}
<i>Nostoc</i> sp. CU150162	11.23±0.14 ^a	6.80±0.88 ^a
<i>Nostoc</i> sp. CU150191	10.53±0.12 ^a	5.20±0.16 ^{ab}
Control	9.5±0.25 ^b	3.80±0.37 ^b

Values are given as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

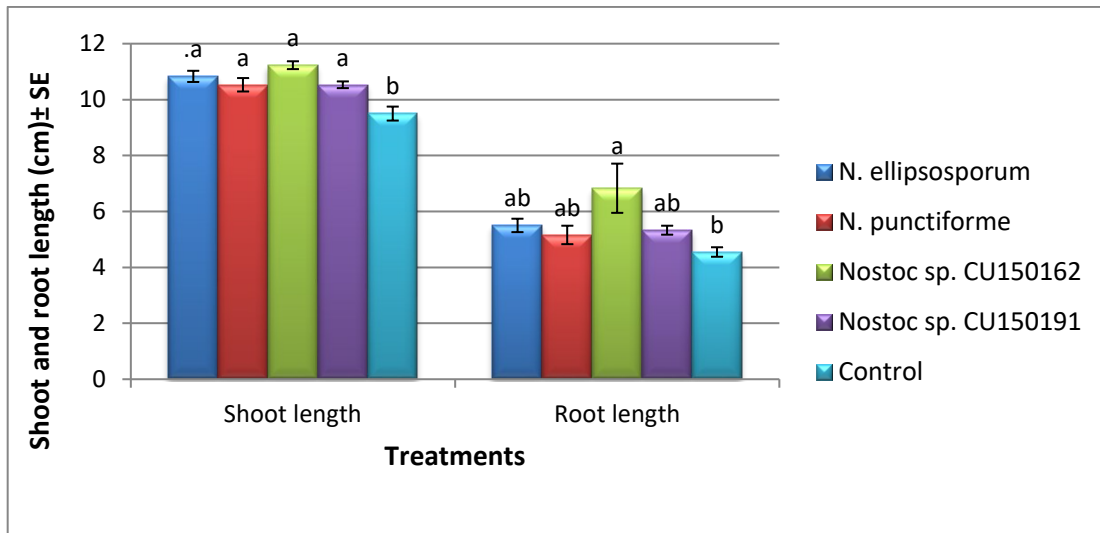


Fig. 7.2C. Effects of bio-priming on the morphological characters of rice variety Annapurna on the 14th day. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

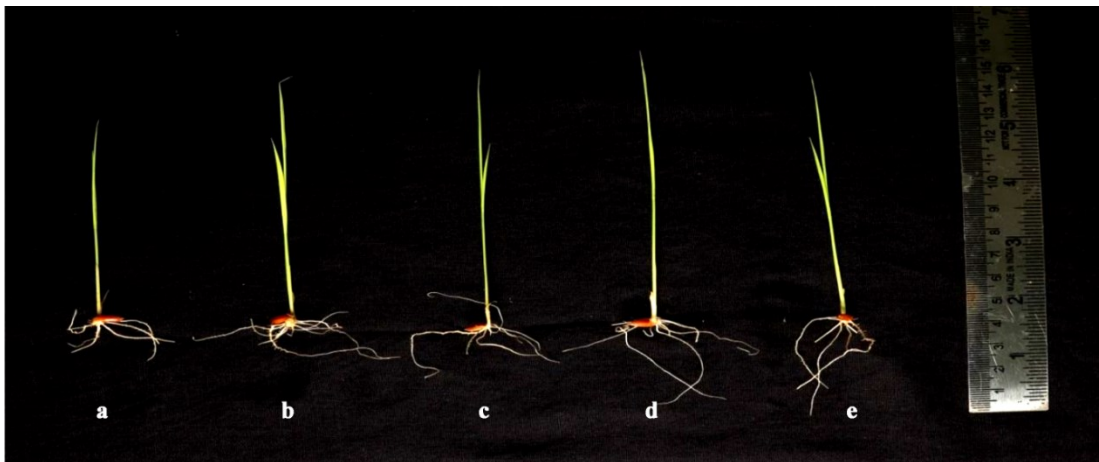


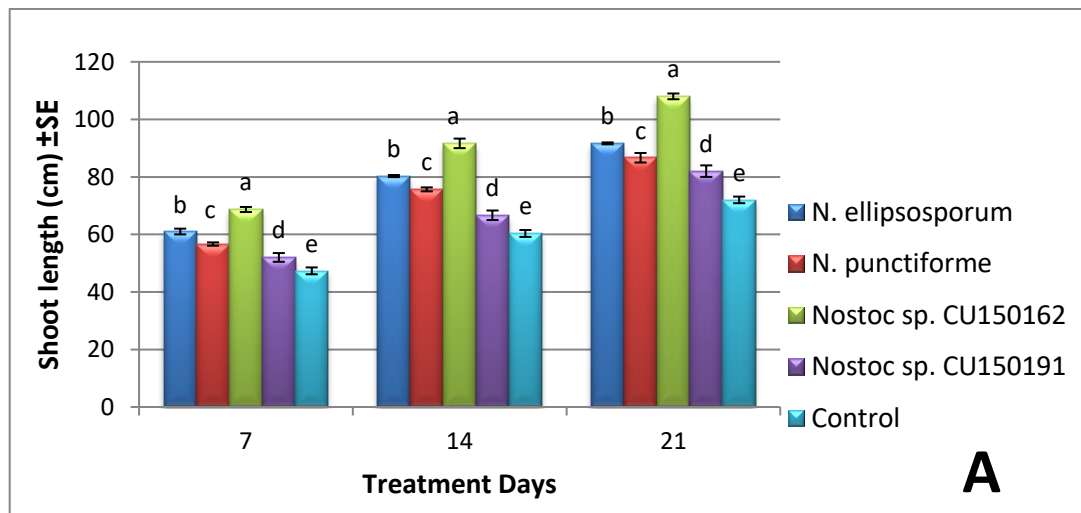
Fig. 7.2D Effects of treatments with a) Control b) *N. punctiforme* FY150188 c) *N. ellipsosporum* FY150125 d) *Nostoc* sp. CU150162 e) *Nostoc* sp. CU150191 on the morphological characters of Annapurna seedlings.

7.2.1.3 Effects of different *Nostoc* spp. on the morphological and biochemical characteristics of rice varieties.

A pot experiment was conducted to determine the effects of *Nostoc* spp. on the growth characteristics of rice varieties in the field. 14-day-old bio-primed seedlings of rice varieties were transplanted to sterilized soil for further analysis of plant growth. After 7 days of transplantation, 1g of wet *Nostoc* inoculum was added

to 1kg of sterilized soil. After 14 days of treatment, *Nostoc* species multiplied in the soil and morphological and biochemical characteristics of Jyothi and Annapurna plants were analysed. On the 7th, 14th and 21st days after the multiplication of the *Nostoc* cultures, the morphological and biochemical characters of plants were determined.

Morphological characters such as shoot length, leaf number, leaf length and leaf breadth were analysed. In the Jyothi variety, the effect of different *Nostoc* spp. on plant height showed a significant difference within the treatments and days of treatment ($p<0.05$). The shoot length of the inoculated plants increased during different intervals (Table. 7.4). Plants treated with *Nostoc* sp. CU150162 showed the highest plant height on the 21st day (108 ± 1.00 cm) followed by *N. elliposporum* FY150125 (91.66 ± 0.88 cm), *N. punctiforme* FY150188 (86.66 ± 1.66 cm) and *Nostoc* sp. CU150191 (82 ± 2.0 cm) (Fig. 7.3A). *Nostoc* spp. treated with the Jyothi variety showed a significant effect on the leaf number, leaf length and leaf breadth compared to the control plants on the 7th, 14th and 21st days (Fig. 7.3B-7.3C). Among various treatments, seedlings treated with *Nostoc* sp. CU150162 were better in leaf number, leaf length and leaf breadth during different incubation periods.



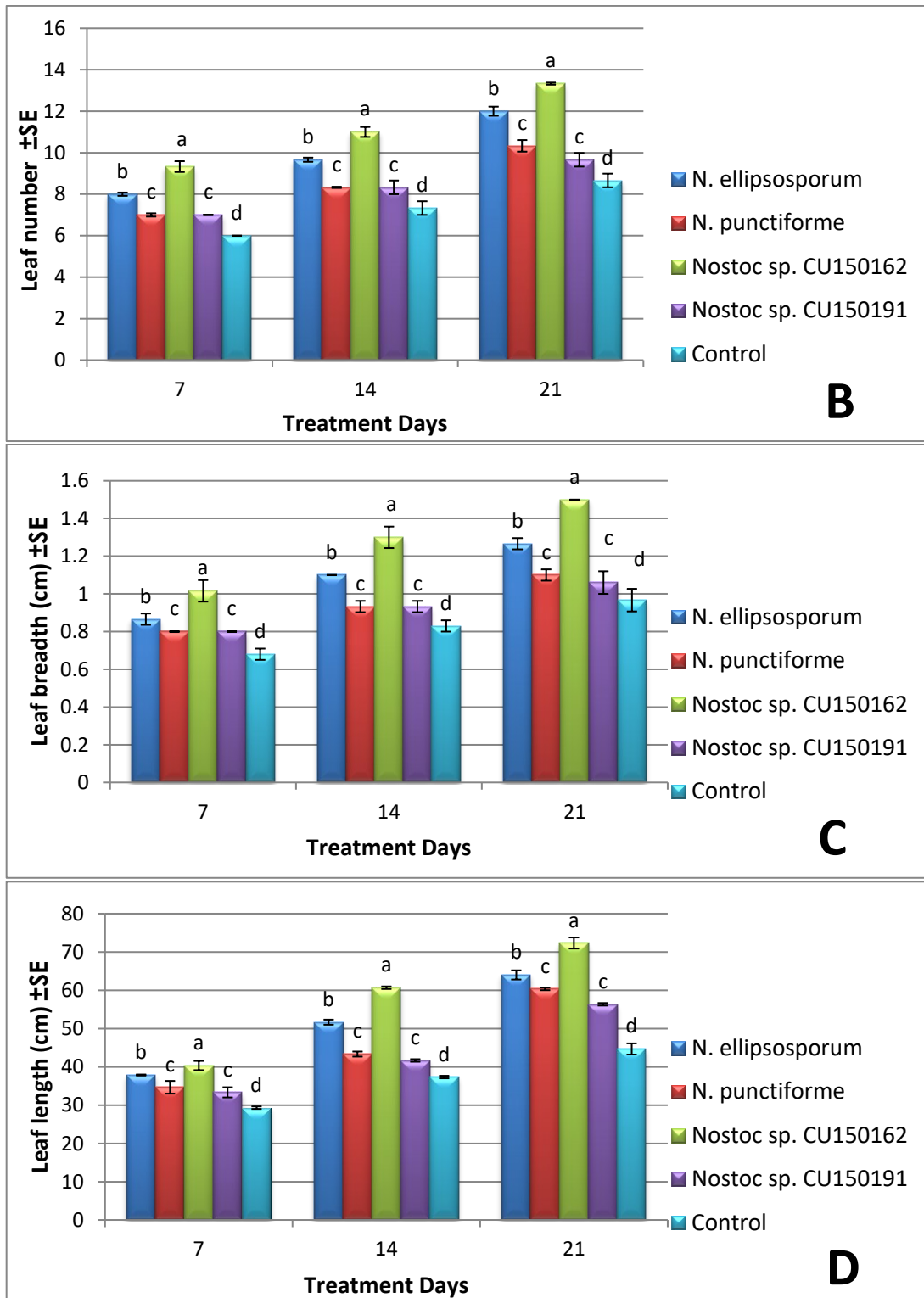


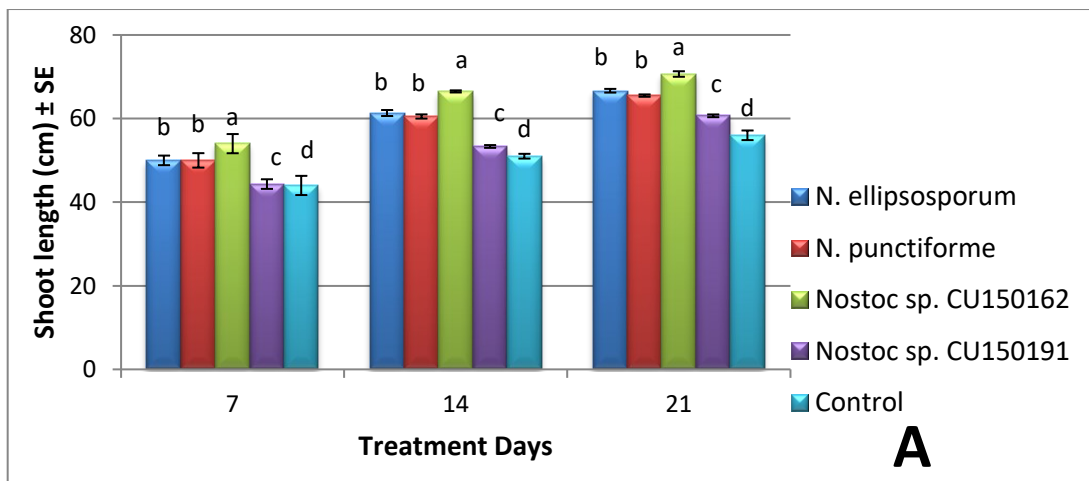
Fig. 7.3A-7.3D Effects of *Nostoc* spp. on the growth parameters of rice variety Jyothi on the 7th, 14th and 21st day of intervals. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$. **A)** Shoot length **B)** Leaf number **C)** Leaf breadth **D)** Leaf length.

Table 7.4 Effects of *Nostoc* spp. on the morphological characters of rice variety Jyothi on the 7th, 14th and 21st day of intervals.

Morphological characters	Days	<i>N. ellipsosporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191	Control
Shoot length (cm±SE)	7	61±1.00 ^b	56.66±0.33 ^{bc}	68.66±0.33 ^a	52±1.52 ^c	47.33±1.20 ^d
	14	80.3±0.57 ^b	75.66±0.66 ^{bc}	91.66±1.66 ^a	66.66±1.66 ^c	60.33±1.20 ^d
	21	91.66±0.88 ^b	86.66±1.66 ^{bc}	108±1.00 ^a	82±2.0 ^c	72±1.15 ^d
Leaf number	7	8.00±0 ^b	7.00±0 ^c	9.33±0.33 ^a	7.00±0 ^c	6.00±0 ^d
	14	9.66±0.33 ^b	8.33±0.33 ^c	11.00±0 ^a	8.33±0.33 ^c	7.33±0.33 ^d
	21	12.00±0.57 ^b	10.33±0.33 ^c	13.33±0.33 ^a	9.66±0.33 ^c	8.66±0.33 ^d
Leaf length (cm±SE)	7	37.86±0.13 ^b	34.66±0.66 ^c	40.33±1.20 ^a	33.33±1.33 ^c	29.33±0.33 ^d
	14	51.66±1.66 ^b	43.33±0.66 ^c	60.66±0.33 ^a	41.66±0.33 ^c	37.33±0.33 ^d
	21	64.00±2.08 ^b	60.33±1.45 ^c	72.33±1.45 ^a	56.33±0.33 ^c	44.66±1.45 ^d
Leaf breadth (cm±SE)	7	0.86±0.03 ^b	0.80±0 ^c	1.016±0.05 ^a	0.80±0 ^c	0.68±0.03 ^d
	14	1.1±0 ^b	0.933±0.03 ^c	1.30±0.05 ^a	0.93±0.03 ^c	0.83±0.03 ^d
	21	1.26±0.03 ^b	1.10±0.03 ^c	1.50±0 ^a	1.06±0.63 ^c	0.967±0.06 ^d

Values are given as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

Significant differences were observed in the morphological characters of the Annapurna rice variety on the 7th, 14th and 21st day of treatments (Table 7.5). Plants treated with *Nostoc* sp. CU150162 produced maximum height compared to the control. The highest shoot length was observed in plants treated with *Nostoc* sp. CU150162 (70.66±0.66 cm), followed by *N. elliposporum* FY150125 (66.66±0.44 cm), *N. punctiforme* FY150188 (65.5±0.28 cm) and *Nostoc* sp. CU150191 (60.66±0.33 cm) during the 21st day (Fig 7.4 A). Variability in the number of leaves, leaf length and leaf breadth were assessed in treated plants of Annapurna. The highest number of leaves (13.33±0.33) was observed on the 21st day in the plants treated with *Nostoc* sp. CU150162 compared to the control plants (7.66±0.33) (Fig 7.4B). Significant differences were observed in the leaf length and leaf breadth of plants on the progressive days of treatments (Fig 7.4C-7.4D). Leaf length and leaf breadth on the 21st day of treatment were; *Nostoc* sp. CU150162 (52±1.15 and 1.3±0.05 cm), *N. elliposporum* FY150125 (44.66±0.16 and 1.18±0.03 cm), *N. punctiforme* FY150188 (43±0.76 and 1.1±0.05 cm), *Nostoc* sp. CU150191 (41.66±0.88 and 1.06±0.03 cm) and control (38.16±1.75 and 0.88±0.04 cm).



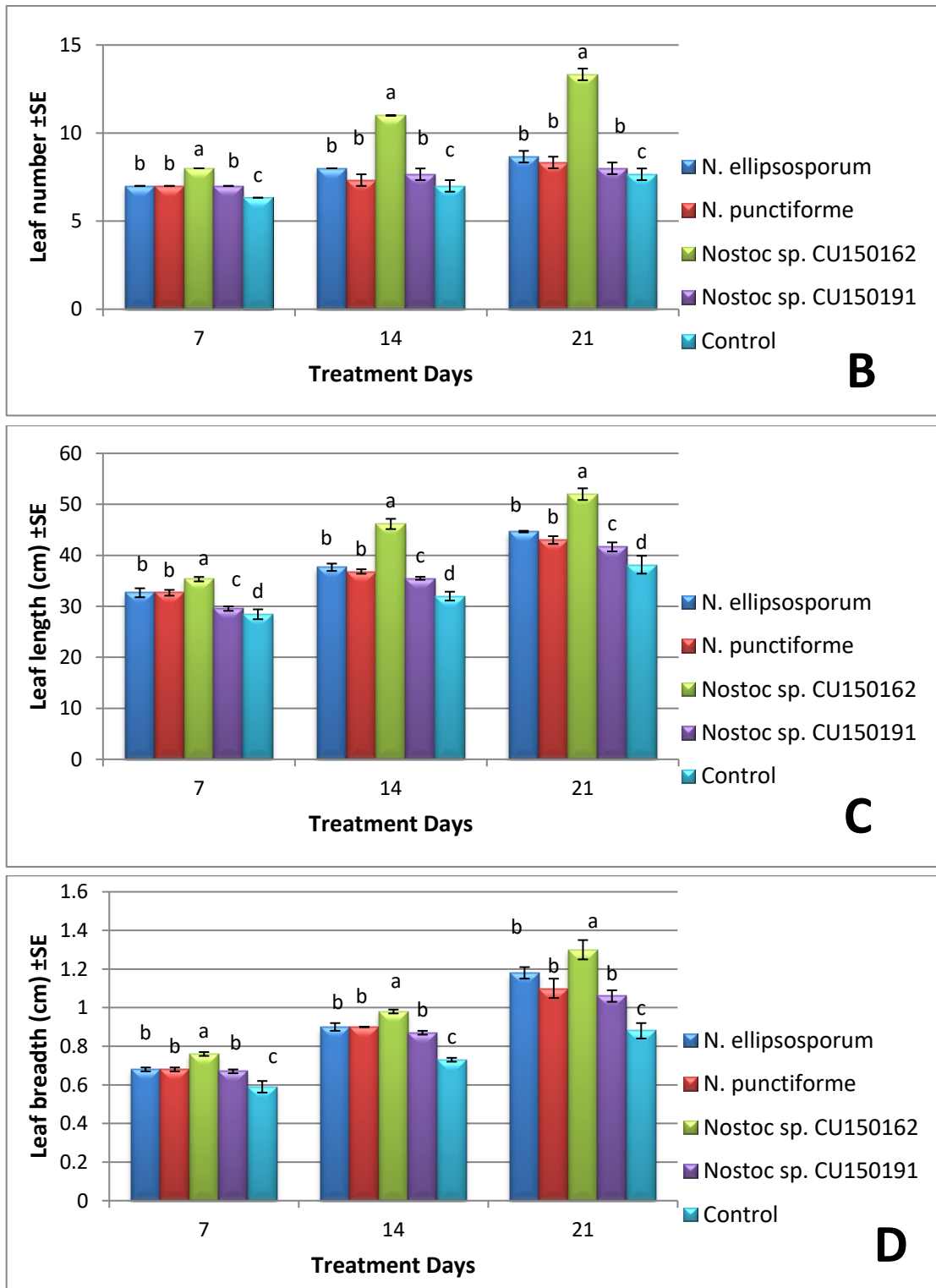


Fig 7.4A-7.4D. Effects of *Nostoc* spp. on the leaf breadth of rice variety Annapurna on the 7th, 14th and 21st day of intervals. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$. A) Shoot length B) Leaf number C) Leaf length D) Leaf breadth.

Table 7.5. Effects of *Nostoc* spp. on the morphological characters of rice variety Annapurna on the 7th, 14th and 21st day of intervals.

Morphological characters	Days	<i>N. ellipsosporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191	Control
Shoot length (cm±SE)	7	50±1.15 ^b	50±1.73 ^b	54±2.30 ^a	44.33±1.15 ^c	44±2.30 ^d
	14	61.33±0.72 ^b	60.5±0.50 ^b	66.50±0.29 ^a	53.33±0.33 ^c	51±0.57 ^d
	21	66.66±0.44 ^b	65.5±0.28 ^b	70.66±0.66 ^a	60.66±0.33 ^c	56±1.15 ^d
Leaf number	7	7.00±0 ^b	7.00±0 ^b	8.00±0 ^a	7.00±0 ^b	6.33±0 ^c
	14	8.00±0 ^b	7.33±0.33 ^b	11.00±0 ^a	7.66±0.33 ^b	7.00±0.3 ^c
	21	8.66±0.33 ^b	8.33±0.33 ^b	13.33±0.33 ^a	8.00±0 ^b	7.66±0.33 ^c
Leaf length (cm±SE)	7	32.66±0.88 ^b	32.66±0.57 ^b	35.33±.44 ^a	29.55±0.44 ^c	28.44±0.97 ^d
	14	37.66±0.73 ^b	36.83±0.44 ^b	46.16±1.0 ^a	35.5±0.29 ^c	32±0.87 ^d
	21	44.66±0.16 ^b	43±0.76 ^b	52±1.15 ^a	41.66±0.88 ^c	38.16±1.75 ^d
Leaf breadth (cm±SE)	7	0.68±0.01 ^b	0.68±0.01 ^b	0.76±0.01 ^a	0.67±0.01 ^b	0.59±0.03 ^c
	14	0.9±0.02 ^b	0.9±0 ^b	0.98±0.01 ^a	0.87±0.01 ^b	0.73±0.01 ^c
	21	1.18±0.03 ^b	1.1±0.05 ^b	1.3±0.05 ^a	1.06±0.03 ^b	0.88±0.04 ^c

Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

Biochemical characteristics such as total chlorophyll, total protein and total sugar were analysed on the 7th, 14th and 21st day after the multiplication of *Nostoc* spp. in soil. Data on biochemical parameters of the Jyothi variety inoculated with different *Nostoc* spp. and control is presented in Table 7.6. The results of the study indicated that Jyothi variety treated with all *Nostoc* spp. showed better results than the control. Among the 4 *Nostoc* spp., *Nostoc* sp. CU150162 showed the highest chlorophyll content on the 21st day (13.92 ± 0.36 mg/g) followed by *N. elliposporum* FY150125 (12.83 ± 0.06 mg/g), *N. punctiforme* FY150188 (12.19 ± 0.17 mg/g), *Nostoc* sp. CU150191 (11.74 ± 0.05 mg/g) and control (10.79 ± 0.10 mg/g) (Fig. 7.5A). Quantitative analysis of the total protein content of the Jyothi variety treated with different *Nostoc* spp. showed a significant effect on treatment intervals. Total protein content was found to be higher in the Jyothi variety inoculated with *Nostoc* sp. CU150162. During the 21st day highest protein content of 1.17 ± 0.03 mg/ml was observed compared to other treatments (Fig. 7.5B). The total protein content was calculated from the standard graph constructed using the gradient concentration of BSA (Fig. 7.5C). The total sugar content was calculated from the standard graph of glucose (1 mg/ml) (Fig. 7.5D). The total sugar content was also observed to be the highest in the Jyothi variety treated with *Nostoc* sp. CU150162 with 23.87 ± 0.15 mg/ml (Fig. 7.5E).

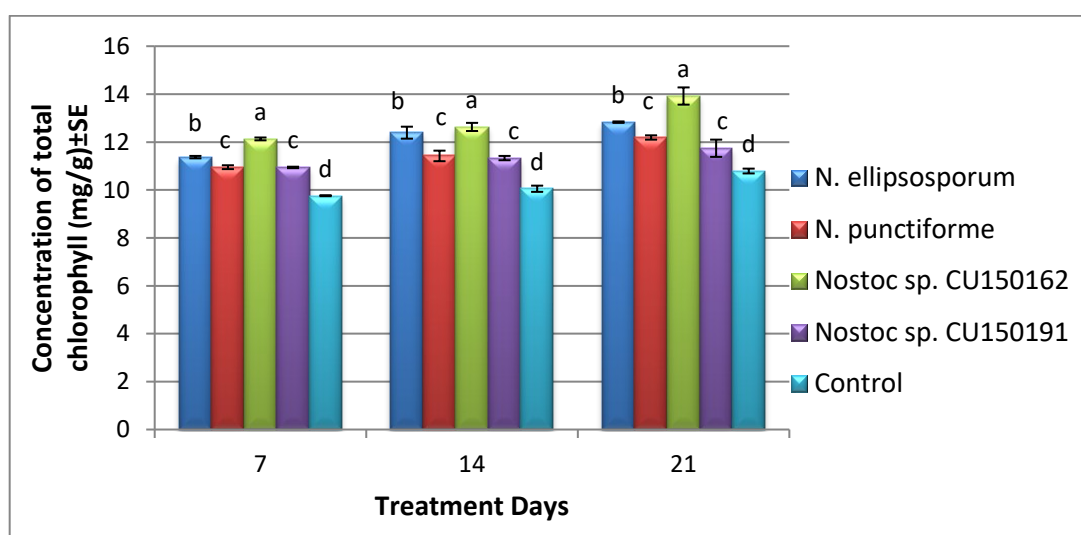


Fig. 7.5A Effects of *Nostoc* spp. on the total chlorophyll content of rice variety Jyothi on the 7th, 14th and 21st day of intervals. Data presented as mean \pm SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

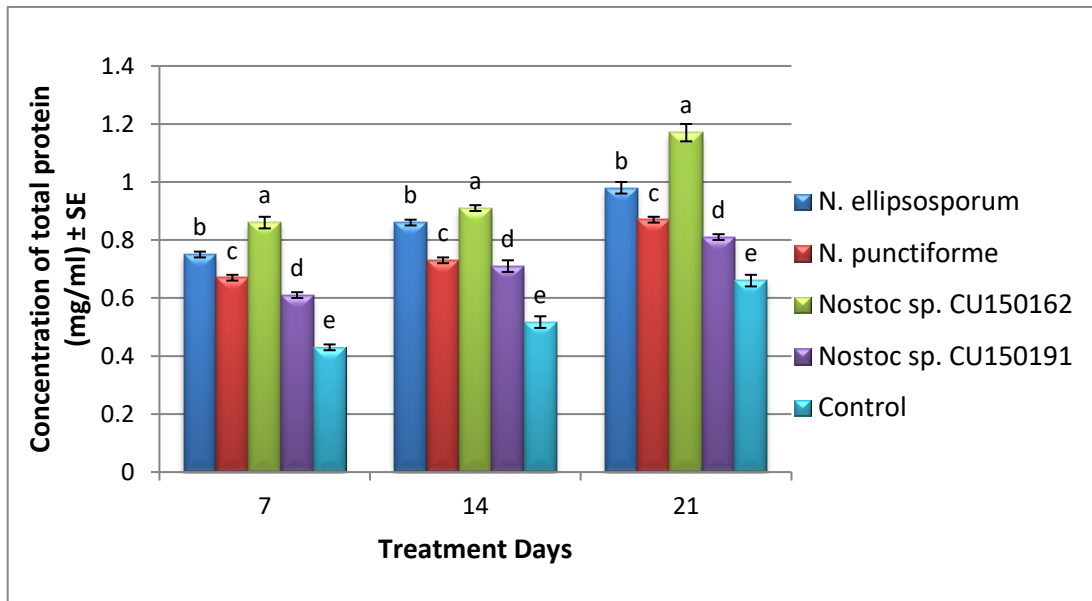


Fig. 7.5B Effects of *Nostoc* spp. on the total protein content of rice variety Jyothi on the 7th, 14th and 21st day of intervals. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

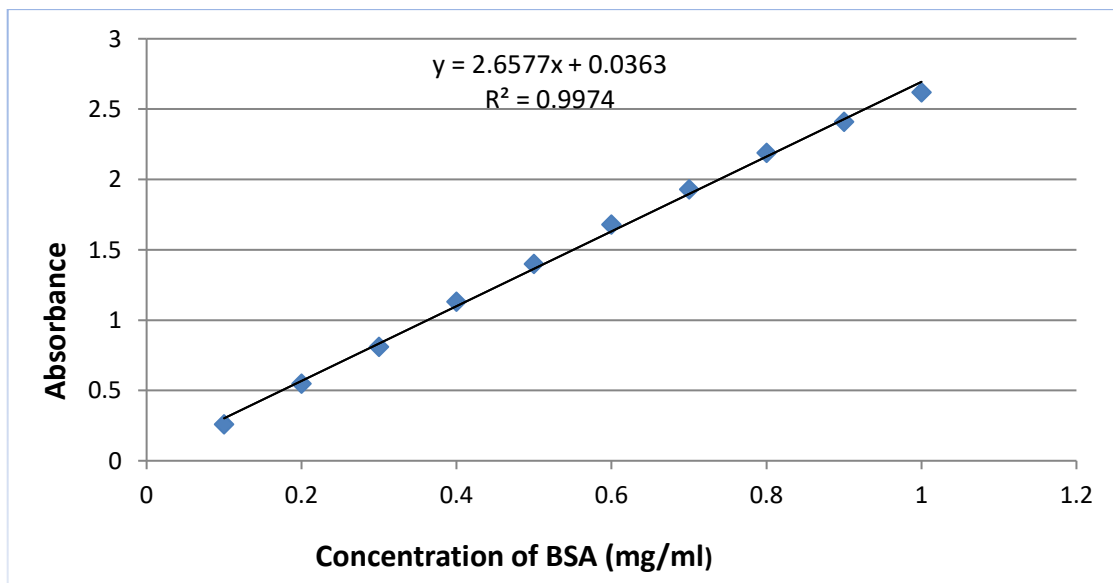


Fig. 7.5C Standard graph prepared by using gradient concentration of BSA for the quantification of protein content.

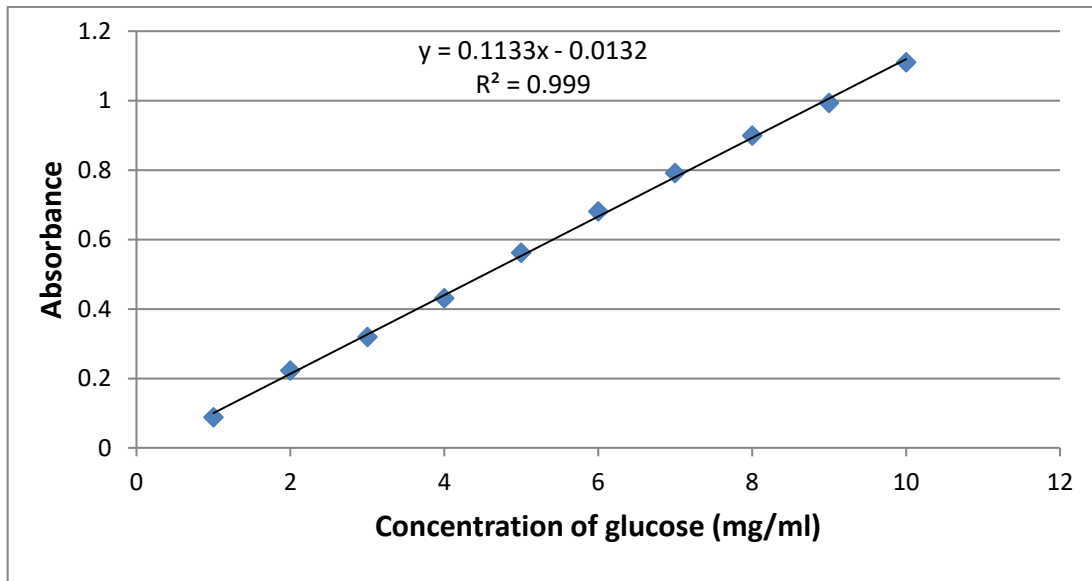


Fig. 7.5D Standard graph prepared by using gradient concentration of glucose for the quantification of total sugar content.

