DEVELOPMENT OF INOCULANT CULTURES OF NITROGEN FIXING CYANOBACTERIA FROM WETLAND ECOSYSTEM OF VELLAYANI

ARYA S.

(2019-11-224)

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2022

DEVELOPMENT OF INOCULANT CULTURES OF NITROGEN FIXING CYANOBACTERIA FROM WETLAND ECOSYSTEM OF VELLAYANI

by

ARYA S.

(2019-11-224)

THESIS

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2022

DECLARATION

I, hereby declare that this thesis entitled **"DEVELOPMENT OF INOCULANT CULTURES OF NITROGEN FIXING CYANOBACTERIA FROM WETLAND ECOSYSTEM OF VELLAYANI"**is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani **ARYA S.**

Date: 13-05-2022 (2019-11-224)

CERTIFICATE

Certified that this thesis entitled **"DEVELOPMENT OF INOCULANT CULTURES OF NITROGEN FIXING CYANOBACTERIA FROM WETLAND ECOSYSTEM OF VELLAYANI"**is a record of research work done independently by Ms. ARYA S. (2019-11-224) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellayani,
Date:13-05-2022

Dr. Chitra N.
(Major advisor)
Assistant Professor
Department of Agricultural Microbiology
RARS(SZ), College of Agriculture
Vellayani, Thiruvananthapuram – 695 522

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. ARYA S(2019-11-224), a candidate for the degree of **Master of Science in Agriculture** with major in Agricultural Microbiology, agree that the thesis entitled **"DEVELOPMENT OF INOCULANT CULTURES OF NITROGEN FIXING CYANOBACTERIA FROM WETLAND ECOSYSTEM OF VELLAYANI"** may be submitted by Ms. ARYA S (2019-11-224) in partial fulfilment of the requirement for the degree.

Dr. Chitra N.
Assistant Professor
Department of Agricultural Microbiology
RARS (SZ)College of Agriculture,Vellayani,
Thiruvananthapuram-695 522

Dr. Anith K. N.
Professor and Head
Department of AgriculturalMicrobiology
College of Agriculture,
Vellayani,
Thiruvananthapuram-695 522

Dr. Anu Rajan S
Assistant Professor
Department of Agricultural Microbiology
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

Dr. Aparna B.
Assistant Professor
Department of Soil science and Agrl.Chemistry
College of Agriculture, Vellayani
Thiruvananthapuram-695 522

ACKNOWLEDGEMENT

First and foremost, praises and thanks to the Almighty, for everything that happens to me…

It is my great pleasure to place a record with deepest sense of gratitude and indebtedness to my major advisor Dr. Chitra N. Assistant Professor, Department of Agricultural Microbiology for the constructive guidance, constant inspiration, abundant encouragement, kind treatment, critical scrutiny of the manuscript and valuable suggestions which render me to accomplish the research work successfully. I extend my sincere gratitude for providing a stress free situation by the open minded approach and for the care and affection bestowed on me throughout the study period.

I convey my heartfelt gratitude to Dr. Anith K. N., Professor and Head, Department of Agricultural Microbiology for the unceasing encouragement, valuable advices and whole hearted approach right from the beginning, who guided me till the completion of the thesis.

I extend my sincere gratefulness to Dr. Anu Rajan S., Assistant Professor, Department of Agricultural Microbiology for the valuable suggestions, technical advices and incessant motivation throughout the research work.

I am extremely thankful to Dr. Aparna B., Assistant Professor, Department of Soil science and Agricultural Chemistry, for the unstinting support, suggestions and passionate approach rendered during the period of research work.

I am deeply grateful to Dr. Sowmiya S., Department of Agricultural Microbiology for generous support, continues and timely advice, constructive criticisms and constant encouragement rendered to me during the course of my study and research work.

I express my special thanks to Bindhuchechi, Subha chechi, Jasmin chechi, Viji chechi, Ajith chettan, Akshaya, Anu sree for their love and support during the period of research work.

I owe my deepest gratitude to my batch mates Gyathri, Safa, Sruthi Suresh and Anju whose cooperation, love, support and affection helped me through many hardships. Without their persistent help and care, this would not have been possible.

I express my sincere thanks to my seniors Nysanthchetta, Yasashwinichechi, Akhilachechi, Siva Priya chechi and Nandhanachechi and lovely junior Sangeetha lekshmi for their help and support.

Words are inadequate to express my thanks to my friends, Arun Thazhath, Sreekumar, Jaseel, Ananya, AmrithaVaranya, Haritha, Shiva, Shyna, Akhilaashokan and Jayasri for their unbound love and bighearted support.

I am most indebted to my beloved Achan, Sri. Sivadasan K., Amma, Girija K., brother, Amrith S., for their unbounding love, unparallel affection, moral support and encouragement throughout my career.

Once again, I express my cordial gratefulness collectively to everyone who helped me during my research work.

Arya S.

CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS USED

et al.	And other co-worker
cm	Centimeter
CRD	Completely randomized design
^{o}C	Degree celsius
Fig.	Figure
g	Gram
ha	Hectare
h	Hours
pH	Negative logarithm of hydrogen ions
EPS	Extracellular polymeric substances
IAA	Indole acetic acid
IBA	Indole butyric acid
GS	Glutamine synthetase
MSO	L-methionine sulfoximine
kg	Kilo gram
Chl*a*	Chlorophyll *a*
ATP	Adenine triphosphate
Fd	Ferredoxin
µL	Microlitre
min	Minutes
nm	Nanometer
mL	Milliliter
psi	Pounds per square inch

rpm	Revolutions per minute
L	Litre
OD	Optical density
µm	micrometer
µg	Microgram
mg	Milligram
PC	Phycocyanin
APC	Allophycocyanin
PE	Phycoerythrin
BSA	Bovine serum albumin
ANOVA	Analysis of variance
SE	Standard error
f.sp.	Forma specialis
SDW	Sterile distilled water
DAT	Days after treatment
ppm	Parts per million
CCA	Complementary chromatic adaptation
%	Percentage
No.	Number
Sl.	Serial
sp.	Species
i.e.	That is
t	Tonne
VLY	Vellayani
ONPG	Ortho-Nitrophenol-β-galactoside

INTRODUCTION

1. INTRODUCTION

Cyanobacteria, commonly known as blue-green algae, are a diverse group of oxygenic photosynthetic gram-negative prokaryotes. They are one of the oldest life forms present on the planet earth. There are around 150 genera and 2000 species with unicellular, colonial, filamentous and branched forms (Yibeltie and Sahile, 2018), which thrive in both aquatic and terrestrial habitats like ponds, lakes and streams.They are capable of tolerating a wide range of temperature, salinity, osmotic potential and pH.

The potential role of cyanobacteria in agriculture through their role as biofertilizers, soil conditioners, plant growth regulators and soil health ameliorators has been well recognized (Vaishampayan *et al.,* 2001). Rice fields inoculated with cyanobacteria can help renew and improve soil structure by providing a variety of substances such as polysaccharides, peptides and lipids (Misra and Kaushik, 1989). India is the world's second largest producer of rice which accounts for an area of 437.89 lakh hectare with a production and yield of 112.91 million tones and 2578 kg ha^{-1} respectively (Annual report 2017-2018). It isan important food crop since rice is a key source calories for more than 60 % of the world's population. Rice is traditionally grown under submerged conditions, which has negative consequences on ecology since it causes water loss through percolation and reduces fertilizer use efficiency (Prasanna and Nayak, 2007). Cyanobacteria is an important biofertilizer used in maintaining the fertility of rice fields (Singh, 1961; Venkataraman, 1975; Roger, 1996).

Cyanobacteria are essential primary producers on a global scale and they play an important role in the nitrogen, carbon, and oxygen biogeochemical cycles. Many species are capable of fixing atmospheric nitrogen, making them the only bacteria with a plant like metabolism. The prokaryotic nature of the cell makes them bacteria like, but the existence of an oxygen evolving photosynthetic machinery gives the advantage of eukaryotic algae (Anand *et al*., 2019). Chemical fertilizers are being replaced by cyanobacteria, which is a more cost effective and environmentally beneficial option. They play potential role in crop production through different mechanisms including as

biofertilizers, enhanced solubilization and mobility of nutrients, improving physico-chemical conditions of soils, mineralization of amino acids etc. Cyanobacteria are well known for the production of plant growth promoting substances such as hormones like gibberellins, cytokinin, auxin or abscisic acids, vitamins, amino acids, polypeptides and exopolysaccharides. These substances help for obtaining maximal growth and yield which is considered as the direct role of cyanobacteria in agriculture. Production of exopolysaccharides by cyanobacteria helps in soil formation and protection from erosion. They play an indirect role in agriculture through the reduction of other microbes which are not suitable for plant growth. They can also produce vitamins, particularly vitamin B, antibiotics and toxins.

Cyanobacteria can fix atmospheric nitrogen either by symbiotic or free-living associations with partners such as water fern *Azolla*, *Gunnera*, cycads etc. Some of the members have specialized cells called heterocyst – modified cells with thick-wall, which are having nitrogenase enzyme for nitrogen fixation. They can supply 20– 30 kg N ha^{-1} to the soil as well as the organic matters (Issa *et al.,* 2014). With the help of phosphatase enzymes, they can also boost phosphorous availability by solubilizing and mobilizing insoluble organic phosphates in the soil. Several cyanobacterial species such as *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima* and *Tolypothrix fertilissima* can be exploited as efficient biofertilizers.

Cyanobacteria are well known to produce varied types of bioactive compounds of antibacterial, antialgal and antiviral potential belonging to group of polyketides, amides, alkaloids, fatty acids, indoles and lipopeptides. The bulk of hazardous chemicals are produced by cyanobacteria belonging to the orders Chroococcales, Oscillatoriales and Nostocales. Among them, *Anabaena* sp. and *Nostoc* sp. among the Nostocales synthesize many secondary metabolites with anticancer, antimalarial, antimicrobial and antifungal activity (Burja *et al*., 2001). *Botrytis cinerea* on strawberries and *Erysiphae polygoni* causing powdery mildew on turnips and damping off in tomato are both reported to be reduced by them. Extracts from *Nostoc muscorum* have been shown to inhibit the growth

of fungal plant pathogens such as *Sclerotinia sclerotiorum* causing cottony rot of vegetables and fruits and *Rhizoctonia solani* causing stem and root rot, according to Kulik (1995). Cyanobacterial species like *Nostoc*, well known cryptophycin producer is the source of natural pesticides against fungi, insects and nematodes (Biondi *et al.,* 2004).

Eco-friendly, low-cost farming with the help of native microorganisms is an example of sustainable agriculture. Microorganisms play an important role in establishing food security without causing environmental difficulties, according to sustainable agriculture techniques. Being a beneficial microbe by the above aspects, cyanobacteria could be exploited as an efficient biofertilizer in rice fields as well as a biocontrol agent against various plant pathogens.

Paddy fields are vital part of Kerala's environment and ecological system. They offer natural flood or drainage channels, save ground water and protect a diverse range of vegetation and fauna. However, a decline in area under rice cultivation was observed from 1980s onwards. Realizing the need for developing better strains of cyanobacteria for biofertilizer and as biocontrol agent which are capable of fixing atmospheric nitrogen, solubilizing phosphorous, production of plant growth promoting substances and with antagonistic activities, the present study was taken up with following objectives.

1. Isolate and characterize cyanobacterial strains with efficient nitrogen fixing capacity from wetland ecosystem of Vellayani.
2. Estimate the plant growth promotion activities of the isolates.
3. Study the potential of selected cyanobacterial isolate for *in vitro*plant growth promotion.
4. Assessing the antagonistic effect of cyanobacterial isolates on different plant pathogenic bacteria and fungi.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Cyanobacteria are the most ancient organisms inhabiting the earth's environment. They are gram negative oxygenic photosynthetic autotrophic microorganisms. Over the past 3 billion years, they have contributed to the production of oxygen in the earth's atmosphere. Cyanobacteria, or blue-green algae, are photosynthetic microorganisms that can be found in variety of habitats, including rivers, seas, soil and bare rock. They exist in different forms such as unicellular, colonies or filaments. Even though they are minute, they can be viewed by our naked eyes when they form colonies like blooms or crusts.

2.1 SIGNIFICANCE OF CYANOBACTERIA

2.1.1 Adaptability to life in extreme environments

Cyanobacteria may thrive in variety of environments including freshwater, brackish water, and industrial wastewater etc. many of the microbial communities can inhabit environmental conditions ranging from hotsprings at temperature of 85.2^{o}C to lower temperatures (Singh*et al*., 2016). Many alkaline lakes support them and some can endure both high pH and high salinity. Filamentous cyanobacteria like *Oscillatoria* sp. and *Spirullina* sp.are known to tolerate acidic condition down to a pH of 2.9 (Sharma *et al*., 2013). Akinetes, also known as "spores," are differentiated cells in cyanobacteria, produced under adverse conditions andcan remain dormant for years, until germinating and forming new filaments when conditions become suitable again.

2.1.2 Contribute to the primary production

The activity of unicellular cyanobacteria, predominantly species of novel kinds, is responsible for a major portion of carbondioxide fixation in the oceans. They are mainly characterized by chlorophyll *a* and phycobiliproteins as important photosynthetic pigments. They produce oxygen as a byproduct of oxygenic photosynthesis (Sharma *et al*., 2013).

2.1.3 Reclamation of salt affected soils

Singh(1961) proposed that cyanobacteria may be utilizedto reclaim usar soils as they form a thick stratum on the soil surface that conserves organic C, N, and P as well as moisture, and convert the Na^{+} clay to Ca^{2+} clay. In such soils, cyanobacteria supply organic matter and nitrogen which aids binding of the soil particles and so enhances soil permeability and aeration. By reducing the pH, electrical conductivity and hydraulic conductivity they can also improve the physico-chemical quality of saline and alkali soils such as soil aggregation.

2.1.4 Bioremediation

Pesticides, crude oil, naphthalene, phenanthrene, phenol and catechol, heavy metals (Singh *et al*., 2011b) and xenobiotics are just few of the environmental toxins that cyanobacteria can accumulate or degrade. They could be utilized for tertiary treatment of urban, agro-industrial effluents reducing eutrophication and metal toxicity problems in aquatic habitats. Pesticides can build up to dangerous levels in their bodies.

2.1.5 Source of bio-energy

Cyanobacteria can convert up to 10% of solar energy uptake into biomass, compared to the 1% conversion done by traditional energy crops like sugarcane or corn with their unique and superior photosynthesis capabilities, shorter growth period, and high oil contents. Through photosynthesis, they absorb carbondioxide and convert it to carbon-rich lipids that can be used in production of bio-fuels. They also produce molecular hydrogen (H_2) which maybe a better and more suitable alternative for fossil fuels. *Anabaena*, *Calothrix*, *Oscillatoria*, *Cyanothece*, *Nostoc*, *Synechococcus*, *Microcystis*, *Gloeobacter*, *Aphanocapsa*, *Chroococcidiopsis* and *Microcoleus* are among the cyanobacterial genera known for their ability to produce H_2 under diverse culture conditions (Singh *et al*., 2011b).

2.2 CYANOBACTERIAL NITROGEN FIXATION

Filamentous cyanobacteria such as, *Anabaena* sp., *Plectonema* sp., *Gleocapsa* sp., *Nostoc* sp., *Lyngbya* sp., *Aulosira* sp., *Gleotheca* sp., etc. fix molecular nitrogen. Some of them do this by forming symbiotic association with plants. The water fern Azolla contains *Anabaena azollae* within small pores of its fronds. Azolla has been used for centuries for enriching rice fields with fixed nitrogen (Singh, 2012). These cyanobacteria fix atmospheric nitrogen by means of specialized cells called heterocysts.

2.2.1 Heterocysts

Heterocysts are specialized nitrogen fixing cells, arranged in a uniform pattern along a filament or at one end of a filament. They form as a result of differentiation of vegetative cells which in heterocystous cyanobacteria are only source of nitrogen fixation. When cyanobacteria are deprived of both nitrate and ammonia, about 5 to 10 % of vegetative cells can convert into heterocyst. During formation of heterocyst, vegetative cells produce thick wall, discards photosystem II, phycobiliproteins and synthesizes nitrogen fixing enzyme, nitrogenase. Photosystem I still operate and generates ATP.

2.2.2 Nitrogen fixation

In nature cyanobacteria shows different patterns of nitrogen fixation based on their strain type and environmental conditions. Being oxygenic photosynthetic, the enzyme nitrogenase is irreversibly inhibited by oxygen. There are different ways by which cyanobacteria can overcome the inhibition of nitrogenase, (a) rapid and continuous consumption of oxygen before it can inhibit nitrogenase, (b) presence of physical barriers to inward oxygen diffusion, (c) spatial and temporal separation of nitrogen fixation and oxygenic photosynthesis, (d) creation of an environment of low partial pressure of oxygen.

Molecular nitrogen can be used as a nitrogen source by the entire group of nitrogen fixing cyanobacteria. Dinitrogen is reduced to ammonia by an enzyme complex

nitrogenase, which is dependent on reduced ferredoxin and must couple to reduction of protons, releasing molecular hydrogen.

$$N_2 + 8Fd_{red} + 8H^+ + 16\ ATP \rightarrow NH_3 + H_2 + 8Fd_{ox} + 16\ ADP + 16\ P_i$$

Filamentous and heterocystous cyanobacteria have nitrogenase activity that is light dependent whereas dark, heterocystous cyanobacteria have either poor nitrogenase activity or full suppression. The failure of heterocysts in the dark to fix atmospheric nitrogen could be related due to lack of oxygen diffusion. Nitrogenase activity is confined to the night in non heterocystous cyanobacteria like *Gleotheca* sp., *Oscillatoria* sp., and almost no activity was reported during day time.

Ammonia excretion by nitrogen fixing cyanobacteria is not a normal physiological function except in some symbiotic relationship but it can be induced by the addition of glutamine synthetase (GS) inhibitors or glutamate analogues like L-methionine-DL-sulfoxymine (MSO) or by producing ammonia liberating mutant strains. Prolonged exposure to MSO can be toxic for rice field cyanobacterium *Anabaena* sp. causing nitrogen starvation and inhibition of protein synthesis (Sinha and Hader, 1996).*Anabaena* sp. has been reported to excrete ammonia in the absence of metabolic inhibitors. Several cyanobacteria can liberate small amount of amino acids, polypeptides and proteins into the medium (Sinha and Hader, 1996).