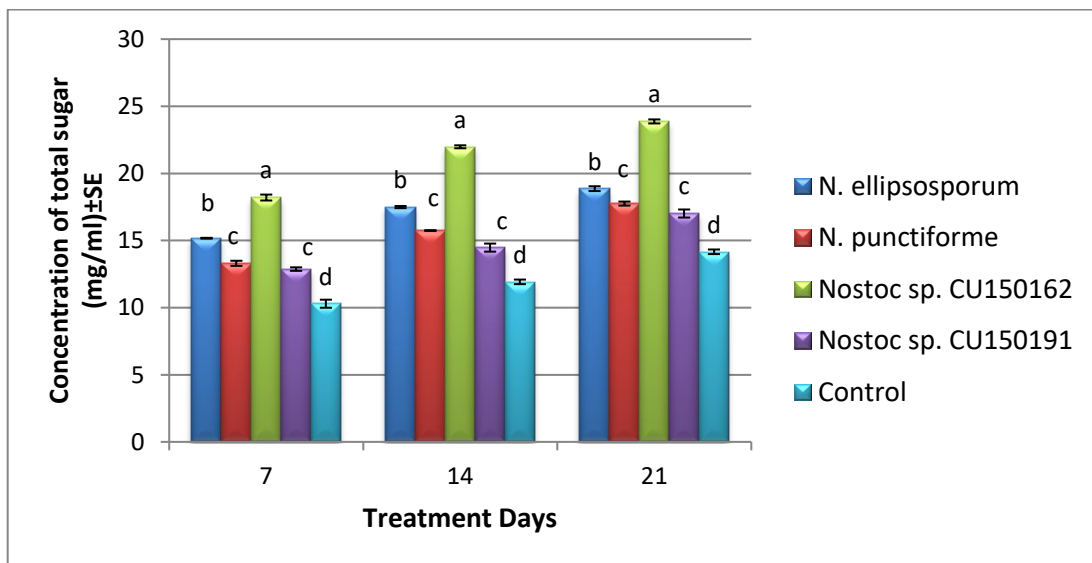


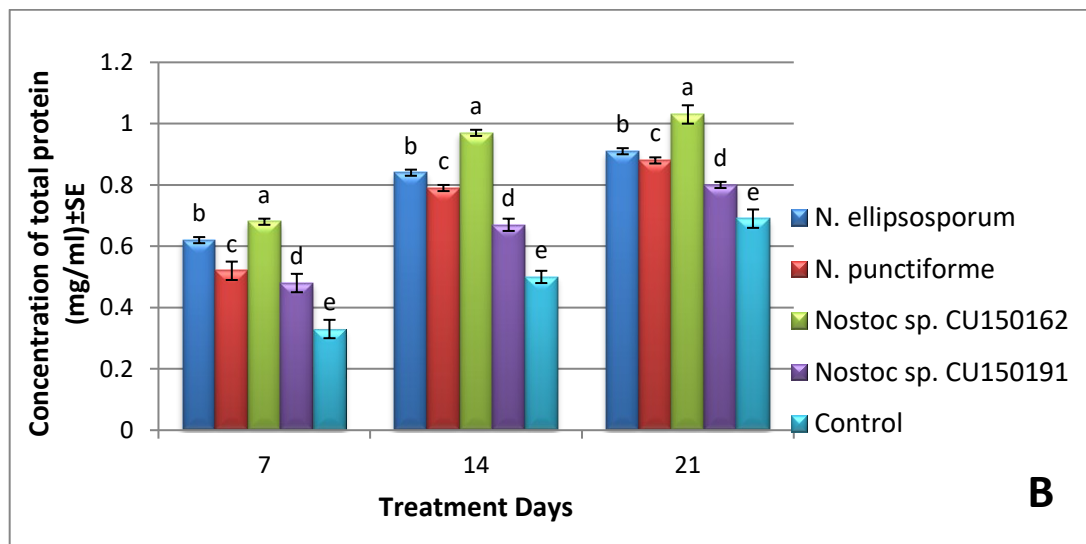
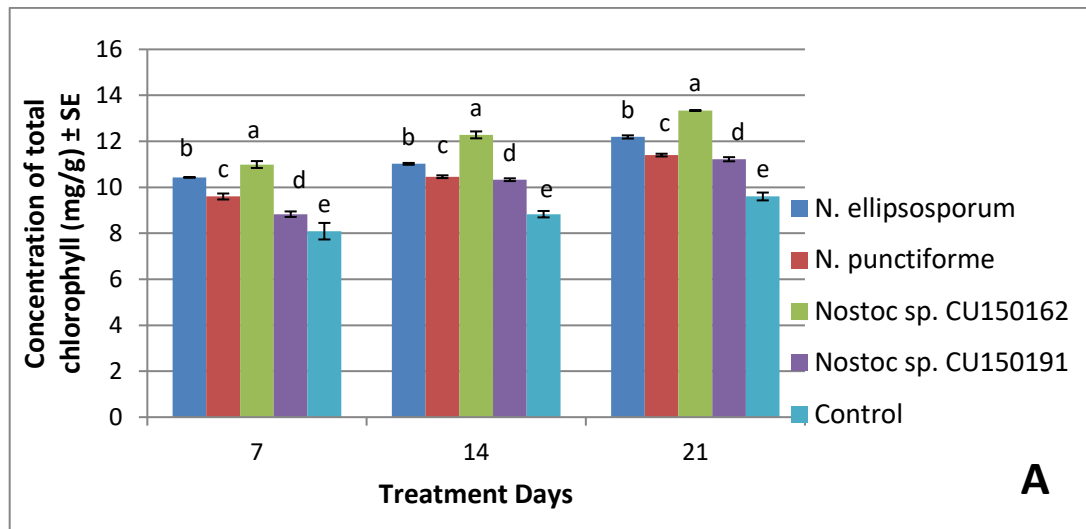
Fig. 7.5E Effects of *Nostoc* spp. on the total sugar content of rice variety Jyothi on the 7th, 14th and 21st day of intervals. Data presented as mean±SE for each treatment. The different letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

Table 7.6 Effects of *Nostoc* spp. on the biochemical parameters of rice variety Jyothi on the 7th, 14th and 21st day of intervals.

Biochemical characters	Days	<i>N. elliposporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191	Control
Total chlorophyll content (mg/g±SE)	7	11.37±0.05 ^b	10.95±0.25 ^c	12.13±0.03 ^a	10.94±0.12 ^c	9.76±0.02 ^d
	14	12.39±0.08 ^b	11.42±0.22 ^c	12.63±0.09 ^a	11.33±0.12 ^c	10.05±0.13 ^d
	21	12.83±0.06 ^b	12.19±0.17 ^c	13.92±0.36 ^a	11.74±0.05 ^c	10.79±0.10 ^d
Total protein content (mg/ml±SE)	7	0.75±0.01 ^b	0.67±0.01 ^c	0.86±0.02 ^a	0.61±0.01 ^d	0.43±0.01 ^e
	14	0.86±0.01 ^b	0.73±0.01 ^c	0.91±0.01 ^a	0.71±0.02 ^d	0.517±0.02 ^e
	21	0.98±0.01 ^b	0.87±0.01 ^c	1.17±0.03 ^a	0.81±0.01 ^d	0.66±0.02 ^e
Total sugar content (mg/ml±SE)	7	15.17±0.03 ^b	13.33±0.19 ^c	18.20±0.22 ^a	12.87±0.29 ^c	10.29±0.14 ^d
	14	17.49±0.08 ^b	15.74±0.02 ^c	21.98±0.11 ^a	14.47±0.30 ^c	11.92±0.17 ^d
	21	18.86±0.18 ^b	17.75±0.15 ^c	23.87±0.15 ^a	17.01±0.3 ^c	14.16±0.17 ^d

Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

As the total chlorophyll content, total protein and total sugar content of the treated plants of Annapurna showed a significant statistical difference at different intervals (Table 7.7). The highest chlorophyll content, total protein and total sugar content were recorded in the Annapurna variety inoculated with *Nostoc* sp. CU150162 with 13.34 ± 0.02 mg/g, 1.03 ± 0.01 mg/ml and 22.62 ± 0.52 mg/ml on the 21st day (Fig.7.6A-7.6C).



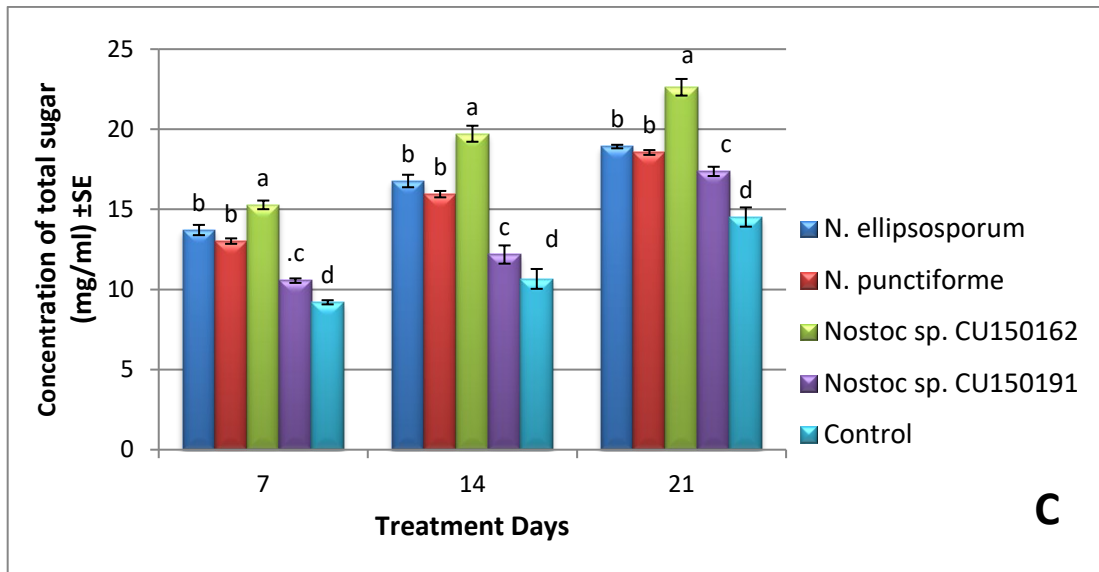


Fig. 7.6A-7.6C Effects of *Nostoc* spp. on the **A)** total chlorophyll content **B)** total protein content **C)** total sugar content of rice variety Jyothi on the 7th, 14th and 21st day of intervals. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

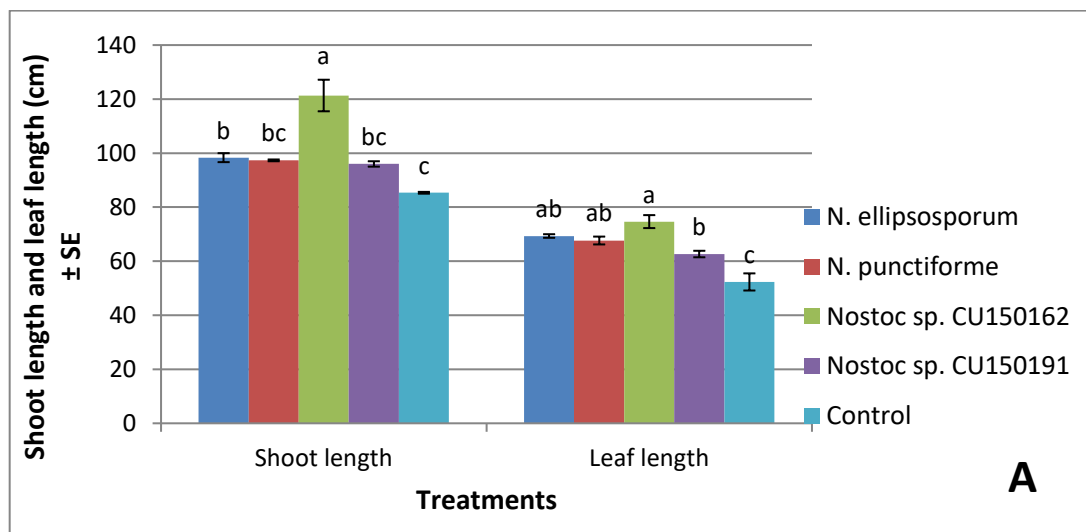
Table 7.7 Effects of *Nostoc* spp. on the biochemical parameters of rice variety Annapurna on the 7th, 14th and 21st day of intervals.

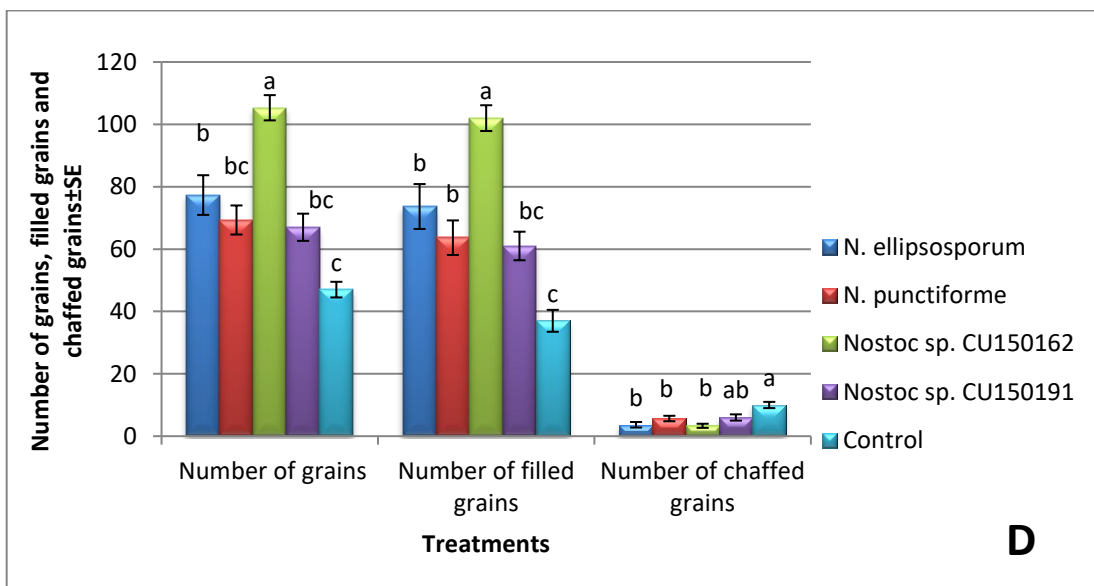
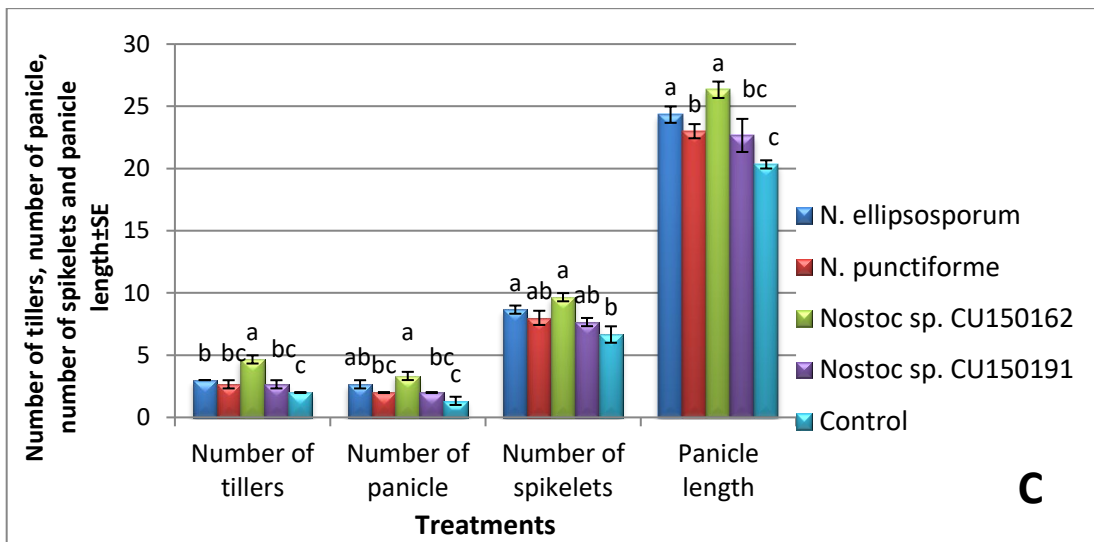
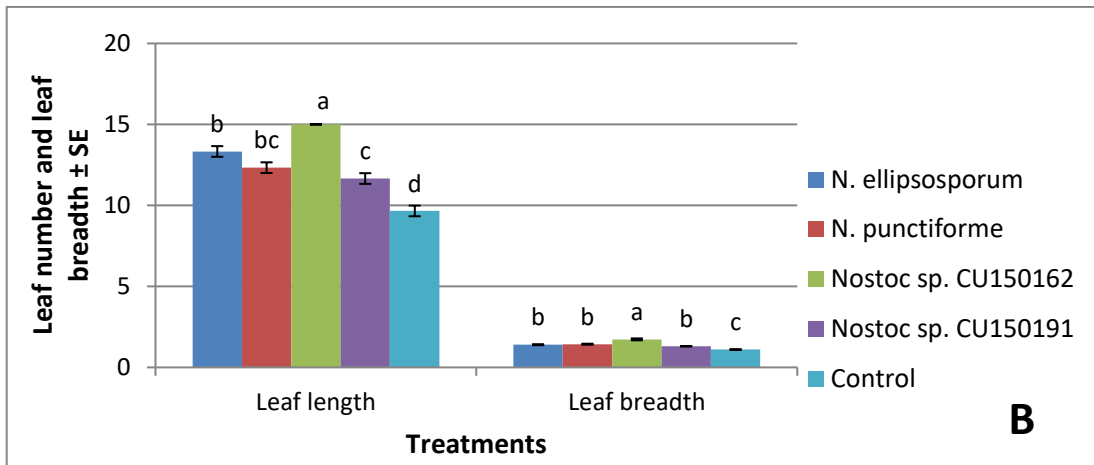
Biochemical characters	Days	<i>N. elliposporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191	Control
Total chlorophyll content (mg/g±SE)	7	10.43±0.0 ^b	9.60±0.13 ^c	10.99±0.15 ^a	8.83±0.20 ^d	8.09±0.36 ^e
	14	11.02±0.04 ^b	10.46±0.06 ^c	12.28±0.19 ^a	10.33±0.06 ^d	8.83±0.14 ^e
	21	12.19±0.07 ^b	11.40±0.06 ^c	13.34±0.02 ^a	11.22±0.09 ^d	9.6±0.17 ^e
Total protein content (mg/ml±SE)	7	0.62±0.01 ^b	0.52±0.03 ^c	0.68±0.01 ^a	0.48±0.03 ^d	0.33±0.03 ^e
	14	0.84±0.01 ^b	0.79±0.01 ^c	0.97±0.01 ^a	0.67±0.02 ^d	0.50±0.02 ^e
	21	0.91±0.01 ^b	0.88±0.01 ^c	1.03±0.01 ^a	0.80±0.01 ^d	0.69±0.03 ^e
Total sugar content (mg/ml±SE)	7	10.76±0.17 ^b	10.03±0.42 ^b	13.01±0.52 ^a	8.54±0.34 ^c	7.14±0.21 ^d
	14	16.77±0.42 ^b	15.95±0.20 ^b	19.72±0.50 ^a	12.18±0.57 ^c	10.66±0.62 ^d
	21	19.81±0.53 ^b	18.55±0.15 ^b	24.42±1.03 ^a	17.76±0.12 ^c	14.20±0.23 ^d

Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

7.2.2 Effects of *Nostoc* spp. on the yield of rice varieties at the harvesting time.

Different morphological and yield characters such as shoot length, leaf number, leaf length, leaf breadth, number of tillers, number of panicles, panicle length, number of grains, number of filled grains, number of chaffed grains and weight of 50 grains were analysed at the harvesting time of Jyothi and Annapurna varieties. All the morphological and yield characters were significantly augmented in the Jyothi variety inoculated with *Nostoc* spp. (Table. 7.8). Among various treatments, plants treated with *Nostoc* sp. CU150162 showed better results followed by *N. elliposporum* FY150125, *N. punctiforme* FY150188 and *Nostoc* sp. CU150191. During the harvesting period the shoot length (121.133 ± 5.8 cm), leaf number (15.00 ± 0 cm), leaf length (67.66 ± 1.45 cm), leaf breadth (1.73 ± 0.06 cm), number of tillers (4.66 ± 0.33), number of panicles (3.66 ± 0.33), panicle length (26.33 ± 0.66), number of spikelets (9.66 ± 0.33), number of grains (105.33 ± 4.05), number of grains filled (102 ± 4.16), number of chaffed grains (3.33 ± 0.66) and weight of 50 grains (1.56 ± 0.01 g) was observed maximum in the Jyothi variety inoculated with *Nostoc* sp. CU150162 (Fig. 7.7A-7.7E). Total chlorophyll, total protein and total sugar were analysed on the 75 DAT (days after transplanting) in the Jyothi variety (Table 7.9). Higher biochemical contents were achieved by the plants treated with *Nostoc* sp. CU150162 followed by *N. elliposporum* FY150125 (Fig. 7.8A-7.8E).





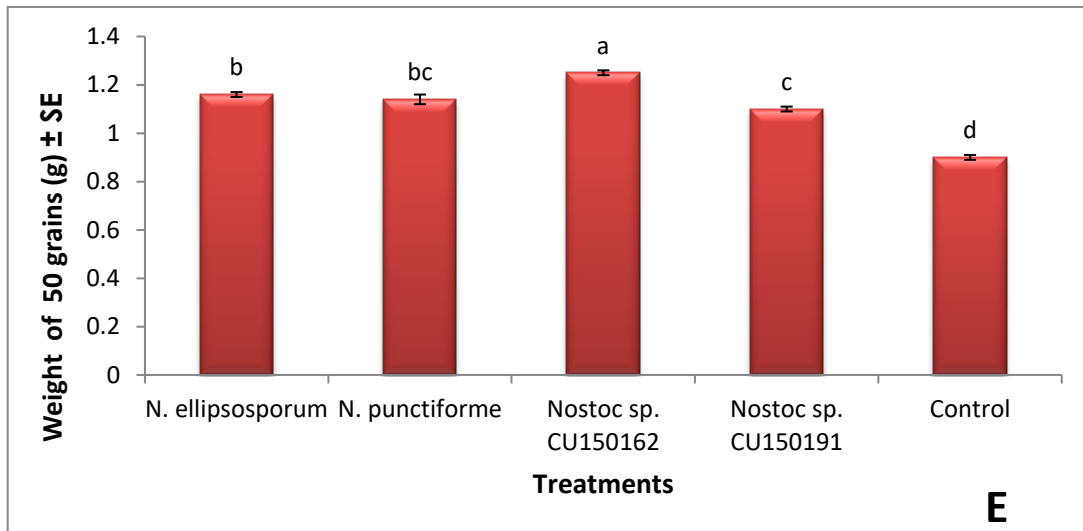


Fig. 7.7A-7.7E Effects of *Nostoc* spp. on the yield parameters of Jyothi variety. **A)** shoot and leaf length **B)** leaf number and leaf breadth **C)** number of tillers, number of panicles, number of spikelets and panicle length **D)** number of grains, number of filled grains and number of chaffed grains **E)** weight of 50 grains. Data presented as mean \pm SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

Table 7.8. Effects of *Nostoc* spp. on yield parameters of rice variety Jyothi on the harvesting time.

Treatments	Shoot length (cm)	Leaf number	Leaf length (cm)	Leaf breadth (cm)	No. of tillers per plant	No. of panicle	Panicle length (cm)	No. of spikelets per panicle	No. of grains	No. of grains filled	No. of chaffed grains	Weight of 50 grains (g)
<i>N. elliposporum</i> FY150125	98.33±1.66 ^b	13.33±0.33 ^b	74.66±2.40 ^{ab}	1.43±0.33 ^b	3±0 ^b	2.6±0.33 ^{ab}	24.33±0.66 ^a	8.66±0.33 ^a	77.33±6.33 ^b	73.33±7.2 ^b	3.66±0.88 ^b	1.30±0.01 ^b
<i>N. punctiforme</i> FY150188	97.33±0.33 ^{bc}	12.33±0.33 ^{bc}	69.33±0.66 ^{ab}	1.43±0.33 ^b	2.66±0.33 ^b	2.0±0 ^{bc}	23±0.57 ^{ab}	8±0 ^{ab}	69.33±4.67 ^b	63.66±5.54 ^b	5.66±0.88 ^b	1.27±0.01 ^{bc}
<i>Nostoc</i> sp. CU150162	121.133±5.8 ^a	15.00±0 ^a	67.66±1.45 ^a	1.73±0.06 ^a	4.66±0.33 ^a	3.66±0.33 ^a	26.33±0.66 ^a	9.66±0.33 ^a	105.33±4.05 ^a	102±4.16 ^a	3.33±0.66 ^b	1.56±0.01 ^a
<i>Nostoc</i> sp. CU150191	96.00±1.00 ^{bc}	11.66±0.33 ^c	62.66±1.20 ^b	1.30±0 ^b	2.66±0.33 ^{bc}	2±0 ^{bc}	22.66±1.33 ^{ab}	7.66±0.33 ^{ab}	67±4.36 ^{bc}	61±4.5 ^{bc}	6±1 ^{ab}	1.23±0.02 ^c
Control	85.33±0.33 ^c	9.66±0.33 ^d	52.33±3.17 ^c	1.10±0 ^c	2±0 ^c	1.33±0.33 ^c	20.33±0.33 ^b	6.66±0.33 ^b	47.66±2.51 ^c	37±3.5 ^c	10±1 ^a	1.11±0.02 ^d

Data presented as mean±SE for each treatment. The different letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

Table 7.9. Effects of *Nostoc* spp. on biochemical parameters of rice variety Jyothi on the 75 DAT.

Treatments	Total chlorophyll content (mg/g±SE)	Total protein content (mg/ml± SE)	Total sugar content (mg/ml± SE)
<i>N. elliposporum</i> FY150125	12.86±0.10 ^b	1.01±0.25 ^b	19.60±0.14 ^b
<i>N. punctiforme</i> FY150188	12.73±0.25 ^b	0.87±0.02 ^c	18.76±0.20 ^b
<i>Nostoc</i> sp. CU150162	14.46±0.13 ^a	1.20±0.01 ^a	24.01±0.09 ^a
<i>Nostoc</i> sp. CU150191	12.19±0.24 ^b	0.83±0.01 ^c	18.18±0.44 ^b
Control	11.15±0.11 ^c	0.72±0.03 ^d	15.52±0.30 ^c

Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

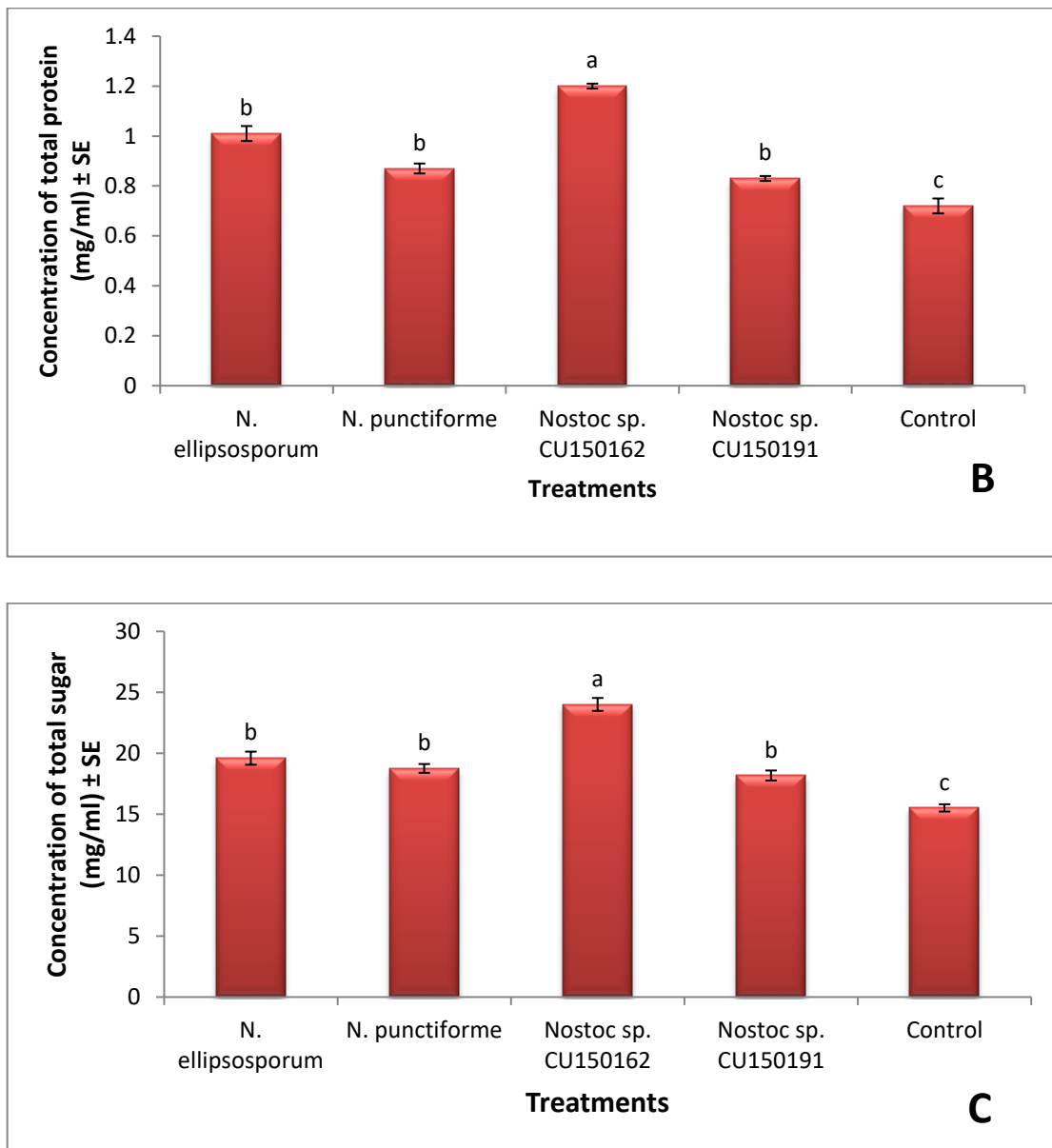


Fig. 7.8A-7.8C Effects of *Nostoc* spp. on the biochemical parameters of Jyothi variety on 75 DAT. **A)** Total chlorophyll content **B)** total protein content **C)** total sugar content. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