2.3 CYANOBACTERIA AS POTENTIAL BIOFERTILIZERS

When applied to seeds, plant surfaces or soil, biofertilizers are compounds containing living microorganismsthat colonizes the rhizosphere or the interior of the plant and promotes development by increasing the supply or availability of primary nutrient or growth stimulus to the target crop (Vessey, 2003). It contains beneficial bacteria and fungi that improve chemical and biological characteristics of soilas well as agricultural production (El-Habbasha*et al*., 2007). Biofertilizers supply nutrients to plants through the natural processes such as nitrogen fixation, solubilizing phosphorus and through the synthesis of growth promoting substances. Biofertilizers can be expected to reduce the

use of chemical fertilizers and pesticides and increase the crop yield by 20-30% (Sahu*et al*.,2012). As a natural biofertilizer, cyanobacteria play an important role in maintaining and enhancing soil fertility, resulting in increased rice growth and yield (Song *et al*., 2005).Their filamentous structure leads to formation of soil pores and produces adhesive substances. They excrete growth promoting substances such as auxins, gibberellins, vitamins, amino acids and increases water holding capacity (Roger and Reynaud, 1982; Rodriguez *et al*., 2006),increases soil biomass after their death and decomposition,decreases soil salinity and prevents weed growth (Saadatnia and Riahi, 2009) and also increases soil phosphate by excretion of organic acids (Wilson, 2006).

Nostoc linkia, Anabaena variabilis, Aulosirafertilisima, Calothrix sp*., Tolypothrix*sp., and *Scytonema*sp. were found to be efficient nitrogen fixing strains in various agro-ecological regions and used for rice production (Prasad and Prasad, 2001). In many fields, nitrogen deprivation is the second limiting factor for plant growth after water and fertilizers are used to compensate for this(Malik *et al*., 2001).

Malliga*et al*. (1996) found that when *Anabaena azollae* was usedas a biofertilizer,it exhibitedlignolysis and released phenolic compounds causing the organism to sporulate profusely. This studydiscusses the benefits of using coirwaste as a carrier for cyanobacterial biofertilizer as well as enzyme studies on lignin degrading ability of cyanobacteria and utilization of lignocellulosic coir waste as a low-cost carrier. Cyanobacteria like *Nostoc*sp*., Anabaena*sp*., Tolypothrix* sp. etcfix atmospheric nitrogen and can be used as inoculants for paddy crop grown both under upland and wetland conditions. In addition to this, few cyanobacteria species can also form symbiotic associations with plants, animals, non-photosynthetic protists etc. Dry green algae contain large quantities of macronutrients, micronutrients and amino acids.

After rice seed transplantation, fresh (100 kg ha^{-1}) and air-dried (10 kg ha^{-1}) cyanobacterial inoculants are applied as biofertilizer (Venkataraman,1981; Richmond, 1986). In rice fields, dried mats of *Anabaena fertilssima* extensively can be used as biofertilizer. The biomass of applied cyanobacterial strains increases with the growth of

rice plants. Depending on the strain, environmental and agroclimatic conditions such as fertilizer application, pH, temperature, light, availability of water in the fields cyanobacterial blooms develop differently in different paddy fields (Kerby *et al*.,1989). Cyanobacterial biofertilizers are applied in combination with nitrogenous fertilizers resulted in a response comparable to additional application of chemical nitrogenous fertilizers (Singh and Singh, 1986). According to Sinha and Kumar (1992), rice plants with single or mixed cyanobacterial biofertilizers had increased length, total fresh weight, chlorophyll *a* (Chl*a*) content and grain production. In comparison to their free-living counterparts, symbiotic cyanobacteria have the benefit of being able to produce the principal fertilizing product ammonia at a relatively low cost as well as higher nitrogen fixation rates and heterocyst frequency.

2.3.1 Plant growth promoting substances

Cyanobacteria plays a major role in plant growth promotion as biofertilizer and soil conditioners (Ashraf *et al*., 2013). They also enhance soil organic content, soil moisture, nitrogen enrichment and extracellular polysaccharides production which improve soil aggregation and phosphate solubilization (Pandey *et al*., 2020). They can produce variety of compounds like amino acids, auxins, gibberellins etc.that can promote the availability of nutrients and aid plant uptake. Free living and symbiotic species of cyanobacteria like *Nostoc* sp*., Calothrix* sp*., Plectonema*sp*., Anabaena* sp*., Cylindrospermum*sp. etc. are known to synthesize indole acetic acid (IAA) (Singh *et al*., 2013). Sood and co workers(2011) reported the interaction of cyanobiont PI 01 and *Nostoc* PCC 9229 with wheat seedlings, in co-culturing experiments. The transverse sections of root in co-cultured seedlings revealed the presence of cyanobionts in it and reported to excrete amino acids, sugars and IAA in the medium. Heterocystouscyanobacteria like *Nostoc calcicola* and *Anabaena vaginicola* reported to produce three auxins (IAA, APA, IBA). They were found to have significant growth promoting effect on variety of vegetable and herbaceous plants (Hashtoudi*et al*., 2013). Inoculation of wheat with cyanobacterial species like *Anabaena* sp. Ck1 and *Chroococcidiopsis*sp*.,* Ck4 resulted in enhancement

of seed germination, shoot length, tillering, number of lateral roots, spike length and grain weight of plant due to release of cytokinin and IAA(Hussain and Hasnain, 2011).

Cyanobacteria operate as a mucilaginous binding agent in soil, mixing with soil to increase the humus content which is beneficial forplant growth. In natural ecosystem, phosphorus, a necessary nutrient for plant growth is the limiting factor for biomass production. Most of the phosphatic fertilizers in the soil profile are in insoluble forms and cannot be used by plants. The role of cyanobacteria is very important for the availability of phosphorous to plants. Cyanobacteria can decompose and mineralize insoluble tricalcium diphosphate, ferric orthophosphate and aluminium phosphate found in soils, sediments or in pure cultures by means of extracellular phosphatases and excretion of organic acids. The involvement of acid phosphatases and alkaline phosphatases in phosphate solubilization was discovered in soil inoculated with cyanobacteria (Prasanna *et al.,* 2013b). The maximum phosphorous uptake by wheat in *Anabaena-Pseudomomas* biofilm was reported by Swarnalaksmi*et al*. (2013).

Nitrogen fixing cyanobacteria and azolla have been important in improving rice productivity. In China and Vietnam, *Azolla-Anabaena* association has been used as effective green manure for flooded crops. This association doubles its biomass in a matter of days and produce more than 2 tonnes per hectare and it can fix more than10 kg N $ha^{-1}day^{-1}$ under ideal conditions (Vaishampayan*et al*.,2001). Prasanna *et al*. (2013b) reported that cyanobacterial species like *Anabaena doliolum, A. torulosa, Nostoc carneum, Oscillatoria* sp., etc. can synthesize extracellular polymeric substances (EPS) which enhance the water holding capacity of soil, soil aggregation and prevent erosion.

Presence of blue green algae in rhizosphere can help in assimilating organic compounds and their association with cropplantscan enhance the yield of crops (Prasanna *et al*., 2013b). Cyanobacterial consortia using different rhizospheric bacteria reported significant enhancement in soil fertility, crop yield, macronutrient enrichment and also efficient C-N sequestration for rice-wheat cropping systems (Prasanna *et al*., 2012a).

2.4 CYANOBACTERIA AS POTENTIAL BIOCONTROL AGENTS.

Cyanobacteria represent an important bioresource which is utilized as a biofertilizer because of their major role in nitrogen fixation, restoration of soil fertility etc. They are also thought to be a good source of novel antifungal and antibacterial chemicals (Singh *et al*., 2016). They also produce cyanotoxins such as aeruginosins, microginins and cyclic peptides like anabaenopeptins, nostopeptolides and anabeanopeptides which have bioactivities like serine protease inhibition (Golakoti*et al*., 2001).

2.4.1 Biocontrol potential against diseases

Tolypothrixtjipanensis produces tjipanazoles which shows a fungicidal activity against *Aspergillus flavus*, well known plant pathogen that attacks groundnut and stored grains (Bonjouklian*et al*., 1991). *Fischerilla*sp. produces fischerillin-A, showed antifungal activity against several plant pathogenic fungi (Hagmann*et al.,* 1996). It can inhibit *Uromyces appendiculatus* causing brown rust, *Erysiphe graminis* causing powdery mildew, 80% inhibition of *Phytophthora infestans* causing late blight of potato and tomato and also *Pyriculariaoryzae* which causes blast disease of rice.

*Oscillatoria redekei*extracts have been discovered to have antimicrobial effect against gram positive bacteria but no activity was found against gram negative bacteria(Mundt *et al*., 2001; Sabin *et al*., 2003). Twenty-two cyanobacterial strains were recovered from fresh water and terrestrial habitat showed action against gram positive bacteria but mild activity against gram negative bacteria. *Microcystis* sp. was tested for its antibacterial property against *Xanthomonas vesicatoria* (Volk *et al.,* 2006; Pandey and Pandey, 2002). Cyanobacterial extractshave been shown to lower the incidence of *Botrytis cineraria* on strawberries, *Erysiphaepolygoni* on turnips that causes powdery mildew and damping off in tomatoes and also inhibit the growth of plant pathogens like *Rhizoctonia solani* and *Sclerotiana sclerotium*. Kulik (1995) reported the extracts of cyanobacteria *Nostoc muscorum* can inhibit the growth of fungal pathogen *Sclerotiana sclerotium* which causes cottony rot of vegetables and *Rhizoctonia solani* causing root and stem rot.

Culture filtrates of *Synechococcusnidulans* reported nematicidal activity against *Meloidegyneincognitia, M. graminicola, Heteroderacajani, Heteroderaavanae* (Hollajer*et al*., 2012). Cyanobacterial extracts of*Synechococcusnidulans, Oscillatoria* sp*., Lyngbya*sp*., Phormidiummolle*etccan cause juvenile mortality and hatching inhibition in nematode, *Meloidogyneincognitia* (Sharma *et al*., 2007; Sharma *et al*., 2008).

2.5 BENEFITS OF CYANOBACTERIAL BIOFERTILIZER IN RICE CULTIVATION

De and Mandal (1956) reported the increased grain and straw yield resulting from the use of cyanobacterial strains as biofertilizer. Other reports suggest that cyanobacteria could reclaim alkaline usar soils in Uttarpradeshandalso they could enhance the availability of nitrogen and phosphorous levels in the soil (Singh and Bisoy, 1989). Plant size, nitrogen content, number of tillers, ears, spikelets and filled grains per panicle have all shown to be beneficial. Application of cyanobacteria like *Aulosirafertilissima* was found to increase in rice yield by 368% and 114% in pots and fields. In addition to increased grain and straw yield, cyanobacterial inoculation could also increase the nitrogen content of grain and straw, plant height, leaf length etc. Venkataraman (1993) reported that the cyanobacterial inoculation is generally more successful in the dry season, during which a saving of 20-30 Kg N ha^{-1} is observed.

After the cell death, cyanobacteria often release the fixed nitrogen in the form of polypeptides, with small quantity of free amino acids, vitamins and auxin like substances either through exudation or by microbial degradation. The transfer of fixed nitrogen to the plants and other organisms had been investigated using ^{15}N tracer technique. The rice crop was reported to contain37% and 51% of nitrogen with ^{15}N labeled *Aulosira*sp. spread over the soil and incorporated in the soil respectively. Because of the organic nature and low C:N ratio of the cyanobacterial material, it provides a better nitrogen source than an organic fertilizer. The susceptibility of cyanobacterial material to decomposition, which varies not only with the strain but also with the physiological condition, determines the availability of cyanobacterial nitrogen to rice.

Cyanobacterial inoculation speeds up the recovery of biological crusts caused by natural and anthropogenic activities. Extracellular polymeric substances (EPS) produced by cyanobacteriaimprove the water holding capacity of soil and reduces soil erosion. *Anabaena doliolum, A. torulosa, Nostoc carneum, N. piscinale, Oscillatoria*sp., *Plectonema*sp., *Schizothrix*sp.and other cyanobacterial species enhance the soil microbial biomass, carbon, nitrogen and humus content hence holding the moisture and aiding soil formation. Cyanobacterial formulations are reported to enhance the contents of nitrogen, potassium, phosphorous and zinc (Prasanna *et al*., 2013b).

Wetland rice fields are the most common human-managed wetland environment on the planet accounting for more than 90% of rice production. Wetland rice fields comprise a major ecosystem for cyanobacteria and nitrogen fixation by them is important to wetland rice field as a biological fertilizer (Ladha and Reddy, 2003).Roger and Ladha (1992) found that nitrogen contribution from cyanobacteria was often higher than the nitrogen inputs from heterotrophic bacteria in rice rhizosphere or heterotrophic bacteria which are free living in the soil.

Inoculation of mixed culture of *Aulosirafertilissima, Nostoc muscorum*and *Anabaena* sp. into a rice soil system enhanced rice grain yield (Ghosh and Saha, 1993). Nitrogen fixation by blue green algae can provide 20-30 kg Nha^{-1} annually and can reduce the use of urea fertilizer by 25-35% (Hashem, 2001). Use of cyanobacterial species in symbiotic association with Azolla can enhance the nitrogen fixation rates (Scott and Marcarelli, 2012).

The mass cultivation and use of cyanobacteria as biofertilizer was initiated in Japan. The term “algalization” (cyanobacterial inoculation) was coined to denote the application of cyanobacterial culture in fields as biofertilizer in small fields. Algalization is beingused in Philippines, Nepal, Iran, China etc. At the field level in India, it is followed in Tamil Nadu, Uttar Pradesh, Andhra Pradesh, Karnataka, Maharashtra and Haryana. Vaishampayan*et al*. (2001) reported that presence of cyanobacteria in the field could sustain the nitrogen concentration and fertility of flooded rice fields for longer period.

Application of cyanobacterial biofertilizers for atleast 3-4 consecutive seasons can enhance the crop yield by 4-33% and also reduce the input of chemical fertilizers. Mishra and Pabbi (2004) created a rural oriented algae biofertilizer system for rice, resulting in low-cost production while maintaining soil yield and health. Gurung and Prasad (2005) reported that the exclusive inoculation of cyanobacteria could increase the grain yield and straw yield of paddy crop by 14% and 10% respectively (Singh *et al.,* 2014).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The experiment on "Development of inoculant cultures of nitrogen fixing cyanobacteria from wetland ecosystem of Vellayani" was carried out in the Department of Agricultural Microbiology, College of Agriculture, Vellayani during the period 2019-2021.

The details of the materials used and methods followed during the course of study are mentioned below.

3.1 ISOLATION OF NITROGEN FIXING CYANOBACTERIA

3.1.1 Collection of samples and isolation of cyanobacteria

Sample collection and isolation was done as per the method outlined by Prasanna and Nayak (2007). Samples were collected in sterile vials of 15 mL and 30 mL volumefrom waterlogged and wetland areas of Vellayani. Sampling bottle were filled to its one third capacity with specimen along with water, the remaining part is left for aeration. BG-11 liquid medium without nitrogen supplement was prepared and sterilized by autoclave at 121^{o}C for 15 minutes under 15 psi pressure.Samples were diluted and inoculated in this sterile BG-11 medium for the enrichment of nitrogen fixing cyanobacteria. The enrichment flasks werechecked for green coloured cyanobacterial growth and viewed microscopically on regular basis. As and when the cyanobacterial growth was observed it was isolated and purified using serial dilution method. Dilutions were prepared upto$10^{-4}$and, one mL of each dilution was transferred to 10 ml nitrogen free BG-11 broth in screw capped vials and incubated at 26±2^{o}C under light condition.The procedure was repeated until a single isolate of cyanobacteria was obtained in a vial.

3.1.2 Culture conditions and maintenance of cyanobacterial isolates

Samples with well-developed colonies after dilution were inoculated into freshly prepared BG-11 broth. Inoculated broth was incubated at 26±2 ^{o}C, at 1200-1500 lux light intensity in16:8 light dark cycles, until cyanobacterial colonies developed. After

establishment of individual culture, they were transferred to liquid media in 250 mL, 500 mL and 1 L flasks, for better biomass production.

In nitrogen free BG-11 media, the cyanobacterial isolates were maintained in batch culture mode under sterile conditions. Regular subculturing into fresh liquid medium at 26±2 ^{o}C in a 16/8 hour light/dark cycle was used to sustain the culture sets (Lopes *et al*., 2012).

3.1.3 Purification of nitrogen fixing cyanobacteria

Solid BG-11 medium was prepared by addition of 2% agar and autoclaved before use. Subcultured isolates were subjected to streaking on agarized BG-11 medium for obtaining discrete colonies under uniform cultivation conditions. After 14 days of incubation, the colonies were picked up and inoculated in freshly prepared BG-11 broth. Axenization of cyanobacterial cultures was done by treating inoculated cultures with antibiotic solution. Antibiotic solution was prepared by dissolving 100mg penicillin and 50mg streptomycin sulphate in 10 mL distilled water. The solution was filtered using 0.45µ filtration assembly and added to the exponential phase of cyanobacterial cultures. It was incubated for 1 or 2 hrs. After incubation, cultures were pelleted and repeatedly washed with sterile water to remove traces of antibiotic and were inoculated into freshly prepared BG-11 medium (Fiore *et al*., 2005).

3.1.4Identificationof nitrogen fixing cyanobacteria

The morphological examination of the cyanobacterial isolates was done. The isolates were viewed under light microscope and their shape, size of the cell, unicellular/multicellular nature and presence or absence of heterocyst were observed and recorded. Identification was carried out by consulting various keys, literature of algal taxonomy.