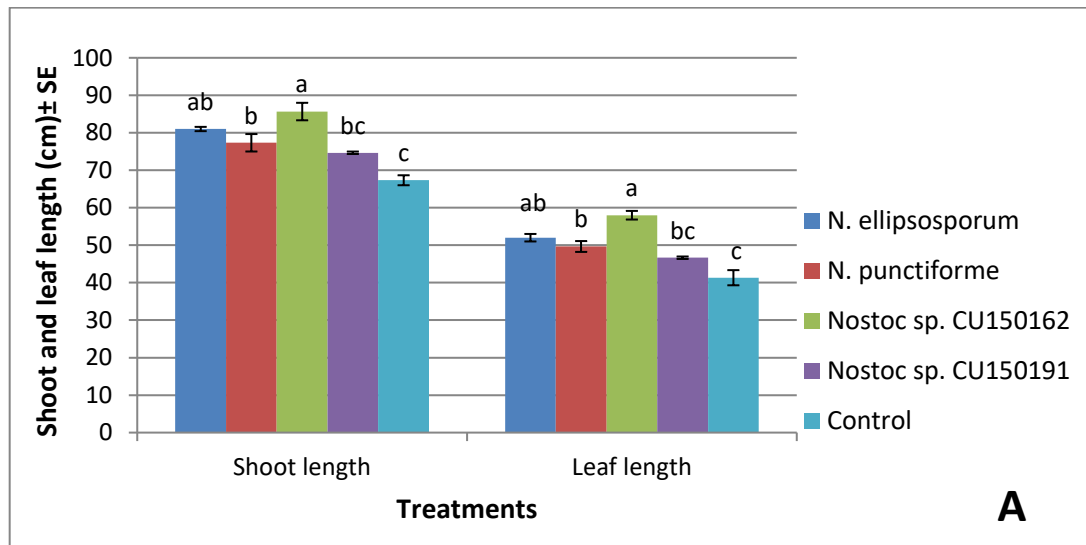


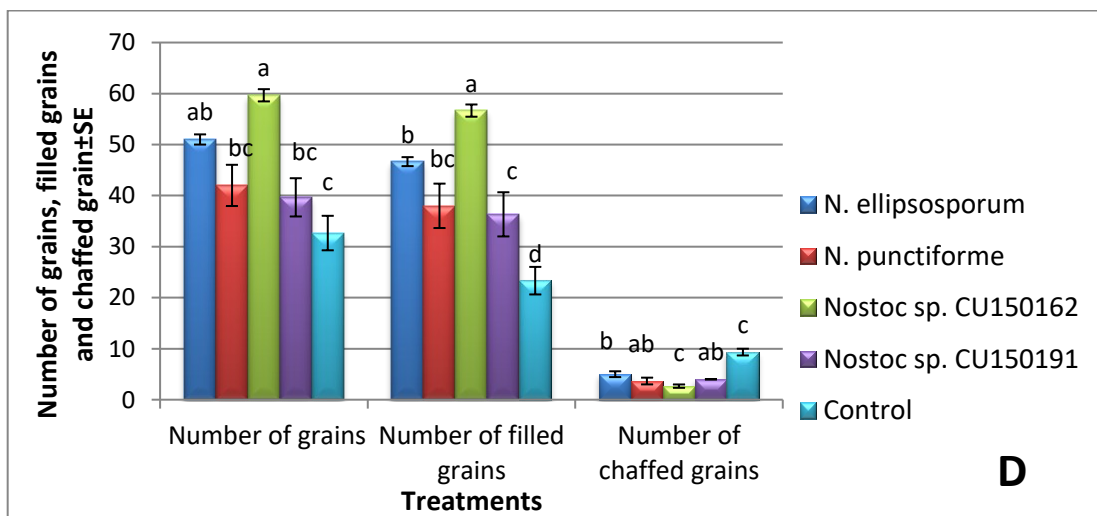
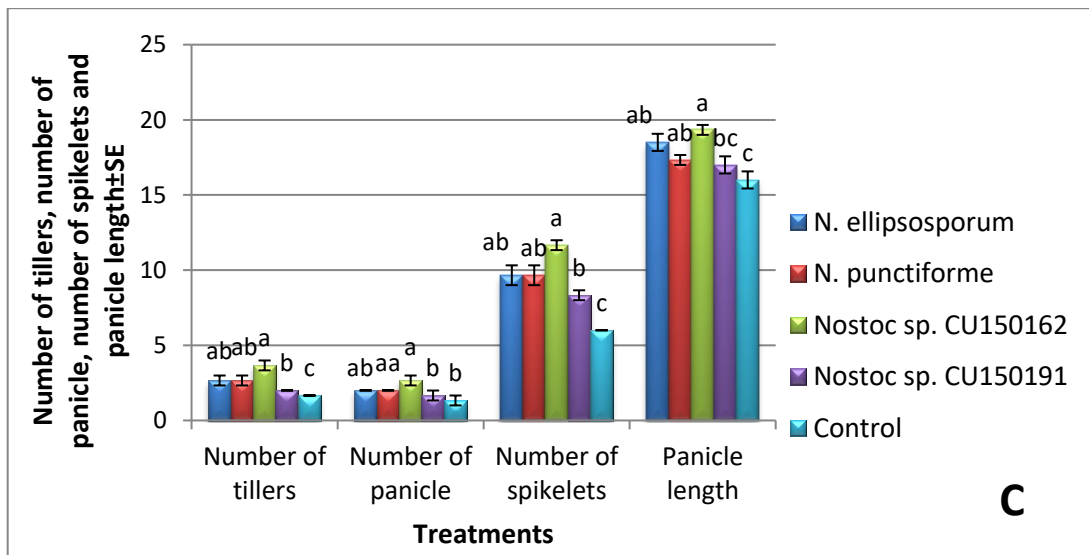
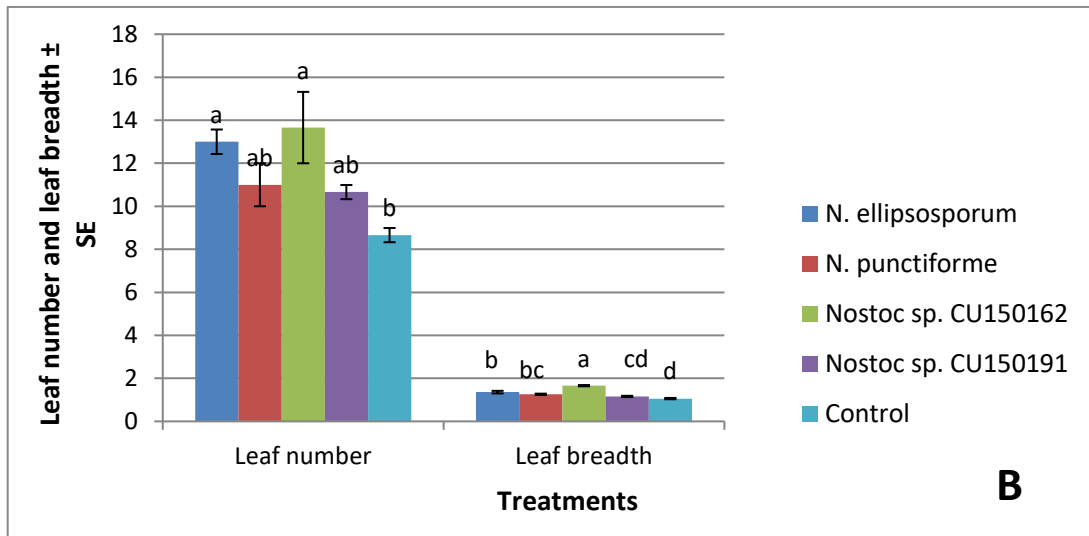
Fig. 7.8D Effects of *Nostoc* spp. on the growth of Jyothi variety. a) *N. elliposporum* FY150125 b) *Nostoc* sp. CU150162 c) *N. punctiforme* FY150188 d) *Nostoc* sp. CU150191 e) Control



Fig. 7.8E Effects of *Nostoc* spp. on the grain yield of Jyothi plants. a) Control b) *N. elliposporum* FY150125 c) *Nostoc* sp. CU150162 d) *Nostoc* sp. CU150191 e) *N. punctiforme* FY150188

Highest shoot length (85.66 ± 2.33 cm), leaf number (13.66 ± 1.66), leaf length (58.00 ± 1.15 cm), leaf breadth (1.66 ± 0.03 cm), number of tillers (3.66 ± 0.33), number of panicles (2.66 ± 0.33), panicle length (19.33 ± 0.33), number of spikelets (11.66 ± 0.33), number of grains (59.66 ± 1.20), number of grains filled (56.66 ± 1.2), number of chaffed grains (2.66 ± 0.33) and weight of 50 grains (1.25 ± 0.01) were observed maximum in the Annapurna variety inoculated with *Nostoc* sp. CU150162 followed by *N. ellipsosporum* FY150125 and *N. punctiforme* FY150188 (Fig. 7.9A-7.9E). The total number of grains, number of filled grains and weight of 50 grains are lowest in the control plants (Table 7.10). Different primary metabolites such as total chlorophyll, total protein and total sugar showed significant variations under the influence of *Nostoc* spp. on the Annapurna variety. The estimated biochemical parameters were lower in the control plants compared to the treated plants on the 75 DAT (Table 7.11). The control plants showed minimum yield compared to all treatments (Fig 7.10A-7.10E).





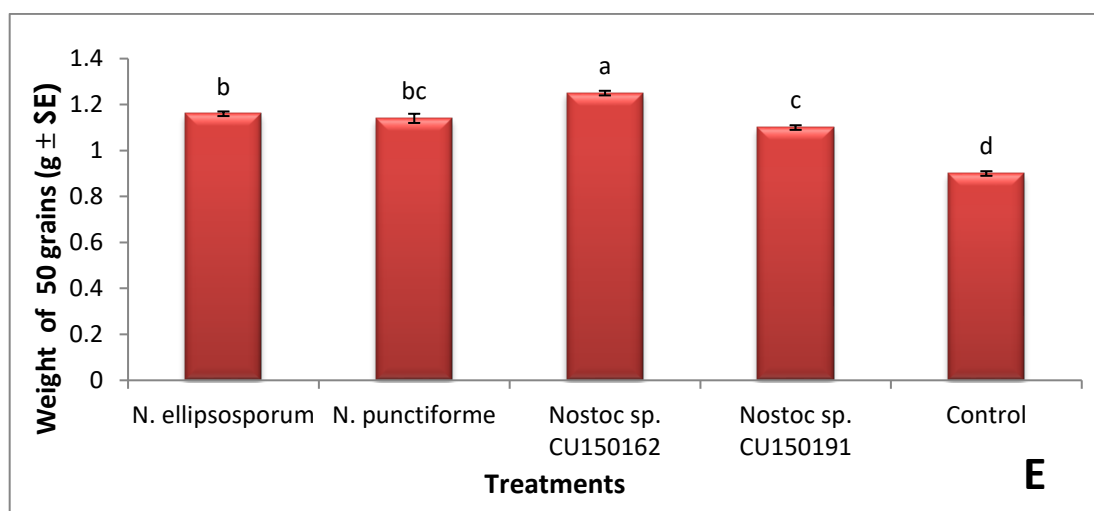


Fig. 7.9A-7.9E. Effects of *Nostoc* spp. on the yield parameters of Annapurna variety. **A)** shoot and leaf length **B)** leaf number and leaf breadth **C)** number of tillers, number of panicles, number of spikelets and panicle length **D)** number of grains, number of filled grains and number of chaffed grains **E)** weight of 50 grains. Data presented as mean \pm SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.

Table 7.10. Effects of *Nostoc* spp. on yield parameters of rice variety Annapurna on the harvesting time.

Treatments	Shoot length (cm)	Leaf number	Leaf length (cm)	Leaf breadth (cm)	No. of tillers per plant	No. of panicle	Panicle length (cm)	No. of spikelets per panicle	No. of grains	No. of grains filled	No. of chaffed grains	Weight of 50 grains (g)
<i>N. elliposporum</i> FY150125	81±0.57 ^{ab}	13.00±0.5 ^a	52.00±1.00 ^{ab}	1.36±0.06 ^b	2.66±0.33 ^{ab}	2.00±0.33 ^{ab}	18.5±0.57 ^{ab}	9.66±0.66 ^{ab}	51±0 ^{ab}	46.66±0.88 ^b	5±0.57 ^b	1.16±0.01 ^b
<i>N. punctiforme</i> FY150188	77.33±2.33 ^b	11.00±1.0 ^{ab}	49.66±1.45 ^b	1.26±0.03 ^{bc}	2.66±0.33 ^{ab}	2.00±0 ^{ab}	17.33±0.33 ^{abc}	9.66±0.66 ^{ab}	42±4.04 ^{bc}	38±4.35 ^b	3.66±0.66 ^{ab}	1.14±0.02 ^{bc}
<i>Nostoc</i> sp. CU150162	85.66±2.33 ^a	13.66±1.6 ^a	58.00±1.15 ^a	1.66±0.03 ^a	3.66±0.33 ^a	2.66±0.33 ^a	19.33±0.33 ^a	11.66±0.33 ^a	59.66±1.20 ^a	56.66±1.2 ^a	2.66±0.33 ^c	1.25±0.01 ^a
<i>Nostoc</i> sp. CU150191	74.66±0.33 ^{bc}	10.66±0.3 ^{ab}	46.66±0.33 ^{bc}	1.16±0.03 ^{cd}	2.00±0 ^b	1.66±0.33 ^{ab}	17.00±0.57 ^{bc}	8.33±0.33 ^b	41.66±4.33 ^{bc}	36.33±4.33 ^{bc}	4.0±0 ^{ab}	1.11±0.01 ^c
Control	67.33±1.33 ^c	8.66±0.33 ^b	41.33±2.02 ^c	1.06±0.03 ^d	1.66±0 ^b	1.33±0.33 ^b	16.00±0.57 ^c	6.00±0 ^c	32.66±3.38 ^c	23.3±2.7 ^c	9.33±0.66 ^a	0.97±0.01 ^d

Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

Table 7.11 Effects of *Nostoc* spp. on biochemical parameters of rice variety Annapurna on 75 DAT.

Treatments	Total chlorophyll content (mg/g±SE)	Total protein content (mg/ml±SE)	Total sugar content (mg/ml±SE)
<i>N. elliposporum</i> FY150125	12.91±0.10 ^{ab}	0.97±0.01 ^b	19.31±0.84 ^b
<i>N. punctiforme</i> FY150188	12.82±0.37 ^{ab}	0.93±0.01 ^{bc}	18.95±0.22 ^b
<i>Nostoc</i> sp. CU150162	14.16±0.13 ^a	1.08±0.01 ^a	22.82±0.18 ^a
<i>Nostoc</i> sp. CU150191	12.22±0.55 ^b	0.85±0.01 ^c	17.96±0.27 ^b
Control	10.44±0.12 ^c	0.75±0.03 ^d	14.92±0.30 ^c

Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p<0.05$.

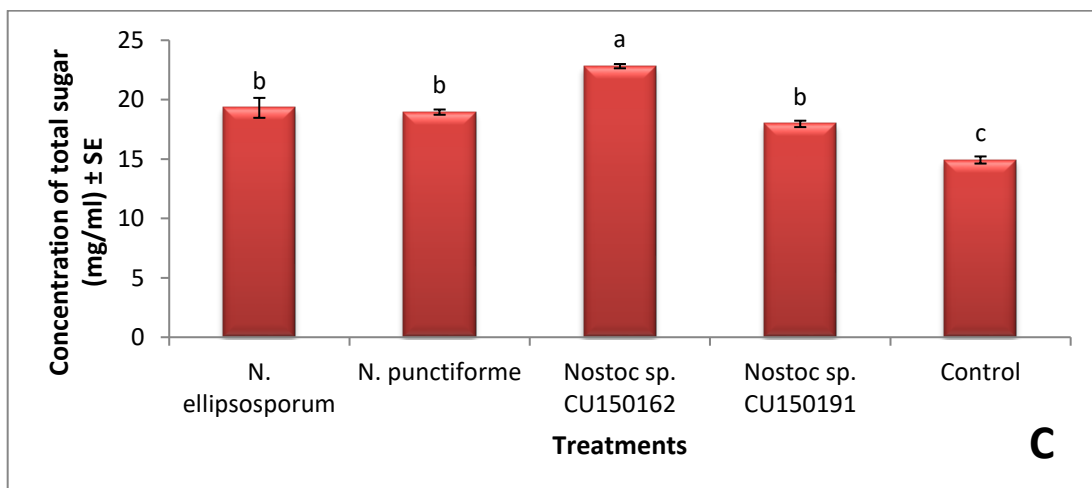
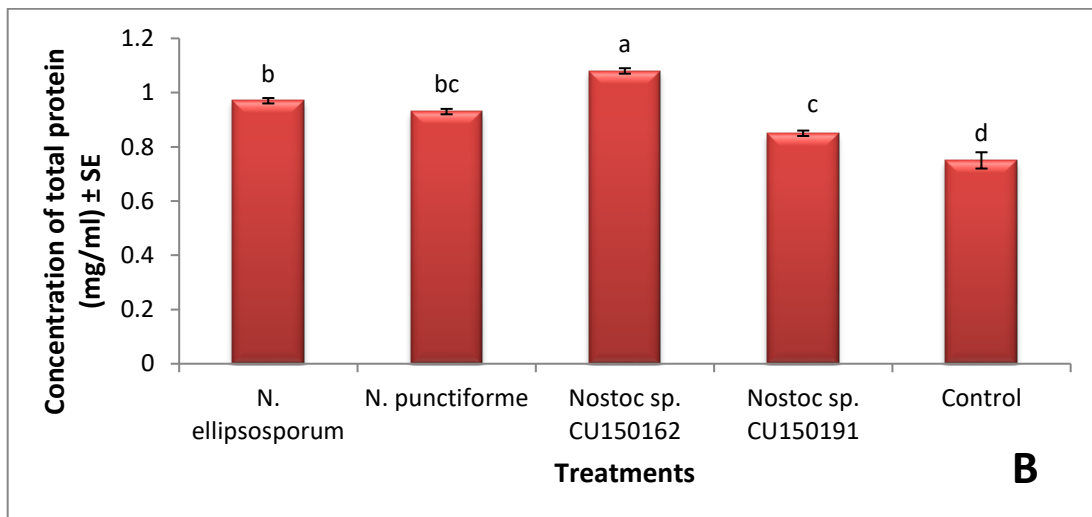
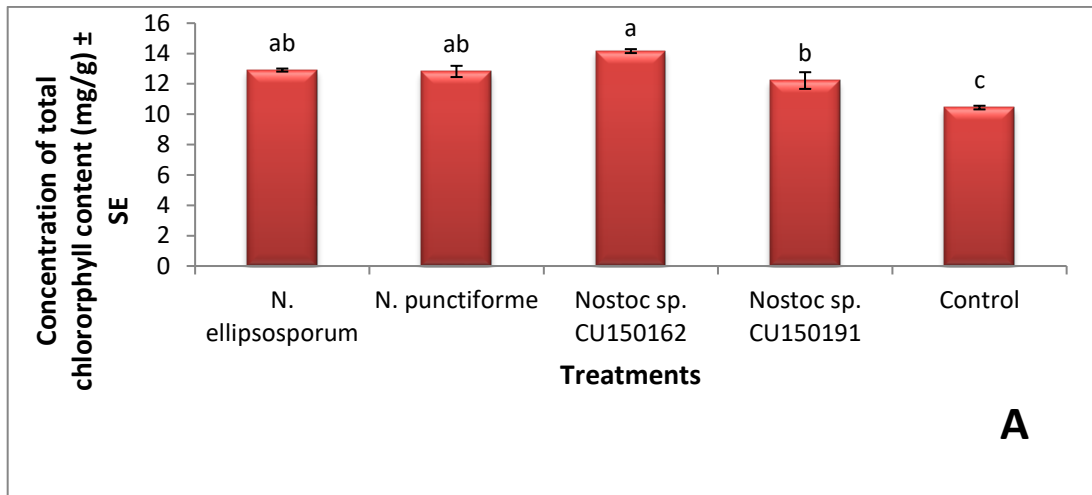


Fig. 7.10A-7.10C Effects of *Nostoc* spp. on the biochemical parameters of Annapurna variety on 75 DAT. **A)** Total chlorophyll content **B)** total protein content **C)** total sugar content. Data presented as mean±SE for each treatment. The letters indicate the different Tukey's grouping and the significant difference is $p < 0.05$.



Fig. 7.10D Effects of *Nostoc* spp. on the growth of Annapurna rice plants. a) Control b) *N. elliposporum* FY150125 c) *N. punctiforme* FY150188 d) *Nostoc* sp. CU150162 e) *Nostoc* sp. CU150191



Fig. 7.10E Effects of *Nostoc* spp. on the grain yield of Annapurna rice. a) Control b) *N. elliposporum* FY150125 c) *Nostoc* sp. CU150162 d) *Nostoc* sp. CU150191 e) *N. punctiforme* FY150188

7.2.2 Effects of *Nostoc* spp. on the elemental composition of rice seeds.

Elemental analysis was done to find out the percentage of different elements present in the grains of different treatments. The percentage of different elements such as carbon, nitrogen, hydrogen and sulphur were analysed from the grains using a CHNS analyser. The grains of the Jyothi variety were treated with the inoculum of different *Nostoc* spp. showed an increase in nitrogen, carbon, hydrogen and sulphur content compared to the seeds of the control plants (Fig.7.11-7.15). The highest percentage of carbon (39.10%), hydrogen (7.18%) and nitrogen (2.06%) were found in the grains of plants that were treated with *Nostoc* sp. CU150162 (Table 7.12). Whereas sulphur content was high in the grains of plants treated with *N. elliposporum* FY150125 (0.22%) followed by *Nostoc* sp. CU150162 (0.10%). In control, nitrogen content was very low compared to other grains of plants treated with *Nostoc* inoculum.

Table 7.12 Elemental analysis of grains of Jyothi variety determined by CHNS analysis.

Treatments	<i>N. elliposporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191	Control
Nitrogen (%)	2.00	1.92	2.06	1.85	1.23
Carbon (%)	38.80	39.10	39.10	38.86	38.62
Hydrogen (%)	7.14	6.85	7.18	6.84	6.62
Sulphur (%)	0.22	0.10	0.10	0.1	0.07
Totals (%)	48.17	47.99	48.43	47.65	46.55

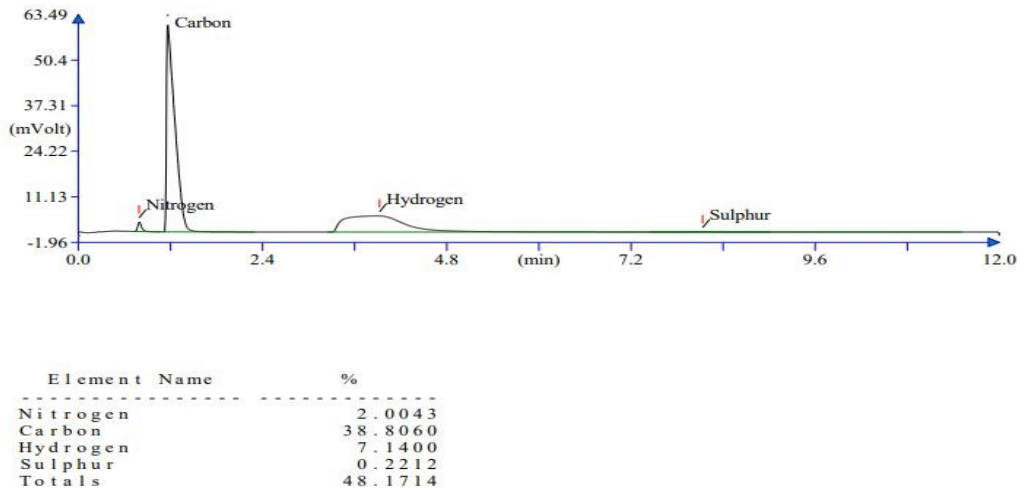


Fig. 7.11 CHNS chromatogram of rice grains of Jyothi variety inoculated with *N. elliposporum* FY150125

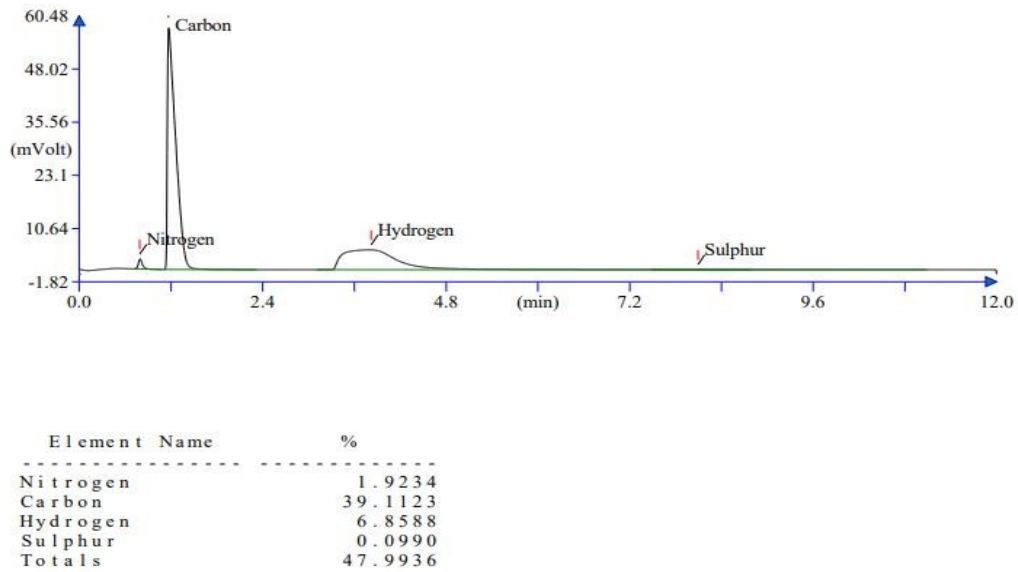
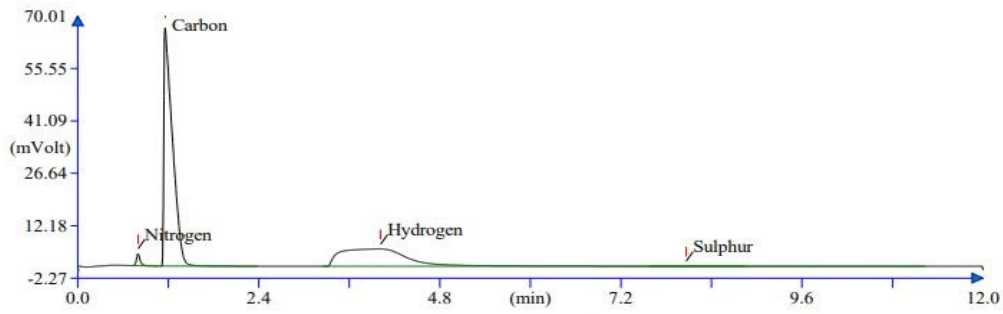
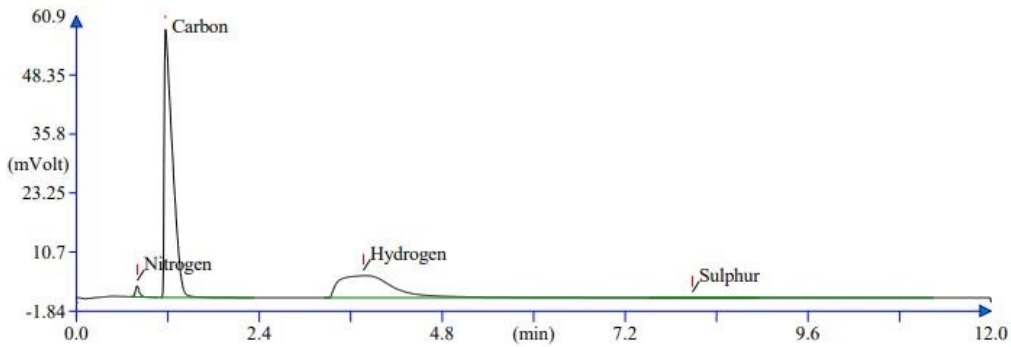


Fig. 7.12 CHNS chromatogram of rice grains of Jyothi variety inoculated with *N. punctiforme* FY150188



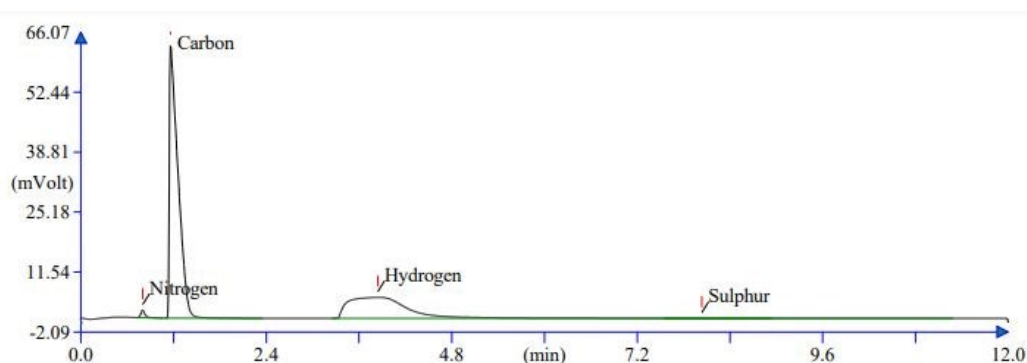
Element Name	%
Nitrogen	2.0587
Carbon	39.1002
Hydrogen	7.1784
Sulphur	0.1023
Totals	48.4396

Fig. 7.13 CHNS chromatogram of rice grains of Jyothi variety inoculated with *Nostoc* sp. CU150162



Element Name	%
Nitrogen	1.8552
Carbon	38.8624
Hydrogen	6.8400
Sulphur	0.0981
Totals	47.6556

Fig. 7.14 CHNS chromatogram of rice grains of Jyothi variety inoculated with *Nostoc* sp. CU150191



Element Name	%
Nitrogen	1.2320
Carbon	38.6236
Hydrogen	6.6225
Sulphur	0.0765
Totals	46.5547

Fig. 7.15 CHNS chromatogram of rice grains of Jyothi variety used as control

Annapurna variety, treated with different *Nostoc* spp., the grains of plants contained a high percentage of element composition (Table 7.13). The composition of carbon, nitrogen, hydrogen and sulphur was higher in the grains of plants treated with *Nostoc* sp. CU150162 is 40.12%, 2.78%, 7.12% and 0.17% respectively. The high percentage of nitrogen present in the grains of all treated plants compared to the control (1.53%) (Fig. 7.16-7.20).