3.2 CHARACTERIZATION OF NITROGEN FIXING CYANOBACTERIA

Morphological characters were identified by microscopic observation. For microscopic observation, actively growing culture was used. Wet mounts were prepared from culture of each isolate and observed under lightmicroscope at 40X magnification. Morphological characters including unicellular/multicellular nature, cell size, shape, colony characteristics, presence of heterocyst, position of heterocyst etc. were observed.

3.2.1. Morphological Characterization nitrogen fixing cyanobacterial isolates.

3.2.1.1 Cell size

Size of the cyanobacterial cells were measured using micrometry and expressed in µm.

3.2.1.2 Cell shape

The cyanobacterial isolates were observed microscopically for recording the morphological character of the cells.

3.2.1.3 Colony characteristics

The isolates were observed microscopically for their colony morphology characters like unicellular, colonial, mucilagenous, sheath.

3.2.1.4Presence of heterocyst

The isolates were observed microscopically for the presence of heterocyst and the position of heterocyst if present.

3.2.2. Biochemical Characterization of nitrogen fixing cyanobacterial isolates.

Biochemical characterization of the isolates was done based on the carbohydrate utilization pattern of the isolates using HiCarbohydrate^{TM} Kit (KB009A / KB009B / KB009C). The inoculation of the kit was done following the kit protocol and the kits were incubated in dark condition to elucidate the carbohydrate utilization pattern of the cyanobacterial isolates under light lighting conditions.

The kit was opened aseptically and 50µL of the cyanobacterial isolates were inoculated in each well by surface inoculation method. Later onthe kit was incubated in 26±2^{o}Cfor 24-48 hours. The results were interpreted according to the chart provided with the kit.

3.2.2.1 Carbohydrate fermentation test.

After incubation, the development of yellow colour from red was considered as positive reaction. If the test was negative, the medium remains red

3.2.2.2 Ortho-nitrophenyl-β-galactosidase test

The development of yellow colour from colourless in the medium was taken as positive reaction and colourless medium was taken as negative reaction.

3.2.2.3 Esculin hydrolysis

The development of black colour of the medium from cream colour was considered as positive reaction and the medium remaining colourless was taken as negative reaction.

3.2.2.4 Citrate utilization

The development of the blue colour of the medium from yellowish green was taken as positive reaction and the medium remaining colourless was taken as negative reaction.

3.2.2.5 Malonate utilization

The development of blue colour of the medium from light green was taken as positive reaction and the medium remaining light green was taken as negative reaction.

3.2.2.6 Taxonomic identification of cyanobacterial isolates based on morphology

Identification was carried out by consulting various keys, literature of algal taxonomy (Desikachary, 1959).

3.2.2.7 Estimation of Chlorophyll a

A known amount (10 mL) of homogenized cyanobacterial culture was centrifuged at 5,000 ×g for 10 min. The cells were resuspended in distilled water and the pelleting process was repeated twice. The pellet obtained was resuspended in 4 mL of methanol and vortexed vigorously. To keep the solvent from evaporating, the test tube's mouth was sealed with aluminium foil. The tubes were incubated for 1 hour in a water bath at 60°C with intermittent shaking. The tubes were cooled and centrifuged for 5 min at 5,000 rpm. The supernatant was moved to another tube and 4mL of the solvent was added and followed by another extraction. Two mL of the solvent was added to the pellet and the process repeated to ensure complete extraction. The supernatants were combined and made up to 10 mL volume with methanol. The optical density (OD) was measured using UV-VIS spectrophotometer at 663 nm and 645 nm (Mackinney, 1941).

3.2.2.8 Estimation of Phycobiliproteins

A known volume (10 mL) of cyanobacteria suspension was homogenized and centrifuged at 3000×g for 5 minutes. Phycobiliprotein was entirely extracted from the pellet by repeated freezing and thawing using 0.05M phosphate buffer. The absorbance of the supernatant was read at 615, 652, and 562 nm in UV-VIS spectrophotometer. The phycobiliproteins were determined (µg mL^{-1}) using the formula (Bennet and Bogorad, 1973).

Phycocyanin (PC) = (A615) – (0.475 × A562) /5.34

Allophycocyanin (APC) = (A652) – (0.208 × A615) /5.09

Phycoerythrin (PE) = (A562) – (2.41 × PC) – (0.849 × APC) /9.6.

3.2.2.9 Estimation of Carotenoids

The washed cyanobacterial culture pellet was suspended in 3mL of acetone and incubated in dark for 45min. The contents were centrifuged for 5 min at 5000×g. The resulting supernatant was refrigerated and the extraction procedure was repeated until the acetone became colourless. The supernatant was collected and acetone was used to dilute it to a final volume of 10mL. The absorbance of the supernatant was measured at 450nm in UV-VIS spectrophotometer (Jenson, 1978)

Carotenoids ($\mu g\ mL^{-1}$) = A_{450} × volume of sample × 10/2500.

3.2.2.10 Estimation of total proteins

The cyanobacterial pellet was cleaned twice with distilled water after centrifuging a known volume (10 mL) of culture solution. The pellet was soaked in a boiling water bath for half an hour after being treated with 10% trichloro acetic acid. The precipitate was produced by centrifugation at 5000 ×g for 15 minutes in a cooling microfuge. For protein quantification, the precipitate was neutralized in a known quantity of 1N NaOH. Protein content was estimated using bovine serum albumin (BSA) as the standard (40 to 200 µg mL^{-1} concentrations of BSA was prepared using reagent I and reagent II and the absorbance was measured at 660 nm)

By comparing the protein content of the sample to a standard graph, the protein content of the sample was determined (Lowry et al., 1951).

3.2.2.11 Antibiotic sensitivity assay

Antibiotic sensitivity of the isolates was assessed using growth inhibition experiment on agar plates with exponentially grown cyanobacterial cultures. Axenically grown cyanobacterial culture was pelleted out, homogenized and mixed with melted cooled BG 11 semisolid agar (0.8 %). This was poured over the 1.2 % BG-11 agar plates of 3 mm thickness. Sterile discs of antibiotics streptomycin(25µg),tetracycline(10 µg),nalidixic acid(30 µg), penicillin(10 µg), erythromycin(15 µg), rifampicin(5µg) and

chloramphenicol(30 µg) wasplaced equidistantly over the top agar containing cyanoabactria. Plates were incubated at 26±2°C for 5 days for proper growth. Antibiotic sensitivity was observed as clear zone around the antibiotic discs and the diameter of the zone was measured in millimeters. (Mishra *et al.,* 2013).

3.3. QUANTITATIVE ESTIMATION OF EXTRACELLULAR AMMONIA PRODUCTION

Extracellular ammonia excretion was determined spectrophotometrically by the method described by Cappuccino and Sherman (1999).

Cyanobacterial isolates of 14 days old was inoculated in 20 mL of N-free BG 11 broth and kept for incubation at 26±2 ^{o}C, at 1200-1500 lux light intensity in16:8 light dark cycles, until the colonies developed. After incubation, the cultures were subjected to centrifugation for 15 minutes at 4500 rpm. The presence of ammonia in supernatant was detected by the development of brown colour on addition of Nessler's reagent. This was taken as positive reaction for ammonia production as described by (Cappuccino and Sherman, 1999). The absorbance of the colour developed was measured at 450 nm and the quantity of ammonia produced was estimated by comparing to the standard curve of ammonium sulphate (0.1 – 1 µmol concentrations of ammonium sulphate solution was prepared using distilled water and absorbance was measured at 450 nm).

3.4 QUANTITATIVE ESTIMATION OF NITROGEN BY MICRO-KJELDAHL METHOD

3.4.1Single Acid Digestion

Cyanobacterial cultures were inoculated into 100mL specific broth and incubated at 26±2 ^{o}C, at 1200-1500 lux light intensity in 16:8 light dark cycles for 14 days, until the colonies developed. After incubation, the samples were kept over a hot plate at 110^{o}Cfor 5 days, until it evaporated down to a quantity of 5 mL. This 5 mL was collected and fed into tubes of digestion unit. A pinch of digestion mixture (100:10:1 ratio of potassium

sulphate, copper sulphate, and selenium powder) was added to the tubes and 10mL concentrated sulphuric acid was added. Clear solution was obtained after the completion of digestion process. Following the completion of digestion, the contents were transferred to a 100mL volumetric flask and made upto 100mL with distilled water.

In Kjeldahl digestion unit, 10mL of digested sample and 10mL of NaOH were added, and 10mL of boric acid with a few drops of mixed indicator was taken in a conical flask. Titration of ammonia collected in a conical flask was done against sulphuric acid.

$$\% \text{ of total N in the sample} = \frac{TV \times N \times 0.014 \times 100 \times 100}{V \times 10}$$

N- Normality of acid (0.02N)

V-Volume of sample.

3.5 ASSESSMENT OF ANTAGONISTIC EFFECT OF THE CYANOBACTERIAL ISOLATES ON PLANT PATHOGENIC BACTERIA AND FUNGI

Disc diffusion method for assessment of antagonistic effect of isolates against plant pathogenic bacteria and fungi was carried out according to the procedure described by Zaidan*et al.* (2005). The bacterial pathogen *Xanthomonas oryzae* and the fungal pathogens *Rhizoctoniasolani*, *Pythiumultimum*, *Phytophthoracapsici*, *Fusariumoxysporum*f.sp. *solani*available at the Department of Agricultural Microbiology, College of Agriculture, Vellayani were used for the assay. The bacterial pathogens were grown for 24 hrs in broth culture to attain 10^8cfumL^{-1}. A lawn ofbacterial pathogen was prepared on nutrientagar plates by swabbing the prepared broth culture.The plates were dried for 15 minutes and then used to test for antagonistic activity. The discs which had been impregnated with 100 µL of cyanobacterial isolates were placed on the plates and the plates were incubated at 26±2 ^{o}C, at 1200-1500 lux light intensity in 16:8 light dark cycles.

The antifungal activity of the isolates was tested by disc diffusion method. The fungal pathogens were grown on PDA plates for 3-5 days and mycelia discs were punched out from the actively growing region of fungal colony using a 5mm sterile cork borer. This was inoculated at the center of PDA plate prepared for disc diffusion method. The discs which had been impregnated with homogenized culture of cyanobacterial isolates were placed on the PDA plates. One positive control was a standard commercial antibiotic (Streptomycin- 5000 µg mL^{-1}) and negative control was sterile water. Depending on the pathogen species utilized in the test, the plateswere subsequently incubated at 28°C for 24-48 hours. After the incubation period, the plates were inspected for the presence of an inhibitory zone and if present was measured in mm.

$$\text{Zone of inhibition (\%)} = \frac{\text{Inhibition zone (mm)} - \text{Diameter of the disc (mm) X 100}}{\text{Diameter of the disc (mm)}}$$

3.6 QUANTITATIVE ESTIMATION OF INDOLE ACETIC ACID (IAA) BY SPECTROPHOTOMETRY

The quantification of IAA was done according to the standard procedure described by Gordon and Weber (1951).

Twenty milliliters of N-free BG-11 broth was prepared and supplemented with 0.1 per cent L-tryptophan (heat labile). The cyanobacterial cultures were inoculated into the broth and kept for incubation at 26±2 °C, at 1200-1500 lux light intensity in16:8 light dark cycles for 14 days, until the colonies developed. After incubation the samples were collected in centrifuge tubes and centrifuged at 4500 rpm for 20 minutes at 4°C.Two millilitres of the supernatant was immediately transferred to a sterile test tube, along with two drops of orthophosphoric acid, and thoroughly mixed. After one minute, 4 mL of Salkowski reagent was added, mixed well using vortex mixer and kept under dark conditions for 25 minutes. The optical density (OD) was measured using UV-VIS spectrophotometer at 530 nm. The amount of IAA in the sample was determined by

comparing to a standard curve prepared with chemical grade indole-3-acetic acid (Standards of concentration 0, 5, 10, 20, 50 and 100 µg mL^{-1} was made in culture medium and the absorbance was measured at 530 nm).

3.7 WEIGHTED AVERAGE RANKING

Weighted average ranking was done for identifying the best cyanobacterial isolates. For the ranking, IAA, extracellular ammonia and nitrogen were considered. The isolate showing the highest value in each parameter were given the 1^{st} rank and so on. The ranks obtained for different parameters were added to obtain weighted average rank. The isolate showing the lowest value in weighted rank was assigned 1^{st} rank, the second lowest was given 2^{nd} and so on.

3.8 *IN-VITRO* PLANT GROWTH PROMOTION STUDY

The isolate selected by weighted average ranking was subjected to roll towel assay. Seeds of rice (*Oryza sativa* L.) was surface sterilized with 1 per cent sodium hypochlorite for 2-3 minute and were given three subsequent washes in sterile water. Surface sterilized seeds were then soaked overnight in the treatment listed below. Completely Randomized Design (CRD) was followed with thirteen treatments and three replications each. Ten seeds per replication were placed on germination paper. The rolled paper towels were kept at 28°C, and the saturated moisture condition was maintained by watering the roll towel every day. The plant growth parameters were observed after seven days.

3.8.1 Treatments

1. T_1 - 10 mL of sterilized distilled water
2. T_2 -10 mL of sterilized BG-11 medium
3. T_3 -10 mL of sterilized BG-11 medium amended with 50 µg mL^{-1} of tryptophan

4. T_4- T_8 -10 mL of sterilized distilled water mixed with 20 µL, 40 µL, 60 µL, 80 µL, 100µL of cyanobacterial culture grown in BG-11 media
5. T_9- T_{13} -10 mL of sterilized distilled water mixed with 20µL, 40µL, 60µL, 80µL, 100µL of cyanobacterial culture grown in BG-11 media amended with 50 µg mL^{-1} of tryptophan.

3.8.2 Observations

3.4.8.1Days taken for germination

Number of days taken for seed germination was observed in all the treatments.

3.4.8.2 Percentage Seed Germination

The percentage of seed germination was calculated using the following formula.

$$\text{Seedgermination (\%)} = \frac{\text{Numberof seeds germinated}}{\text{Totalnumber of seeds}} \times 100$$

3.4.8.3Length of Shoot

The shoot length was measured after seven days from the base of the stem to the tip of the longest leaf and expressed in centimetres.

3.4.8.4Length of Root

The root length was measured after seven days from the base of the stem to the tip of the root. It was expressed in centimetres.

3.4.8.5 Dry Weight of Shoot

The shoot dry weight was measured and expressed in mg.

3.4.8.6 Dry Weight of Root

The plant samples were dried by keeping in hot air oven at 55^{o}C for 24 h. The root dry weight was measured in mg.

3.4.8.7 Shoot root ratio

The shoot root ratio was calculated using the dry weight of root and shoot using the formula,

$$\text{Shoot root ratio} = \frac{\text{Dryweight of shoot}}{\text{Dry weight of root}}$$

3.4.8.8 Seedling Vigour Index

The seedling vigour index was calculated using the following formula (Abdul-Baki and Anderson, 1973),

Seedling Vigour Index I = Germination Percentage × (Shoot length + Root length)

Seedling Vigour Index II = Germination Percentage × (Shoot dry weight+ Root dry weight)

3.9STATISTICAL ANALYSIS

The collected data were subjected to statistical analysis using Analysis of Variance Technique (ANOVA) under Completely Randomized Design (CRD).The data on mean, Standard Error (SE) and Critical Difference (CD) were obtained using GRAPES (Gopinath *et al*., 2021).

RESULTS

4. RESULTS

Nitrogen fixing cyanobacteria were isolated from wetland ecosystem of Vellayani region. The succeeding results were obtained through the statistical analysis done with the data from the experiments carried-out during the course of study.

4.1 ISOLATION OF CYANOBACTERIA CAPABLE OF FIXING NITROGEN

Ten isolates of cyanobacteria capable of fixing atmospheric nitrogen were isolated from rice fields and wetland ecosystem of Vellayani area in N free BG-11 liquid medium. The isolates were given code numbers from VLY 1- VLY 10.

4.2 MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF THE ISOLATES

4.2.1 Morphological characters of the isolates

Morphological characters of cyanobacterial isolates including unicellular/multi cellular nature, cell size, cell shape, and presence and position of heterocyst were observed and recorded in Table. 1. This included six unicellular and four filamentous cyanobacterial genera with their cell size/filament length ranging from 0.5 – 30 µm. The cell shape of VLY 1, VLY 2, VLY 3, VLY 4, VLY 6, and VLY 9 was unicellular whereas the cell shape of VLY 5, VLY 7, VLY 8 and VLY 10 was filamentous in nature. The isolates VLY 8 (Intercalary) and VLY 10 (Terminal) showed the presence of heterocyst (Desikachary, 1959).(Plate 1,2)

Table 1. Morphological characteristics of cyanobacterial isolates

Sl. No.	Isolates	Cell size / Filament length (µm)	Shape	Unicellular (U), Colonial(C), Mucilagenous (M), Sheath (S), Filamentous(F), Non filamentous(NF)	Presence of heterocyst
1	VLY 1	1.5 - 2.0	Oblong	Unicellular, Non filamentous	No
2	VLY 2	0.6 - 0.8	Spherical	Unicellular,Non filamentous	No
3	VLY 3	1.0 - 1.3	Spherical	Colonial, Mucilagenous, Non filamentous	No
4	VLY 4	1.8 - 2.1	Ovoid	Colonial, Mucilagenous, Non filamentous	No
5	VLY 5	2.0 - 20.0	Cylindrical	Filamentous	No
6	VLY 6	2.0 - 7.5	Spherical	Cells in pair or as four, Mucilagenous,	No
7	VLY 7	11.0 - 15.0	Cylindrical	Filamentous, Sheath	No
8	VLY 8	25.0 - 30.0	Spherical	Filamentous	Yes(Intercalary)
9	VLY 9	5.0 - 8.0	Spherical	Cells in pair or as four, Mucilagenous,Nonfilamentous	No
10	VLY 10	11.0 - 15.0	Cylindrical	Filamentous, Sheath	Yes(Basal)

4.2.2 Biochemical characterization of the isolates

The carbohydrate utilization pattern of the isolates was studied using 35 different carbohydrates in HiCarbohydrate Kit. All the isolates showed utilization of Galactose, Sucrose, L-Arabinose, Malonate utilization, ONPG as mentioned in Table 2. In fructose utilization test, VLY 9 alone showed positive reaction. VLY 6 showed positive reaction in lactose test. Isolates like VLY 6, VLY 9, VLY 2, VLY 10, VLY 1 and VLY 7 showed positive reaction for lactose, fructose, mannitol, adonitol, xylitol and sorbose utilization tests. In the case of rhamnose utilization test, VLY 4 and VLY 5 showed positive reaction. In the case of Esculin hydrolysis test, all isolates are observed to have positive reaction except VLY 9. In citrate utilization test, all isolates are shown to produce positive reaction except VLY3, VLY 4, VLY 7. In cellobiose and melezitose utilization test, VLY 1, VLY 2 and VLY 6 showed positive reaction whereas in ∞ Methyl-D-glucoside test. VLY 1 and VLY 5 showed positive reaction. The isolates showed negative reaction for lactose, raffinose, melibiose, inulin, sodium gluconate, glycerol, salicin, inositol, sorbitol, arabitol, erythritol, ∞ Methyl-D-glucoside utilization tests.(Plate 3)

4.2.3 Taxonomic identification of cyanobacterial isolates

4.2.3.1 Tentative Identification based on morphology

The identified species of blue green algae are structurally different in shape, size and structure. The key characters for the identification based on Desikachary (1959) is given in Table 3.

Table 2. Biochemical characterization of cyanobacterial isolates

Sl. No	Isolates	Xylose	Maltose	Fructose	Dextrose	Galactose	Trehalose	Sucrose	L-Arabinose	Dulcitol	D-Arabinose	Mannitol	Adonitol	Xylitol	ONPG	∞ Methyl-D-mannoside	Rhamnose	Malonate utilization	Cellobiose	Melezitose	Esculin hydrolysis	Citrate utilization	Sorbose	Mannose
1	VLY 1	-	+	-	+	+	-	+	+	-	+	-	-	+	+	+	-	+	+	+	+	+	-	-
2	VLY 2	-	+	-	+	+	+	+	+	-	+	+	-	-	+	-	-	+	+	+	+	+	-	-
3	VLY 3	-	-	-	+	+	+	+	+	-	+	-	-	-	+	-	-	+	-	-	+	+	-	+
4	VLY 4	-	+	-	+	+	-	+	+	-	+	-	-	-	+	-	+	+	-	-	+	-	-	+
5	VLY 5	+	-	-	+	+	-	+	+	-	+	-	-	-	+	+	+	+	-	+	+	+	-	+
6	VLY 6	+	-	-	-	+	-	+	+	+	+	-	-	-	+	-	-	+	+	+	+	+	-	-
7	VLY 7	-	+	-	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-	+	-	+	-
8	VLY 8	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	+	-	-	-	+	-	+
9	VLY 9	-	-	+	+	+	-	+	+	-	+	-	-	-	+	-	-	+	-	-	+	+	-	+
10	VLY10	-	-	-	-	+	-	+	+	-	+	-	+	+	+	-	-	+	-	-	+	+	-	-

Table 3. Taxonomic identification of cyanobacterial isolates using taxonomic keys in Desikachary (1959).

SL. No.	Isolates	Characters	Reference
1	VLY 1	Amorphous colonies are formed by a large number of oblong, flattened cells that are irregularly distributed within mucilage. Individual cells have no characteristic mucilage sheath.	*Aphanotheca*sp.Desikachary,1959,Cyanophyta 104, pl.22, fig 4
2	VLY 2	Cells are spherical resulting in irregularly shaped microscopic colonies.	*Aphanocapsa* sp.Desikachary, 1959, Cyanophyta 110, pl 23, fig 1
3	VLY 3	Mainly colonial in nature, unicellular fast-growing cultures with viscous mucilagenous sheath.	*Microcystis* sp.Desikachary, 1959, Cyanophyta 86, pl 17, fig 10
4	VLY 4	Cells unicellular, typically differentiated into base and apex. solitary or in groups placed in parallel within a whitish sheath, slightly oval cells.	*Dermocarpa*sp. Desikachary, 1959, Cyanophyta 170,pl 33, fig 19
5	VLY 5	Cells filamentous, with rigid and straight trichome (usually no sheath), or flexible. Trichomes cylindrical and not in bundles.	*Oscillatoria* sp. Desikachary, 1959, Cyanophyta 244, pl 42, fig 6
6	VLY 6	Inside a mucilagenous membrane, single cells or groups of cells normally have an even number of cells. Cells are spherical, however they are often hemispherical with a dense sheath.	*Chroococcus* sp. Desikachary, 1959, Cyanophyta 122, pl 24, fig 5
7	VLY 7	Trichomes that are thick and straight, with a hard sheath. Filaments are usually unbranched, not in bundles and without heterocysts.	*Lyngbya* sp. Desikachary, 1959, Cyanophyta 288, pl 48, fig 4
8	VLY 8	Trichomes with spherical cells and intercalary heterocysts. Cells may be cylindrical, or bent, but overall look much like a string of beads. Some species have colourless mucilage.	*Anabaena* sp.Desikachary, 1959, Cyanophyta 397, pl 72 fig 3
9	VLY 9	Cells unicellular or forming colonies, not forming filament like growth and may be single, or in small group of 2-4. Sheath of individual cells distinct.	*Chrococcus* sp.Desikachary, 1959, Cyanophyta 122, pl 24, fig 2
10	VLY10	Attached trichomes larger at the base and tapering, each with a basal heterocyst. Trichomes may occur singly or arranged in groups but single trichomes always separate.	*Calothrix* sp.Desikachary, 1959, Cyanophyta 532, pl 110, fig 8

4.2.4 Estimation of chlorophyll *a* in the cyanobacterial isolates

The quantitative estimation of chlorophyll *a*ranged between 5.372±0.721 mgmL^{-1}and 0.688±0.057 mg mL^{-1}.The maximum chlorophyll *a*content was recorded in *Aphanotheca* sp. VLY 1 (5.372±0.721 mg mL^{-1}) which was on par with *Aphanocapsa* sp. VLY 2 (5.363±0.238 mg mL^{-1}) and the minimum concentration of chlorophyll *a* was recorded by *Chroococcus* sp. VLY 6(0.688±0.057 mg mL^{-1})(Table 4) (Plate 4).

4.2.5 Quantitative Estimation of phycobiliproteins in the cyanobacterial isolates

The maximum content ofphycobiliproteins, phycocyanin (0.036±0.014 µg mL^{-1}), Allophycocyanin (0.037±0.005 µg mL^{-1}), Phycoerythrin (0.013±0.001 µgmL^{-1}) was shown by *Aphanocapsa* sp. VLY 2 which was on par with *Chroococcus* sp. VLY 6 for both Phycocyanin (0.036±0.022 µgmL^{-1}) and Allophycocyanin (0.030±0.013 µgmL^{-1}) with *Aphanotheca* sp. VLY 1 for Phycoerythrin (0.009±0.002 µgmL^{-1}). There was no significant difference between the concentration of Phycocyanin for all the isolates. The least concentration of Allophycocyanin (0.015±0.002 µgmL^{-1}) was shown by both *Lyngbya* sp. VLY 7 and *Anabaena* sp. VLY 8 and the minimum concentration of Phycoerythrin (0.005±0.001 µgmL^{-1}) was shown by *Lyngbya* sp. VLY 7 (Table 5).

4.2.6 Estimation of carotenoids in cyanobacterial isolates

The maximum concentration of carotenoids was recorded by *Calothrix* sp. VLY 10 with 0.006 µg mL^{-1}which was followed by *Dermocarpa* sp. VLY 4 with 0.005 µgmL^{-1}. The lowest concentration of carotenoids was reported by *Chroococcus* sp. VLY 6 (0.001 µgmL^{-1}) and *Lyngbya* sp. VLY 7(0.001 µgmL^{-1})(Table 6) (Plate 5).

Table 4. Chlorophyll *a*content in cyanobacterial isolates

Sl.No.	Isolates	Chlorophyll *a* (mg mL^{-1})
1	*Aphanotheca* sp. VLY 1	5.37 ± 0.72^{a}
2	*Aphanocapsa* sp. VLY 2	5.36 ± 0.23^{a}
3	*Microcystis* sp. VLY 3	4.35 ± 0.54^{b}
4	*Dermocarpa* sp. VLY 4	2.57 ± 0.15^{b}
5	*Oscillatoria* sp. VLY 5	2.10 ± 0.10^{e}
6	*Chroococcus* sp. VLY 6	0.68 ± 0.05^{f}
7	*Lyngbya* sp. VLY 7	3.04 ± 0.39^{cd}
8	*Anabaena* sp. VLY 8	3.28 ± 0.24^{c}
9	*Chroococcus* sp. VLY 9	3.09 ± 0.57^{cd}
10	*Calothrix* sp. VLY 10	4.35 ± 0.24^{b}

Table 5. Phycobiliproteins in cyanobacterial isolates

Sl. No.	Isolates	Phycocyanin ($\mu g\ mL^{-1}$)	Allophycocyanin ($\mu g\ mL^{-1}$)	Phycoerythrin ($\mu g\ mL^{-1}$)
1	*Aphanotheca* sp. VLY 1	0.02±0.00	0.02 ± 0.00^{bcd}	0.01 ± 0.00^{ab}
2	*Aphanocapsa* sp. VLY 2	0.03±0.01	0.03 ± 0.00^{a}	0.01 ± 0.00^{a}
3	*Microcystis* sp. VLY 3	0.02±0.00	0.02 ± 0.00^{bc}	0.01 ± 0.00^{bc}
4	*Dermocarpa* sp. VLY 4	0.01±0.00	0.01 ± 0.00^{cd}	0.01 ± 0.00^{bc}
5	*Oscillatoria* sp. VLY 5	0.02±0.00	0.02 ± 0.00^{cd}	0.00 ± 0.01^{c}
6	*Chroococcus* sp. VLY 6	0.03±0.02	0.03 ± 0.01^{ab}	0.01 ± 0.01^{bc}
7	*Lyngbya* sp. VLY 7	0.01±0.00	0.01 ± 0.00^{d}	0.01 ± 0.00^{bc}
8	*Anabaena* sp. VLY 8	0.01±0.00	0.01 ± 0.00^{d}	0.01 ± 0.00^{bc}
9	*Chroococcus* sp. VLY 9	0.01±0.01	0.02 ± 0.00^{cd}	0.01 ± 0.00^{bc}
10	*Calothrix* sp. VLY 10	0.01±0.00	0.01 ± 0.00^{cd}	0.01 ± 0.00^{bc}

Table 6. Carotenoids incyanobacterial isolates

Sl.No.	Isolates	Carotenoids ($\mu g\ mL^{-1}$)
1	*Aphanotheca* sp. VLY 1	0.0019^{f}
2	*Aphanocapsa* sp. VLY 2	0.0020^{e}
3	*Microcystis* sp. VLY 3	0.0015^{h}
4	*Dermocarpa* sp. VLY 4	0.0054^{b}
5	*Oscillatoria* sp. VLY 5	0.0029^{d}
6	*Chroococcus* sp. VLY 6	0.001^{i}
7	*Lyngbya* sp. VLY 7	0.001^{i}
8	*Anabaena* sp. VLY 8	0.0017^{g}
9	*Chroococcus* sp. VLY 9	0.003^{c}
10	*Calothrix* sp. VLY 10	0.006^{a}

4.2.7 Estimation of total proteins in cyanobacterial isolates

Among the isolates, total proteins content was highest in the isolate *Oscillatoria* sp. VLY 5 (236.32±0.153 µg mL^{-1}). The lowest protein content was in *Lyngbya* sp. VLY 7(102.57±0.120 µg mL^{-1})(Table 7) (Plate 6).

4.2.8 Antibiotic sensitivity assay of cyanobacterial isolates

The results are presented in Table 8. *Aphanotheca* sp. VLY 1 showed sensitivity to rifampicin(5 µg), streptomycin(25 µg) and tetracycline(10 µg) with zone of inhibition of 14mm, 12mm, 8 mm respectively. *Oscillatoria* sp. VLY 5 was sensitive against rifampicin(5 µg) and tetracycline(10 µg) with a zone of 10mm and 13mm. *Lyngbya*sp. VLY 7 and *Chroococcus*sp. VLY 9 was sensitive to rifampicin (5 µg) with a zone of 9mm and 11mm. The rest of the isolates were resistant to these antibiotics at the specific concentrations (Plate 7,8).

4.3 ESTIMATION OF EXTRACELLULAR AMMONIA PRODUCTION BY THE ISOLATES

The maximum extracellular ammonia production was recorded in *Chroococcus*sp. VLY 6 (171.984±4.362 µmol mL^{-1}) which was on par with*Anabaena*sp. VLY 8 (168.588±1.353 µmol mL^{-1}). The lowest value was recorded in *Calothrix* sp. VLY 10 with 111.746±5.532 µmol mL^{-1} (Table 9).

Table 7. Total protein content of the cyanobacterial isolates

Sl.No.	Isolates	Total protein ($\mu g\ mL^{-1}$)
1	*Aphanotheca* sp. VLY 1	227.47 ± 0.49^{c}
2	*Aphanocapsa* sp. VLY 2	233.49 ± 0.15^{b}
3	*Microcystis* sp. VLY 3	158.53 ± 0.24^{g}
4	*Dermocarpa* sp. VLY 4	222.50 ± 0.14^{d}
5	*Oscillatoria* sp. VLY 5	236.32 ± 0.15^{a}
6	*Chroococcus* sp. VLY 6	179.72 ± 0.12^{f}
7	*Lyngbya* sp. VLY 7	102.57 ± 0.12^{j}
8	*Anabaena* sp. VLY 8	181.59 ± 0.23^{e}
9	*Chroococcus* sp. VLY 9	129.38 ± 0.32^{h}
10	*Calothrix* sp. VLY 10	119.75 ± 0.17^{i}

Table 8. Antibiotic sensitivity assay of the cyanobacterial isolates

Isolates	Rifampicin (5 µg)	Streptomycin (25 µg)	Tetracycline (10 µg)
Aphanotheca sp. VLY 1	S (14mm)	S (12mm)	S (8mm)
Oscillatoria sp. VLY 5	S (10mm)	R	S (13mm)
Lyngbya sp. VLY 7	S (9mm)	R	R
Chrococcus sp. VLY 9	S (11mm)	R	R

(R) Resistant, (S) Sensitive

The values in bracket indicates the zone of inhibition in mm.

Table 9. Extracellular ammonia production by the cyanobacterial isolates

SL.No.	Isolates	Extracellular ammonia ($\mu mol\ mL^{-1}$)
1	*Aphanotheca* sp. VLY 1	155.30 ± 2.88^{bc}
2	*Aphanocapsa* sp. VLY 2	157.81 ± 3.34^{b}
3	*Microcystis* sp. VLY 3	153.82 ± 3.78^{bcd}
4	*Dermocarpa* sp. VLY 4	147.91 ± 2.43^{cde}
5	*Oscillatoria* sp. VLY 5	140.24 ± 0.92^{e}
6	*Chroococcus* sp. VLY 6	171.98 ± 4.36^{a}
7	*Lyngbya* sp. VLY 7	145.55 ± 12.90^{de}
8	*Anabaena* sp. VLY 8	168.58 ± 1.35^{a}
9	*Chroococcus* sp. VLY 9	155.89 ± 2.22^{bc}
10	*Calothrix* sp. VLY 10	111.74 ± 5.53^{f}

4.4 ASSESSING THE NITROGEN CONTENT OF THE ISOLATES BY ESTIMATION OF NITROGEN BY MICRO-KJELDAHL METHOD

The highest nitrogen content was reported by *Oscillatoria*sp. VLY 5 (0.280±0.01mg mL^{-1}) which was on par with *Dermocarpa*sp. VLY 4, *Aphanocapsa*sp. VLY 2 and *Aphanotheca*sp. VLY 1. The lowest nitrogen content was reported by the isolate *Calothrix*sp. VLY 10 (0.200±0.010 $mgmL^{-1}$) which was on par with *Chroococcus*sp. VLY 9 and *Microcystis*sp. VLY 3. (Table 10).

4.5 ASSESSMENT OF ANTAGONISTIC EFFECT OF ISOLATES ON DIFFERENT PLANT PATHOGENIC BACTERIA AND FUNGI

4.5.1 Antagonistic effect of isolates against *Xanthomonasoryzae*

Two isolates were found to inhibit the growth of plant pathogenic bacteria *Xanthomonasoryzae*. *Aphanotheca* sp. VLY 1 showed a zone of inhibition of 7.1mm and *Microcystis*sp. VLY 3 showed a zone of 8.2mm. The remaining isolates were not found to inhibit the growth *Xanthomonasoryzae.* Antibiotic solution (Streptomycin– 5000 ppm) which was used as positive control recorded 10.2 mm (Table 11)(Plate 9).

4.5.2 Antagonistic effect of isolates against plant pathogenic fungi.

The isolates were not found to inhibit the growth of fungal pathogens viz *Rhizoctonia solani*, *Pythium ultimum*, *Phytophthora capsici*, *Fusarium oxysporum* f.sp. *solani*.

Table 10. Nitrogen content of the cyanobacterial isolates

SL.No.	Isolates	Nitrogen content ($mg\ mL^{-1}$)
1	*Aphanotheca* sp. VLY 1	0.26 ± 0.01^{ab}
2	*Aphanocapsa* sp. VLY 2	0.25 ± 0.02^{ab}
3	*Microcystis* sp. VLY 3	0.20 ± 0.01^{c}
4	*Dermocarpa* sp. VLY 4	0.26 ± 0.01^{ab}
5	*Oscillatoria* sp. VLY 5	0.28 ± 0.01^{a}
6	*Chroococcus* sp. VLY 6	0.24 ± 0.01^{b}
7	*Lyngbya* sp. VLY 7	0.24 ± 0.01^{b}
8	*Anabaena* sp. VLY 8	0.25 ± 0.01^{b}
9	*Chroococcus* sp. VLY 9	0.21 ± 0.01^{c}
10	*Calothrix* sp. VLY 10	0.20 ± 0.01^{c}

Table 11. Antagonistic activities of the isolates on plant pathogenic bacteria *Xanthomonas* sp.