Table 7.13 Elemental analysis of rice grains of Annapurna variety determined by CHNS analysis

Treatments	<i>N. ellipsosporum</i> FY150125	<i>N. punctiforme</i> FY150188	<i>Nostoc</i> sp. CU150162	<i>Nostoc</i> sp. CU150191	Control
Nitrogen (%)	2.72	2.50	2.78	2.46	1.53
Carbon (%)	39.52	39.66	40.12	39.63	39.14
Hydrogen (%)	6.77	6.81	7.12	6.65	6.56
Sulphur (%)	0.12	0.10	0.17	0.12	0.08
Totals (%)	49.13	49.07	50.19	48.88	47.33

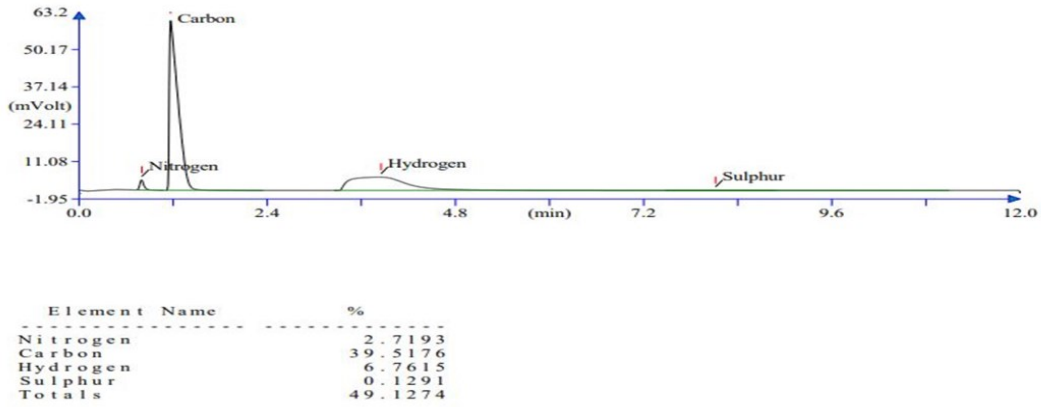


Fig. 7.16 CHNS chromatogram of rice grains of Annapurna variety inoculated with *N. elliposporum* FY150125

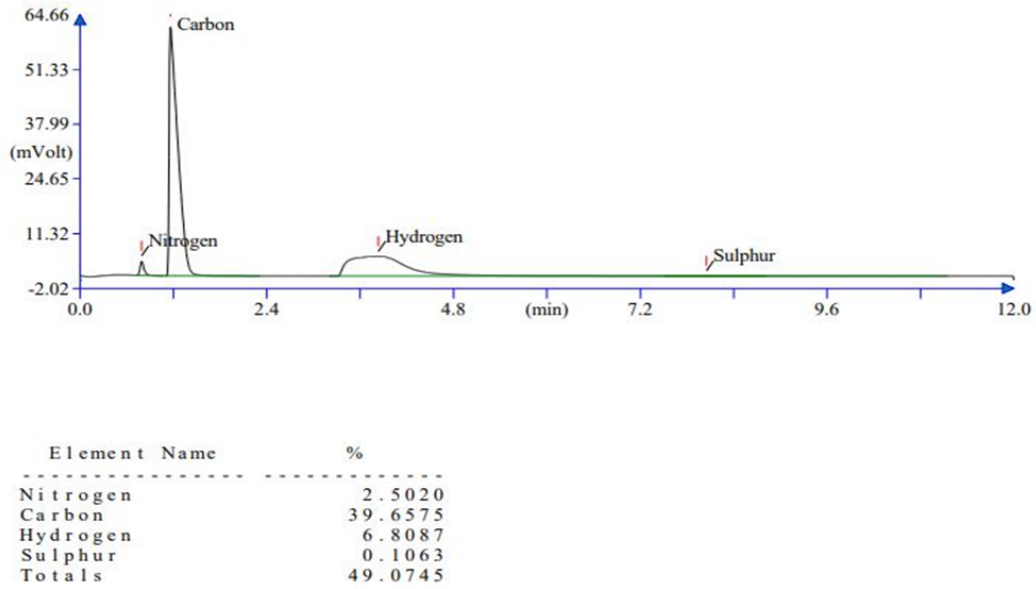
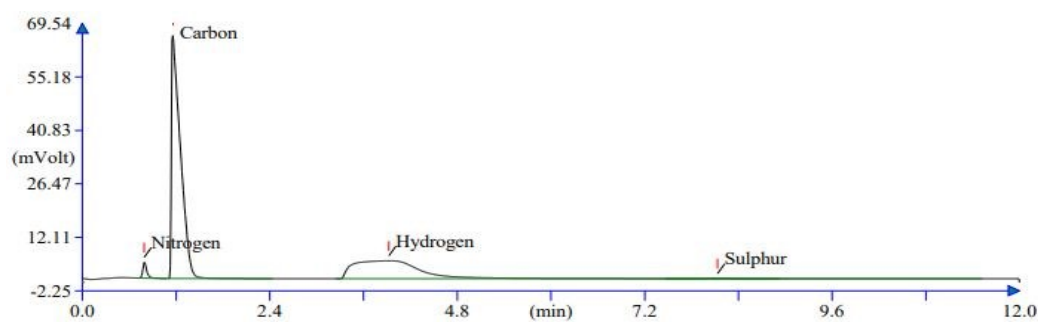
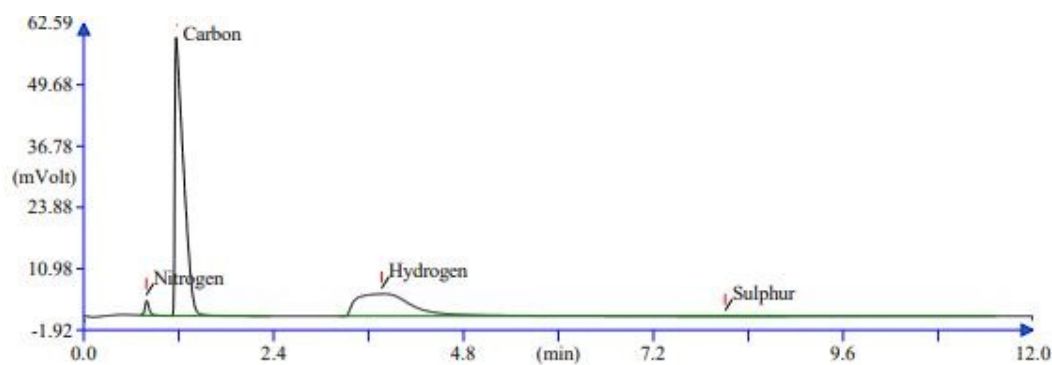


Fig 7.17 CHNS chromatogram of rice grains of Annapurna variety inoculated with *N. punctiforme* FY150188



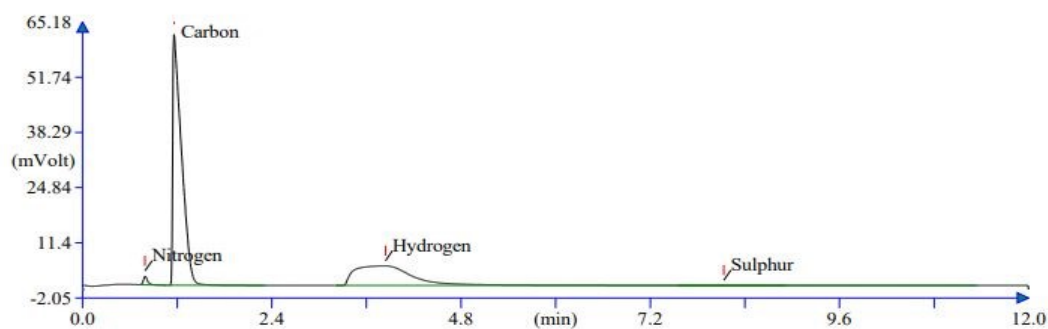
Element Name	%
Nitrogen	2.7826
Carbon	40.1157
Hydrogen	7.1206
Sulphur	0.1726
Totals	50.1916

Fig. 7.18 CHNS chromatogram of rice grains of Annapurna variety inoculated with *Nostoc* sp. CU150162



Element Name	%
Nitrogen	2.4616
Carbon	39.6395
Hydrogen	6.6572
Sulphur	0.1210
Totals	48.8793

Fig 7.19 CHNS chromatogram of grains of Annapurna variety inoculated with *Nostoc* sp. CU150191



Element Name	%
Nitrogen	1.5337
Carbon	39.1446
Hydrogen	6.5647
Sulphur	0.0872
Totals	47.3302

Fig. 7.20 CHNS chromatogram of rice grains of Annapurna variety control plant

Discussion

The use of chemical fertilizer is inevitable for obtaining optimum crop yield. However, it has been reported that continuous use of chemical fertilizers may disturb soil biological activities, e.g., enhancing the decomposition of soil organic matter, resulting in the degradation of soil structure (Chen, 2006). Biofertilizers are often referred as microbial inoculants, including bacteria (*Azotobacter*), cyanobacteria (blue-green algae), and mycorrhizal fungi, which play an essential role in providing nutrients for plants, regulating the dynamics of organic matter and improving soil biological activities (Koller *et al.*, 2012; Singh *et al.*, 2016). Nitrogen-fixing cyanobacteria are potential microorganisms used as biofertilizers to increase soil fertility and agricultural productivity. They can fix atmospheric N₂, improve the physical and chemical properties of soil, and produce some bioactive compounds that enhance plant growth (Kaushik, 2014). Heterocystous cyanobacteria improve crop production by acting as natural fertilizers as they increase both the carbon and nitrogen status of soils (Lange *et al.*, 1986). They can degrade a wide range of pollutants and perform various roles in the soil ecosystem to sustain soil fertility. According to Song *et al.* (2005), cyanobacteria can control nitrogen deficiency in plants, improve the aeration of the soil, and water holding capacity and

add vitamin B12. Furthermore, cyanobacteria are the dominant microorganisms in biological soil crusts and have been shown to promote soil stability and reduce water loss (Bu *et al.*, 2014). They can produce exopolysaccharides, which form a biofilm layer on which consequently other organisms are colonized and recruited. Regarding these features, cyanobacteria are suggested as good eco- and agro-friendly organisms to restore, maintain, and improve soil and land stability, fertility, quality, and productivity. The inoculation of cyanobacteria on plants or seeds influences various metabolic processes in plants since they activate the production of defense proteins that lead to greater immunity of plants against pathogens (Gupta *et al.*, 2013)

One of the major challenges facing agriculture today is producing enough food for an ever-growing world population. Rice is one of the most important cereal crops in the world and the major source of food for millions of households in dozens of countries. Rice plants (*Oryza sativa* L.) require a large quantity of water and mineral nutrients, including nitrogen and phosphorus, for their growth, development, and grain production (Qi *et al.*, 2020). To meet the crop's demands, around 16–17 kg N/ton of N fertilizer are used (Choudhury and Kennedy, 2004). *Nostoc* can survive on the surface of soil and rocks, and fix up to 20–25 kg/ha atmospheric nitrogen. Nitrogen fixation capability is not the only factor contributing to growth promotion, and the existence of several other bioactive chemicals, like plant growth regulators such as auxin, cytokinin, etc., also plays an important role in crop production (Hashtroudi *et al.*, 2013). The application of *Nostoc* spp. as a biofertilizer has been researched in rice, wheat, and peas (Sholkamy *et al.*, 2015; Takacs *et al.*, 2019). Likewise, in this study, *Nostoc* spp. were used as inoculants to enhance the yield of rice varieties Annapurna and Jyothi by pot experiment. The results suggested that the application of *Nostoc* spp. was effective in enhancing the growth of both rice varieties. Similar reports regarding the yield of rice plants due to the application of cyanobacteria have also been reported (Begum *et al.*, 2011).

The positive impact of the cyanobacterial filtrate on the germination process has been reported by several researchers. They demonstrated a significant increase

in seed germination of some crop plants such as wheat, sorghum, lentils and sugar beet as a result of the treatment with the culture filtrate or cell extract of *Nostoc muscorum* (Adam, 1999; Aly *et al.*, 2008). Numerous studies confirmed that the inoculation of cyanobacteria directly in the soil or by seed immersion or priming causes an increase in the germination rate, better development of plants and a higher productive yield in a wide variety of cereal, horticultural and vegetable crops (Rodríguez *et al.*, 2006; Saadatnia and Riahi, 2009). Moreover, the bio-priming of cucumber seeds with cyanobacterial extract revealed a remarkable promoter effect in the early stages of plant development and also acted as a biocontrol agent against damping-off caused by *Pythium ultimum* in cucumber seedbeds (Toribio *et al.*, 2021). In addition, Hassan and Morcos (2006) observed an enhancement in the seed germination of rice that was treated with the culture filtrates of *Nostoc calcicola*. Similarly, in the current study, it has been shown that bio-priming of seeds with *Nostoc* spp. enhances the seed germination and the morphological characteristics of rice seedlings in both rice varieties. The treatment of rice seeds *Pusa suganth* with *Calothrix* sp. showed better results in seedlings. The results showed two-fold increases in root length of inoculated seedlings to control. This study also corroborated our results, in which the bio-priming of rice seeds with *Nostoc* spp. enhances the seedling growth compared to hydro-primed seeds.

In the present study, it has been shown that using the cyanobacteria, *N. ellipso sporum*, FY150125 *N. punctiforme* FY150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191 as a biofertilizer, improved the growth and yield of rice varieties Jyothi and Annapurna similar to previous reports for rice, wheat, and pea (Karthikeyan *et al.*, 2007; Osman *et al.*, 2010; Dash *et al.*, 2016). Additionally, Chittapun *et al.* (2018) observed the effects of cyanobacteria (*N. carneum* and *N. commune*) and chemical fertilizer on growths and yields of rice (*Oryza sativa* L.) cv. Pathum Thani 1 in pot trials. The experimental set-up consisted of 1 kg soil with 1 g wet weight of *Nostoc* inoculum was used. Similarly, the current study also utilized 1 kg of sterilized soil with 1 g of wet *Nostoc* inoculum. The plants inoculated with *Nostoc* spp. showed a significant increase in root length, shoot length, number of grains and total weight of 100 grains. In addition, the treatment was applied with the

combination of *Nostoc* spp. resulted in better rice production in terms of quantity. The study also suggested that using half of the recommended dose (16–20–0 of 78.12 kg ha⁻¹ and 46–0–0 of 31.25 kg ha⁻¹) of chemical fertilizer with cyanobacteria was better for rice production.

A comparative analysis of pot experiments showed that there is a significant difference between the plants treated with *Nostoc* spp. and control in their morphological characters, especially in shoot length. Positive effects of *Nostoc punctiforme* on morphological characters such as shoot length, root length, and weight of rice plants were previously reported by Alvarez *et al.* (2020) under laboratory conditions. *N. punctiforme* is a model organism of plant-cyanobacteria symbiosis and the roots of plants treated with *N. punctiforme* showed double in length and weight than control plants in hydroponic conditions. In the current study, *N. punctiforme* FY150188 was used as a treatment to assess the effect of species on rice growth. Similarly, our results also corroborated with the findings of Alvarez *et al.*, (2020). Rice varieties Annapurna and Jyothi treated with *N. punctiforme* FY150188 showed better results than the control plants. Additionally, the findings of our results indicated that, the inoculation of plants with *Nostoc* spp. significantly improved all the yield characters especially the weight of grains. These findings corroborated well with the observations of Salama (2015) that individual or combinations of *Nostoc* sp. and *Anabaena* sp. significantly increased the crop yield and the weight of rice. Biofertilization with *N. entophytum* led to higher contents of chlorophyll-a, carotenoids and protein in pea plants (Osman *et al.*, 2010). Similarly, in this study, the inoculation with *Nostoc* spp. also significantly increased the chlorophyll, protein and sugar contents of rice plants.

Elemental analysis of grains showed the presence of different elements in a plant material is not only beneficial for plants but also the source of essential elements for those who consume it. Previously, most of the studies were mainly focused on the yield of crops. In our study, we aimed not only at the quantity of grains but also the quality of grains also analysed. The higher percent of carbon and hydrogen in rice grains means the higher quantity of carbohydrates, which provide

energy to the consumers (Karthikeyan and Kumaravel, 2016). Our results showed a high percentage of carbon, hydrogen, nitrogen and sulphur in the grains of treated plants than control. Two-fold increase in the sulphur and nitrogen was reported in the grains of plants treated with *Nostoc* sp. CU150162 than control. The results also concluded that, inoculation of rice plants with *Nostoc* spp. not only augmenting the yield of rice but also improving the quality of the grains.

CHAPTER 8

SUMMARY AND CONCLUSION

The cyanobacteria *Nostoc* is highly diverse in morphology, functional properties, biotic relations, and habitat distribution. They occur as gelatinous masses of variable shapes and colors in rice fields, freshwater lakes, ponds, streams, rock surfaces, in wet and dry soils, but rarely exist in marine habitats. The majority of the species are free-living and few species exhibit symbiotic associations with angiosperms, gymnosperms and bryophytes. The present investigation was focused on the morphological, biochemical and molecular characterization of *Nostoc* spp. and their assessment as a biofertilizer for rice varieties Annapurna and Jyothi.

The diversity of *Nostoc* spp. has not been completely explored in Kerala State. Hence, the present investigation is focussed exclusive on the biodiversity and taxonomy of *Nostoc* spp. from Kerala. The climate of Kerala State is ideal for the growth of *Nostoc* spp. The cyanobacterial samples were collected from different geographical areas of Kerala. A total of thirty-nine *Nostoc* spp. were identified. Among them, thirty-four species were identified up to the species level and the remaining five species were identified up to the genus level using the keys and descriptions of standard taxonomic manuals. *Nostoc* spp. can be found in a wide range of habitats including rock surfaces (epilithic), soil of paddy fields (epipsammic), attached to plants (epiphytic), freshwater bodies (benthic) and bark of trees (corticolous).

Nostoc spp. existing diverse colours in nature, including brown, blackish green, pale green, dark green, reddish brown and violet. The colour of *Nostoc* thallus on the substratum depends on the pigments present in the cells. During this study, most of the species were collected from rock surfaces of the Western Ghats region of Kerala. Due to the great topographic heterogeneity and strong rainfall tremendous species variability of *Nostoc* exists in the Western Ghats regions of Kerala. The highest number of *Nostoc* spp. was observed from Sholayar (Thrissur-10 species) followed by Vagamon (Idukki-6 species) and Kakkayam (Kozhikode-5 species). Among the identified *Nostoc* spp., 25 species are epilithic, 6 species are epipsammic, 2 species co-occur in epilithic and epiphytic, 2 species co-occur in

epilithic and epipsammic, 2 species co-occur in epilithic and corticolous, 1 species co-occur in epilithic, epipsammic and benthic and 1 species co-occur in epilithic, epiphytic and epipsammic habitat.

Nostoc aureum f. *variabilis*, *N. bornetii*, *N. caeruleum*, *N. calcicola*, *N. carneum*, *N. citrisporum*, *N. comminutum*, *N. commune*, *N. edaphicum*, *N. ellipso sporum*, *N. ellipso sporum* var. *violaceum*, *N. flagelliforme*, *N. fuscescens*, *N. gelatinosum*, *N. hatei*, *N. humifusum*, *N. indistinguendum*, *N. interbryum*, *N. kihlmanii*, *N. linckia*, *N. locularis*, *N. microscopicum*, *N. minutum*, *N. muscorum*, *N. nylstromicum*, *N. oryzae*, *N. paludosum*, *N. passerinianum*, *N. pruniforme*, *N. punctiforme*, *N. sphaericum*, *N. sphaeroides*, *N. tibeticum*, *N. undulatum*, *Nostoc* sp. CU150162, *Nostoc* sp. CU150191, *Nostoc* sp. CU167213, *Nostoc* sp. CU150122 and *Nostoc* sp. CU150172 were the identified *Nostoc* species. *N. indistinguendum*, *N. locularis* and *N. tibeticum* are new records of the cyanobacterial flora of India. A total of 11 *Nostoc* spp. were reported as new to cyanobacterial flora of Kerala. *N. aureum* f. *variabilis*, *N. citrisporum*, *N. edaphicum*, *N. flagelliforme*, *N. fuscescens*, *N. indistinguendum*, *N. kihlmanii*, *N. locularis*, *N. pruniforme*, *N. sphaericum* and *N. tibeticum* are the new addition to cyanobacterial flora of Kerala. Most of the diversity studies of *Nostoc* spp. concentrated on the paddy fields of Kerala.

Among the species identified using morphological characters, four were selected for further studies based on their quick isolation procedure, culturing and convenience for mass cultivation. Axenic cultures of the selected species were done by continuous sub-culturing in BG-11₀ medium. 16S rRNA gene sequencing was used for the correct identification of species and the evaluation of relationships between them. Molecular characterization based on 16S rRNA gene sequencing confirmed that the selected species were *Nostoc ellipso sporum* FY150125, *N. punctiforme* FY150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191. The sequences of the amplified genes were submitted in NCBI and accession numbers were obtained. Phylogenetic analysis using neighbor-joining and maximum likelihood method for the selected *Nostoc* spp., along with sequences retrieved from NCBI, showed the evolutionary distance and interrelationship among the species. In the neighbor-joining tree, *N. ellipso sporum* FY150125, *N. punctiforme* FY150188 and *Nostoc* sp. CU150191 is grouped in cluster 1 and cluster 2 consists of *Nostoc* sp. CU150162.

Nostoc produces a wide range of secondary metabolites with antibacterial, antifungal, antitumor, enzyme inhibiting, immunostimulant, nematicidal, insecticidal and herbicidal activities. Biochemical characterization of *Nostoc* spp. done by analysing the chlorophyll-a content and GC-MS analysis to develop a chemotaxonomic profile of *Nostoc* spp. *Nostoc* sp. CU150162 showed maximum chlorophyll content with 3.71 ± 0.08 $\mu\text{g/ml}$ on the 21st day. GC-MS analysis of the methanolic extract of *Nostoc* spp. showed the presence of 24 compounds belonging to different chemical groups, including methyl esters of fatty acids, steroids/triterpenes, alkanes, diterpenes, and acyclic diterpene alcohols. Phytol, heptadecane, lupeol, methyl palmitate, methyl stearate, neophytadiene, propyl hexanoate, isobutyl caproate, 9, 12-octadecadienoic acid, methyl ester, 10-octadecenoic acid, methyl ester and guanosine were the major bioactive compounds identified from *Nostoc* spp. Neophytadiene, phytol and heptadecane are the major bioactive compounds detected in all selected *Nostoc* spp. Lupeol, propyl hexanoate, and isobutyl caproate are reported for the first time in *N. ellipsosporum*. Guanosine is an alkaloid constructed with a guanine base, which is present in *N. punctiforme* with 75.86% of quantity, and also in *Nostoc* sp. CU150191 (18.97%).

Systematics of the genus *Nostoc* is difficult; however, an attempt was made to classify them based on secondary metabolites. Cluster analysis using PAUP was performed on *Nostoc* spp. based on the presence of major bioactive compounds. The dendrogram derived from the active compounds generated two main clusters. Cluster 1 consists of *N. ellipsosporum* FY150125, *N. punctiforme* FY150188 and *Nostoc* sp. CU150191. Cluster 2 consists of *Nostoc* sp. CU150162, which contains more bioactive compounds compared to other selected *Nostoc* spp. Principal component analysis and agglomerative hierarchical clustering of the compounds were also performed based on the relative percentage of the identified compounds from *Nostoc* spp. using XLSTAT statistical software. PCA was used to correlate the percentage of bioactive compounds with *Nostoc* spp. Dendrogram constructed by heat map, clustering *Nostoc* spp. in 2 clusters and cluster 1 consists of spp. *N. ellipsosporum*, *N. punctiforme* and *Nostoc* sp. CU150191 and cluster 2 consist of *Nostoc* sp. CU150162. A Venn diagram was also generated using the software Biovinci 2.0 to represent similar and dissimilar compounds in *Nostoc* spp. In the

Venn diagram, it was clear that three compounds (Phytol, neophytadiene and heptadecane) were identified in all selected species.

Nostoc spp. can be used as a biofertilizer in paddy fields due to its nitrogen-fixing capacity and they can release various bioactive compounds into the soil that are beneficial to the growth of rice. Being the most important food crop, improving the productivity and quality of rice is always crucial. Bio-priming is a novel method of seed treatment that attracted much attention for its potential to induce seed germination and enhance seedling growth. In this study, four selected species of *Nostoc* were co-cultivated with rice seeds and analysed the effect of the species on paddy growth. The selected four species were *N. elliposporum* FY150125 and *Nostoc* sp. CU150162 were epipsammic whereas *N. punctiforme* FY150188 and *Nostoc* sp. CU150191 as epilithic. These four species showed fast growth and adapted better to the growth medium. Bio-priming of rice seeds, Jyothi and Annapurna with *Nostoc* spp. enhanced the germination percentage and seedling growth than hydro-primed seeds. Morphological (shoot length, leaf number, leaf length and leaf breadth) and biochemical characters (total chlorophyll, total protein and total sugar content) of rice varieties treated with *Nostoc* spp. were monitored on the 7th, 14th and 21st days after the multiplication of *Nostoc* spp. in soil and also assessed during the harvesting time. Compared to the control plants, the vegetative and biochemical characteristics of the rice varieties inoculated with *Nostoc* spp. showed higher performance. Maximum growth of the rice varieties significantly increased in the plants treated with *Nostoc* sp. CU150162, followed by *N. elliposporum* FY150125, *N. punctiforme* FY150188 and *Nostoc* sp. CU150191.

Yield parameters such as total number of grains and weight of grains were higher in treated plants, proving that the potential of four strains as biofertilizers. Previous studies mainly focused on the productivity of rice using the inoculation of cyanobacteria, but our research focused on the quantity as well as the quality of the grains. In this investigation, the elemental contents of grains (percentage of carbon, nitrogen, hydrogen and sulphur) of treated plants were analysed using a CHNS analyzer. The percentage of all the analyzed elements was higher in the grains of

inoculated plants. Two-fold increases in the nitrogen and sulphur were reported in the grains of plants treated with *Nostoc* sp. CU150162 than control. The results also concluded that, inoculation of rice plants with *Nostoc* spp. not only augmented the yield of rice but also improved the quality of the grains. These findings suggested that *Nostoc* spp. are potential candidates for their application to develop eco-friendly and sustainable agricultural practices. Among the treatments, *Nostoc* sp. CU150162 has emerged as a potential biofertilizer that is economic and environment friendly.

Conclusion

According to the research findings, it is concluded that Kerala State is very rich in the biodiversity of *Nostoc* species. Especially, Western Ghats regions (Biodiversity hotspot) of Kerala are abundant with epilithic *Nostoc* species. In this study, a total of 39 *Nostoc* spp. were identified from different districts of Kerala, among them the majority of the species were identified as epilithic from the Western Ghats regions. This is the first study from Kerala State reported with 39 species of *Nostoc*. *N. indistinguendum*, *N. locularis* and *N. tibeticum* are new reports of the cyanobacterial flora of India. Among the identified *Nostoc* spp. from Kerala, four species were selected for molecular and biochemical characterization. Molecular characterization of the selected species confirmed that the species were *Nostoc elliposporum* FY150125, *N. punctiforme* FY150188, *Nostoc* sp. CU150162 and *Nostoc* sp. CU150191. Biochemical profiling using GC-MS analysis of the *Nostoc* spp. assisted in detecting a wide range of compounds with broad commercial applications. Lupeol, propyl hexanoate and isobutyl caproate are reported for the first time in *N. elliposporum*. Assessment of selected *Nostoc* spp. as biofertilizer for rice varieties Jyothi and Annapurna showed that, *Nostoc* spp. promoted crop yield by 10-45%. It has also been concluded that, the inoculation of plants with *Nostoc* spp. used in this study enhanced the grain quality. Thus, inoculation with beneficial *Nostoc* spp. has emerged as an innovative, eco-friendly technology to feed the global population more sustainably.

Recommendations

The current work will provide the baseline information on the taxonomy of *Nostoc* spp. which would facilitate future research on the diversity and ecology of *Nostoc* spp. from Kerala. A systematic study of *Nostoc* spp. in Kerala State needs to be further explored and has a long way to go. To elucidate the phylogenetic relationship among *Nostoc* spp., more conserved genes such as *rbcL*, *nifD* and *HetR* can be opted. Research on polyphasic analysis of *Nostoc* spp. from Kerala State could be conducted without any limitations. In the present work, *Nostoc* sp. CU150162 enhances the growth of rice plants in comparison to the control plants. Hence, the possibility of utilizing the *Nostoc* sp. CU150162 as a biofertilizer with minimal use of chemical fertilizers and pesticides could be explored. Bioprocess technology could also be applied for pilot-scale and large-scale production of *Nostoc* as a source of biofertilizer and thus promote sustainable agricultural practices. Studies on metabolic pathways involved in the growth-promoting process could be an exciting area to be examined. These studies would be greatly beneficial to the farmers and agriculturalists cultivating rice and other crop plants. *Nostoc* spp. are considered to be an important species in nitrogen fixation for harnessing soil health as well as for assuring economic, environmental, and agronomic benefits. Studies on the endophytic colonization of roots of rice and other crop plants with *Nostoc* spp. and their role in promoting growth could also be investigated. Mass spectrometric analysis of *Nostoc* in various solvents could help in identifying novel compounds with bioactive potential. The associated role of *nif* genes and their regulatory mechanism in growth promotion can also be studied. The application of *Nostoc* strains in the agriculture field can reduce farmer's dependence on chemical fertilizers and pesticides, as well as reduce farming costs and environmental hazards.

REFERENCES

- Abarzua, S., Jakubowski, S., Eckert, S. & Fuchs, P. (1999). Biotechnological investigation for the prevention of marine biofouling II. Blue-green algae as potential producers of biogenic agents for the growth inhibition of microfouling organisms. *Botanica Marina*, 42, 459-465.
- Abdel-Hafez, S. I., Abo-Elyousr, K. A., & Abdel-Rahim, I. R. (2015). Fungicidal activity of extracellular products of cyanobacteria against *Alternaria porri*. *European Journal of Phycology*, 50(2), 239-245.
- Abdul-Baki, A. A. & Anderson, J. D. (1973). Vigour determination in *Soybean* seed by multiple criteria. *Crop Science*, 13 (6), 630-633.
- Abo-Shady, A. M., Osman, M. E. A. H., Gaafar, R. M., Ismail, G. A., & El-Nagar, M. M. (2023). Cyanobacteria as a Valuable Natural Resource for Improved Agriculture, Environment, and Plant Protection. *Water, Air, & Soil Pollution*, 234(5), 313.
- Adam, M. S. (1999). The promotive effect of the cyanobacterium *Nostoc muscorum* on the growth of some crop plants. *Acta Microbiologica Polonica*, 48(2), 163-171.
- Adams, D. G. (2000b). Heterocyst formation in cyanobacteria. *Current Opinion in Microbiology*, 3(6), 618-624.
- Adams, D. G., Bergman, B. I. R. G. I. T. T. A., Nierzwicki-Bauer, S. A., Rai, A. N., & Schüßler, A. (2006). Cyanobacterial-plant symbioses. The prokaryotes: a handbook on the biology of bacteria, 1, 331-363.
- Adams, D.G. (2000a). Symbiotic interactions. In: Whitton BA, Potts M (eds) Ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, pp. 669.
- Adams, D.G., & Duggan, P.S. (2012). "Signalling in cyanobacteria-plant symbioses," in *Signaling and Communication in Plant Symbiosis* eds S. Perotto and F. Balauška (Berlin Heidelberg: Springer). 11, 93-121.
- Afify, A. H., Hauka, F. I. A., El-Zawawy, H. A. H., & Abou Elatta, A. E. A. (2023). Characterization of Cyanobacterial Strains Isolated from Soils Polluted with Insecticides. *Journal of Agricultural Chemistry and Biotechnology*, 73-78.
- Al-Amin, A., Parvin, F., Chakraborty, J., & Kim, Y.I. (2021). Cyanobacteria mediated heavy metal removal: a review on the mechanism, biosynthesis, and removal capability. *Environmental Technology Reviews*, 10(1), 44-57.
- Ali, M. A., Naveed, M., Mustafa, A., & Abbas, A. (2017). The good, the bad, and the ugly of rhizosphere microbiome. *Probiotics and Plant Health*, 253-290.
- Allakhverdiev, S. I., Sakamoto, A., Nishiyama, Y., Inaba, M., & Murata, N. (2000). Ionic and osmotic effects of NaCl-induced inactivation of photosystems I and II in *Synechococcus* sp. *Plant physiology*, 123(3), 1047-1056.

- Álvarez, C., Navarro, J. A., Molina-Heredia, F. P., & Mariscal, V. (2020). Endophytic colonization of Rice (*Oryza sativa* L.) by the symbiotic strain *Nostoc punctiforme* PCC. *Molecular Plant-Microbe Interactions*, 33, 1040-1045.
- Alwathnani, H. A., & Perveen, K. (2012). Biological control of fusarium wilts of tomato by antagonist fungi and cyanobacteria. *African Journal of Biotechnology*, 11(5), 1100-1105.
- Aly, M. H. A., El-All, A., Azza, A. M., & Mostafa, S. S. (2008). Enhancement of sugar beet seed germination, plant growth, performance and biochemical components as contributed by algal extracellular products. *Journal of Agricultural Chemistry and Biotechnology*, 33(12), 8223-8242.
- Amma, P. A., R. S. Aiyer & N. Subramoney. 1966. Occurrence of blue-green algae in acid soils of Kerala. *Agricultural Research Journal Kerala* 4, 141-142.
- Amudha, P., Jayalakshmi, M., Pushpabharathi, N., & Vanitha (2018). Identification Of Bioactive Components in Enhalus Acoroides Seagrass Extract by Gas Chromatography–Mass Spectrometry. *Asian Journal of Pharmaceutical and Clinical Research*, 11(10), 313-315.
- Anagnostidis, K. & Komárek, J. (1985). Modern approach to the classification system of Cyanophytes 1 - Introduction. *Archiv für Hydrobiologie- Supplement: Algological Studies*, 38-39, 291-302.
- Anagnostidis, K. & Komárek, J. (1988). Modern approach to the classification system of cyanophytes 3 - Oscillatoriales. *Archiv für Hydrobiologie- Supplement: Algological Studies*. 50-53, 327-472.
- Anagnostidis, K. & Komarek, J. (1999). *Süßwasserflora von mitteleuropa Tiel 1: Chroococcales*. Spektrum Akademischer Verlag, pp. 548.
- Anand, N & Hopper, R. S. S. (1987). Blue-green algae from rice fields in Kerala State, India. *Hydrobiology*, 144 (3), 223-232.
- Anand, N. (1989). Handbook of Blue-Green Algae of Rice Fields of South India. Dehradun: Bishen Singh Mahendra Pal Singh. pp. 77.
- Anand, N., & Subramanian, T. D. (1994). Distribution of natural populations of blue green algae in a rice field. *Phykos*, 33(1), 163-169.
- Anand, N., (1981). Certain viewpoints on the renaming of Cyanophyta. *Current Science*. 50, 535-536.
- Anand, N., Thajuddin, N., & Dadheech, P. K. (2019). Cyanobacterial taxonomy: Morphometry to molecular studies. In *Cyanobacteria*. Academic Press. pp. 64.
- Ananya, A. K. (2016). Fatty acid profiling and antioxidant potential of total polar lipid content of cyanobacterium *Nostoc muscorum*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(2), 159-163.
- Ananya, A., & Kamal, A. (2016). Fatty acid profiling and antioxidant potential of total polar lipid content of cyanobacterium *Nostoc muscorum*. *International Journal of Pharmacy and Pharmaceutical Sciences*, 8(2), 159-163.

- Andersen, K., & Shanmugam, K. T. (1977). Energetics of biological nitrogen fixation: determination of the ratio of formation of H₂ to NH⁴⁺ catalysed by nitrogenase of *Klebsiella pneumoniae* in vivo. *Microbiology*, 103(1), 107-122.
- Anderson, S. L., Rovnyak, D., & Strein, T. G. (2017). Identification of Edible Oils by Principal Component Analysis of ¹H NMR Spectra. *Journal of Chemical Education*, 94(9), 1377-1382.
- 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, Ed. Dhar, A., Department of Ocean Development, Technical Publication 9, New Delhi, India. pp. 243–246
- Arima, H., Horiguchi, N., Takaichi, S., Kofuji, R., Ishida, K. I., Wada, K., & Sakamoto, T. (2012). Molecular genetics and chemotaxonomic characterization of the terrestrial cyanobacterium *Nostoc commune* and its neighboring species. *FEMS Microbiology Ecology*, 79(1), 34-45.
- Armstrong, L., Vaz, M. G. M. V., Genuário, D. B., Fiore, M. F., & Debonisi, H. M. (2019). Volatile compounds produced by cyanobacteria isolated from mangrove environment. *Current microbiology*, 76, 575-582.
- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24(1), 1.
- Ashtekar, P. V., & Kamat N. D. (1980). Nostocales of Marathwada, Maharashtra. *Phykos*, 19(1), 89-93.
- Ausubel, F., Brent, R., Kingston, R., Moore, D., Seidman, J., Smith, J., & Struhl, K. (1995). Phenol extraction and ethanol precipitation of DNA. *Short Protocols in Molecular Biology*, 3rd Ed.; John Wiley and Sons, Inc.: Canada, 2-3.
- Bakieva, G. R., Khaibullina, L. S., Gaisina, L. A., & Kabirov, R. R. (2012). Ecological-floristic analysis of soil algae and cyanobacteria on the Tra-Tau and Yurak-Tau Mounts, Bashkiria. *Eurasian Soil Science*, 45, 873-881.
- Balasubramaniam, V., Gunasegavan, R. D. N., Mustar, S., Lee, J. C., & Noh, M. F. M. (2021). Isolation of industrial important bioactive compounds from microalgae. *Molecules*. 26(4), 1-45.
- Banerji, J. C. (1938). Studies on the Myxophyceae of Lower Bengal – II. *Journal of the Department of Science, University of Calcutta* 1, 95-109.
- Bano, A. Z. R. A., & Siddiqui, P. J. (2004). Characterization of five marine cyanobacterial species with respect to their pH and salinity requirements. *Pakistan Journal of Botany*, 36(1), 133-144.
- Bano, I., & Deora, G. S. (2020). Preliminary phytochemical screening and gc-ms analysis for identification of bioactive compounds from *Abutilon fruticosum* guill and perr. A rare and endemic plant of Indian Thar Desert, *International Journal of Pharma Sciences and Research*, 11(6), 2671-2679.

-
- Barman, N., Satpati, G. G., & Pal, R. (2015). A morphotaxonomic account of cyanobacterial diversity of Indian Sundarbans. *Journal of Algal Biomass Utilization*, 6(3), 39-46.
- Barton, J. W., Kuritz, T., O'Connor, L. E., Ma, C. Y., Maskarinec, M. P., & Davison, B. H. (2004). Reductive transformation of methyl parathion by the cyanobacterium *Anabaena* sp. strain PCC7120. *Applied Microbiology and Biotechnology*, 65, 330-335.
- Basumatary, R. R., Das, M., Das, R., & Bhuyan, S. I. (2022). Cyanobacterial diversity in different sites and seasons of Gossaigaon subdivision, Assam, India. *Proceedings of the International Academy of Ecology and Environmental Sciences*, 74-88.
- Beale, D. J., Pinu, F. R., Kouremenos, K. A., Poojary, M. M., Narayana, V. K., Boughton, B. A., Kanojia, K., Dayalan, S., Jones, O. A. & Dias, D. A. (2018). Review of recent developments in GC-MS approaches to metabolomics-based research. *Metabolomics*, 14, 1-31.
- Beebe, K. R., Pell, R. J., & Seasholtz M. B. (1998). Chemometrics, a practical guide. John Wiley and Sons, NewYork.
- Begum, Z. N. T., Mandal, R. & Islam, S. (2011). Effect of cyanobacterial biofertilizer on the growth and yield components of two HYV of rice. *Journal of Algal Biomass Utilization*, 2, 1-9.
- Begum, Z. T., Mandal, R., & Amin, F. B. (2008). Quantification and nitrogen fixation of cyanobacteria in rice field soils of Bangladesh. *Bangladesh Journal of Botany*, 37(2), 183-188.
- Behrendt, L., Brejnrod, A., Schliep, M., Sørensen, S. J., Larkum, A. W., & Kühl, M. (2015). Chlorophyll f-driven photosynthesis in a cavernous cyanobacterium. *The ISME Journal*, 9(9), 2108-2111.
- Bekker, A., Holland, H. D., Wang, P. L., Rumble, D., Stein, H. J., Hannah, J. L., Coetzee, L. L., and Beukes, N. J. (2004). Dating the rise of atmospheric oxygen. *Nature*, 427, 117-120.
- Bendre, A. M. & S. Kumar. (1975). Cyanophyceae of Meerut. *Phykos*, 14, 11-27.
- Bergman, B., & Rai, A. N. (1993). Cyanobacterial- Plant Symbioses. *Symbiosis*, 14, 61-81.
- Bergman, B., Rai, A. N., & Rasmussen, U. (2007). Cyanobacterial associations Associative and endophytic nitrogen-fixing bacteria and cyanobacterial associations, 257-301.
- Bhadury, P., & Wright, P. C. (2004). Exploitation of marine algae: biogenic compounds for potential antifouling applications. *Plantae*, 219, 561-578.
- Bhakta, S., Das, S. K., & Adhikary, S. P. (2010). Freshwater algae of Sikkim. *The Journal of Indian Botanical Society*, 89(1&2), 169-184.
- Bhakta, S., Pattanaik, L., Dutta, P., Sahu, E., & Bastia, A. K. (2014). Diversity of corticolous algae from Similipal Biosphere Reserve, Mayurbhanj, Odisha. *Phykos*, 44(1), 9-16.
-

- Bharadwaja, Y. (1933). Contributions to our knowledge of the Myxophyceae of India. *Annals of Botany*, 47(185), 117-143.
- Bharti, A., Prasanna, R., Kumar, G., Kumar, A., & Nain, L. (2019). Co-cultivation of cyanobacteria for raising nursery of chrysanthemum using a hydroponic system. *Environmental Biology of Fishes*, 31, 3625-3635.
- Bhateja, P., Mathur, T., Pandya, M., Fatma, T., & Rattan, A. (2006). Activity of blue- green microalgae extracts against *in vitro* generated *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Fitoterapia*, 77, 233-235.
- Bihana, S., Dhiman, A., Singh, G., Satija, S. (2018). Gas chromatography mass spectroscopy analysis of bioactive compounds in the whole plant parts of ethanolic extract of *Asclepias curassavica* L. *International Journal of Green Pharmacy*, 12(2),107-121.
- Biondi, N., Piccardi, R., Margheri, M. C., Rodolfi, L., Smith, G. D., & Tredici, M. R. (2004). Evaluation of *Nostoc* strain ATCC 53789 as a potential source of natural pesticides. *Applied and environmental microbiology*, 70(6), 3313-3320.
- Bisen, K., Keswani, C., Mishra, S., Saxena, A., Rakshit, A., & Singh, H. B. (2015). Unrealized potential of seed biopriming for versatile agriculture. *Nutrient use efficiency: from basics to advances*, 193-206.
- Bold, H. C. & Wynne, M. J., (1978). Introduction to the Algae-structure and reproduction, Prentice-Hall, Englewood Cliffs, New Jersey. pp. 706.
- Borah, D., Vimala, N., & Thajuddin, N., (2016). Biochemical composition and chemotaxonomy of cyanobacteria isolated from Assam, North-East India. *Phykos*, 46 (2), 33-45.
- Bornet, E. & Flahault, C. (1888b). Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France (quatrième et dernier fragment). *Annales des Sciences Naturelles Botanique* 7, 177-262.
- Bornet, E. & Flahaut, C. (1888a). Note sur deux nouveaux genres d'algues perforantes. *Journal de Botanique*, 2, 161-165.
- Bortoli, S., Oliveira-Silva, D., Krüger, T., Dörr, F. A., Colepicolo, P., Volmer, D. A., & Pinto, E. (2014). Growth and microcystin production of a Brazilian *Microcystis aeruginosa* strain (LTPNA 02) under different nutrient conditions. *Revista Brasileira de Farmacognosia*, 24, 389-398.
- Bourrelly, P. (1970). Les Algues d'eau douce -3: Les Algues bleues et rouges, Paris, N. Boubée et Cie. pp. 512.
- Boyd, M. R., Gustafson, K. R., McMahon, J. B., Shoemaker, R. H., O'Keefe, B. R., Mori, T., & Henderson, L. E. (1997). Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. *Antimicrobial Agents and Chemotherapy*, 41(7), 1521-1530.

- Bu, C., Wu, S., Yang, Y., & Zheng, M. (2014). Identification of factors influencing the restoration of cyanobacteria-dominated biological soil crusts. *PLoS One*, 9(3), e90049.
- Büdel, B. (1999). Ecology and diversity of rock-inhabiting cyanobacteria in tropical regions. *European Journal of Phycology*, 34(4), 361-370.
- Büdel, B., Bendix, J., Bicker, F. R., & Allan Green, T. G. (2008). Dewfall as a water source frequently activates the endolithic cyanobacterial communities in the granites of Taylor Valley, Antarctica 1. *Journal of Phycology*, 44(6), 1415-1424.
- Burja, A. M., Banaigs, B., Abou-Mansour, E., Burgess, J. G., & Wright, P. C. (2001). Marine cyanobacteria a prolific source of natural products. *Tetrahedron*, 57(46), 9347-9377.
- Cai, F., Yu, G., Liu, Y., Sun, Y., & Li, R. (2021). Description of two new species of *Nostoc* from China based on the polyphasic approach. *Fottea*, 21(2), 259-271.
- Cameron, R. E. (1962). Species of *Nostoc vaucher* occurring in the Sonoran Desert in Arizona. *Transactions of the American Microscopical Society*, 81(4), 379-384.
- Case, R. J., Boucher, Y., Dahllöf, I., Holmström, C., Doolittle, W. F., & Kjelleberg, S. (2007). Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. *Applied and Environmental Microbiology*, 73(1), 278-288.
- Castenholz, R. W., & Garcia-Pichel, F. (2012). Cyanobacterial responses to UV radiation. In *Ecology of cyanobacteria II: Their diversity in space and time* (pp. 481-499). Dordrecht: Springer Netherlands.
- Castenholz, R.W. (2001). Oxygenic photosynthetic bacteria. In: Boone D.R. and Castenholz R.W. (eds), *Bergey's Manual of Systematic Bacteriology* (2nd ed.), Volume 1, Springer-Verlag, New York, pp. 600.
- Castenholz, R.W., & Waterbury, J.B. (1989). Oxygenic photosynthetic bacteria. Group I. Cyanobacteria, in *Bergey's Manual of Systematic Bacteriology*, Vol. 3 (J.T. Staley, M.P. Bryant, N. Pfennig, J.G. Holt, Eds). Williams & Wilkins, Baltimore. pp. 1710-1789.
- Caudales, R., & Wells, J. M. (1992). Differentiation of free-living *Anabaena* and *Nostoc* cyanobacteria on the basis of fatty acid composition. *International Journal of Systematic and Evolutionary Microbiology*, 42(2), 246-251.
- Caudales, R., Wells, J. M. & Butterfield, J. E. (2000). Cellular fatty acid composition of cyanobacteria assigned to subsection II, order Pleurocapsales. *International Journal of Systematic and Evolutionary Microbiology*, 50, 1029-1034.
- Celis-Pla, P. S., Rearte, T. A., Neori, A., Masojídek, J., Bonomi-Barufi, J., Alvarez-Gomez, F., & Figueroa, F. L. (2021). A new approach for cultivating the cyanobacterium *Nostoc calcicola* (MACC-612) to produce biomass and bioactive compounds using a thin-layer raceway pond. *Algal Research*, 59, 102421.
- Chakraborty, S., Vijayan, K., Nair, C. V., Santra, S. C., & Bhattacharya, T. (2008). Isolation and characterization of high quality DNA from marine benthic macroalgae. *Journal of Environmental Biology*, 29, 907-910.

- Chamizo, S., Mugnai, G., Rossi, F., Certini, G., & De Philippis, R. (2018). Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. *Frontiers in Environmental Science*, 6, 49.
- Chaudhary, V., Prasanna, R., Nain, L., Dubey, S. C., Gupta, V., Singh, R., & Bhatnagar, A. K. (2012). Bioefficacy of novel cyanobacteria-amended formulations in suppressing damping off disease in tomato seedlings. *World Journal of Microbiology and Biotechnology*, 28, 3301-3310.
- Chellappa, S. L., Marinho, I. R. & Chellappa, N. T. (2004). Freshwater phytoplankton assemblages and the bloom of toxic cyanophyceae of Campo Grande reservoir of Rio Grande do Norte State of Brazil. *Indian Hydrobiology*, 7, 151-171.
- Chen, J. H. (2006). The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. In *International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use*, Land Development Department Bangkok Thailand. 16 (20), 1-11.
- Chittapun, S., Limbipichai, S., Amnuaysin, N., Boonkerd, R., & Charoensook, M. (2018). Effects of using cyanobacteria and fertilizer on growth and yield of rice, Pathum Thani I: a pot experiment. *Journal of Applied Phycology*, 30, 79-85.
- Chittora, D., Meena, M., Barupal, T., Swapnil, P., & Sharma, K. (2020). Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochemistry and biophysics reports*, 22, 100737.
- Choi, G. G., Bae, M. S., Ahn, C. Y., & Oh, H. M. (2008). Induction of axenic culture of *Arthrospira (Spirulina) platensis* based on antibiotic sensitivity of contaminating bacteria. *Biotechnology letters*, 30, 87-92.
- Choudhary, K., & Bimal, R. (2010). Distribution of nitrogen-fixing cyanobacteria (Nostocaceae) during rice cultivation in fertilized and unfertilized paddy fields. *Nordic Journal of Botany*, 28(1), 100-103.
- Choudhury, A. T. M. A., & Kennedy, I. R. (2004). Prospects and potentials for systems of biological nitrogen fixation in sustainable rice production. *Biology and Fertility of Soils*, 39, 219-227.
- Chua, M., Erickson, T. E., Merritt, D. J., Chilton, A. M., Ooi, M. K., & Muñoz-Rojas, M. (2020). Bio-priming seeds with cyanobacteria: effects on native plant growth and soil properties. *Restoration Ecology*, 28, S168-S176.
- Cramer, G. R., Urano, K., Delrot, S., Pezzotti, M., & Shinozaki, K. (2011). Effects of abiotic stress on plants: a systems biology perspective. *BMC plant biology*, 11(1), 1-14.
- Dadheech, N. (2010). Desiccation tolerance in cyanobacteria. *African Journal of Microbiology Research*, 4, 1584-1593.
- Dahms, H.-U., Xu, Y. & Pfeiffer, C. (2006). Antifouling potential of cyanobacteria: a mini-review. *Biofouling*, 22, 317-327.

- Daines, A.M. (2003). The synthesis of naturally occurring Vitamin K and Vitamin K analogues. *Current Organic Chemistry*, 7, 1625-1634.
- Damerval, T., Guglielmi, G., Houmard, J., & De Marsac, N. T. (1991). Hormogonium differentiation in the cyanobacterium *Calothrix*: a photoregulated developmental process. *The Plant Cell*, 3(2), 191-201.
- Dasgupta, M., & Ahmed, S. U. (2013). Some potential rice field BGA isolates from Sonitpur district Assam, North east India. *Journal of Natural Product and Plant Resources*, 3(4), 17-23.
- Dash, N. P., Kumar, A., Kaushik, M. S., & Singh, P. K. (2016). Cyanobacterial (unicellular and heterocystous) biofertilization to wetland rice influenced by nitrogenous agrochemical. *Journal of Applied Phycology*, 28, 3343-3351.
- Davis, E. L., Meyers, D. M., Dullum, C. J., & Feitelson, J. S. (1997). Nematicidal activity of fatty acid esters on soybean cyst and root-knot nematodes. *Journal of Nematology*, 29 (4), 677-684.
- Davydov, D. (2021). Cyanobacterial diversity of the northern Polar Ural Mountains. *Diversity*, 13(11), 607.
- De Caire, G. Z., De Cano, M. S., De Mule, M. C. Z., & De Halperin, D. R. (1990). Antimycotic products from the cyanobacterium *Nostoc muscorum* against *Rhizoctonia solani*. *Phyton, Buenos Aires*, 51(1), 1-4.
- Delis, C., Krokida, A., Georgiou, S., Peña-Rodríguez, L.M., Kavroulakis, N., & Ioannou, E. (2011). Role of lupeol synthase in *Lotus japonicus* nodule formation. *New Phytol*, 189, 335-346.
- Dembitsky, V. M., & Rezanka, T. (2005). Metabolites produced by nitrogen-fixing *Nostoc* species. *Folia Microbiologica*, 50(5), 363-391.
- Dembitsky, V. M., & Srebnik, M. (2002). Variability of hydrocarbon and fatty acid components in cultures of the filamentous cyanobacterium *Scytonema* sp. isolated from microbial community “black cover” of limestone walls in Jerusalem. *Biochemistry (Moscow)*, 67, 1276-1282.
- Dembitsky, V. M., Shkrob, I., & Dor, I. (1999). Separation and identification of hydrocarbons and other volatile compounds from cultured blue-green alga *Nostoc* sp. by gas chromatography–mass spectrometry using serially coupled capillary columns with consecutive nonpolar and semipolar stationary phases. *Journal of Chromatography*, 862(2), 221-229.
- Desikachary, T.V. (1959). Cyanophyta. Monographs on Algae. Indian Council of Agricultural Research. pp. 686.
- Devi, A.G., Dorycanta, H., & Singh, N.I. (1999). Cyanobacteria. Flora of Rice Fields Soils of Manipur. *Phykos*, 38, 13-18.
- Devi, K. M., & Mehta, S. K. (2016). Antimicrobial activities of freshwater cyanobacterium, *Nostoc* sp. from Tamdil Wetland of Mizoram, India: an identification of bioactive compounds by GC-MS. *International Journal of Pharmaceutical Sciences and Research (IJPSR)*, 7(3), 1179-1191.

- Devi, S., Rani, N., & Sagar, A. (2020). GC-MS Analysis and antioxidant activity of two species of cyanobacteria isolated from Drang salt mine of district Mandi, Himachal Pradesh, India. *Plant Archives*, 20, 7505-7510.
- Devi, T. E., & Parthiban, R. (2020). Hydrothermal liquefaction of *Nostoc ellipsosporum* biomass grown in municipal wastewater under optimized conditions for bio-oil production. *Bioresource Technology*, 316, 123943.
- Dey, H. S., Tayung, K., & Bastia, A. K. (2010). Occurrence of nitrogen-fixing cyanobacteria in local rice fields of Orissa, India. *Ecoprint: An International Journal of Ecology*, 17, 77-85.
- Deyab, M. A., El-Sheekh, M. M., Hasan, R. S., Elsadany, A. Y., & Abu Ahmed, S. E. S. (2021). Phytochemical components of two cyanobacterial local strains. *Scientific Journal for Damietta Faculty of Science*, 11(1), 67-75.
- Dhanya, V., & Ray, J.G. (2015). Ecology and Diversity of Cyanobacteria in Kuttanadu paddy Wetlands, Kerala, India. *American Journal of Plant Sciences*, 6, 2924–2938.
- Dhar, D.W., Prasanna, R., & Singh, B. (2007). Comparative performance of three carrier based blue green algal biofertilizers for sustainable rice cultivation. *Journal of Sustainable Agriculture*, 30, 41-50.
- Díez, B., & Ininbergs, K. (2014). Ecological importance of cyanobacteria. *Cyanobacteria: an economic perspective*, 41-63.
- Dikhoba, P. M., Mongalo, N. I., Elgorashi, E. E. & Makhafola, T. J. (2019). Antifungal and anti-mycotoxigenic activity of selected South African medicinal plants species. *Heliyon*, 5(10), 2668.
- Dillon, J. G., & Castenholz, R. W. (1999). Scytonemin, a cyanobacterial sheath pigment, protects against UVC radiation: implications for early photosynthetic life. *Journal of Phycology*, 35(4), 673-681.
- Dixit, S., & Singh, D. P. (2014). An evaluation of phycoremediation potential of cyanobacterium *Nostoc muscorum*: characterization of heavy metal removal efficiency. *Journal of Applied Phycology*, 26, 1331-1342.
- Dixit, S.C. (1936). The Myxophyceae of the Bombay Presidency, India - I. *Proceedings of the National Institute of Sciences of India*, 3, 93-106.
- Dixon, R., & Kahn, D. (2004). Genetic regulation of biological nitrogen fixation. *Nature Reviews Microbiology*, 2(8), 621-631.
- Dodds, W. K., Gudder, D. A., & Mollenhauer, D. (1995). The ecology of *Nostoc*. *Journal of Phycology*, 31(1), 2-18.
- Dominic, T. K., & Madhusoodanan, P. V. (1999). Cyanobacteria from extreme acidic environments. *Current science*, 77(8), 1021-1023.
- Durai, P., Batool, M., & Choi, S. (2015). Structure and effects of cyanobacterial lipopolysaccharides. *Marine Drugs*, 13(7), 4217-4230.
- Easa, P.S. (2004). Biodiversity documentation for Kerala. Part 1: Algae KFRI. pp. 06.

- Egbuna, C., Mukherjee, M., Rao, G. N., Gido, L. J. F. J., & Tijjani, H. (2018). Introduction to phytochemistry. In *Phytochemistry*. Apple Academic Press. pp. 3-36.
- Ehling-Schulz, M., & Scherer, S. (1999). UV protection in cyanobacteria. *European Journal of Phycology*, 34(4), 329-338.
- Eily, A. N., Pryer, K. M., & Li, F. W. (2019). A first glimpse at genes important to the *Azolla–Nostoc* symbiosis. *Symbiosis*, 78, 149-162.
- Elagamey, E., Abdellatef, M. A., & Flefel, H. E. (2023). Cyanobacteria: A Futuristic Effective Tool in Sustainable Agriculture. pp. 1-22.
- El-Bestawy, E. A., Abd El-Salam, A. Z., & Mansy, A. E. R. H. (2007). Potential use of environmental cyanobacterial species in bioremediation of lindane-contaminated effluents. *International Biodeterioration & Biodegradation*, 59(3), 180-192.
- Elenkin, A. A. (1936). Monographie algarum Cyanophycearum aquidulcium et terrestrium infinibus URSS inventatum, vol 1. Acad Nauk URSS Moscow, Leningrad, pp. 675.
- El-Hameed, M. M. A., Abuarab, M. E., Al-Ansari, N., Mottaleb, S. A., Bakeer, G. A., Gyasi-Agyei, Y., & Mokhtar, A. (2021). Phycoremediation of contaminated water by cadmium (Cd) using two cyanobacterial strains (*Trichormus variabilis* and *Nostoc muscorum*). *Environmental Sciences Europe*, 33(1), 1-10.
- El-Sheek, M. M., Zayed, M. A., & Elmossel, F. K. (2018). Effect of cyanobacteria isolates on rice seeds germination in saline soil. *Baghdad Science Journal*, 15(1), 0016-0016.
- El-Sheekh, M. M., Deyab, M. A., Hasan, R. S., Abu Ahmed, S. E., & Elsadany, A. Y. (2022). Biological control of Fusarium tomato-wilt disease by cyanobacteria *Nostoc* spp. *Archives of Microbiology*, 204(1), 116.
- Engle, S., Whalen, S., Joshi, A., & Pollard, K. S. (2017). Unboxing cluster heatmaps. *BMC bioinformatics*, 18(2), 1-15.
- Erişoğlu, M., & Sakallıoğlu, S. (2010). An investigation of effects on hierarchical clustering of distance measurements. *Selcuk Journal of Applied Mathematics*, 39-53.
- Esch, C. (2014). A native cyanobacteria, *Nostoc*, as a biofertilizer. Western Kentucky University.
- Faisal, M., Hameed, A., & Hasnain, S. (2005). Chromium-resistant bacteria and cyanobacteria: impact on Cr (VI) reduction potential and plant growth. *Journal of Industrial Microbiology and Biotechnology*, 32(11-12), 615-621.
- Farrag, D., Mehesen, A., Kasem, M., & El-Dein, O. (2017). Impact of cyanobacteria filtrate, compost tea and different rates of nitrogen fertilizer on growth, fruit yield and quality of cantaloupe plants. *Microbiology Research Journal International*, 18, 1-10.
- Fay, P. (1992). Oxygen relations of nitrogen fixation in cyanobacteria. *Microbiological reviews*, 56(2), 340-373.

-
- Felsenstein, J. (1985). Phylogenies and the comparative method. *The American Naturalist*, 125(1), 1-15.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *Journal of Molecular Evolution*, 17, 368-376.
- Felsenstein, J. (1989). PHYLIP—phylogeny inference package (version 3.2). *Cladistics*, 5, 164-166.
- Fernández, A., & Gómez, S. (2008). Solving non-uniqueness in agglomerative hierarchical clustering using multidendrograms. *Journal of Classification*, 25, 43-65.
- Ferroni, L., Klisch, M., Pancaldi, S., & Häder, D. P. (2010). Complementary UV-absorption of mycosporine-like amino acids and scytonemin is responsible for the UV-insensitivity of photosynthesis in *Nostoc flagelliforme*. *Marine Drugs*, 8(1), 106-121.
- Fiore, M. F., Moon, D. H., Tsai, S. M., Lee, H., & Trevors, J. T. (2000). Miniprep DNA isolation from unicellular and filamentous cyanobacteria. *Journal of Microbiological Methods*, 39(2), 159-169.
- Fitriati, E., Setiawati, W., Salamah, A., & Hendrayanti, D. (2016). Influence of *Nostoc* strains CPG24 and GIA13a on germination of paddy seedling in various media. In *AIP Conference Proceedings* (Vol. 1729, No. 1). AIP Publishing.
- Forlani, G., Pavan, M., Gramek, M., Kafarski, P., & Lipok, J. (2008). Biochemical bases for a widespread tolerance of cyanobacteria to the phosphonate herbicide glyphosate. *Plant and cell physiology*, 49(3), 443-456.
- Fritsch, F.E. (1912). Freshwater algae. In: National Antarctic Expedition 1901-1904 *Natural History*. Vol. VI. Zoology and Botany [Part 3]. (Anon. Eds), London, pp. 1-66.
- Fritsch, F.E. (1959). The structure and reproduction of the algae. Volume II. Foreword, Phaeophyceae, Rhodophyceae, Myxophyceae. pp. 268.
- Gaikwad, S. A., & Nalawade, S. P. (2020). GCMS analysis for separation and identification of bioactive compounds from freshwater cyanobacterium *Nostoc fuscescence*. *Journal of Advanced Scientific Research*, 11(04), 327-330.
- Galhano, V., De Figueiredo, D. R., Alves, A., Correia, A., Pereira, M. J., Gomes-Laranjo, J., & Peixoto, F. (2011). Morphological, biochemical and molecular characterization of *Anabaena*, *Aphanizomenon* and *Nostoc* strains (Cyanobacteria, Nostocales) isolated from Portuguese freshwater habitats. *Hydrobiologia*, 663, 187-203.
- Gantar, M. (2000). Mechanical damage of root provides enhanced colonization of wheat endorhizosphere by N₂-fixing cyanobacterium *Nostoc* sp. strain 2S9B. *Biology and Fertility of Soils*, 32, 250-255.
- Gantar, M., & Elhai, J. (1999). Colonization of wheat para-nodules by the N₂-fixing cyanobacterium *Nostoc* sp. Strain 2S9B. *New Phytologist*, 141, 373-379
- Gantar, M., & Svirčev, Z. (2008). Microalgae and cyanobacteria: food for thought 1. *Journal of phycology*, 44(2), 260-268.
-

- Gantar, M., Kerby, N. W., & Rowell, P. (1991). Colonization of wheat (*Triticum vulgare* L.) by N₂-fixing cyanobacteria: II. An ultrastructural study. *New Phytologist*, 118, 485-492.
- Gao, K. (1998). Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. *Journal of Applied Phycology*, 10, 37-49.
- Garcia-Pichel, F. (2009). Cyanobacteria. In *Encyclopedia of Microbiology, Third Edition*, Elsevier. pp. 124.
- Garcia-Pichel, F., López-Cortés, A., & Nübel, U. (2001). Phylogenetic and morphological diversity of cyanobacteria in soil desert crusts from the Colorado Plateau. *Applied and Environmental Microbiology*, 67(4), 1902-1910.
- Garlapati, D., Chandrasekaran, M., Devanesan, A., Mathimani, T., & Pugazhendhi, A. (2019). Role of cyanobacteria in agricultural and industrial sectors: an outlook on economically important byproducts. *Applied Microbiology and Biotechnology*, 103(12), 4709-4721.
- Gaysina, L.A., Saraf, A. & Singh, P. (2018) Cyanobacteria in Diverse Habitats. In: Mishra, A.K., Tiwari, D.N. and Rai, A.N., Eds., *Cyanobacteria: From Basic Science to Applications*, Academic Press, Cambridge, pp. 1-28.
- Geethu, V., & Shamina, M. (2021). Filamentous cyanobacteria from Western Ghats of North Kerala, India. *Bangladesh Journal of Plant Taxonomy*, 28(1), 83-95.
- Geitler, L. (1932). Cyanophyceae. In: Rabenhorst's Kryptogamen Flora von Deutschland, vol 14. Akad Verlagsges, Leipzig, pp. 1196.
- Ghaneian, M.T., Ehrampoush, M.H., Jebali, A., Hekmatimoghaddam, S., & Mahmoudi, M. (2015). Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. *Environmental Health Engineering and Management*, 2(1), 13-16.
- Ghasemi Y., Moradian A., Mohagheghzadeh A. Shokravi, S., & Morowvat, M. H. (2004). Antifungal and antibacterial activity of the microalgae collected from paddy fields of Iran: characterization of antimicrobial activity of *Chroococcus dispersus*. *Journal of Biological Sciences*, 7, 904-910
- Ghose, S. L. (1919). The myxophyceae of Lahore. *Journal of Indian Botanical Society*, 1(1), 8-13.
- Ghosh, A., & Bhadury, P. (2021). Genome description of *Nostoc ellipsosporum* strain NOK (Nostocales, Cyanobacteria) isolated from an arsenic contaminated paddy field of the Bengal Delta Plains. *IOP SciNotes*, 2(3), 034003.
- Giovannoni, S. J., Turner, S., Olsen, G. J., Barns, S., Lane, D. J., & Pace, N. R. (1988). Evolutionary relationships among cyanobacteria and green chloroplasts. *Journal of Bacteriology*, 170(8), 3584-3592.
- Gkelis, S., Rajaniemi, P., Vardaka, E., Moustaka-Gouni, M., Lanaras, T., & Sivonen, K. (2005). *Limnothrix redekei* (Van Goor) Meffert (Cyanobacteria) strains from Lake Kastoria, Greece form a separate phylogenetic group. *Microbial Ecology*, 49, 176-182.