Sl.No.	Isolates	Zone of inhibition (mm)	Percentage inhibition
1.	*Aphanotheca* sp. VLY 1	7.12[c]	42.4
2.	*Aphanocapsa* sp. VLY 2	0.00[d]	0.00
3.	*Microcystis* sp. VLY 3	8.20[b]	64.0
4.	*Dermocarpa* sp. VLY 4	0.00[d]	0.00
5.	*Oscillatoria* sp. VLY 5	0.00[d]	0.00
6.	*Chroococcus* sp. VLY 6	0.00[d]	0.00
7.	*Lyngbya* sp. VLY 7	0.00[d]	0.00
8.	*Anabaena* sp. VLY 8	0.00[d]	0.00
9.	*Chroococcus* sp. VLY 9	0.00[d]	0.00
10.	*Calothrix* sp. VLY 10	0.00[d]	0.00
11.	Antibiotic solution	10.20[a]	104.00
12.	Sterile water	0.00[d]	0.00

4.6 QUANTITATIVE ESTIMATION OF INDOLE ACETIC ACID PRODUCED BY THE ISOLATES

The maximum IAA production was reported by *Chroococcus*sp. VLY 6 (7.10±0.23µg mL^{-1}), followed by *Calothrix*sp.VLY 10 (6.87±0.01 µg mL^{-1}). The lowest IAA production was reported by *Lyngbya*sp.VLY 7 (1.47±0.01µg mL^{-1}) (Table 12).

4.7 WEIGHTED AVERAGE RANKING FOR THE SELECTION OF BEST CYANOBACTERIAL ISOLATE

The isolate *Chroococcus*sp. VLY 6 was selected as the superior one capable of fixing atmospheric nitrogen among the ten isolates based on the weighted average ranking (Table 13).

Table 12. Indole acetic acid production by the cyanobacterial isolates.

Sl.No.	Isolates	IAA production ($\mu g\ mL^{-1}$)
1	*Aphanotheca* sp. VLY 1	4.18 ± 0.00^{g}
2	*Aphanocapsa* sp. VLY 2	4.87 ± 0.01^{e}
3	*Microcystis* sp. VLY 3	3.70 ± 0.02^{h}
4	*Dermocarpa* sp. VLY 4	6.57 ± 0.02^{c}
5	*Oscillatoria* sp. VLY 5	4.52 ± 0.01^{f}
6	*Chroococcus* sp. VLY 6	7.10 ± 0.23^{a}
7	*Lyngbya* sp. VLY 7	1.47 ± 0.01^{i}
8	*Anabaena* sp. VLY 8	3.67 ± 0.00^{h}
9	*Chroococcus* sp. VLY 9	5.94 ± 0.01^{d}
10	*Calothrix* sp. VLY 10	6.87 ± 0.01^{b}

Table 13. Weighted average ranking of the cyanobacterial isolates based on growth promotion aspects.

SL.No.	Isolates	Weighted average ranking
1	*Aphanotheca* sp. VLY 1	3
2	*Aphanocapsa* sp. VLY 2	2
3	*Microcystis* sp. VLY 3	8
4	*Dermocarpa* sp. VLY 4	3
5	*Oscillatoria* sp. VLY 5	5
6	*Chroococcus* sp. VLY 6	1
7	*Lyngbya* sp. VLY 7	9
8	*Anabaena* sp. VLY 8	5
9	*Chroococcus* sp. VLY 9	5
10	*Calothrix* sp. VLY 10	8

4.8 *IN VITRO* PLANT GROWTH PROMOTION STUDY USING THE SELECTED ISOLATE

The plant growth parameters such as germination percentage, number of days taken for germination, plant height, dry weight, seedling vigour index and shoot root ratio are presented (Plate 10).

Days taken for germination were similar in all the treatments. Maximum germination percentage was observed in seeds bioprimedwith 40µL of cyanobacterial culture grown in medium amended with tryptophan (96.66±5.77 % of seed germination) followed by bio primed seedswith 60µL, 80 µL 100 µL of cyanobacterial culture grown in medium amended with tryptophan (93.33±11.54 %). The germination percentage was minimum inseeds bio primed with sterile distilled water (80±10.00 %)(Table 14).

The effect of cyanobacterial culture treatment on shoot length and root length of rice was also observed. The seeds bioprimed with 40 µL of cyanobacterial culture grown in medium amended with tryptophan showed maximum shoot length of 6.83±0.42 cm and root length of 12.23±1.12 cm. The minimum was reported by the seeds bioprimed with sterile distilled water with shoot length of 5.75±0.33 cm and root length of 7.65±2.23 cm. All the seeds bioprimed with cyanobacterial cultures had significant shoot length and root length when compared to seeds bioprimed with sterile distilled water (Table 15).

The maximum dry weight of shoot was reported in seeds bio primed with 10mL sterile distilled water mixed with 40 µL of cyanobacterial culture medium having 5 mg mL^{-1} with shoot dry weight of 4.76±0.03 mg and root dry weight of 2.67±0.02 mg. All the plants with cyanobacterial treatments showed significantly greater shoot and root dry weight compared to control (Table 16).

Significantly higher shoot root ratio was observed in seeds bioprimed with sterile distilled water(2.38±0.01) which was on par with seeds treated with sterile BG-11 media

and sterile BG-11 media amended with 5mg mL^{-1} of tryptophan. The lowest shoot root ratio was reported by seeds treated with 100µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan with 1.73±0.02 (Table 17).

Highest seedling vigour index-I was reported by seeds bioprimed with 40 µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan (1781±223.71) which was followed by seeds bioprimed with 80µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan (1757±67.26). The minimum was reported by the control (1340±252.33). Maximum seedling vigour index-II was recorded by seeds bio primed with 10mL sterile distilled water mixed with 40 µL of cyanobacterial culture medium having 5 mg mL^{-1}, with 713.82±4.85 followed by seeds bio primed with 10mL sterile distilled water mixed with 60 µL of cyanobacterial culture medium having 5 mg mL^{-1} (692.67±2.78) (Table 17) (Plate 11, 12)

Table 14. Effect of biopriming with *Chroococcus* sp. VLY 6 on germination percentage and days taken for germination of rice seeds

Sl.No.	Treatments	Seed germination	
		Percentage (%)	Days taken
1	T_1-10 mL SDW (Control)	80.00±10.00	2
2	T_2-10 mL BG-11	80.00±10.00	2
3	T_3-10 mL BG-11 (T)	80.00±10.00	2
4	T_4-10 mL SDW+20 µL C BG-11	90.00±0.00	2
5	T_5-10 mL SDW+40 µl C BG-11	83.33±5.77	2
6	T_6-10 mL SDW+60 µL C BG-11	90.00±0.00	2
7	T_7-10 mL SDW+80 µL C BG-11	83.33±5.77	2
8	T_8-10 mL SDW+100 µL C BG-11	86.66±5.77	2
9	T_9-10 mL SDW+20 µL C BG-11(T)	86.66±5.77	2
10	T_{10}-10 mL SDW+40 µL C BG-11(T)	96.66±5.77	2
11	T_{11}-10 mL SDW+60 µL C BG-11(T)	93.33±11.54	2
12	T_{12}-10 mL SDW+80 µL C BG-11(T)	93.33±11.54	2
13	T_{13}-10 mL SDW+100 µL C BG-11(T)	93.33±11.54	2

SDW – Sterile distilled water

BG-11(T) – BG-11 media amended with 50 μgmL^{-1}tryptophan

C BG-11 – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media

C BG-11(T) – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media amended with 50μgmL^{-1} oftryptophan

Table 15. Effect of *Chroococcus*sp. VLY 6 on shoot length and root length of bio-primed rice seeds on 7th DAT*

Sl.No.	Treatments	Shoot length (cm)	Root length (cm)
1	T_1-10 mL SDW (Control)	5.75±0.33	7.65±2.23^{c}
2	T_2-10 mL BG-11	6.05±0.23	8.96±1.07^{bc}
3	T_3-10 mL BG-11 (T)	6.14±0.72	11.21±0.46^{ab}
4	T_4-10 mL SDW+20 µL C BG-11	5.88±0.80	10.71±0.99^{ab}
5	T_5-10 mL SDW+40 µl C BG-11	6.14±0.85	11.08±1.14^{ab}
6	T_6-10 mL SDW+60 µL C BG-11	6.13±0.19	11.13±1.04^{ab}
7	T_7-10 mL SDW+80 µL C BG-11	6.05±1.17	10.26±1.12^{ab}
8	T_8-10 mL SDW+100 µL C BG-11	6.36±0.23	10.50±2.03^{ab}
9	T_9-10 mL SDW+20 µL C BG-11(T)	6.35±0.80	10.06±0.70^{ab}
10	T_{10}-10 mL SDW+40 µL C BG-11(T)	6.83±0.42	12.23±1.12^{a}
11	T_{11}-10 mL SDW+60 µL C BG-11(T)	6.62±0.33	10.83±1.16^{ab}
12	T_{12}-10 mL SDW+80 µL C BG-11(T)	6.40±0.05	10.26±0.85^{ab}
13	T_{13}-10 mL SDW+100 µL C BG-11(T)	6.51±0.36	11.03±2.02^{ab}

SDW – Sterile distilled water

BG-11(T) – BG-11 media amended with 50 µgmL^{-1} tryptophan

C BG-11 – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media

C BG-11(T) – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media amended with 50 µgmL^{-1}oftryptophan

Table 16. Effect of rice bio-primed seeds on fresh weight and dry weight on 7th DAT*

Sl.No.	Treatments	Dry weight (mg)	
		Shoot	Root
1	T_1-10 mL SDW (Control)	3.808±0.003^{h}	1.597±0.011^{h}
2	T_2-10 mL BG-11	3.834±0.041^{gh}	1.621±0.015^{h}
3	T_3-10 mL BG-11 (T)	3.908±0.033^{g}	1.646±0.009^{h}
4	T_4-10 mL SDW+20 µL C BG-11	3.862±0.033^{gh}	1.733±0.019^{g}
5	T_5-10 mL SDW+40 µl C BG-11	3.894±0.026^{g}	1.773±0.006^{fg}
6	T_6-10 mL SDW+60 µL C BG-11	3.908±0.019^{g}	1.801±0.014^{f}
7	T_7-10 mL SDW+80 µL C BG-11	4.081±0.035^{f}	2.041±0.085^{e}
8	T_8-10 mL SDW+100 µL C BG-11	4.206±0.075^{e}	2.268±0.074^{d}
9	T_9-10 mL SDW+20 µL C BG-11(T)	4.420±0.115^{d}	2.425±0.006^{c}
10	T_{10}-10 mL SDW+40 µL C BG-11(T)	4.762±0.030^{a}	2.674±0.024^{a}
11	T_{11}-10 mL SDW+60 µL C BG-11(T)	4.683±0.022^{ab}	2.608±0.009^{b}
12	T_{12}-10 mL SDW+80 µL C BG-11(T)	4.621±0.023^{bc}	2..603±0.020^{b}
13	T_{13}-10 mL SDW+100 µL C BG-11(T)	4.544±0.038^{c}	2.621±0.010^{ab}

SDW – Sterile distilled water

BG-11(T) – BG-11 media amended with 50 µgmL^{-1} tryptophan

C BG-11 – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media

C BG-11(T) – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media amended with 50 µgmL^{-1}oftryptophan

*DAT-Days After Treatment

Table 17. Effect of priming shoot root ratio and seedling vigourindex on 7th DAT*

Sl. No.	Treatments	Shoot root ratio	Seedling vigour Index-I	Seedling vigour Index-II
1	T_1-10 mL SDW (Control)	2.38±0.0.01^{a}	1340±252.33	432.35±0.94^{gh}
2	T_2-10 mL BG-11	2.36±0.04^{a}	1484±282.37	418.30±33.68^{h}
3	T_3-10 mL BG-11 (T)	2.37±0.02^{a}	1631±157.66	444.29±2.92^{g}
4	T_4-10 mL SDW+20 µL C BG-11	2.22±0.00^{b}	1392±319.60	496.42±15.86^{f}
5	T_5-10 mL SDW+40 µl C BG-11	2.19±0.02^{b}	1572±87.75	483.34±2.29 ^{f}
6	T_6-10 mL SDW+60 µL C BG-11	2.17±0.02^{b}	1655±179.65	490.13±8.60^{f}
7	T_7-10 mL SDW+80 µL C BG-11	2.00±0.09^{c}	1619±473.72	551.01±8.60^{e}
8	T_8-10 mL SDW+100 µL C BG-11	1.85±0.02^{d}	1572±426.38	582.66±13.43^{d}
9	T_9-10 mL SDW+20 µL C BG-11(T)	1.82±0.04^{de}	1710±103.32	616.26±10.66^{c}
10	T_{10}-10 mL SDW+40 µL C BG-11(T)	1.78±0.01^{ef}	1781±223.71	713.82±4.85^{a}
11	T_{11}-10 mL SDW+60 µL C BG-11(T)	1.79±0.00^{de}	1741±79.73	692.67±2.78^{b}
12	T_{12}-10 mL SDW+80 µL C BG-11(T)	1.77±0.02^{ef}	1757±67.26	686.22±1.02^{b}
13	T_{13}-10 mL SDW+100 µL C BG-11(T)	1.73±0.02^{f}	1715±108.97	680.67±2.68^{b}

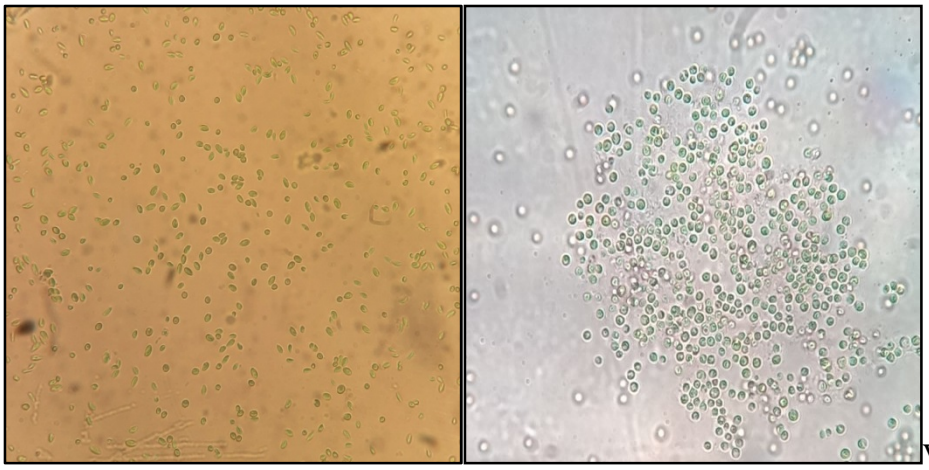
SDW – Sterile distilled water

BG-11(T) – BG-11 media amended with 50 µgmL^{-1} tryptophan

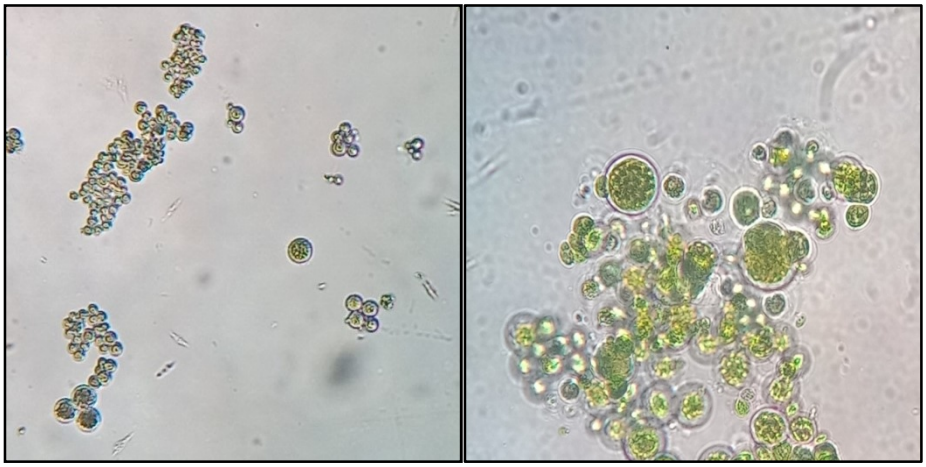
C BG-11 – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media

C BG-11(T) – Culture of *Chroococcus* sp. VLY 6 grown in BG-11 media amended with 50 µgmL^{-1} oftryptophan

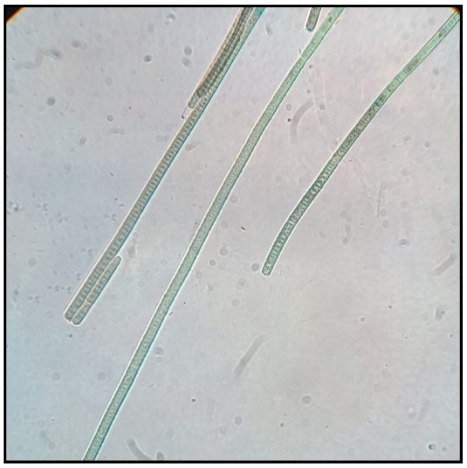
*DAT-Days After Treatment



VLY 1(*Aphanotheca* sp.) VLY 2 (*Aphanocapsa* sp.)

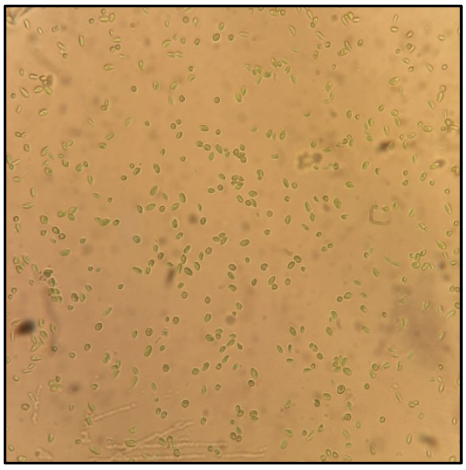


VLY 3 (*Microcystis* sp.) VLY 4 (*Dermocarpa* sp.)

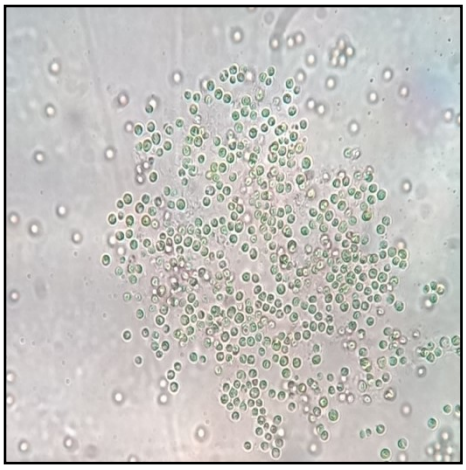


VLY 5 (*Oscillatoria* sp.)

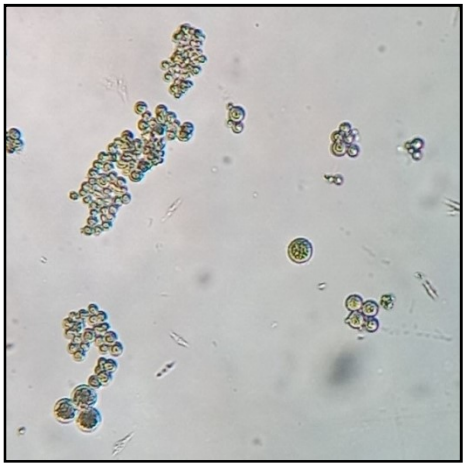
Plate 1. Microscopic view of nitrogen fixing cyanobacterial isolates (VLY 1-VLY 5)



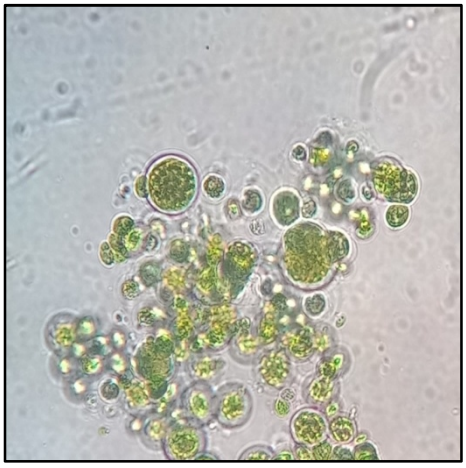
VLY 1 (*Aphanotheca* sp.)



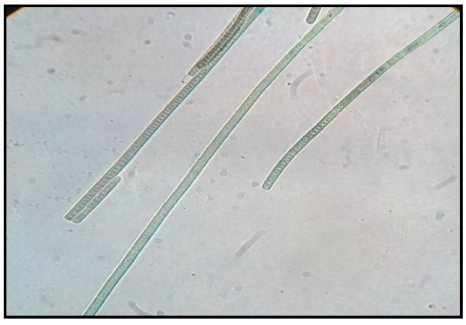
VLY 2 (*Aphanocapsa* sp.)



VLY 3 (*Microcystis* sp.)

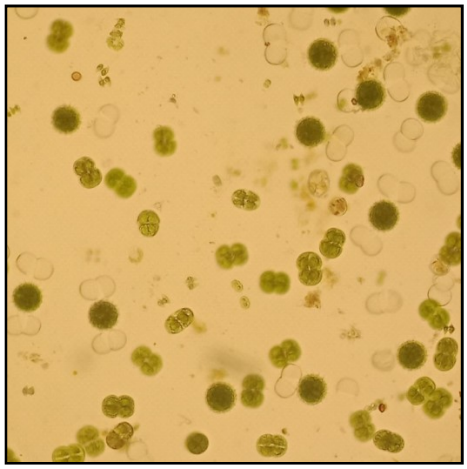


VLY 4 (*Dermocarpa* sp.)

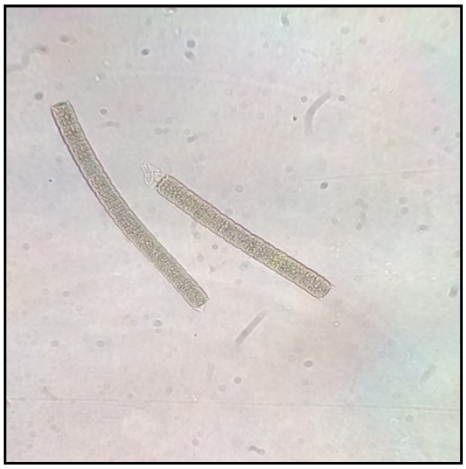


VLY 5 (*Oscillatoria* sp.)

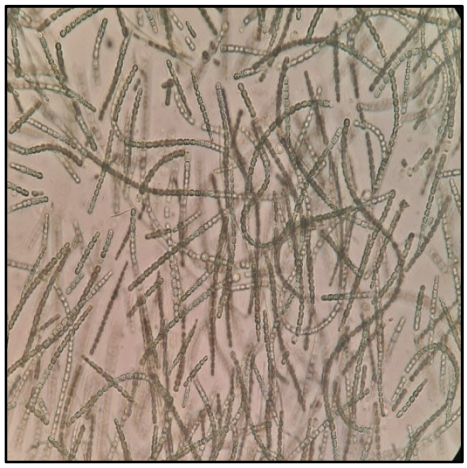
Plate 1. Microscopic view of nitrogen fixing cyanobacterial isolates (VLY 1- VLY 5)



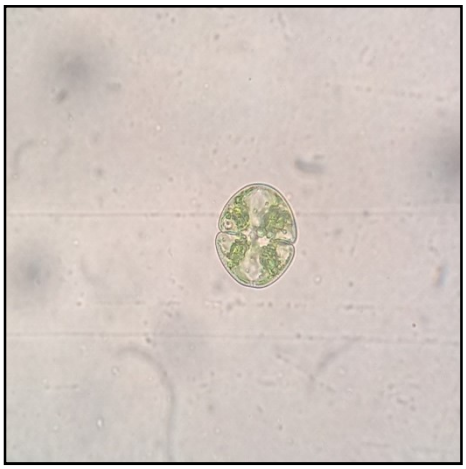
VLY 6 (*Chroococcus* sp.)



VLY 7 (*Lyngbya* sp.)



VLY 8 (*Anabaena* sp.)



VLY 9 (*Chroococcus* sp.)

VLY 10 (*Calothrix* sp.)

Plate 2. Microscopic view of nitrogen fixing cyanobacterial isolates (VLY 6-VLY 10)

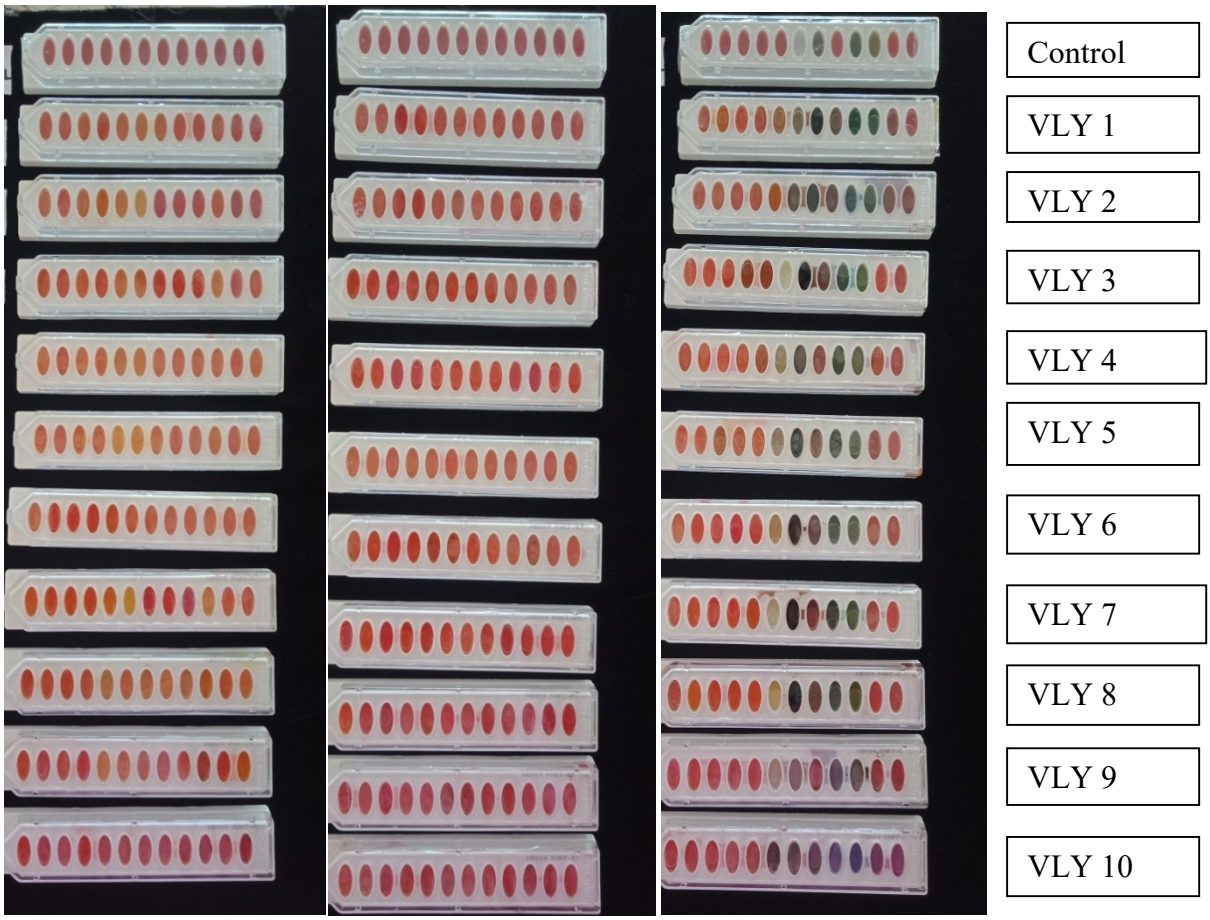


Plate 3. Biochemical tests done for cyanobacterial isolates using KB009HiCarbohydrate™ Kit (KB009A / KB009B / KB009C).

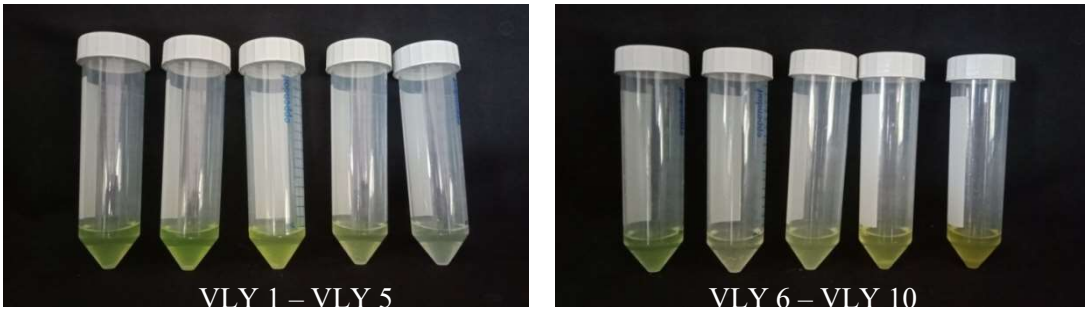


Plate 4. Chlorophyll *a* estimation of the cyanobacterial isolates

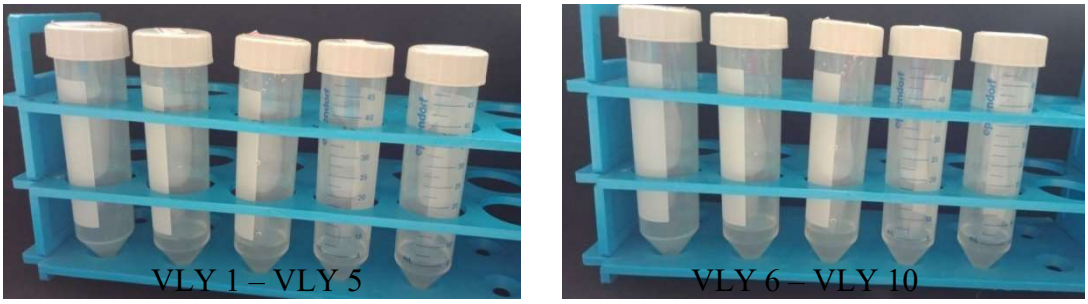


Plate 5. Carotenoid estimation of the cyanobacterial isolates

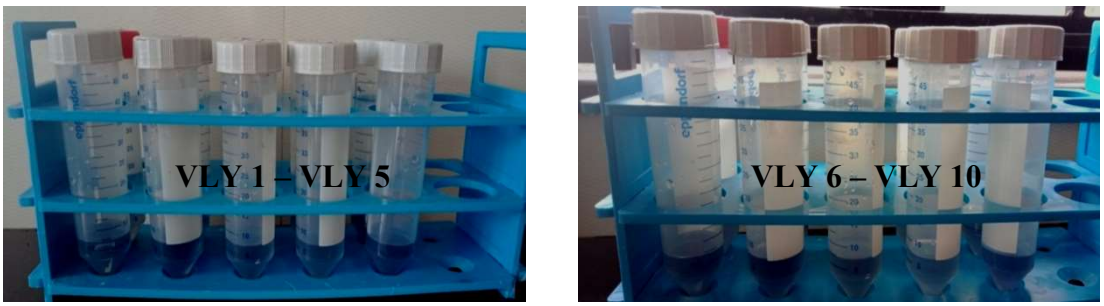
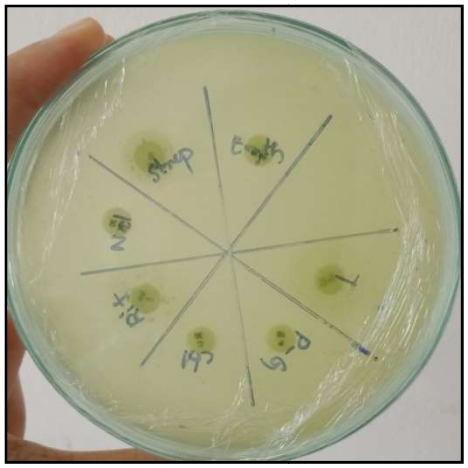
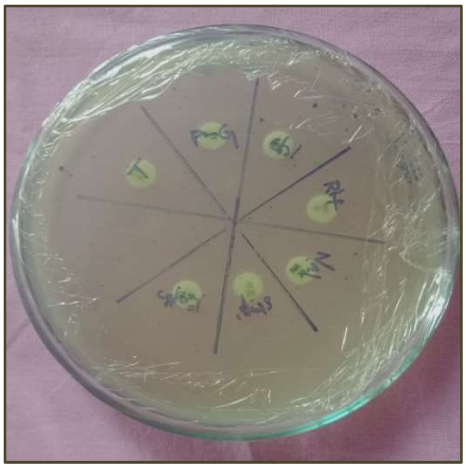


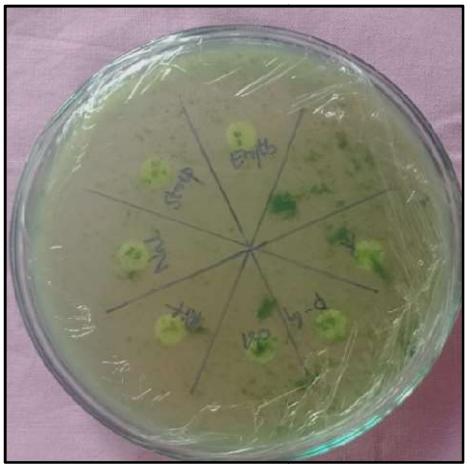
Plate 6. Total protein estimation of the cyanobacterial isolates



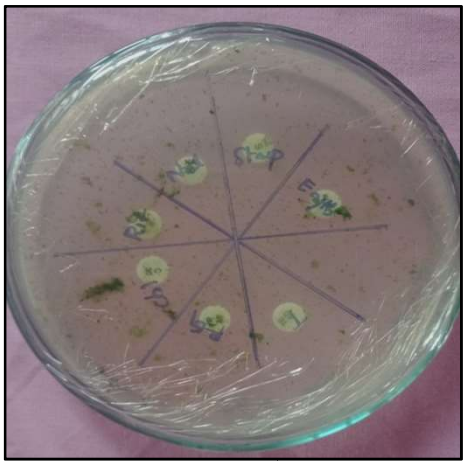
Aphanotheca sp VLY 1



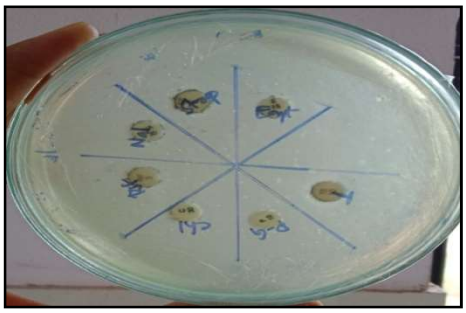
Aphanocapsa sp VLY 2



Microcystis sp VLY 3

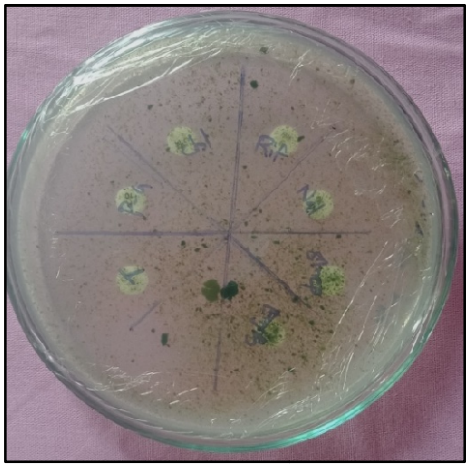


Dermocarpa sp VLY 4

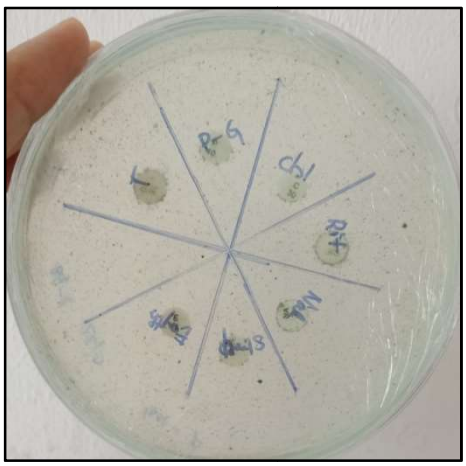


Oscillatoria sp VLY 5

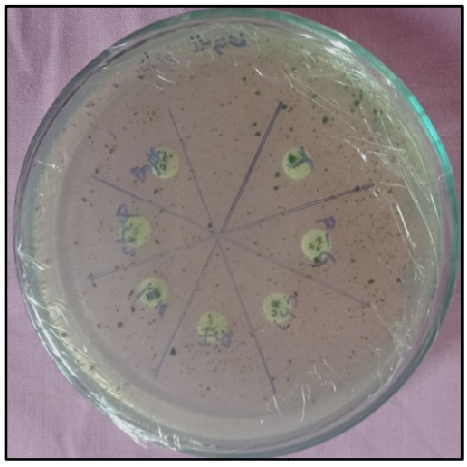
Plate 7. Antibiotic sensitivity assay of cyanobacterial isolates (VLY 1 – VLY 5)