- Golubic, S. (1979). Taxonomy of stromatolite building cyanophytes. In: Walter MR (ed.) *Stromatolites, Development in sedimentology*, vol 20. Elsevier, New York, pp.127-140.
- Golubic, S., & Campbell, S. E. (1979). Analogous microbial forms in recent subaerial habitats and in Precambrian cherts: *Gloeothece coerulea* Geitler and *Eosynechococcus moorei* Hofmann. *Precambrian Research*, 8, 201-217.
- Gomes, A. F. D. E. (2012). Algal studies on the diversity and distribution of blue green algae (BGA) from selected paddy field habitats of Goa. Thesis of University of Goa.
- Gomont, M. (1892). Monographie des Oscillatoriées (Nostocacées homocystées). *Annales des Sciences Naturelles; Botanique*, 7(15), 263-368.
- Gonçalves, A. L. (2021). The use of microalgae and cyanobacteria in the improvement of agricultural practices: a review on their biofertilising, biostimulating and biopesticide roles. *Applied Sciences*, 11(2), 871.
- Gopaldaswamy, G., Karthikeyan, C. V., Raghu, R., Udayasuriyan, V., & Apte, S. K. (2007). Identification of acid-stress-tolerant proteins from promising cyanobacterial isolates. *Journal of Applied Phycology*, 19, 631-639.
- Govindjee, & Shevela, D. (2011). Adventures with cyanobacteria: A personal perspective. *Frontiers in Plant Science*, 2, 1-17
- Goyal, S. K., Sharma, B. M., & Gupta, R. S. (1984). Algal flora of rice field soils of Jammu and Kashmir State. *Phykos*, 23, 59-64.
- Grover, I.S. and Pandhol, R.K. (1975). Algal flora of paddy fields of Ludhiana and its adjacent areas. *Phykos*, 14(1&2), 89-97.
- Grzesik, M., Romanowska-Duda, Z., & Kalaji, H. M. (2017) Effectiveness of cyanobacteria and green algae in enhancing the photosynthetic performance and growth of willow (*Salix viminalis* L.) plants under limited synthetic fertilizers application. *Photosynthetica*, 55, 510-521.
- Gugger, M., Lyra, C., Suominen, I., Tsitko, I., Humbert, J. F., Salkinoja-Salonen, M. S., & Sivonen, K. (2002). Cellular fatty acids as chemotaxonomic markers of the genera *Anabaena*, *Aphanizomenon*, *Microcystis*, *Nostoc* and *Planktothrix* (cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology*, 52(3), 1007-1015.
- Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59(3), 307-321.
- Guiry, M. D. & Guiry, G. M. (2022). AlgaeBase. World-wide electronic publication, University of Galway. Ireland, Galway. <https://www.algaebase.org>.
- Guiry, M. D., & Guiry, G. M. (2023). AlgaeBase. World-wide electronic publication, University of Galway. Ireland, Galway. <https://www.algaebase.org>

- Gupta, D., Ip, T., Summers, M. L., & Basu, C. (2015). 2-Methyl-3-buten-2-ol (MBO) synthase expression in *Nostoc punctiforme* leads to over production of phytols. *Bioengineered*, 6(1), 33-41.
- Gupta, P. (2012). Algae of India Volume 1. A checklist of Cyanoprokaryota (Cyanophyceae). Salt Lake, Kolkata: Botanical Survey of India, Ministry of Environment & Forests. pp. 180.
- Gupta, P. (2018). Occurrence of *Nostoc* Vaucher ex Bornet and Flahault in Water Bodies of Maldah District, West Bengal. ANSDN [Internet]. [cit. 4 Sep. 2022 cit.://anushandhan.com/index.php/ANSDHN/article/view/1255].
- Gupta, R. K. & Das, S. K. (2019). Flora of Karnataka-a checklist. Volume – 1: Algae, Fungi, Lichens, Bryophytes & Pteridophytes Karnataka biodiversity board. pp. 1-189.
- Gupta, R.K. (2021). Algal flora of Jharkhand. Salt Lake City, Kolkata: Botanical Survey of India Ministry of Environment. *Forest and Climate Change*. pp. 396.
- Gupta, S. K., & Chakraborty, A. P. (2020). Cyanobacterial biofertilizer for sustainable agriculture and environment. *International Journal of Creative Research Thoughts*, 8, 4-10.
- Gupta, V., Ratha, S.K., Sood, A., Chaudhary, V., & Prasanna, R. (2013). New insights into the biodiversity and applications of cyanobacteria (blue-green algae)-Prospects and challenges. *Algal Research*, 2(2), 79-97.
- Hagemann, M. (2011). Molecular biology of cyanobacterial salt acclimation. *FEMS microbiology reviews*, 35(1), 87-123.
- Hagemann, M., Henneberg, M., Felde, V.J., Berkowicz, S.M., Raanan, H., Pade, N., Felix-Henningsen, P. & Kaplan, A., (2017). Cyanobacterial populations in biological soil crusts of the northwest Negev Desert, Israel-effects of local conditions and disturbance. *FEMS Microbiology Ecology*, 93(6), 219-230.
- Hagmann, L., & Jüttner, F. (1996). Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. *Tetrahedron letters*, 37(36), 6539-6542.
- Halim, R., Harun, R., Webley, P. A., & Danquah, M. K. (2013). Bioprocess engineering aspects of biodiesel and bioethanol production from microalgae. *Advanced Biofuels and Bioproducts*, 601-628.
- Hamidi, S. S., Akbari, E., & Motameni, H. (2019). Consensus clustering algorithm based on the automatic partitioning similarity graph. *Data & Knowledge Engineering*, 124, 101754.
- Han, D., Bi, Y., & Hu, Z. (2004). Advance in study on *Nostoc* (cyanobacteria) and its applications. *Gaojishu Tongxun (Chenese)*, 14, 106-110.
- Han, D., Deng, Z., Lu, F., & Hu, Z. (2013). Biology and biotechnology of edible *Nostoc*. *Handbook of microalgal culture: Applied phycology and biotechnology*, 433-444.

-
- Han, D., Fan Y. & Hu, Z. (2009). An evaluation of four phylogenetic markers in *Nostoc*: Implications for cyanobacterial phylogenetic studies at the intrageneric level. *Current Microbiology*, 58, 170-176
- Han, D., Fan, Y., & Hu, Z. (2009). An evaluation of four phylogenetic markers in *Nostoc*: implications for cyanobacterial phylogenetic studies at the intrageneric level. *Current Microbiology*, 58, 170-176.
- Han, X., Zeng, H., Bartocci, P., Fantozzi, F., & Yan, Y. (2018). Phytohormones and effects on growth and metabolites of microalgae: a review. *Fermentation*, 4(2), 1-15.
- Hariot, P. (1891). Le genre *Polycoccus* Kützing. *Journal de Botanique [Morot]*, 5, 29-32.
- Harman, G. E., & Taylor, A. G. (1988). Improved seedling performance by integration of biological control agents at favorable pH levels with solid matrix priming. *Phytopathology*, 78(5), 520-525.
- Haroun, S. A., & Hussein, M. H. (2003). The promotive effect of algal biofertilizers on growth, protein pattern and some metabolic activities of *Lupinus termis* plants grown in siliceous soil. *Asian Journal of Plant Sciences*, 2, 944-951.
- Hartmann, L. S., & Barnum, S. R. (2010). Inferring the evolutionary history of mo-dependent nitrogen fixation from phylogenetic studies of *nifK* and *nifDK*. *Journal of Molecular Evolution*, 71, 70-85.
- Hasan, M. A. (2020). Cyanobacteria from rice field and comparative study of their performances as biofertilizer on rice plants. *Journal of Global Biosciences*, 9, 8078-8087.
- Hashtroudi, M. S., Ghassempour, A., Riahi, H., Shariatmadari, Z., & Khanjir, M. (2013). Endogenous auxins in plant growth-promoting Cyanobacteria-*Anabaena vaginicola* and *Nostoc calcicola*. *Journal of applied phycology*, 25, 379-386.
- Hassan, E. S. A., & Morcos, M. M. (2006). Bio-enhancement effect of cyanobacteria on rice seeds germination and seedlings growth. *Journal of Soil Sciences and Agricultural Engineering*, 31(8), 5367-5378.
- Hauer, T. (2008). Epilithic cyanobacterial flora of Mohelenská hadcová steppe Nature Reserve (western Moravia, Czech Republic) 70 years ago and now. *Fottea*, 8(2), 129-132.
- Hayes, P. K., El Semary, N. A. & Sanchez-Baracaldo, P. (2007). The taxonomy of cyanobacteria: Molecular insights into a difficult problem. In: Brodie J, Lewis J. (eds) *Unravelling the algae: The past, present and future of algal systematic*. CRC Press/Taylor and Francis Group, Boca Raton, pp. 93-101.
- Hedge, J. E., & Hofreiter, B. T. (1962). Determination of total carbohydrate by anthrone method. *Carbohydrate Chemistry*, 17.
- Henson, B. J., Watson, L. E., & Barnum, S. R. (2004). The evolutionary history of nitrogen fixation, as assessed by *NifD*. *Journal of Molecular Evolution*, 58, 390-399.
-

- Hensyl, W.R. (1989) *Bergey's Manual of Systematic Bacteriology*, Williams & Wilkins, London, pp. 1780.
- Hernandez-Muniz, W. & Stevens, E. (1986). Characterization of the motile hormogonia of *Mastigocladus laminosus*. *Journal of Bacteriology*, 169(1), 218-223.
- Higa, T. (1991). Effective microorganisms: a biotechnology for mankind, in *Proceedings of the First International Conference on Kyusei Nature Farming*, eds J. F. Parr, S. B. Hornick, and M. E. Simpson (Washington, DC: U.S. Department of Agriculture), 8-14.
- Higo, A., Suzuki, T., Ikeuchi, M., & Ohmori, M. (2007). Dynamic transcriptional changes in response to rehydration in *Anabaena* sp. PCC 7120. *Microbiology*, 153(11), 3685-3694.
- Hill, D. R., Hladun, S. L., Scherer, S., & Potts, M. (1994). Water stress proteins of *Nostoc commune* (Cyanobacteria) are secreted with UV-A/B-absorbing pigments and associate with 1, 4-beta-D-xylanxylanohydrolase activity. *Journal of Biological Chemistry*, 269(10), 7726-7734.
- Hoagland, K.D., Roemer, S.C., & Rosowski, J.R. (1982). Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). *American Journal of Botany*, 69, 188-213.
- Hoiczky, E. (2000). Gliding motility in cyanobacteria: observations and possible explanations. *Archives of microbiology*, 174, 11-17.
- Honda, D., Yokota, A., & Sugiyama, J. (1999). Detection of seven major evolutionary lineages in cyanobacteria based on the 16S rRNA gene sequence analysis with new sequences of five marine *Synechococcus* strains. *Journal of Molecular Evolution*, 48, 723-739.
- Hong, J. W., Kim, O. H., Jo, S. W., Kim, H., Jeong, M. R., & Yoon, H. S. (2018). Characterization of alkane-producing *Nostoc* sp. isolated from a summer bloom for biofuel potential. *Journal of Biobased Materials and Bioenergy*, 12(3), 244-251.
- Horie, T. (2019). Global warming and rice production in Asia: Modeling, impact prediction and adaptation. *Proceedings of the Japan Academy, Series B*, 95(6), 211-245.
- Horiike, T. (2016). An introduction to molecular phylogenetic analysis. *Reviews in Agricultural Science*, 4, 36-45.
- Hossain, M. M., & Nakamoto, H. (2003). Role for the cyanobacterial HtpG in protection from oxidative stress. *Current Microbiology*, 46, 0070-0076.
- Hrouzek, P., Lukešová, A., Mareš, J., & Ventura, S. (2013). Description of the cyanobacterial genus *Desmonostoc* gen. nov. including *D. muscorum* comb. nov. as a distinct, phylogenetically coherent taxon related to the genus *Nostoc*. *Fottea*, 13(2), 201-213.
- Huber-Pestalozzi, G. (1938). Das Phytoplankton des Süßwassers. Systematik und Biologie. 1. Teil: Blaualgen, Bakterien, Pilze. – E. Schweizerbart, Stuttgart. pp. 342.

- Huo, D., Li, H., Cai, F., Guo, X., Qiao, Z., Wang, W., Yu, G., & Li, R. (2021). Genome evolution of filamentous cyanobacterium *Nostoc* species: from facultative symbiosis to free living. *Microorganisms*, 9, 2015.
- Hussain, A., Krischke, M., Roitsch, T., & Hasnain, S. (2010). Rapid determination of cytokinins and auxin in cyanobacteria. *Current Microbiology*, 61, 361-369.
- Ibraheem, I. (2007). Cyanobacteria as alternative biological conditioners for bioremediation of barren soil. *Egyptian Journal of Phycology*, 8(1), 99-117.
- Ismail, A. E. W. A., & Ismail, M. M. (2011). Antagonistic activity of some fungi and cyanobacteria species against *Rhizoctonia solani*. *International Journal of Plant Pathology*, 2(3), 101-114.
- Ismail, G., & Abo-Hamad, S. (2017). Effect of different *Anabaena variabilis* (Kütz) treatments on some growth parameters and physiological aspects of *Hordeum vulgare* L. and *Trigonella foenum-graecum* L. *Egyptian Journal of Botany*, 57(3), 507-516.
- Jacq, V., & Roger, P. (1977). Decrease of seed losses due to sulphate reduction by pretreatment of rice with Cyanophyceae.
- Jain, P. K. (2018). Blue-green Algal Flora of Alwar and Its Environ. RBSA Publishers. pp. 357.
- Jaiswal, A.G. (2017). Algae of north-west Khandesh. Solapur: Laxmi Book Publication. pp. 448.
- Jaki, B., Orjala, J., Heilmann, J., Linden, A., Vogler, B., & Sticher, O. (2000). Novel Extracellular Diterpenoids with Biological Activity from the Cyanobacterium *Nostoc commune*. *Journal of Natural Products*, 63(3), 339-343.
- Jensen, B., Knudsen, I.M., Madsen, M. and Jensen, D.F. 2004. Biopriming of infected carrot seed with an antagonist, *Clonostachys rosea*, selected for control of seedborne *Alternaria* spp. *Phytopathology* 94, 551-560.
- Jensen, K.S. (2014). Ecophysiology of gelatinous *Nostoc* colonies: unprecedented slow growth and survival in resource-poor and harsh environments. *Annals of Botany*, 114(1), 17-33.
- Jha, M. N., & Mishra, S. K. (2005). Biological responses of cyanobacteria to insecticides and their insecticide degrading potential. *Bulletin of Environmental Contamination and Toxicology*, 75(2), 374-381.
- Jiménez, J. C., Borrero, A. C., Becerra-Absalón, I., Urizar, E. P., Santamaría, K. M., & Ortega, P. M. (2023). Description of two new species of *Nostoc* (Nostocales, Cyanobacteria) from central Mexico, using morphological, ecological, and molecular attributes. *Journal of Phycology*, 59(6), 1237-1257.
- Johannes, E., & Litaay, M. S. (2015). The bioactivity of hexadecanoic acid compound isolated from hydroid *Aglaophenia cupressina* Lamoureaux as antibacterial agent against *Salmonella typhi*. *International Journal of Biological and Medical Research*, 4(1), 5469-5472.

- Johansson, C., & Bergman, B. (1994). Reconstitution of the symbiosis of *Gunnera manicata* Linden: cyanobacterial specificity. *New Phytologist*, 126(4), 643-652.
- John, J., & Francis, M. S. (2013). Illustrated algal flora of Kerala. Vol. I. Kochi: Science Books. pp. 281.
- Jose, J. & Francis, M.S. (2013). An Illustrated Algal Flora of Kerala. Vol. 1: Idukki District, Green Carmel Scientific Books, Kochi, pp. 40–281.
- Joshi, A., Sharma, A. P., & Pant, M. C. (1981). Limnological studies in a subtropical system (Lake Sattal, India). *Acta Hydrochimica et Hydrobiologica*, 9(4), 407-425.
- Juttner, F., Todorova, A.K., Walch, N., Von Philipsborn, W. (2001). Nostocyclamide M: a cyanobacterial cyclic peptide with allelopathic activity from *Nostoc* 31. *Phytochemistry*, 57, 613-619.
- Kaczyńska, A., Łoś, M., & Węgrzyn, G. (2013). An improved method for efficient isolation and purification of genomic DNA from filamentous cyanobacteria belonging to genera *Anabaena*, *Nodularia* and *Nostoc*. *Oceanological and Hydrobiological Studies*, 42, 8-13.
- Kajiyama, S. I., Kanzaki, H., Kawazu, K., & Kobayashi, A. (1998). Nostofungicidine, an antifungal lipopeptide from the field-grown terrestrial blue-green alga *Nostoc commune*. *Tetrahedron Letters*, 39(22), 3737-3740.
- Kallas, T., Rebiere, M. C., Rippka, R., & Tandeau de Marsac, N. (1983). The structural *nif* genes of the cyanobacteria *Gloeotheca* sp. and *Calothrix* sp. share homology with those of *Anabaena* sp., but the *Gloeotheca* genes have a different arrangement. *Journal of Bacteriology*, 155(1), 427-431.
- Kaneda, T. O. S. H. I. (1991). Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiological Reviews*, 55(2), 288-302.
- Kant, R., Sarma, K., Saini, A., Singh, J., Ziyaul, N., & Kumar, S. (2020). Diversity of the genus *Nostoc vaucher* (Nostocales, Cyanoprokaryota) from Tripura, India. *The Journal of Indian Botanical Society*, 100(1&2), 16-29.
- Kant, S. & Gupta, P. (1998). Algal flora of Ladakh. Jodhpur: Scientific Publishers (India). pp. 341.
- 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(1), 23-30.
- Karthikeyan, R., & Kumaravel, S. (2016). Study on Phenolic content, Antioxidant Activity and CHNS elemental analysis of *Amorphophallus sylvaticus*. *International Journal of Agricultural and Life Sciences*, 2(1), 12-17.
- Kauff, F., & Büdel, B. (2011). Phylogeny in cyanobacteria: an overview. *Progress in Botany*, 72, 209-224.
- Kaushik, B. D. (1998). Use of cyanobacterial biofertilizers in rice cultivation: a technology improvement. *Cyanobacterial Biotechnology*, 2, 211-222.

-
- Kaushik, B. D. (2014). Developments in cyanobacterial biofertilizer. In *Proceedings of Indian National Science Academy*, 80(2), pp. 379-388.
- Kenyon, C. (1972a). Fatty acid composition of unicellular strains of blue-green algae. *Journal of Bacteriology*, 109(2), 827-834.
- Kenyon, C. N., Rippka, R. & Stanier, R. Y. (1972b). Fatty acid composition and physiological properties of some filamentous blue-green algae. *Archives of Microbiology*, 83, 216.
- Kerner, H., Campbell, J., & Strickland, M. (2022). Introduction to machine learning. In *Machine Learning for Planetary Science*, Elsevier, pp. 24.
- Khajepour, F., Hosseini, S. A., Ghorbani Nasrabadi, R., & Markou, G. (2015). Effect of light intensity and photoperiod on growth and biochemical composition of a local isolate of *Nostoc calcicola*. *Applied Biochemistry and Biotechnology*, 176, 2279-2289.
- Kharkongor, D., & Ramanujam, P. (2014). Diversity and species composition of subaerial algal communities in forested areas of Meghalaya, India. *International Journal of Biodiversity*, 2014, 1-10.
- Khush, G. S. (2005). What it will take to feed 5.0 billion rice consumers in 2030. *Plant molecular biology*, 59, 1-6.
- Kimani, D. (2016). Biodiesel and hydrogen production: A study of *Nostoc* sp. In pulp and paper waste water. Umeå University, 37.
- Kluge, M., Mollenhauer, D., Mollenhauer, R., & Kape, R. (1992). *Geosiphon pyriforme*, an endosymbiotic consortium of a fungus and a cyanobacterium (*Nostoc*), fixes nitrogen. *Botanica acta*, 105(5), 343-344.
- Knoll, A. H. (2008). Cyanobacteria and earth history -In: Herrero A. & Flores E. (eds), The cyanobacteria: molecularbiology,genomics and evolution, CaisterAcademicPress, Norfolk, 1-19.
- Knothe, G. (2010). Biodiesel and renewable diesel: a comparison. *Progress in Energy and Combustion Science*. 36, 364-73.
- Koller, M., Salerno, A., Tuffner, P., Koinigg, M., Böchzelt, H., Schober, S., Pieber, S., Schnitzer, H., Mittelbach, M. & Braunegg, G. (2012). Characteristics and potential of micro algal cultivation strategies: a review. *Journal of Cleaner Production*, 37, 377-388.
- Kollmen, J., & Strieth, D. (2022). The beneficial effects of cyanobacterial co-culture on plant growth. *Life*, 12(2), 223.
- Komárek, J. & Anagnostidis, K. (1986). Modern approach to the classification system of the cyanophytes 2. Chroococcale-*Algological Studies*, 43, 157-226.
- Komárek, J. & Anagnostidis, K. (1989) Modern Approach to the Classification System of Cyanophytes 4. Nostocales. *Archiv für Hydrobiologie*, 82, 247-345.
-

- Komárek, J. & Anagnostidis, K. (1998). Cyanoprokaryota. 1. Chroococcales. In Ettl H., Gärtner G., Heynig H. & Mollenhauer, D. (eds.) Süßwasserflora von Mitteleuropa. Bd 19. Jena, Gustav Fischer. pp. 548.
- Komárek, J. & Anagnostidis, K. (2005). Cyanoprokaryota.2. Oscillatoriales.–In: Büdel B., Krienitz L., Gärtner G. & Schagerl M.(eds), Süßwasser flora von Mitteleuropa 19/2, Elsevier/Spektrum, Heidelberg. pp. 759.
- Komárek, J. (1989). Modern approach to the classification system of cyanophytes. 4. Nostocales. *Archiv für Hydrobiologie Supplement*, 8, 247-345.
- Komárek, J. (2006). Cyanobacterial taxonomy: current problems and prospects for the integration of traditional and molecular approaches. *Algae*, 21(4), 349-375.
- Komárek, J. (2013). Süßwasserflora von Mitteleuropa In: Freshwater Flora of Central Europe, Bd. 19/3: Cyanoprokaryota 3. Teil/3rd part: Heterocytous Genera. *Süßwasserflora von Mitteleuropa. Spektrum Akademischer Verlag, Heidelberg*. pp. 1-1130.
- Komárek, J., Kaštovský, J., Mareš, J., & Johansen, J. R. (2014). Taxonomic classification of cyanoprokaryotes (cyanobacterial genera), using a polyphasic approach. *Preslia*, 86(4), 295-335.
- Komárek, J., Komárková, J., & Kling, H. (2003). Filamentous Cyanobacteria. In: J.D. Wehr & R.G. Sheath (eds.). Freshwater algae of the United States. Academic Press, Amsterdam, pp. 117-196.
- Kondrateva, N.V. (1962). Novyi vyd synio-zelenykh vodorostey – *Nostoc edaphicum* sp. n. [A new species of blue-green algae – *Nostoc edaphicum* sp. n.]. *Ukrainian Botanical Journal*, 19(1), 58-65.
- Kondrateva, N.V. (1968). Sino-zeleni vodorosti-Cyanophyta [Blue-green algae - Cyanophyta]. In *Viznanik prisnovodnykh Vodorostej Ukrajinskoj RSR*, 1(2), 1-524.
- Krüger, G. H. J., De Wet, H., Kock, J. L. F., & Pieterse, A. J. H. (1995). Fatty acid composition as a taxonomic characteristic for *Microcystis* and other coccoid cyanobacteria (blue-green alga) isolates. *Hydrobiologia*, 308, 145-151.
- Kulasooriya, S. A., & Magana-Arachchi, D. N. (2016). Nitrogen fixing cyanobacteria: their diversity, ecology and utilisation with special reference to rice cultivation. 44(2), 111-118.
- Kulasooriya, S.A., & Magana-Arachchi, D.N. (2016). Nitrogen fixing cyanobacteria: their diversity, ecology and utilisation with special reference to rice cultivation. *Journal of the National Science Foundation of Sri Lanka*, 4(2), 111-118
- Kulik, M. M. (1995). The potential for using cyanobacteria (blue green algae) and algae in the cyanobacterial control of plant pathogenic bacteria and fungi. *European Journal of Plant Pathology*, 101, 585-599.
- Kultschar, B., & Llewellyn, C. (2018). Secondary metabolites in cyanobacteria. *Secondary Metabolites-Sources and Applications*, 64.

-
- Kumar, J., Singh, D., Tyagi, M. B., & Kumar, A. (2019). Cyanobacteria: applications in biotechnology. In *Cyanobacteria*. Academic Press. pp. 327-346.
- Kumar, K., Mella-Herrera, R. A., & Golden, J. W. (2010). Cyanobacterial heterocysts. *Cold Spring Harbor perspectives in biology*, 2(4), a000315.
- Kumar, M., & Mugunthan, M. (2018). Evaluation of three DNA extraction methods from fungal cultures. *Medical journal armed forces India*, 74(4), 333-336.
- Kumar, M., Prasanna, R., Bidyarani, N., Babu, S., Mishra, B.K., Kumar, A., Adak, A., Jauhari, S., Yadav, K., Singh, R., & Saxena, A.K. (2013). Evaluating the plant growth promoting ability of thermotolerant bacteria and cyanobacteria and their interactions with seed spice crops. *Scientia Horticulturae Amsterdam*. 164, 94-101.
- Kumar, M., Prasanna, R., Bidyarani, N., Babu, S., Mishra, B.K., Kumar, A., Adak, A., Jauhari, S., Yadav, K., & Singh, R. (2013). Evaluating the plant growth promoting ability of thermotolerant bacteria and cyanobacteria and their interactions with seed spice crops. *Scientia Horticulture*, 164, 94-101.
- Kumar, M., Saxena, R., Parihar, S. S., Rai, P. K., & Tomar, R. S. (2018). Molecular characterization and phylogeny of some cyanobacterial strains isolated from soil and freshwater ecosystem. *Journal of Pure and Applied Microbiology*, 12(2), 897-904.
- Kumar, S. J., & Sridhar, S. (2012). Antibacterial and antifungal activity of paddy-fields cyanobacteria from the Poondi Village, Thanjavur, Tamil Nadu. *International Journal of Pharmacology Research*, 4(2), 64-67.
- Kumar, S. R. S., & Rao K. V. B. (2012). Biological nitrogen fixation: A Review. *International Journal of Advancement in Life Sciences Research*, 1, 1-6.
- Kumar, S., Nei, M., Dudley, J., & Tamura, K. (2008). MEGA: biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in bioinformatics*, 9(4), 299-306.
- Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular biology and evolution*, 33(7), 1870-1874.
- Kützing, F.T. (1850). *Tabulae phycologicae*; order, Abbildungen der Tange.
- Kvídařová, J., Elster J. & Šimek M. (2011). *In situ* response of *Nostoc commune* s.l. colonies to desiccation in Central Svalbard, Norwegian High Arctic. *Fottea*, 11, 87-97.
- Ladha, J. K., Barraquio, W. L., & Revilla, L. (1997). Isolation of endophytic diazotrophic bacteria from wetland rice. In opportunities for biological nitrogen fixation in rice and other non-legumes: papers presented at the second working group meeting of the frontier project on nitrogen fixation in rice held at the National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan, 13–15 October 1996. Springer Netherlands. pp. 15-24.
-

- Lakatos, M., Bilger, W., & Büdel, B. (2001). Carotenoid composition of terrestrial cyanobacteria: response to natural light conditions in open rock habitats in Venezuela. *European Journal of Phycology*, 36(4), 367-375.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. *Nucleic Acid Techniques In Bacterial Systematics*. John Wiley and Sons, New York, pp. 115-175.
- Lange, O. L., Kilian, E., & Ziegler, H. (1986). Water vapour uptake and photosynthesis of lichens: performance differences in species with green and blue-green algae as phycobionts. *Oecologia*, 71, 104-110.
- Le Manach, S., Duval, C., Marie, A., Djediat, C., Catherine, A., Edery, M., Bernard, C. & Marie, B. (2019). Global metabolomic characterizations of *Microcystis* spp. highlights clonal diversity in natural bloom-forming populations and expands metabolite structural diversity. *Frontiers in microbiology*, 10, 791.
- Le Moigne, D., Demay, J., Reinhardt, A., Bernard, C., Kim Tiam, S., & Marie, B. (2021). Dynamics of the Metabolome of *Aliinostoc* sp. PMC 882.14 in Response to Light and Temperature Variations. *Metabolites*, 11(11), 745.
- Lea-Smith, D. J., Ortiz-Suarez, M. L., Lenn, T., Nürnberg, D. J., Baers, L. L., Davey, M. P., & Howe, C. J. (2016). Hydrocarbons are essential for optimal cell size, division, and growth of cyanobacteria. *Plant Physiology*, 172(3), 1928-1940.
- Lee, D. H., Zo, Y. G., & Kim, S. J. (1996). Non-radioactive method to study genetic profiles of bacterial communities by PCR-single strand conformation polymorphism. *Applied and Environmental Microbiology*, 62, 3112-3120.
- Lehtimäki, J., Lyra, C., Suomalainen, S., Sundman, P., Rouhiainen, L., Paulin, L., & Sivonen, K. (2000). Characterization of *Nodularia* strains, cyanobacteria from brackish waters, by genotypic and phenotypic methods. *International Journal of Systematic and Evolutionary Microbiology*, 50(3), 1043-1053.
- Lemmermann, E. (1900). Beiträge zur Kenntnis der Planktonalgen. III. Neue Schwebalgen aus der Umgegend von Berlin. *Berichte der deutsche botanischen Gesellschaft*, 18, 24-32.
- Li, D., & Liu, Y. (2003). Research review on *Nostoc commune* Vaucher in China in past decade. *Shuisheng Shengwu Xuebao (Chinese)*, 27, 408-412.
- Li, R., & Watanabe, M. M. (2001). Fatty acid profiles and their chemotaxonomy in planktonic species of *Anabaena* (Cyanobacteria) with straight trichomes. *Phytochemistry*, 57(5), 727-731.
- Li, R., & Watanabe, M. M. (2004). Fatty acid composition of planktonic species of *Anabaena* (Cyanobacteria) with coiled trichomes exhibited a significant taxonomic value. *Current microbiology*, 49, 376-380.
- Li, R., Wilhelm, S. W., Carmichael W. W. and Watanabe, M. M. (2008). Polyphasic characterization of water bloom forming *Raphidiopsis* species (cyanobacteria) from central China. *Harmful Algae*, 7, 146-153.