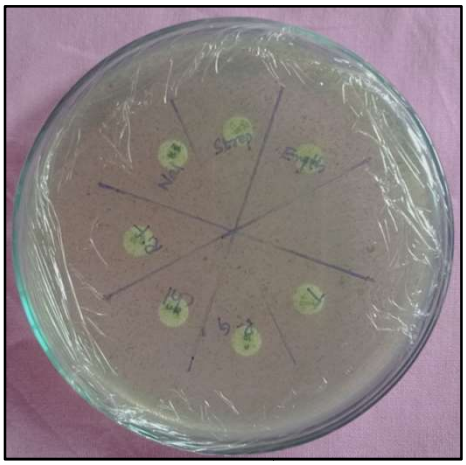
Chroococcus sp VLY 6



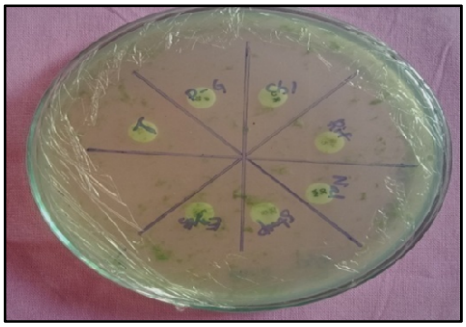
Lyngbya sp VLY 7



Anabaena sp VLY 8



Chroococcus sp VLY 9



Calothrix sp VLY 10

Plate 8. Antibiotic sensitivity assay of cyanobacterial isolates (VLY 6 – VLY 10)

Plate 9. Experimental set up of *in vitro* study for growth promotion in rice using roll towel method.

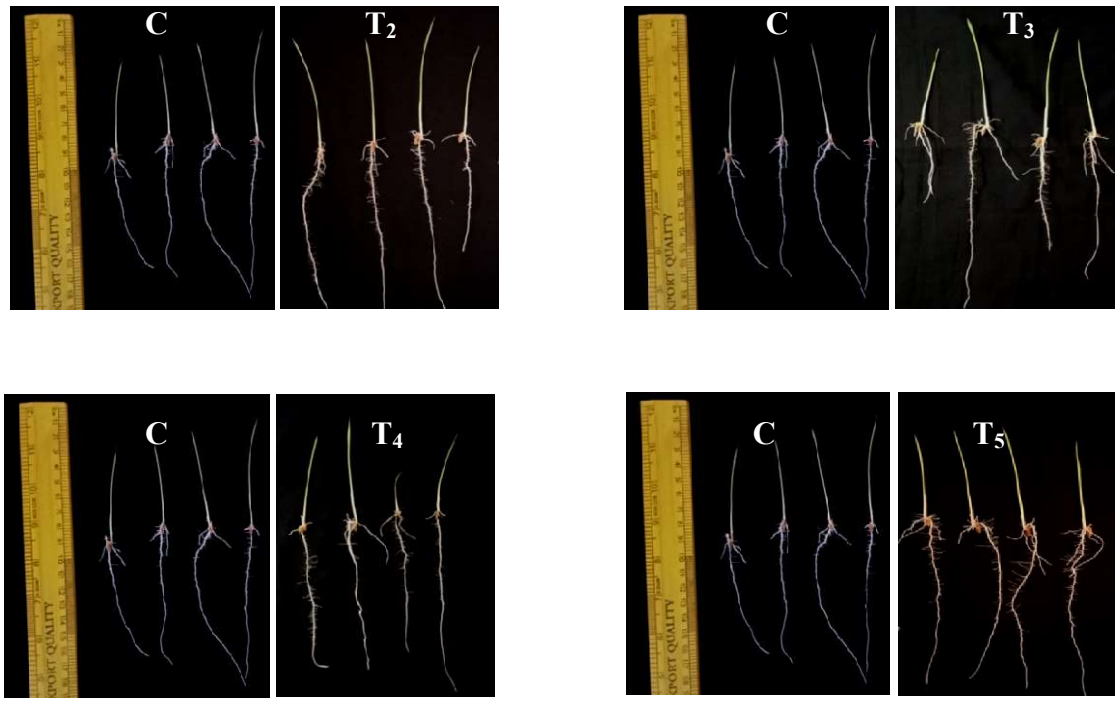


Plate 10. Effect of biopriming of rice seeds with isolate *Chroococcus* sp. VLY 6 on 7th DAT

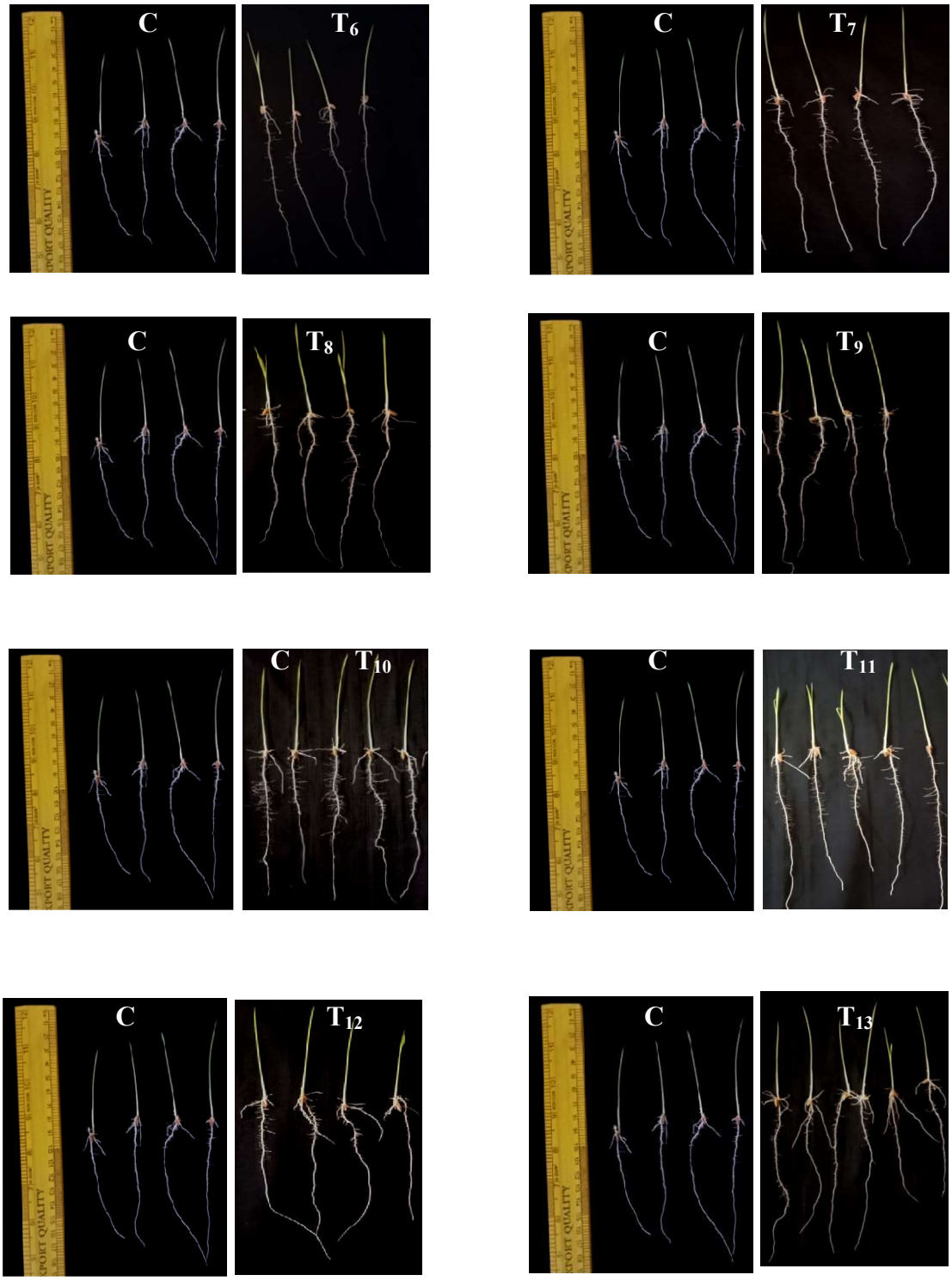
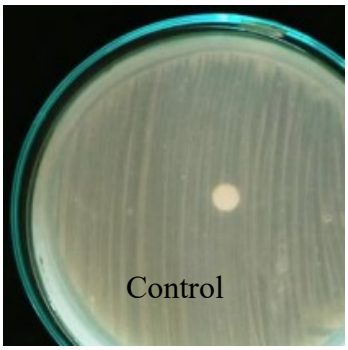
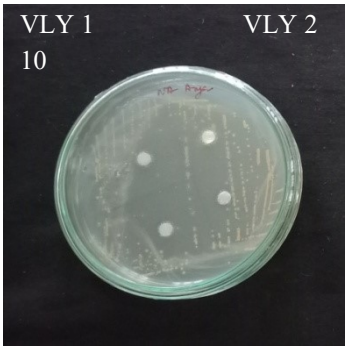
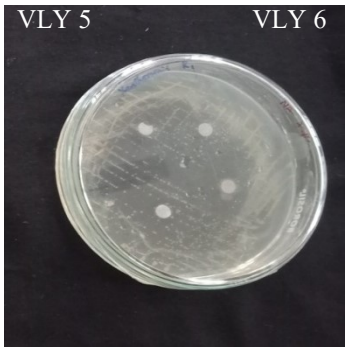


Plate 11. Effect of biopriming of rice seeds with isolate *Chroococcus* sp. VLY 6 on 7th DAY







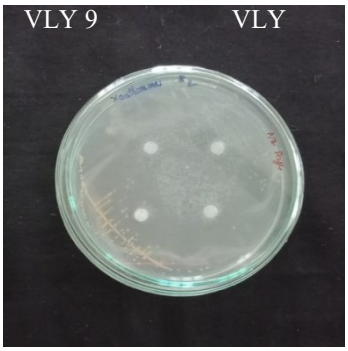


Plate 12. Antagonistic activity of cyanobacterial isolates against *Xanthomonas oryzae*.

DISCUSSION

5. DISCUSSION

Rice (*Oryza sativa* L.) is the most important food crop of India, is associated with wet, humid climate, though it is not a tropical plant.Rice is a staple food for billions of people around the world and it provides 31% of calorie requirement for this population(Singh, 2010). It is well known that the rice plant requires more nutrients to produce more yield(Singh, 2010). It is believed to be a descendent of wild grass that was likely cultivated in the foothills of Eastern Himalayas. In a country like India, more than 80% farmers belong small and marginal category and most of them cannot afford costly chemical fertilizers. Nitrogen is one of the essential nutrients required during the ripening stage of rice to maintain the green leaf as well as the photosynthetic activity. The total nitrogen content of almost 80% of rice growing soils ranged from 0.08 to 0.15% with about 7 % of the total soil N is mineralized during the cropping season and are made available to crop. The amount of N mineralized in rice soils during a cropping season ranged from 57 to 80 kg ha^{-1} (Kundu and Latha, 1995). After water, nitrogen deficit is the second limiting factor for plant growth, and fertilizers are used to compensate for this shortfall (Malik *et al*., 2001).

Nitrogen fixing cyanobacteria are a major component of the microbial community in wetland soils, particularly in paddy fields, where they operate as a natural biofertilizer and contribute to fertility. They are the largest and most widely distributed group which have the inherent capacity to fix atmospheric N_2directly into ammonium (NH_4^+) with the help of enzyme nitrogenase (Sinha *et al*.,1996). The biofertilizers like cyanobacteria can make rice cultivation more viable and reduce the hazards caused due to synthetic fertilizers. They are able to synthesize about 40% of the total organic matter synthesized annually on this planet (Singh, 2010).

Cyanobacteria are distributed abundantly in paddy fields of many countries like Austraila, Egypt, India, Indonesia, Iraq, Southeast Asia etc. Tropical and subtropical regions were found to have the highest prevalence of N_2 fixing cyanobacteria.The potential of free-living and symbiotic cyanobacteria, particularly the *Azolla-Anabaena* symbiosis, for wetland soils has been identified. Efficient nitrogen-fixing strains such as *Nostoc* sp., *Anabaena* sp., *Calothrix* sp., *Tolypothrix* sp., and *Scytonema* sp. have

been found and used for rice production in various agroecological zones (Prasad and Prasad, 2001). The cyanobacterial strains commonly used as biofertilizer inoculants in paddy can fix atmospheric nitrogen at the rate of 25 kg N ha^{-1} (Sahu*et al*.,2012).

It is documented that microbes including cyanobacteria have adequate potential in mitigating soil, water and air pollution. They contribute to soil fertility by atmospheric nitrogen fixation and act as supplementary biofertilizer in agriculture. Since most blue-green algae are cosmopolitan species which occupy diverse environmental conditions, exploration of their diversity and ecology of them from paddy zones of Kerala has global relevance towards the development of sustainable paddy farming in the world. The current study was conducted by collecting samples from wetland areas of Vellayani region and isolating N_2 fixing cyanobacteria. The ecology of blue green algae remain unexplored in the zone and this report could help in exploiting the blue green algal community of Vellayani.

Ten cyanobacterial isolates were obtained from wetland areas of Vellayani area using nitrogen free BG-11 media and they were characterized morphologically (Desikachary, 1959). Soil pH was known to have a selective effect on indigenous algal flora and their succession and abundance in soil (Prasanna and Nayak, 2007). Various reports are available on the isolation of nitrogen fixing cyanobacteria using N-free BG-11 media from wetland ecosystem like ricefields(Thilak*et al*., 2020; Reddy *et al*.,1993; Khan *et al*., 1994; De Fatima fiore*et al*., 2005; Mitsui and Cao, 1988).

In the present study, there was ten nitrogen fixing cyanobacterial isolates and were identified to belong to the genus *Aphanotheca* sp., *Aphanocapsa* sp., *Microcystis* sp., *Dermocarpa* sp., *Oscillatoria* sp., *Chrococcus* sp., *Lyngbya* sp., *Anabaena* sp., *Calothrix* sp. based on taxonomy. This included unicellular, colonial and filamentous cyanobacterial genera with their cell size/filament length ranging from 0.5 – 30 µm. Two filamentous cyanobacteria were heterocystous belonging to genus *Anabaena*and *Calothrix*. A survey of blue-green algae occurring in rice fields in Kerala has been carried out by Anand and Kumar Hopper(1987). They identified thirty taxa and ten were recorded for the first time in rice field environment. The taxa included unicellular, non-heterocystous filamentous, hetrocystous filamentous and

heterotrichous forms. First report of blue-green algal community of Kuttanad in relation to different soil-regions, seasons and crop growth stages was done by Vijayan and Ray(2015). A total of 64 species, with Oscillatoriales as the dominants (38%) was observed by them.

Lopes*et al*.(2012) reported the morphological description of forty-four cyanobacterial isolates and distinguished nineteen different morphospecies belonging to nine genera based on cell shape, cell size, presence / absence of sheaths. The isolates belonged to chrococcales, oscillatoriales and nostocales.Vanlalveni and Lalfakzuala (2021), identified ten nitrogen-fixing cyanobacteria belonging to six taxa from a wetland paddy area in Mizoram (*Fischerella*sp.*, Scytonema*sp.*, Calothrix*sp.*, Nostoc*sp.*, Anabaena*sp. and *Westiellopsis*sp.). They were characterized morphologically by using keys of Desikachary (1959).

Oscillatoria sp.VLY 5, *Dermocarpa* sp. VLY 4 showedutilization of Rhamnose. Kawaguchi and Decho, 2000 conducted a study for biochemical characterization of EPS from *Syechocystis*sp. and *Oscillatoria* sp. where they detected the presence of hexoses (glucose, galactose, mannose), pentoses (ribose, xylose, arabinose), deoxy sugars (rhamnose and fucose) and acidic sugars (glucuronate and galacturonate).

Chlorophyll, phycobiliproteins (phycocyanin, allophycocyanin, and phycoerythrin), and carotenoids are all pigments produced by cyanobacteria. These pigments are used for commercial purposes like natural coloring agent, drugs, antioxidants in cosmetics etc. In this study, the maximum chlorophyll *a* content was reported by *Aphanotheca*sp. VLY 1 (5.372±0.721 mg mL^{-1}) followed by *Aphanocapsa*sp. VLY 2 with 5.363±0.238 mg mL^{-1} (Figure 1). Jeevananthame*t al*.(2019) reported that chlorophyll *a* content of *Synechococcus elongatus* and *Phormidium fragile* with 10.39±0.36 mg mL^{-1} and 28.00±0.067 mg mL^{-1} respectively. Sundaram and Sowmya(2011) reported the chlorophyll *a* content of *Nostoc muscorum, Synechococcus* PCC 7492, *Spirulina platensis*, *Anabaena cylindrica* under organic stress condition was reported to range between 3.68 to 4.6 mg mL^{-1}, 3.24 to 4.02 mg mL^{-1}, 3.23 to 4.37 mg mL^{-1}, 3.31 to 4.37 mg mL^{-1} respectively.