- Li, Y., Lin, Y., Loughlin, P. C., & Chen, M. (2014). Optimization and effects of different culture conditions on growth of *Halomicronema hongdechloris*—a filamentous cyanobacterium containing chlorophyll f. *Frontiers in plant science*, 5, 67.
- Liaimer, A., Jensen, J. B., & Dittmann, E. (2016). A genetic and chemical perspective on symbiotic recruitment of cyanobacteria of the genus *Nostoc* into the host plant *Blasia pusilla* L. *Frontiers in microbiology*, 7, 1693.
- Lin, Y., Hirai, M., Kashino, Y., Koike, H., Tuzi, S., & Satoh, K. (2004). Tolerance to freezing stress in cyanobacteria, *Nostoc commune* and some cyanobacteria with various tolerances to drying stress. *Polar Bioscience*, 17, 56-68.
- Lipman, C. B. (1941). The successful revival of *Nostoc commune* from a herbarium specimen eighty-seven years old. *Bulletin of the Torrey Botanical Club*, 664-666.
- Liu P., Liu, Z. H., Wang, C. B., Guo, F., Wang, M., & Zhang, Y.F. (2012). Effects of three long-chain fatty acids present in peanut (*Arachis hypogaea* L.) root exudates on its own growth and the soil enzymes activities, *Allelopathy Journal*, 29 (1), 13-24.
- Liu, A., Zhu, T., Lu, X., & Song, L. (2013). Hydrocarbon profiles and phylogenetic analyses of diversified cyanobacterial species. *Applied Energy*, 111, 383-393.
- Liu, C. H., & Huang, H. Y. (2012). Antimicrobial activity of curcumin- loaded myristic acid microemulsions against *Staphylococcus epidermidis*. *Chemical and Pharmaceutical Bulletin*, 60(9), 1118-112.
- Liu, S., Ruan, W., Li, J., Xu, H., Wang, J., Gao, Y., & Wang J., (2008). Biological control of phytopathogenic fungi by fatty acids, *Mycopathologia*, 166 (2), 93-102.
- Liu, X. J., & Chen, F. (2003). Nitrogen fixation behavior of a terrestrial heterocystous cyanobacterium, *Nostoc flagelliforme*, as affected by available nutrients in liquid suspension culture. *Journal of Phycology*, 39, 37-37.
- López, M. A. R., Chávez, M. M. G., Orteg, N. C. C., & Sánchez, M. A. Z., (2012). Activity of the main fatty acid components of the hexane leaf extract of *Ricinus communis* against *Spodoptera frugiperda*. *African Journal of Biotechnology*, 11(18), 4274-4278.
- López-Cortés, A., Garcia-Pichel, F., Nübel, U., & Vázquez-Juárez, R. (2001). Cyanobacterial diversity in extreme environments in Baja California, Mexico: a polyphasic study. *International Microbiology*, 4, 227-236.
- Lowry, O., Rosebrough, C. N., Farr, A., & Randall R. (1951). Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265-275.
- Ludwig, W. & Klenk, H.P. (2001). Overview: A phylogenetic backbone and taxonomic framework for prokaryotic systematic. In: Boone DR, Castenholz RW (eds) The archaea and the deeply branching and phototrophic bacteria, *Bergey's manual of systematic bacteriology*, 2nd edition. Springer, New York, pp. 49-65.
- Ludwig, W., & Schleifer, K. H., (1994). Bacterial phylogeny based on 16S and 23S rRNA sequence analysis. *FEMS Microbiology Reviews*, 15, 155-173.

- Lyra, C., Suomalainen, S., Gugger, M., Vezie, C., Sundman, P., Paulin, L., & Sivonen, K. (2001). Molecular characterization of planktic cyanobacteria of *Anabaena*, *Aphanizomenon*, *Microcystis* and *Planktothrix* genera. *International Journal of Systematic and Evolutionary Microbiology*, 51(2), 513-526.
- Ma, W. J., Chen, X. F., Lou, W. J., Liu, H., Wang, B., Wu, Q., Zhao, Y., Peng, T. & Chen, P. (2016). An effective method for high purity genomic DNA extraction from *N. flagelliforme*. *SCIREA Journal of Agriculture*, 1(2), 169-185.
- Mackinney, G. (1941). Absorption of light by chlorophyll solutions. *Journal of biological chemistry*, 140(2), 315-322.
- Magana-Arachchi, D. N., Wanigatunge, R. P., & Jeyanandarajah, P. (2008). Setting up a polymerase chain reaction assay for the detection of toxic cyanobacteria. *Journal of the National Science Foundation of Sri Lanka*. 36(3) 229-233.
- Magana-Arachchi, D., & Wanigatunge, R. (2011). A simple and rapid DNA extraction method for Cyanobacteria and Monocots. *Ceylon Journal of Science*, 40 (1), 59-63.
- Mahmood, A., Turgay, O. C., Farooq, M., & Hayat, R. (2016). Seed biopriming with plant growth promoting rhizobacteria: a review. *FEMS microbiology ecology*, 92(8), 112.
- Mahmoud, S. A., Abd El-Aty, A. M., Kandil, H., & Siam, H. S. (2019). Influence of different algal species application on growth of spinach plant (*Spinacia oleracea* L.) and their role in phytoremediation of heavy metals from polluted soil. *Plant Archives*, 19, 2275-2281.
- Makra, N., Gell, G., Juhász, A., Soós, V., Kiss, T., Molnár, Z., Ördög, V., Vörös, L., & Balázs, E. (2019). Molecular taxonomic evaluation of *Anabaena* and *Nostoc* strains from the Mosonmagyaróvár Algal Culture Collection. *South African Journal of Botany*, 124, 80-86.
- Manjunath, M., Prasanna, R., Nain, L., Dureja, P., Singh, R., Kumar, A., & Kaushik, B. D. (2010). Biocontrol potential of cyanobacterial metabolites against damping off disease caused by *Pythium aphanidermatum* in solanaceous vegetables. *Archives of Phytopathology and Plant Protection*, 43(7), 666-677.
- Maqubela, M. P., Mkeni, P. N. S., Malam Issa, 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 Soil*, 315, 79-92.
- Marrez, D. A., & Sultan, Y. Y. (2016). Antifungal activity of the cyanobacterium *Microcystis aeruginosa* against mycotoxigenic fungi. *Journal of Applied Pharmaceutical Science*, 6(11), 191-198.
- Mastrangelo, A., Ferrarini, A., Rey-Stolle, F., Garcia, A., & Barbas, C. (2015). From sample treatment to biomarker discovery: A tutorial for untargeted metabolomics based on GC-(EI)-Q-MS. *Analytica chimica acta*, 900, 21-35.
- Mazhar, S., Cohen, J. D., & Hasnain, S. (2013). Auxin producing non-heterocystous Cyanobacteria and their impact on the growth and endogenous auxin homeostasis of wheat. *Journal of Basic Microbiology*, 53(12), 996-1003.

- Mazor, G., Kidron, G. J., Vonshak, A., & Abeliovich, A. (1996). The role of cyanobacterial exopolysaccharides in structuring desert microbial crusts. *FEMS Microbiology Ecology*, 21(2), 121-130.
- Mazur, B. J., Rice, D., & Haselkorn, R. (1980). Identification of blue-green algal nitrogen fixation genes by using heterologous DNA hybridization probes. *Proceedings of the National Academy of Sciences*, 77(1), 186-190.
- McGregor, G. B. (2018). Freshwater cyanobacteria of north-eastern Australia: 3. Nostocales. *Phytotaxa*, 359(1), 1-166.
- Meeks, J. C., & Elhai, J. (2002). Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. *Microbiology and molecular biology reviews*, 66(1), 94-121.
- Meeks, J. C., & Elhai, J. (2002). Regulation of cellular differentiation in filamentous cyanobacteria in free-living and plant-associated symbiotic growth states. *Microbiology and Molecular Biology Reviews*. 66(1), 94-121.
- Mei, W. A. N. G., Yi-Nong, X. U., Gui-Zhen, J. I. A. N. G., Liang-Bi, L. I., & Ting-Yun, K. U. A. N. G. (2000). Membrane lipids and their fatty acid composition in *Nostoc flagelliforme* cells. *Journal of Integrative Plant Biology*, 42(12), 1263-1266.
- Mendhekar, S. V. (2023). Cyanobacteria: Isolation, Purification and Principles. *International Journal of Current Science Research and Review*, 6(4), 2688-2696.
- Mesfin, M., Johansen, J. R., Pietrasiak, N., & Baldarelli, L. M. (2020). *Nostoc oromo* sp. nov. (Nostocales, Cyanophyceae) from Ethiopia: a new species based on morphological and molecular evidence. *Phytotaxa*, 433(2), 81-93.
- Milne, I., Lindner, D., Bayer, M., Husmeier, D., McGuire, G., Marshall, D. F., & Wright, F. (2009). TOPALi v2: a rich graphical interface for evolutionary analyses of multiple alignments on HPC clusters and multi-core desktops. *Bioinformatics*, 25, 126-127.
- Milovanović, I., Mišan, A., Simeunović, J., Kovač, D., Jambrec, D., & Mandić, A. (2015). Determination of volatile organic compounds in selected strains of cyanobacteria. *Journal of Chemistry*, 1-6.
- Mishra, A. K., Shukla, E., & Singh, S. S. (2013). Phylogenetic comparison among the heterocystous cyanobacteria based on a polyphasic approach. *Protoplasma*, 250, 77-94.
- Miyashita, H., Ikemoto, H., Kurano, N., Adachi, K., Chihara, M., & Miyashi, S. (1996). Chlorophyll d a major pigment. *Nature*, 383, 402.
- Moffitt, M. C., Blackburn, S. I., & Neilan, B. A. (2001). rRNA sequences reflect the ecophysiology and define the toxic cyanobacteria of the genus *Nodularia*. *International Journal of Systematic and Evolutionary Microbiology*, 51(2), 505-512.
- Mohsen, A. A. M., Salama, A.S.A., & El-Saadony, F. M. A. (2016). The effect of foliar spray with cyanobacterial extracts on growth, yield and quality of lettuce plants (*Lactuca sativa* L.). *Middle East Journal of Agriculture Research*, 5, 90-96.

- Mollenhauer, D. (1970). Beiträge zur Kenntnis der Gattung *Nostoc*. *Abh Senckenb Naturf Ges*, 24, 1-80.
- Mollenhauer, D., Bengtsson, R., & Lindstrøm, E. A. (1999). Macroscopic cyanobacteria of the genus *Nostoc*: a neglected and endangered constituent of European inland aquatic biodiversity. *European Journal of Phycology*, 34(4), 349-360.
- Mondal, M., Halder, G., Oinam, G., Indrama, T., & Tiwari, O.N. (2019). Bioremediation of organic and inorganic pollutants using microalgae, New and future developments in microbial biotechnology and bioengineering. Elsevier, pp. 223-235.
- Moore, R. E. (1996). Cyclic peptides and depsipeptides from cyanobacteria: a review. *Journal of Industrial Microbiology*, 16, 134-143.
- Morin, N., Vallaey, T., Hendrickx, L., Natalie, L., & Wilmotte, A. (2010). An efficient DNA isolation protocol for filamentous cyanobacteria of the genus *Arthrospira*. *Journal of Microbiological Methods*, 80(2), 148-154.
- Morone, J., Lopes, G., Preto, M., Vasconcelos, V., & Martins, R. (2020). Exploitation of filamentous and picoplanktonic cyanobacteria for cosmetic applications: Potential to improve skin structure and preserve dermal matrix components. *Marine drugs*, 18(9), 486.
- Mundt, S., Kreitlow, S., & Jansen, R. (2003). Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB051. *Journal of Applied Phycology*, 15, 263 -267.
- Mundt, S., Kreitlow, S., Novotony, A., & Effmert, U. (2001). Biochemical and pharmacological investigations of selected cyanobacteria. *International Journal of Environment and Health*, 203, 327-334.
- Muñoz-Rojas, M., Chilton, A., Liyanage, G. S., Erickson, T. E., Merritt, D. J., Neilan, B. A., & Ooi, M. K. J. (2018). Effects of indigenous soil cyanobacteria on seed germination and seedling growth of arid species used in restoration. *Plant and Soil*, 429, 91-100.
- Murata, N., Wada, H. & Gombos, Z. (1992). Modes of fatty-acid desaturation in cyanobacteria. *Plant and Cell Physiology*, 33, 933-41.
- Mutale-Joan, C., Redouane, B., Najib, E., Yassine, K., Lyamlouli, K., Laila, S., Zeroual, Y., & El Arroussi, H. (2020). Screening of microalgae liquid extracts for their biostimulant properties on plant growth, nutrient uptake and metabolite profile of *Solanum lycopersicum* L. *Scientific Reports*, 10, 2820.
- Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., & Bux, F., (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production, *Bioresource Technology*, 102 (1), 57-70.
- Muthukumar, C., Muralitharan, G., Vijayakumar, R., Panneersevam, A., & Thajuddin, N. (2007). Cyanobacterial biodiversity from different freshwater ponds of Thanjavur, Tamilnadu (India). *Acta Botanica Malacitana*, 32, 17-25.
- Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. & Kent, J., (2000). Biodiversity hotspots for conservation priorities. *Nature*, 403, 853-858

- Nagappan, S., Bhosale, R., Nguyen, D. D., Pugazhendhi, A., Tsai, P. C., Chang, S. W. & Kumar, G. (2020). Nitrogen-fixing cyanobacteria as a potential resource for efficient biodiesel production. *Fuel*, 279, 118440.
- Nasser, K. M., & Sureshkumar, S. (2013). Interaction between microalgal species richness and environmental variables in Peringalkuthu Reservoir, Western Ghats, Kerala. *Journal of Environmental Biology*, 34(6), 1001.
- Nayak, S. & Prasanna, R. (2007). Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. *Applied Ecology and Environmental Research*, 5(2), 103-113
- Nayak, S., Prasanna, R., Pabby, A., Dominic, T. K., & Singh, P. K. (2004). Effect of urea, blue green algae and *Azolla* on nitrogen fixation and chlorophyll accumulation in soil under rice. *Biology and Fertility of Soils*, 40, 67-72.
- Nayak, S., Prasanna, R., Prasanna, B.M. & Sahoo, D.B. (2007): Analysing diversity among Indian isolates of *Anabaena* (Nostocales, Cyanophyta) using morphological, physiological and biochemical characters. *World Journal of Microbiology and Biotechnology*, 23, 1575-1584.
- Nei, M., & Kumar, S. (2000). *Molecular evolution and phylogenetics*. Oxford University Press, USA. pp. 352.
- Neilan, B. A., Burns, B. P., Relman, D. A., & Lowe, D. R. (2002). Molecular identification of cyanobacteria associated with stromatolites from distinct geographical locations. *Astrobiology*, 2, 271-280.
- Neilan, B. A., Jacobs, D., Therese, D. D., Blackall, L. L., Hawkins, P. R., Cox, P. T., & Goodman, A. E. (1997). rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. *International Journal of Systematic and Evolutionary Microbiology*, 47(3), 693-697.
- Nelissen, B., De Baere, R., Wilmotte, A., & De Wachter, R. (1996). Phylogenetic relationships of nonaxenic filamentous cyanobacterial strains based on 16S rRNA sequence analysis. *Journal of Molecular Evolution*, 42, 194-200.
- Netscher, T. (2007). Synthesis of Vitamin E. *Vitamins and Hormones*, 76, 155-202.
- Neustupa, J., & Škaloud, P. (2008). Diversity of subaerial algae and cyanobacteria on tree bark in tropical mountain habitats. *Biologia*, 63, 806-812.
- Niimura, Y., & Nei, M. (2005). Evolutionary dynamics of olfactory receptor genes in fishes and tetrapods. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 6039-6044.
- Nikam, T. D., Nehul, J. N., Gahile, Y. R., Auti, B. K., Ahire, M. L., Nitnaware, K. M., Josh, B.N. & Jawali, N. (2010). Cyanobacterial diversity in Western Ghats region of Maharashtra, India. *Bioremediation, Biodiversity and Bioavailability*, 7(1), 70-80.
- Nilsson, M., Bhattacharya, J., Rai, A. N., & Bergman, B. (2002). Colonization of roots of rice (*Oryza sativa*) by symbiotic *Nostoc* strains. *New Phytologist*, 156(3), 517-525.

- Nowruzi, B., Haghghat, S., Fahimi, H., & Mohammadi, E. (2018). Nostoc cyanobacteria species: a new and rich source of novel bioactive compounds with pharmaceutical potential. *Journal of Pharmaceutical Health Services Research*, 9(1), 5-12.
- Nowruzi, B., Khavari-Nejad, R. A., Sivonen, K., Kazemi, B., Najafi, F., & Nejadstari, T. (2012). Identification and toxigenic potential of a *Nostoc* sp. *Algae*, 27(4), 303-313.
- Nübel, U., Garcia-Pichel, F., & Muyzer, G. (1997). PCR primers to amplify 16S rRNA genes from cyanobacteria. *Applied and environmental microbiology*, 63(8), 3327-3332.
- Nübel, U., Garcia-Pichel, F., Clavero, E., & Muyzer, G. (2000). Matching molecular diversity and ecophysiology of benthic cyanobacteria and diatoms in communities along a salinity gradient. *Environmental Microbiology*, 2(2), 217-226.
- O'Callaghan, M. (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied microbiology and biotechnology*, 100(13), 5729-5746.
- Obana, S., Miyamoto, K., Morita, S., Ohmori, M., & Inubushi, K. (2007). Effect of *Nostoc* sp. on soil characteristics, plant growth and nutrient uptake. *Journal of applied phycology*, 19, 641-646.
- Obrecht, Z., Kerby, N. W., Gantar, M., & Rowell, P. (1993). Effects of root-associated N 2-fixing cyanobacteria on the growth and nitrogen content of wheat (*Triticum vulgare* L.) seedlings. *Biology and fertility of soils*, 15, 68-72.
- Ogunlesi, M., Okiei, W., Ofor, E., Osibote, A. E. (2009). Analysis of the essential oil from the dried leaves of *Euphorbia hirta* Linn (Euphorbiaceae), a potential medication for asthma. *African Journal of Biotechnology*, 8(24), 7042 -7050.
- Ojit, S. K., Indrama, T., Gunapati, O., Avijeet, S. O., Subhalaxmi, S. A., Silvia, C. H., & Sharma, G. D. (2015). The response of phycobiliproteins to light qualities in *Anabaena circinalis*. *Journal of Applied Biology and Biotechnology*, 3(3), 001-006.
- Ördög, V., Stirk, W. A., Lenobel, R., Bancířová, M., Strnad, M., Van Staden, J., & Németh, L. (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, 16, 309-314.
- Osman, M. E. H., El-Sheekh, M. M., El-Naggar, A. H., & Gheda, S. F. (2010). Effect of two species of cyanobacteria as biofertilizers on some metabolic activities, growth, and yield of pea plant. *Biology and fertility of soils*, 46, 861-875.
- Osman, M.E.H., El-Sheekh, M.M., Metwally, A.M., Ismail, A.A., Ismail, M.M. (2011). Antagonistic activity of some fungi and cyanobacteria species against *Rhizoctonia solani*. *International Journal of Plant Pathology*. 2(3), 101-114.
- Ozdemir, G., Ulku Karabay, N., Dalay, M.C. & Pazarbasi, B. (2004). Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, 18(9), 754-757.
- Ozturk, B.Y., Asikkuttu, B., Akkoz, C. and Atici, T. 2018. Molecular and morphological characterization of several cyanobacteria and chlorophyta species isolated from

-
- Lakes in Turkey. *Turkish Journal of Fisheries and Aquatic Sciences*. 19(8), 635-643.
- Pabbi, S. (2015). Blue green algae: a potential biofertilizer for rice. *The algae world*, 449-465.
- Paerl, H. W. (2014). Mitigating harmful cyanobacterial blooms in a human-and climatically impacted world. *Life*, 4(4), 988-1012.
- Palic, R., Stojanovic, G., Alagic, S., Nikolic, M. & Lepojevic, Z. (2002). Chemical composition and antimicrobial activity of the essential oil and CO₂ extracts of the oriental tobacco, Prilep. *Flavour and Fragrance*, 17(5), 323-326.
- Pan, S., Jeevanandam, J., & Danquah, M. (2019). *Grand Challenges in Marine Biotechnology*. Springer Science and Business. Benefits of algal extracts in sustainable agriculture, pp. 501-553.
- Pan, X. J., Chang, F. Y., Kang, L. J., Li, G. B., Li, D. H., Liu, Y. D., Shen, Y. W. & Wei, Z. H. (2008). Morphological characteristics and phylogenetic relationship of *Anabaena* species from Lakes Dianchi and Erhai, China. *Hydrobiologia*, 614, 353-362.
- Papke, U., Gross, E.M., & Francke, W. (1997). Isolation, identification and determination of the absolute configuration of fischerellin B. A new algicide from the freshwater cyanobacterium *Fischerella muscicola* (Thuret.). *Tetrahedron Letters*, 38, 379-382.
- Parukutty, P. R. (1940). The Myxophyceae of the Travancore state, India. *Proceedings of the Indian Academy of Sciences – Section B*, 11 (3), 177-124
- Pawar, S. T., & Puranik, P. R. (2008). Screening of terrestrial and freshwater halotolerant cyanobacteria for antifungal activities. *World Journal of Microbiology and Biotechnology*, 24, 1019-1025.
- Pearson, R., (2010). The safety of fatty acid methyl esters and their acceptability as immediate previous cargoes to be used in foods after further processing. *The European Oleochemical and Allied Products Group*, 1-8.
- Pedrini, S., Gibson-Roy, P., Trivedi, C., Gálvez-Ramírez, C., Hardwick, K., Shaw, N., Frischie, S., Laverack, G. & Dixon, K. (2020). Collection and production of native seeds for ecological restoration. *Restoration Ecology*, 28, S228-S238.
- Peramuna, A. (2013). *Characterization of neutral lipid production of Nostoc punctiforme*, University of California State, Northridge.
- Peramuna, A., & Summers, M. L. (2014). Composition and occurrence of lipid droplets in the cyanobacterium *Nostoc punctiforme*. *Archives of Microbiology*, 196(12), 881-890.
- Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R., & Tamagnini, P. (2009). Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. *FEMS microbiology reviews*, 33(5), 917-941.
- Pérez, G., Cerecetto, V., & Irisarri, P. (2020). Potential cyanobacterial inoculants for rice described from a polyphasic approach. *Agrocien-cia Uruguay*, 24(2), 52.
-

- Philip M. (2020). *Cyanobacterial diversity of Western Ghat forests of Kerala, India*. PhD (Biology) Thesis. University of Calicut, Kerala, India.
- Piotrowski, K., Romanowska-Duda, Z., & Grzesik, M., Cyanobacteria, Asahi SL and Biojodis as stymulants improving growth and development of the *Sida hermaphrodita* L. Rusby plants under changing climate conditions. *Przemysł Chemiczny* 2016, 95, 1569-1573.
- Porter, R. D. (1988). DNA transformation. In *Methods in enzymology*, Academic Press. 167, pp. 703-712.
- Potts, M. (2000). *Nostoc*. In: Whitton A, Potts M, eds. *The ecology of cyanobacteria*. Dordrecht, The Netherlands: Kluwer. Academic Publishers, pp. 465-504.
- Potts, M. (2002). *Nostoc*. *The ecology of cyanobacteria: their diversity in time and space*, pp. 465-504.
- Potts, M., Olie, J. J., Nickels, J. S., Parsons, J., & White, D. C. (1987). Variation in phospholipid ester-linked fatty acids and carotenoids of desiccated *Nostoc commune* (cyanobacteria) from different geographic locations. *Applied and Environmental Microbiology*, 53(1), 4-9.
- Potts, M., Slaughter, S. M., Hunneke, F. U., Garst, J. F., & Helm, R. F. (2005). Desiccation tolerance of prokaryotes: application of principles to human cells. *Integrative and Comparative Biology*, 45(5), 800-809.
- Poza-Carrión, C., Fernández-Valiente, E., Piñas, F. F., & Leganés, F. (2001). Acclimation of photosynthetic pigments and photosynthesis of the cyanobacterium *Nostoc* sp. strain UAM206 to combined fluctuations of irradiance, pH, and inorganic carbon availability. *Journal of Plant Physiology*, 158(11), 1455-1461.
- Prabha, R., Singh, D. P., & Yadav, S. K. (2019). Seed biopriming with potential microbial inoculants as sustainable options for stress management in crops. *Microbial Interventions in Agriculture and Environment. Soil and Crop Health Management*, 3, 211-224.
- Prasad, B. N., & Mehrotra, R. K. (1980). Blue green algae of paddy fields of U.P. *Phykos*, 19, 121-128.
- Prasad, B. N., & Mehrotra, R. K. (1978). Some New Addition to Cyanophycean Flora of India. *Journal of Indian Botanical Society*, 51(1), 98-101.
- Prasad, S.R., Kamble, U. R., Sripathy, K. V., Udaya Bhaskar, K., & Singh, D. P. (2016). Seed bio-priming for biotic and abiotic stress management. *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 1: Research Perspectives*, 211-228.
- Prasanna, R., & Nayak, S. (2007). Influence of diverse rice soil ecologies on cyanobacterial diversity and abundance. *Wetlands Ecology and Management*, 15, 127-134.
- Prasanna, R., Babu, S., Bidyarani, N., Kumar, A., Triveni, S., Monga, D., Mukherjee, A. K., Kranthi, S., Gokte-Narkhedkar, N., & Adak, A. (2014). Prospecting cyanobacteria-fortified composts as plant growth promoting and biocontrol agents in cotton. *Experimental Agriculture*, 51, 42-65.

- Prasanna, R., Babu, S., Rana, A., Kabi, S.R., Chaudhary, V., Gupta, V., Kumar, A., Shivay, Y. S., Nain, L. and Pal, R. K. (2013). Evaluating the establishment and agronomic proficiency of cyanobacterial consortia as organic options in wheat-rice cropping sequence. *Experimental Agriculture*, 49(3), 416-434.
- Prasanna, R., Bidyarani, N., Babu, S., Hossain, F., Shivay, Y.S., & Nain, L. (2015). Cyanobacterial inoculation elicits plant defense response and enhanced Zn mobilization in maize hybrids. *Cogent Food & Agriculture*, 1(1), 998507.
- Prasanna, R., Joshi, M., Rana, A., Shivay, Y. S., & Nain, L. (2012). Influence of co-inoculation of bacteria-cyanobacteria on crop yield and C–N sequestration in soil under rice crop. *World Journal of Microbiology and Biotechnology*, 28, 1223-1235.
- Prasanna, R., Kanchan, A., Ramakrishnan, B., Ranjan, K., Venkatachalam, S., Hossain F, Shivay, Y.S., Krishnan, P. & Nain, L. (2016). Cyanobacteria-based bioinoculants influence growth and yields by modulating the microbial communities favourably in the rhizospheres of maize hybrids. *European Journal of Soil Biology*, 75, 15-23.
- Prasanna, R., Nain, L., Ancha, R., Srikrishna, J., Joshi, M., & Kaushik, B. D. (2009). Rhizosphere dynamics of inoculated cyanobacteria and their growth-promoting role in rice crop. *Egyptian Journal of Biology*, 11, 26-36.
- Prasanna, R., Nain, L., Tripathi, R., Gupta, V., Chaudhary, V., Middha, S., Joshi, M., Ancha, R. & Kaushik, B.D., (2008). Evaluation of fungicidal activity of extracellular filtrates of cyanobacteria—possible role of hydrolytic enzymes. *Journal of basic microbiology*, 48(3), 186-194.
- Prescott, G. W. (1982). *Algae of the Western Great Lakes area*: Koenigstein, Germany, Otto Koeltz Science Publishers, pp 977.
- Prinsep, M. R., Thomson, R. A., West, M. L., & Wylie, B. L. (1996). Tolypodiol, an antiinflammatory diterpenoid from the cyanobacterium *Tolypothrix nodosa*. *Journal of Natural Products*, 59(8), 786-788.
- Priya, H., Prasanna, R., Ramakrishnan, B., Bidyarani, N., Babu, S., Thapa, S., & Renuka, N. (2015). Influence of cyanobacterial inoculation on the culturable microbiome and growth of rice. *Microbiological Research*, 171, 78-89.
- Punj, G., & Stewart, D. W. (1983). Cluster analysis in marketing research: Review and suggestions for application. *Journal of marketing research*, 20(2), 134-148.
- Qi, D., Wu, Q., & Zhu, J. (2020). Nitrogen and phosphorus losses from paddy fields and the yield of rice with different water and nitrogen management practices. *Scientific Reports*, 10, 1-12.
- Raghavan, P. S., Potnis, A. A., Bhattacharyya, K., Salaskar, D. A., & Rajaram, H. (2020). Axenic cyanobacterial (*Nostoc muscorum*) biofilm as a platform for Cd (II) sequestration from aqueous solutions. *Algal Research*, 46, 101778.
- Ragno, G., De Luca, M., & Ioele, G. (2007). An application of cluster analysis and multivariate classification methods to spring water monitoring data. *Microchemical Journal*, 87(2), 119-127.

-
- Rai, A. N., & Bergman, B. (2000). Creation of new nitrogen-fixing cyanobacterial associations (special issue–biology and environment), in *Proceedings of the Royal Irish Academy*, 102B, 65-68.
- Rai, A. N., Bergman, B., & Rasmussen, U. (2002). *Cyanobacteria in symbiosis*. Kluwer Academic Publication.
- Rai, A. N., Söderbäck, E., & Bergman, B. (2000). Cyanobacterium-plant symbioses. *New Phytologist*, 147(3), 449-481.
- Rai, S. V., & Rajashekhar, M. (2016). Effect of pH, Salinity and Temperature on the Growth of Six Species of Cyanobacteria Isolated from Arabian Sea Coast of Karnataka. *International Journal of Bio Sciences & Technology*, 9(1), 1-6.
- Rajakumar, N. (2004). A review on the quantum of phytoplanktonic primary production of a polluted freshwater pond. *Indian Hydrobiology*, 7, 61-65.
- Rajaniemi, P., Hrouzek, P., Kaštovská, K., Willame, R., Rantala, A., Hoffmann, L., Komárek, J., & Sivonen, K. (2005). Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). *International Journal of Systematic and Evolutionary Microbiology*, 55, 11-26.
- Ram, A. T., & Shamina, M. (2016). Biodiversity of epilithic cyanobacteria from Kerala, India. In *Proceedings of the 25th Swadeshi Science Congress*, Kalady, 309-318.
- Ram, A. T., & Shamina, M. (2017). Cyanobacterial diversity from seven mangrove environments of Kerala, India. *World News of Natural Sciences*, 9.
- Ram, A.T. & Paul, P. T. (2022). Mangrove-Associated Cyanobacteria of *Nostoc* spp. (Nostocales, Nostocaceae) from Kerala State, India. *International Journal on Algae*, 24(2), 145-158.
- Ram, A.T. (2022). Ecology and diversity of mangrove-associated cyanobacteria of Southern Kerala, India. Thesis of University of Calicut, Kerala, India.
- Ramadan, K. M., El-Beltagi, H. S., Shanab, S. M., El-Fayoumy, E. A., Shalaby, E. A., & Bendary, E. S. (2021). Potential antioxidant and anticancer activities of secondary metabolites of *Nostoc linckia* cultivated under Zn and Cu stress conditions. *Processes*, 9(11), 1972.
- Raman, V.B., Samuel, L.A., Saradhi, M.P, Rao, B. N., Krishna, N. V., Sudhakar, M., & Radhakrishnan, T.M. (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. *Asian Journal of Pharmaceutical and Clinical Research*, 5(2), 99-106.
- Rana, A., Joshi, M., Prasanna, R., Shivay, Y. S., & Nain, L. (2012). Biofortification of wheat through inoculation of plant growth promoting rhizobacteria and cyanobacteria. *European Journal of Soil Biology*, 50, 118-126.
- Rao, C.B. (1937). The Myxophyceae of the United Provinces, India - III. *Proceedings of the National Institute of Sciences of India*, 6(6), 339-375.
-

- Rao, D. L. N., & Burns, R. G. (1990). The effect of surface growth of blue-green algae and bryophytes on some microbiological, biochemical, and physical soil properties. *Biology and Fertility of Soils*, 9, 239-244.
- Rastogi, R.P., & Sinha, R.P. (2009). Biotechnological and industrial significance of cyanobacterial secondary metabolites. *Biotechnology advances*, 27(4), 521-539.
- Rawat, D., & Bhargava, S. (2011). Bioactive compounds from *Nostoc* species. *Current Research in Pharmaceutical Sciences*, 48-54.
- Raymond, J., Siefert, J. L., Staples, C. R., & Blankenship, R. E. (2004). The natural history of nitrogen fixation. *Molecular Biology and Evolution*, 21(3), 541-554.
- Řeháková, K., Johansen, J. R., Casamatta, D. A., Xuesong, L., & Vincent, J. (2007). Morphological and molecular characterization of selected desert soil cyanobacteria: three species new to science including *Mojavia pulchra* gen. et sp. nov. *Phycologia*, 46(5), 481-502.
- Renaut, J., Sasson, A., Pearson, H. W., & Stewart, W. D. P. (1975). Nitrogen-fixing algae in Morocco. pp. 229-246.
- Rencher, A. C. (2005). A review of “methods of multivariate analysis, Wiley interscience publication, pp. 708.
- Řezanka, T., Dor, I., Prell, A., & Dembitsky, V. M. (2003). Fatty acid composition of six freshwater wild cyanobacterial species. *Folia Microbiologica*, 48, 71-75.
- Rikkinen, J. (2017). Cyanobacteria in terrestrial symbiotic systems. *Modern Topics in the Phototrophic Prokaryotes: Environmental and Applied Aspects*, 243-294.
- Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., & Stanier, R. Y. (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. *Microbiology*, 111(1), 1-61.
- Rodgers, G. A., Bergman, B., Henriksson, E., & Udriș, N. (1979). Utilization of blue-green algae as biofertilizers. *Plant Soil*, 52, 99-107.
- Rodríguez, A. A., Stella, A. M., Storni, M. M., Zulpa, G., & Zaccaro, M. C. (2006). Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L. *Saline systems*, 2, 1-4.
- Roger, P. A., & Reynaud, P. A. (1982). Free-living blue-green algae in tropical soils. In *Microbiology of tropical soils and plant productivity*. Dordrecht: Springer, Netherlands. pp. 168.
- Rogers, S. L., & Burns, R. G. (1994). Changes in aggregate stability, nutrient status, indigenous microbial populations, and seedling emergence, following inoculation of soil with *Nostoc muscorum*. *Biology and Fertility of Soils*, 18, 209-215.
- Romano, I., Bellitti, M. R., Nicolaus, B., Lama, L., Manca, M. C., Pagnotta, E., & Gambacorta, A. (2000). Lipid profile: a useful chemotaxonomic marker for classification of a new cyanobacterium in *Spirulina* genus. *Phytochemistry*, 54(3), 289-294.

- Rosales-Loaiza, N., Aiello-Mazzarri, C., Gómez, L., Arredondo, B., & Morales, E. (2017). Nutritional quality of biomass from four strains of *Nostoc* and *Anabaena* grown in batch cultures. *International Food Research Journal*, 24(5), 2212-2219.
- Rossi, F., & De Philippis, R. (2015). Role of cyanobacterial exopolysaccharides in phototrophic biofilms and in complex microbial mats. *Life*, 5(2), 1218-1238.
- Roy, S.S., Dasgupta, R., & Bagchi, A. (2014) A reviews on phylogenetic analysis: a journey through modern era. *Computational Molecular Biology*, 4, 39-45.
- Ruffing, A. M. (2014). Improved free fatty acid production in cyanobacteria with *Synechococcus* sp. PCC 7002 as host. *Frontiers in bioengineering and biotechnology*, 2, 17.
- Saadatnia, H., & Riahi, H. (2009). Cyanobacteria from paddy fields in Iran as a biofertilizer in rice plants. *Plant, Soil and Environment*, 55(5), 207-212.
- Sachs, J. (1874). *Lehrbuch der Botanik*, vol XVI, 4th edn. W. Engelmann, Leipzig, pp. 928.
- Saha B. C., & Racine F. M. (2011) Biotechnological production of mannitol and its applications. *Applied Microbiology and Biotechnology*, 89, 879-891.
- Saha, K. C., & Mandal, L. N. (1979). Distribution of nitrogen fixing blue-green algae in some rice soils of West Bengal. *Journal of the Indian Society of Soil Science*, 27(4), 470-477.
- Saha, S. K., Das, R., Bora, K. N., & Uma, L. (2007). Biodiversity of epilithic cyanobacteria from freshwater streams of Kakoijana reserve forest, Assam, India. *Indian Journal of Microbiology*, 47, 219-232.
- Sahrawat, K. Á. (2000). Elemental composition of the rice plant as affected by iron toxicity under field conditions. *Communications in soil science and plant analysis*, 31(17-18), 2819-2827.
- Sahu, J.K., Nayak, H. & Adhikary, S.P. (1996). Blue Green Algae in rice fields of Orissa state. I. Distribution pattern of cyanobacteria in different agroclimatic zones. *Phykos*, 35, 93-110.
- Saitou, N., & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular biology and evolution*, 4(4), 406-425.
- Salama, A. (2015). Response of rice plants to inoculation with indigenous strains of cyanobacterial along with different levels of inorganic n-fertilizers. *Advances in Biochemical Engineering/Biotechnology*, 1, 1-14.
- Salem, O. M., Hoballah, E. M., Ghazi, S. M., & Hanna, S. N. (2014). Antimicrobial activity of microalgal extracts with special emphasize on *Nostoc* sp. *Life Science Journal*, 11(12), 752-758.
- Samad, L. K., & Adhikary, S. P. (2008). Diversity of micro-algae and cyanobacteria on building facades and monuments in India. *Algae*, 23(2), 91-114.
- Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). *Molecular cloning: a laboratory manual* (No. Ed. 2). Cold spring harbor laboratory press. pp. 1546.

-
- Sant'Anna, C. L., Azevedo, M. T. D. P., Branco, L. H. Z., Komárek, J. (2007). New Aerophytic Morphospecies of *Nostoc* (Cyanobacteria) from Sao Paulo State, Brazil. *Hoehnea*, 34(1), 95-101.
- Sarma, T. A., Ahuja, G., & Khattar, J. I. S. (2004). Nutrient stress causes akinete differentiation in cyanobacterium *Anabaena torulosa* with concomitant increase in nitrogen reserve substances. *Folia microbiologica*, 49, 557-561.
- Sato, N., & Murata, N. (1988). Membrane lipids. In *Methods in enzymology*, Academic Press. pp 251-259.
- Scherer, S., & Potts, M. (1989). Novel water stress protein from a desiccation-tolerant cyanobacterium: purification and partial characterization. *Journal of Biological Chemistry*, 264(21), 12546-12553.
- Schleifer, K. H. (2009). Classification of Bacteria and Archaea: past, present and future. *Systematic and Applied Microbiology*, 32(8), 533-542.
- Schön, A., Fingerhut, C., & Hess, W. R. (2002). Conserved and variable domains within divergent rnaP RNA gene sequences of *Prochlorococcus* strains. *International Journal of Systematic and Evolutionary Microbiology*, 52(4), 1383-1389.
- Schopf, J. W. (1968). Microflora of the Bitter Springs Formation, Late Precambrian, central Australia. *Journal of Paleontology*. 42, 651-88.
- Schwartz, R. E., Hirsch, C. F., Sesin, D. F., Flor, J. E., Chartrain, M., Fromtling, R. E., Harris, G. H., Salvatore, M. J., Liesch, J. M., & Yudin, K. (1990). Pharmaceuticals from Cultured Algae. *Journal of Industrial Microbiology*, 5, 113-123.
- Schwarz, D., Orf, I., Kopka, J., & Hagemann, M. (2013). Recent applications of metabolomics toward cyanobacteria. *Metabolites*, 3(1), 72-100.
- Seaward, M. R. (1988). Contribution of lichens to ecosystems. *CRC handbook of lichenology*, 2, 107-129.
- Sebastian, S., & Joseph, A. (2013). Filamentous algae of a hill stream of Kerala, India, 4(3), 35-39.
- Sekar, R., Kaczmarzky, L. T., & Richardson, L. L. (2009). Effect of freezing on PCR amplification of 16S rRNA genes from microbes associated with black band disease of corals. *Applied and environmental microbiology*, 75(8), 2581-2584.
- Sen, N., & Naskar, K. (2003). *Algal flora of Sundarbans mangals*. Daya Books.
- Serpa, R., & Calderón, A. (2006). Effect of different nitrogen sources on the Carotenoid and chlorophyll content of four Peruvian strains of *Dunaliella salina* Teod. *Ecología Aplicada*, 5(1&2), 93-99.
- Setta, N. G. A., Hani Saber, A. A., & Galal, H. R. (2018). Potentiality of algae extracts in alleviating stresses effect on common bean under Upper Egypt conditions. *Advances in Environmental Biology*, 12, 28-37.

- Shalaby, T. A., & El-Ramady, H. (2014). Effect of foliar application of bio-stimulants on growth, yield, components, and storability of garlic (*Allium sativum* L.). *Australian Journal of Crop Science*, 8, 271.
- Shariatmadari, Z., Riahi, H., Hashtroudi, M. S., Ghassempour, A., & Aghashariatmadary, Z. (2013). Plant growth promoting cyanobacteria and their distribution in terrestrial habitats of Iran. *Soil Science and Plant Nutrition*, 59, 535-547.
- Sharma, N., Palia, P., Chaudhary, A., Verma, K. & Kumar, I. (2020). A review on pharmacological activities of lupeol and its triterpene derivatives. *Journal of drug delivery*, 10(5), 325-332.
- Shiels, K., Browne, N., Donovan, F., Murray, P., & Saha, S. K. (2019). Molecular characterization of twenty-five marine cyanobacteria isolated from coastal regions of Ireland. *Biology*, 8(3), 59.
- Shiozaki, T., Nagata, T., Ijichi, M., & Furuya, K. (2015). Nitrogen fixation and the diazotroph community in the temperate coastal region of the northwestern North Pacific. *Biogeosciences*, 12(15), 4751-4764.
- Shishido, T. K., Humisto, A., Jokela, J., Liu, L., Wahlsten, M., Tamrakar, A., & Sivonen, K. (2015). Antifungal compounds from cyanobacteria. *Marine drugs*, 13(4), 2124-2140.
- Sholkamy, E. N., El-Komy, H. M., & Ali, H. M. (2015). Enhancement of soybean (*Glycine max* L.) growth by biofertilizers of *Nostoc muscorum* and *Nostoc rivulare*. *Pakistan Journal of Botany*, 47, 1199-1204.
- Shrestha, J., Kandel, M., Subedi, S., & Shah, K. K. (2020). Role of nutrients in rice (*Oryza sativa* L.): A review. *Agrica*, 9(1), 53-62.
- Shukla, E., Singh, S. S., Singh, P., & Mishra, A. K. (2012). Chemotaxonomy of heterocystous cyanobacteria using FAME profiling as species markers. *Protoplasma*, 249, 651-661.
- Silva, N. M., Branco, L. H. Z., & Necchi Júnior, O. (2012). Corticolous cyanobacteria from tropical forest remnants in northwestern São Paulo State, Brazil. *Brazilian Journal of Botany*, 35, 169-179.
- Silvester, W. B., & Mcnamara, P. J. (1976). The Infection Process and Ultrastructure of the *Gunnera-Nostoc* Symbiosis. *New Phytologist*, 77, 135-141.
- Silvia, C., Devi, W.I., Oinam, G., Singh, O.A., Singh, K.O., Indrama, T., Sharma, A.S., Khangembam, R., Devi, A.T., Miranda, L., Tiwari, O.N., & Kalita M.C. (2015). Pigment production and growth metabolites amongst *Nostoc* strains of unexplored areas of Manipur, India falling under Indo-Burma Biodiversity Hotspots. *International Journal of Advanced Research*, 3(10), 1752-1761.
- Singh Y., Singh G., Singh D.P., & Khattar J.I.S. (2022). A Checklist of Blue-Green Algae (Cyanobacteria) From Punjab, India. *Journal of Threatened Taxa*, 14(3), 20758-20772.
- Singh, A. K., Singh, P. P., Tripathi, V., Verma, H., Singh, S. K., Srivastava, A. K., & Kumar, A. (2018). Distribution of cyanobacteria and their interactions with

- pesticides in paddy field: A comprehensive review. *Journal of environmental management*, 224, 361-375.
- Singh, A. P., Asthana, R. K., Kayastha, A. M., & Singh, S. P. (2005). A comparison of proline, thiol levels and GAPDH activity in cyanobacteria of different origins facing temperature-stress. *World Journal of Microbiology and Biotechnology*, 21, 1-9.
- Singh, B. V., Choudhary, K. K., Dhar, D. W. & Singh, P. K. (2001). Occurrence of some Nostocales from 24 Parganas, West Bengal. *Phykos*, 40 (1&2), 83-87.
- Singh, D. P., Prabha, R., Yandigeri, M. S., & Arora, D. K. (2011). Cyanobacteria-mediated phenylpropanoids and phytohormones in rice (*Oryza sativa*) enhance plant growth and stress tolerance. *Antonie Leeuwenhoek*, 100, 557-568.
- Singh, H., Pal, S., & Bhattacharya, A. (2017). Oxidative stress caused by use of pre-emergent herbicides in wheat seedlings. *International Journal of Current Microbiology and Applied Sciences*, 6(12), 2580-2586.
- Singh, J. S., Kumar, A., Rai, A. N., & Singh, D. P. (2016). Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Frontiers in microbiology*, 7, 529.
- Singh, N. K., Dhar, D. W., & Tabassum, R. (2016). Role of Cyanobacteria in Crop Protection. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*. 86, 1-8.
- Singh, P. K. (1988). Biofertilization of rice crop. In Biofertilization Potentialities and Problems, Eds. Sena SP, Palit PC, Plant Physiology Forum, Calcutta, India. pp.109-114
- Singh, P., Shaikh, Z. M., Gaysina, L. A., Suradkar, A., & Samanta, U. (2016). New species of *Nostoc* (cyanobacteria) isolated from Pune, India, using morphological, ecological and molecular attributes. *Plant Systematics and Evolution*, 302, 1381-1394.
- Singh, P., Singh, S. S., Mishra, A. K., & Elster, J. (2013). Molecular phylogeny, population genetics and evolution of heterocystous cyanobacteria using *nifH* gene sequences. *Protoplasma*, 250, 751-764.
- Singh, P., Šnokhousová, J., Saraf, A., Suradkar, A., & Elster, J. (2020). Phylogenetic evaluation of the genus *Nostoc* and description of *Nostoc neudorfense* sp. nov., from the Czech Republic. *International Journal of Systematic and Evolutionary Microbiology*, 70(4), 2740-2749.
- Singh, R. K., Tiwari, S. P., Rai, A. K., & Mohapatra, T. M. (2011). Cyanobacteria: an emerging source for drug discovery. *The Journal of antibiotics*, 64(6), 401-412.
- Singh, R. N. (1961). Role of blue-green algae in nitrogen economy of Indian agriculture. Indian Council of Agricultural Research, New Delhi. pp. 175.
- Singh, R., Singh, R. P., & Singh, D. V. (2015). Distribution of cyanobacteria (blue-green algae) in rice fields of Varanasi. *International Journal of Advanced Research*, 3(8), 1055-1060.

- Singh, S. (2014). A review on possible elicitor molecules of cyanobacteria: Their role in improving plant growth and providing tolerance against biotic or abiotic stress. *Journal of Applied Microbiology*, 117, 1221-1244.
- Singh, S. C., Sinha, R. P., & Hader, D. P. (2002). Role of lipids and fatty acids in stress tolerance in cyanobacteria. *Acta Protozoologica*, 41(4), 297-308.
- Singh, S. S., Kunui, K., Minj, R. A., & Singh, P. (2014). Diversity and distribution pattern analysis of cyanobacteria isolated from paddy fields of Chhattisgarh, India. *Journal of Asia-Pacific Biodiversity*, 7(4), 462-470.
- Singh, Y., Singh, G., Singh, D. P., & Khattar, J. I. S. (2022). A checklist of blue-green algae (Cyanobacteria) from Punjab, India. *Journal of threatened Taxa*, 14(3), 20758-20772.
- Sinha, R. P., & Hader, D. P. (1996). Invited Review Photobiology and Ecophysiology of Rice Field Cyanobacteria. *Photochemistry and Photobiology*, 64(6), 887-896.
- Sinha, R. P., Klisch, M., Helbling, E. W., & Häder, D. P. (2001). Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. *Journal of Photochemistry and Photobiology B: Biology*, 60(2-3), 129-135.
- Smith, G.M. (1938). *Cryptogamic Botany*, Vol.1, Mc Graw Hill, NewYork. pp. 545.
- Song T., Mårtensson L., Eriksson T., Zheng W., & Rasmussen U. (2005). Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *FEMS Microbial Ecology*, 54(1), 131-140.
- Spencer, D. F., & Linquist, B. A. (2014). Reducing rice field algae and cyanobacteria abundance by altering phosphorus fertilizer applications. *Paddy and water environment*, 12, 147-154.
- Srinivas, M., & Aruna, M. (2016). Diversity of Blue Green Algae from Paddy Fields. *International Journal of Sciences*, 5(5), 27-30.
- Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiology Today*, 33, 152-155.
- Stal, L. J. (1995). Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist*, 131(1), 1-32.
- Stamatakis, A., Ludwig, T., & Meier, H. (2005). RAxML-III: a fast program for maximum likelihood-based inference of large phylogenetic trees. *Bioinformatics*, 21, 456-463.
- Starmach, K. (1966). *Epibolium fonticolum* sp. nova and other algae in a small spring. *Acta Hydrobiologica* 8(3/4), 301-308.
- Stitzenberger, E. (1860). Dr. L Rabenhoosts *Algew Sachens*, manual of phycology. *Chronica Botanica*, Waltham, MA, pp. 243-262.
- Subashchandrabose, S. R., Ramakrishnan, B., Megharaj, M., Venkateswarlu, K., & Naidu, R. (2013). Mixotrophic cyanobacteria and microalgae as distinctive biological agents for organic pollutant degradation. *Environment international*, 51, 59-72.

- Subramanian, G., & Uma, L. (1996). Cyanobacteria in pollution control. *Journal of Scientific and Industrial Research*, 55, 685-692.
- Suresh, L., Veerabah R.M. & Gnanasingh, S.R. (2010). GC-MS analysis of ethanolic extract of *Zanthoxylum rhetsa* (roxb.) dc spines. *Journal of herbal medicine and toxicology*, 4, 191-192.
- Svenning, M. M., Eriksson, T., & Rasmussen, U. (2005). Phylogeny of symbiotic cyanobacteria within the genus *Nostoc* based on 16S rDNA sequence analyses. *Archives of microbiology*, 183, 19-26.
- Syiem, M. B., Nongbri, B. B., Pinokiyo, A., Bhattacharjee, A., Nongrum, N. A., & Hynniewta, L. (2010). Significance of cyanobacterial diversity in different ecological conditions of Meghalaya, India. *Journal of Applied and Natural Science*, 2(1), 134-139.
- Taj, T., Sultana, R., Haleema Shahin, D. H., Chakraborty, M. & Ahmed, M. G. (2021). Phytol a phytoconstituent, its chemistry and pharmacological actions. *Gis Science Journal*, 8(1), 395-406.
- Takács, G., Stirk, W. A., Gergely, I., Molnár, Z., van Staden, J., & Ördög, V. (2019). Biostimulating effects of the cyanobacterium *Nostoc piscinale* on winter wheat in field experiments. *South African Journal of Botany*, 126, 99-106.
- Tamasauskas, D., Sakalauskas, V., & Kriksciuniene, D. (2012). Evaluation framework of hierarchical clustering methods for binary data. *12th International Conference on Hybrid Intelligent Systems (HIS)*, 421-426.
- Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences of the United States of America*, 101, 11030-11035.
- Tarawat, S., Incharoensakdi, A., & Monshupanee, T. (2020). Cyanobacterial production of poly (3-hydroxybutyrate-co-3-hydroxyvalerate) from carbon dioxide or a single organic substrate: improved polymer elongation with an extremely high 3-hydroxyvalerate mole proportion. *Journal of applied Phycology*, 32, 1095-1102.
- Tassara, C., Zaccaro, M. C., Storni, M. M., Palma, M., & Zulpa, G. (2008). Biological control of lettuce white mold with cyanobacteria. *International Journal of Agriculture and Biology*, 10(5), 487-492.
- Temina, M., Rezankova, H., Rezanka, T., & Dembitsky, V. M. (2007). Diversity of the fatty acids of the *Nostoc* species and their statistical analysis. *Microbiological Research*, 162(4), 308-321.
- Teneva, I., Stoyanov, P., Mladenov, R., & Dzhambazov, B. (2012). Molecular and phylogenetic characterization of two species of the genus *Nostoc* (Cyanobacteria) based on the *cpcB-IGS-cpcA* locus of the phycocyanin operon. *Journal of Bioscience and Biotechnology*, 1(1), 9-19.
- Teuscher, E., Lindequist, U., Mundt, S. (1992). Cyanobakterien, Quellen biogener Wirkstoffe. *Pharm. Ztg. Wiss.*, 137, 57-69.

- Thacker, R. W., & Paul, V. J. (2004). Morphological, chemical, and genetic diversity of tropical marine cyanobacteria *Lyngbya* spp. and *Symploca* spp. (Oscillatoriales). *Applied and Environmental Microbiology*, 70(6), 3305-3312.
- Thajuddin, N., & Subramanian, G. (2005). Cyanobacterial biodiversity and potential application in biotechnology. *Current Science*, 89, 47-57.
- Thilak, T. S., Madhusoodanan, P. V., Pradeep, N. S., & Prakashkumar, R. (2020). Isolation and taxonomy of the blue-green algae (Cyanobacteria), *Nostoc* and *Anabaena* in Kerala State, India. *Acta Botanica Hungarica*, 62(1-2), 163-174.
- Thuret, G. (1875). Essai de classification des Nostochinées. *Annales des Sciences Naturelles; Botanique*, 6(1), 372-382.
- Tillett, D., & Neilan, B.A. 2000. Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. *Journal of Phycology*, 36, 251-258.
- Tindall, B. J., Rosselló-Móra, R., Busse, H. J., Ludwig, W., & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *International journal of systematic and evolutionary microbiology*, 60(1), 249-266.
- Tiwari, O. N., & Singh, H. T. (2005). Biodiversity of cyanobacteria in Loktak lake and rice fields of Manipur, having acidic properties. *Proceedings of the National Academy of Sciences, India*. 75(B) III, 209-213.
- Tiwari, O. N., Singh, B. V., Mishra, U. P. A. S. A. N. A., Singh, A. K., Dhar, D. W., & Singh, P. K. (2005). Distribution and physiological characterization of cyanobacteria isolated from arid zones of Rajasthan. *Tropical Ecology-Allahabad*, 46(2), 165.
- Tomaselli, L., Lamenti, G., Bosco, M., & Tiano, P. (2000). Biodiversity of photosynthetic micro-organisms dwelling on stone monuments. *International Biodeterioration & Biodegradation*, 46(3), 251-258.
- Tonk, L., Visser, P. M., Christiansen, G., Dittmann, E., Snelder, E. O., Wiedner, C., & Huisman, J. (2005). The microcystin composition of the cyanobacterium *Planktothrix agardhii* changes toward a more toxic variant with increasing light intensity. *Applied and Environmental Microbiology*, 71(9), 5177-5181.
- Toribio, A. J., Jurado, M. M., Suárez-Estrella, F., López, M. J., López-González, J. A., & Moreno, J. (2021). Seed biopriming with cyanobacterial extracts as an eco-friendly strategy to control damping off caused by *Pythium ultimum* in seedbeds. *Microbiological Research*, 248, 126766.
- Torres-Maza, A., Yupanqui-Bacilio, C., Castro, V., Aguirre, E., Villanueva, E., & Rodríguez, G. & (2020). Comparison of the hydrocolloids *Nostoc commune* and *Nostoc sphaericum*: Drying, spectroscopy, rheology and application in nectar. *Scientia Agropecuaria*, 11(4), 583-589.
- Tóth, J., Gergely, I., Berzsenyi, Z., & Ördög, V. (2019). Influence of *Nostoc entophyllum* and *Tetracystis* sp. on Winter Survival of Rapeseed. *Journal of Agricultural Science and Technology B*, 9, 251-271.

- Tripathy, P., Roy, A., & Adhikary, S. P. (1997). Survey of epilithic blue-green algae (cyanobacteria) from temples of India and Nepal. *Algological Studies/ Archiv für Hydrobiologie, Supplement Volumes*, 43-57.
- Turner, J. S., & Robinson, N. J. (1995). Cyanobacterial metallothioneins: biochemistry and molecular genetics. *Journal of industrial microbiology*, 14, 119-125.
- Turner, S., Pryer, K. M., Miao, V. P., & Palmer, J. D. (1999). Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis 1. *Journal of Eukaryotic Microbiology*, 46(4), 327-338.
- Ukpatu, J., Udoinyang, E., & Udoh, J. P. (2015). The use of agglomerative hierarchical cluster analysis for the assessment of mangrove water quality of Okoro River Estuary, Southeastern Nigeria. *International Journal of Geology, Agriculture and Environmental Science*, 3 (6),17-24.
- Umamaheswari, N. A. (2005). Isolation and characterisation of nostocales cyanobacteria of the paddy fields of Kerala, University of Calicut, Kerala, India.
- Umar, W., Ayub, M. A., Rehman, M. Z. U., Ahmad, H. R., Farooqi, Z. U. R., Shahzad, A., Rehman, U., Mustafa, A. & Nadeem, M., (2020). Nitrogen and phosphorus use efficiency in agroecosystems. *Resources Use Efficiency in Agriculture*, 213-257.
- Vaishampayan, A., Sinha, R. P., Hader, D. P., Dey, T., Gupta, A. K., Bhan, U., & Rao, A. L. (2001). Cyanobacterial biofertilizers in rice agriculture. *The Botanical Review*, 67, 453-516.
- Vandamme, P., Pot, B., Gillis, M., De Vos, P., Kersters, K., & Swings, J. (1996). Polyphasic taxonomy, a consensus approach to bacterial systematics. *Microbiological reviews*, 60(2), 407-438.
- Vargas, M. A., Rodriguez, H., Moreno, J., Olivares, H., Campo, J. D., Rivas, J., & Guerrero, M. G. (1998). Biochemical composition and fatty acid content of filamentous nitrogen-fixing cyanobacteria. *Journal of Phycology*, 34(5), 812-817.
- Vasyurenko, Z. P., & Frolov, A. F. (1986). Fatty acid composition of bacteria as a chemotaxonomic criterion. *Journal of Hygiene, Epidemiology, Microbiology, and Immunology*, 30(3), 287-293.
- Venkataraman, G. S. (1972). *Algal biofertilizers and rice cultivation*. Today & Tomorrow's Printers & Publishers. pp. 75.
- Verma, D. C., & Asthana, D. K. (2005). Studies on the distribution of blue green algae in paddy fields of Uttaranchal State. *Geophytology*, 35(1&2), 91-96.
- Vijayan, D., & Ray, J. G. (2015). Ecology and diversity of cyanobacteria in Kuttanadu paddy wetlands, Kerala, India. *American Journal of plant sciences*, 6(18), 2924-2938.
- Volk, R. B., & Furkert, F. H. (2006). Antialgal, antibacterial and antifungal activity of two metabolites produced and excreted by cyanobacteria during growth. *Microbiological Research*, 161(2), 180-186.
- Wagh, G. S., & Jadhav, M. J. (2019). Diversity of Cyanobacteria in the cultivated fields of Ahmednagar District (MS) India. *Bioscience Discovery*, 10(3), 122-125.

- Wang, Y. N., Wang, H. X., Shen, Z. J., Zhao, L. L., Clarke, S. R., Sun, J. H., & Shi, G. L. (2009). Methyl palmitate, an acaricidal compound occurring in green walnut husks. *Journal of Economic Entomology*, 102(1), 196-202.
- Wangwibulkit, S., Limsuwan, C., & Chuchird, N. (2008). Effects of salinity and pH on the growth of blue-green algae, *Oscillatoria* sp. and *Microcystis* sp., isolated from pacific white shrimp (*Litopenaeus vannamei*) ponds. *Journal of Fisheries and Environment*, 32(1), 1-9.
- Wanigatunge, R. P., Magana-Arachchi, D. N., Chandrasekharan, N. V., & Kulasooriya, S. A. (2014). Genetic diversity and molecular phylogeny of cyanobacteria from Sri Lanka based on 16S rRNA gene. *Environmental Engineering Research*, 19(4), 317-329.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of bacteriology*, 173(2), 697-703.
- Whitton, B. A. (2000). Introduction to the Cyanobacteria—in Whitton BA & Potts M. (eds.): *The Ecology of Cyanobacteria*.
- Whitton, B. A. (2008). Cyanobacterial diversity in relation to the environment. In *Algal toxins: nature, occurrence, effect and detection*. Springer, Netherlands. pp. 17-43.
- Whitton, B.A. (1987). The biology of Rivulariaceae. In: Fay P, Van Baalen C (eds) *The cyanobacteria*. Elsevier, Amsterdam, pp. 513-534.
- Whitton, B.A., & Potts, M. (2012). Introduction to the cyanobacteria. In *Ecology of cyanobacteria II: their diversity in space and time*. Dordrecht: Springer Netherlands. pp. 1-13.
- Wijffels, R. H., Kruse, O., & Hellingwerf, K. J. (2013). Potential of industrial biotechnology with cyanobacteria and eukaryotic microalgae. *Current Opinion in Biotechnology*, 24(3), 405-413.
- Wilkinson, L., & Friendly, M. (2009). The history of the cluster heat map. *The American Statistician*, 63(2), 179-184.
- Wilson, L. T. (2006). Cyanobacteria: a potential nitrogen source in rice fields. *Texas Rice*, 6, 9-10.
- Woese, C. R. (1987). Bacterial evolution. *Microbiological Reviews*, 51, 221-271.
- Woese, C. R., and G. E. Fox. (1977). Phylogenetic structure of the prokaryotic domain: the primary kingdoms. *Proceedings of the National Academy of Sciences of the United States of America*, 74, 5088-5090.
- Wu, X., Zarka, A., & Boussiba, S. (2000). A simplified protocol for preparing DNA from filamentous cyanobacteria. *Plant Molecular Biology Reporter*, 18, 385-392.
- Yaday, V. K., Singh, D. V., & Singh, R. P. (2021). Biodiversity of Cyanobacteria in 'Usar' Lands of districts Azamgarhand Varanasi, Eastern Uttar Pradesh (India). *Asian Journal of Plant and Soil Sciences*, 6(1), 91-102.
- Yang, J., Kloepper, J. W. & Ryu, C. M., (2010). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science*, 14, 1-4.

- Yanni, Y.G., & Abdallah, F.E. (1990). Role of algalization in rice growth, yield and incidence of infestation with the stem borer *Chilo agamemnon* Bles and the leaf miner *Hydrellia prosternalis* deeming in the Nile Delta. *World Journal of Microbiology and Biotechnology*, 6, 383-389.
- Yarza, P., Yilmaz, P., Pruesse, E., Glöckner, F. O., Ludwig, W., Schleifer, K. H., Whitman, W. B., Euzéby, J., Amann, R., & Rosselló-Móra, R. (2014). Uniting the classification of culture and uncultured bacteria and Archea using 16S rRNA gene sequences. *Nature Reviews Microbiology*, 12, 635-645.
- Yasin, D., Fatma, T., Zafaryab, M., Ahmad, N., Aziz, N., & Rizvi, M. M. A. (2018). Exploring the bio-efficacies of methanolic extracts of *Nostoc muscorum* and *Calothrix brevissima* with their Characterization Using GC-MS. *The Natural Products Journal*, 8(4), 305-316.
- Yerel, S. (2010). Water quality assessment of porsuk river, Turkey. *Journal of Chemistry*, 7, 593-599.
- Yu, F., Lu, S., Yu, F., Shi, J., McGuire, P.M., & Wang, R., (2008). Cytotoxic activity of an octadecenoic acid extract from *Euphorbia kansui* (Euphorbiaceae) on human tumour cell strains. *Journal of Pharmacy and Pharmacology*, 60, 253–259.
- Yu, H. F., & Liu, R. (2013). Effect of UV-B radiation on the synthesis of UV-absorbing compounds in a terrestrial cyanobacterium, *Nostoc flagelliforme*. *Journal of Applied Phycology*, 25, 1441-1446.
- Yu, Z., & Morrison, M. (2004). Comparisons of different hypervariable regions of rrs genes for use in fingerprinting of microbial communities by PCR-denaturing gradient gel electrophoresis. *Applied and Environmental Microbiology*, 70(8), 4800-4806.
- Zapomelova, E., Rehakova, K., Jezberova, J. & Komarkova, J. (2010). Polyphasic characterization of eight planktonic *Anabaena* strains (cyanobacteria) with reference to the variability of *Anabaena* populations observed in the field. *Hydrobiologia*, 639, 99-113.
- Zeeshan, M., & Prasad, S. M. (2009). Differential response of growth, photosynthesis, antioxidant enzymes and lipid peroxidation to UV-B radiation in three cyanobacteria. *South African Journal of Botany*, 75(3), 466-474.
- Zehr, J. P., Mellon, M. T., & Hiorns, W. D. (1997). Phylogeny of cyanobacterial *nif* H genes: evolutionary implications and potential applications to natural assemblages. *Microbiology*, 143, 1443-1450.
- Zhou, Y., Bao, J., Zhang, D., Li, Y., Li, H. & He, H. (2020). Effect of heterocystous nitrogen-fixing cyanobacteria against rice sheath blight and the underlying mechanism. *Applied Soil Ecology*, 153, 103580.
- Zulpa, G., Zaccaro, M.C., Boccazzi, F., Parada, J.L., & Storni, M. (2003). Bioactivity of intra and extracellular substances from cyanobacteria and lactic acid bacteria on wood blue stain fungi. *Biological Control*, 27, 345-348.