Tomlinson *et al*. (2016) reported that chlorophyll content of samples taken from cyanobacterial blooms ranged from 16 – 115 mgmL^{-1}. In the case of *Anabaena sphaerica*, chlorophyll production under nitrate depletion condition was observed to be 5.5±0.027mgg^{-1}, 12.23±0.181mgg^{-1}, 9.58±0.028mgg^{-1}, 6.71±0.003mgg^{-1}, 6.12±0.0116 mgg^{-1}at a time interval of 0, 14, 21, 28, 56 days respectively. He also observed the chlorophyll production of *Phormidiumvalderianum* under nitrate depletion condition to be 6.21±0.038mgg^{-1}, 7.73±0.173mgg^{-1}, 9.37±0.055mgg^{-1}, 4.41± 0.038mgg^{-1}, 3.94±0.025 mgg^{-1}at a time interval of 0, 14, 21 and 28 and 56 days respectively. Mishra *et al*. (2013) reported chlorophyll *a* concentration among twelve hetrocystous cyanobacterial strains range between 1.97 µg mL^{-1} l to 6.56 µg mL^{-1}.

Phycobiliproteins are the pigments involved in complementary chromatic adaptation (CCA), an acclimation process employed by some cyanobacteria in response to changes in ambient light.CCA results in optimal growth and development of the cyanobacteria in adverse light conditions(Bordowitz and Montgomery, 2008). Phycobiliproteins are widely used as a natural colorant in food products like chewing gums, milk products, ice creams etc. (Jeevananthame*t al*., 2019). In this study, all the isolates were analyzed for phycobiliproteins present in them and the maximum phycobiliproteins was reported by *Aphanocapsa*sp. VLY 2, with 0.036±0.014 mg mL^{-1}, 0.037±0.005 mg mL^{-1} and 0.017±0.002 mg mL^{-1} of phycocyanin, allophycocyanin and phycoerythrin respectively (Figure 2). A similar study conducted by Nascimento *et al*. (2020)reported the concentration of phycocyanin varied from 0.231 mg mL^{-1} to 0.321 mg mL^{-1} for *Anabaena variabilis* and 0.231 mg mL^{-1} to 0.283 mg mL^{-1} for *Nostoc* sp. The concentration of allophycocyanin varied from 0.183 mg mL^{-1} to 0.347 mg mL^{-1} for *Anabaena variabilis* and 0.213 mg mL^{-1} to 0.364 mg mL^{-1} for *Nostoc* sp. The concentration of phycoerythrin ranging from 0.096 mg mL^{-1} to 0.151 mg mL^{-1} for *Anabaena variabilis* and 0.094 mg mL^{-1} to 0.153 mg mL^{-1} for *Nostoc* sp. Study conducted by Mishra *et al*.(2013) reported phycocyanin concentration among twelve heterocystous cyanobacterial strains range between 1.1 µg mL^{-1}to 9.29 µg mL^{-1}.

Another study done by Jeevananthame*t al*. (2019) recorded the concentration of phycocyanin for *Phormidium* sp., *Synechococcus elongatus*, *Lyngbyadigietii*, *Nostoc carneum* to be 0.014±0.09 mgmL^{-1}, 0.002±0.01 mg mL^{-1}, 0.017 mg mL^{-1},0.012 mg

mL^{-1} respectively. The concentration of allophycocyanin in *Lyngbyadiguetii* and *Cylindrospermummuscicola* was reported to be 0.025 mg mL^{-1} and 0.015 mg mL^{-1} respectively. Similiarily the concentration of phycoerythrin in *Oscillatoria subbrevis*, *Cylindrospermummuscicola*, *Phormidium fragile*, *Phormidium*sp. was reported to be 0.048 mg mL^{-1} and 0.015 mg mL^{-1}, 0.032 mg mL^{-1}, 0.0015 mg mL^{-1} respectively.

Prokaryotes and eukaryotes including cyanobacteria produce carotenoids, which are the most commonly occurring pigment. More than 750 structurally distinct yellow, orange, and red-colored molecules have been identified to date. (Jeevananthame*t al*., 2019). The quantitative estimation of carotenoids was carried out for all the isolates and the superior carotenoid production was reported by *Calothrix* sp. VLY 10, with 0.006 µg mL^{-1} followed by *Oscillatoria*sp. VLY 5, with 0.005 µg mL^{-1} (Figure 3.). Study carried out by Jeevananthame*t al*. (2019) reported the concentration of carotenoids among the five cyanobacterial species (*Synechococcus elongatus*, *Synechococcusaeruginosus*, *Oscillatoria subbrevis*, *Phormidium*sp.,*Phormidium fragile*) ranged between 0.93±0.25 $\mu g g^{-1}$ to7.49±0.21$\mu g g^{-1}$. Study conducted by Sundaram and Sowmya, 2011 reported the concentration of carotenoidsin*Nostoc muscorum*, *Synechococcus* PCC 7492, *Spirulina platensis*, *Anabaena cylindrica* under organic stress condition was reported to range between 2.51 mg mL^{-1} to 3.68 mg mL^{-1}, 2.44 mg mL^{-1} to 3.54 mg mL^{-1}, 3.46 mg mL^{-1} to 4.42 mg mL^{-1}, 2.26 mg mL^{-1} to 3.24 mg mL^{-1} respectively. Study conducted by Mishra *et al*.(2013) reported carotenoid concentration among twelve hetrocystous cyanobacterial strains range between 12.81 µg mL^{-1} to 55.40 µg mL^{-1}. In actively growing cyanobacteria, when complementary chromatic adaptation occur it will result in the decrease of net intracellular levels of individual phycobiliproteins. This reduction in phycobiliproteins may be due to accelerated phycobiliprotein degradation in light or deceleration in the rate of phycobiliprotein synthesis(Bennett and Bogorad, 1973).

The biochemical characterization of cyanobacteria was studied. Total proteinswas estimated as explained by Jeevananthame*t al*.(2019) and the superior protein content was reported by the isolate *Oscillatoria*sp. VLY 5 with 236±0.153 µg mL^{-1}followed by *Aphanocapsa*sp. VLY 2 (233.498±0.153µg mL^{-1}) (Figure 4.). Study was

conducted by Jeevananthame*t al*. (2019) showed the maximum total protein for *Synechococcusaeruginosus* (203.51±15.26µg mL^{-1}) followed by *Oscillatoria subbrevis* (142.81±3.72µg mL^{-1}), *Phormidium fragile* (135.04±013.17µg mL^{-1}). Study conducted by Chakraborty (2014) reported the protein content of *Anabaena sphaerica* under nitrate depletion was 146.16±0.039mgg^{-1}, 275.94±0.053 $mgg^{-1}$and 287.45±0.601 mgg^{-1}at a time interval of 14, 21 and 28 days respectively. Another study conducted by Okmen*et al*. (2011) reported the total protein content of *Anabaena*sp. GO3, *Anabaena*sp. GO5, *Synechocystis* sp. GO8, *Anabaena* sp. GO10 was 760µg mL^{-1}, 730 µg mL^{-1}, 810µg mL^{-1}, 850 µg mL^{-1}respectively. Similar study conducted by Kawaguchi and Decho (2000) reported the protein concentration in EPS of *Synechocystis*sp. and *Oscillatoria*sp.upto be 3.53±0.35 µgmg^{-1} and 6.33±1.53 µgmg^{-1} respectively. Study conducted by Mishra *et al*. (2013) reported cellular protein among twelve hetrocystous cyanobacterial strains range between 56.00 µg $mL^{-1}$to 194.08µg mL^{-1}.

The study of antibiotic susceptibility patterns and the hunt for resistant strains are both part of the investigation of cyanobacteria's antibiotic sensitivity. Some cyanobacteria harbor antibiotic resistant gene and some strains exhibit antibacterial activity *i.e*., they have developed ways to defend themselves from the toxic action of bacteria which shows that they are hot spots for evolution of antibiotic resistance.Antibiotic sensitivity of different cyanobacteria was extensively studied by many researchers (Mishra*et al.,* 2013, Prasanna *et al*., 2010, Urbach*et al*., 2008). Some cyanobacterial strains are resistant to some antibiotics such aspenicillins and ampicillin (Prasanna *et al*., 2010) while the cyanobacterium *Thermosynechococcus elongatus* carries a penicillin binding protein gene (Urbach*et al*., 2008). In this study, antibiotic sensitivity analysis of ten cyanobacterial isolates were tested against antibiotics like streptomycin (10 µg), tetracycline (10 µg), naldixic acid (30 µg), penicillin (10µg), erythromycin (15µg), rifampicin (5µg) and chloramphenicol (30µg). The isolate *Aphanotheca*sp. VLY 1, showed sensitivity against streptomycin (10 µg), rifampicin (5 µg) and tetracycline (10 µg) with a zone of inhibition of 12mm, 14mm, 8mm respectively. *Oscillatoria* sp. VLY 5 showed sensitivity with an inhibition zone of 13mm and 10 mm against tetracycline (10 µg) and rifampicin (5

µg) respectively. *Lyngbya*sp. VLY 7 and *Chroococcus*sp. VLY 9 showed sensitivity against rifampicin (5µg) with a zone of 9mm and 11 mm respectively.This was in accordance with Mishra *et al*. (2013) were all the twelve cyanobacterial strains(*Calothrix brevissima*, *Nostoc spongiaeforme*, *Nostoc muscorum*, *Westiellopsis*sp., *Cylindrospermummusicola*, *Nostoc calcicola*, *Anabaena*sp., *Anabaena doliolum*, *Scytonemabohnerii*, *Anabaena oryzae*, *Hapalosiphonwelwitschii*, *Anabaena* sp. PCC 7120)showed sensitivity against streptomycin (10 µg) and tetracycline (10 µg). Cyanobacterial genera like *Anabaena doliolum*, *Anabaena* sp., *Calothrix brevissima*, *Nostoc spongiaeforme*, *Nostoc muscorum*, *Anabaena* sp. PCC 7120, *Anabaena oryzae*, *Calothrix brevissima* showed susceptibility towards rifampicin. Kannaiyan *et al*. (2003) also reported that certain cyanobacterial strains were sensitive against streptomycin. Prasanna *et al*. (2010) reported susceptibility of *Anabaena* sp., *Nostoc* sp. and *Synechococcus* sp. to 30-5µg disc of tetracycline.

In this study, all the isolates were resistant to nalidixic acid, erythromycin, penicillin, chloramphenicol at the given concentrations. This is consistent with prior research that found *Gleocapsa*sp. and *Chrococcidiopsis*sp. to be resistant to ampicillin, carbenicillin, and penicillin (10 mg L^{-1}) (Reynold and Franche, 1986). Study conducted by Dias *et al*. (2015) assessed the antibiotic susceptibility of fresh water cyanobacteria (*Microcystis aeruginosa*, *Aphanizomenon gracile*, *Chrisosporumbergi*, *Planktothrixagaradhii*) and reported that none of the isolates were susceptible to nalidixic acid. According to our results, *Microcystis* sp. VLY 3 was resistant to tetracycline (10 µg) and nalidixic acid (30 µg) and similar results was suggested by Dias *et al*. (2015). All the nine strains of *Microcystis aeruginosa* exhibited large MIC (Minimum Inhibitory Concentration) value (>1.6 mgL^{-1}). Prabaharan*et al*. (1994) also reported that a marine cyanobacteria, *Phormidiumvalderianum* confers resistance to ampicillin upto a concentration of 2mg mL^{-1}. Padmapriya and Anand (2010) suggested the ability to produce β-lactamases by *Lyngbya spiralis*, *Anabaena variabilis* and *Calothrix membranacea* thereby resisting β-lactams. Studies have reported that a strain of *Anabaena* sp. and a strain of *Synechococcus* sp. were resistant to 30µg/disk of nalidixic acid. They also described a susceptible phenotype to the same dose of this antibiotic for another strain of

Anabaena sp. and two strain of *Nostoc* sp. (Prasanna *et al*., 2010). Multiple antibiotic resistance of cyanpbacterial isolates can be used as markers for cyanobacterial strain identification in field experiments.

The process of ammonium excretion by cyanobacteria has been explored to enhance the efficiency of nitrogen transfer from cyanobacteria to plants, particularly in rice farming. Ammonia production by cyanobacteria is a natural process, but it can be induced experimentally by using specific inhibitors or genetic modifications etc. In this study, cyanobacterial isolate *Chroococcus*sp.VLY 6 was recorded to produce highest extracellular ammonia (171.984±4.362 µmol mL^{-1}) followed by*Anabaena*sp. VLY 8 with 168.588±1.353 µmol mL^{-1} (Figure 5.). Kleiner, 1985 reported the extracellular ammonium concentration of *Nostoc muscorum* at about 260 µmol. Cyanobacterial species like *Anabaena variabilis* ED92-2 was reported to excrete ammonium at a specific rate of 56 µmol mg Chl a^{-1} h^{-1}. Another species of*Anabaena* sp. ATCC 33047 was shown to excrete ammonium at a specific range of 0 – 60 µmol mg Chl a^{-1} h^{-1} (Grizeau*et al*., 2015). Ammonia excretion by cyanobacteria is influenced by a number of factors including light intensity and duration, aeration, carbon concentration in culture medium, cell density etc. Cyanobacterial cell immobilization is also reported to improve the extracellular ammonia production. *Anabaena* sp. are reported to efficient ammonia excretors in aerobic conditions (Grizeau*et al.,* 2016).The results of the present study also indicate the same.

The nitrogen content of the cyanobacterial isolates was estimated using micro-Kjeldahl method and can be used for comparative evaluation of nitrogen fixation by the isolates. In this study, the isolate showing highest nitrogen fixing capacity was *Oscillatoria*sp. VLY 5 with 0.280 mg mL^{-1} followed by *Dermocarpa*sp. VLY 4 with 0.267 mg mL^{-1} (Figure 6.). Pereira *et al*. (2009) assessed the nitrogen fixation rates of cyanobacteria from rice fields of chile and reported that *Microcheatetenera*,*Nostoc* sp. 1, *Nostoc linckia*, *Anabaena iyengarii* var *tenuis*, *Nostoc ellipsosporum*, *Gloeotrichianatans*, *Anabaena fertilissima* and *Nostoc commune* with 0.48 µmoles, 0.55 µmoles, 0.64 µmoles, 0.66 µmoles, 0.76 µmoles, 0.85 µmoles, 1.55 µmoles and 4.54 µmoles respectively. Carpenter and Romans (1991) reported that the mean rate of nitrogen fixation for cyanobacterium *Trichodesmium*sp. is within the range of 10 -

50 mg N $m^{-2}day^{-1}$. Grimm and Petrone(1997) also reported the nitrogen fixation for periphyton and cyanobacterial mats in various aquatic ecosystems. He showed that cyanobacterial mat in salt marshy ecosystem, *Nostoc* in seasonal river ecosystem fix nitrogen at the rate of 7.1 mg N m^{-2} day^{-1} and 11N m^{-2} day^{-1} respectively.

Biopesticides and their products based on microorganisms are environmentally beneficial, and their application has been investigated for a long time. Kulik (1995) reported that the nitrogen fixing cyanobacteria are also considered potential biocontrol agents against plant pathogens. Cyanobacterial species like *Lyngbya majuscule, Microcystis aeruginosa and Plectonemaboryanum* were tested for the antibacterial properties towards *Xanthomonas vesicatoria*causing bacterial leaf spot in chilli. All these strains were reported to have antibacterial properties against *Xanthomonas vesicatoria* (Pandey and Pandey, 2002). This was in agreement with the present study were antibacterial property of *Microcystis*sp. VLY 3 and *Aphanotheca*sp. VLY 1 against *Xanthomonas oryzae* was observed. Singh *et al*. (2016) also reported that bioactive compounds from cyanobacteria exhibits fungicidal and also antibacterial properties. Tjipanazoles, produced by *Tolypothrixtjipianensis* exhibits fungicidal efficacy against *Aspergillus flavus* which is a wellknowngroundnut and stored grains pathogen.

Khairy and El-Kassas (2010) reported that ethyl acetate extract of *Anabaena flos aquae*, *Anabaena variabilis* and *Oscillatoria angustissima* inhibited eight gram positive and gram-negative bacteria (*Bacillus subtilis*, *Bacillus cereus*, *Saccharomyces aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Vibrio fluvialis*). They also inhibited filamentous fungi (*Aspergillus niger* and *Aspergillus flavus*) and minimal antagonistic activity was observed against tested yeasts (*Candida albicans* and *Candida tropicalis*).De Caire*et al*. (1990) reported the antagonistic activity of *Nostoc muscorum* against *Rhizoctonia solani*. Tantawy (2011) reported the biocontrol potential of *Nostoc muscorum* and *Spirullina platensis* against soil pathogenic fungus (*Fusarium oxysporum* and *Rhizoctonia solani*). Rizk (2006) reported the antagonistic activity of *Phormidium fragile*, *Nostoc muscorum*, *N. calcicola* and *Anabaena flos-aquae* against sugarbeet pathogens like *Fusarium verticilloides*, *Rhizoctonia solani* and *Sclerotium rolfsii*.

The role of cyanobacteria in production of plant growth promoter like auxin is widespread. Among auxin,IAA is acknowledged as a critical component that has a direct impact on plant growth. IAA formation *via* indole-3-pyruvic acid and indole-3-acetic aldehyde is found in majority of organisms. The addition tryptophan has influenced directly for accumulation and release of IAA (Prasanna *et al*., 2010). Kulik (1995) and Adam (1999) reportedthat the nitrogenase and nitrate reductase activities of the nitrogen-fixing cyanobacterium could be responsible for the growth enhancement in response to the application of *Nostoc muscorum*. In the present study, the superior IAA production (Figure 7.) was reported by *Chroococcus*sp. VLY 6(7.106±0.231 µg mL^{-1}) which was followed by *Calothrix*sp. VLY 10 (6.874±0.015 µg mL^{-1}) and the lowest IAA production was reported by *Lyngbya*sp. VLY 7 (1.477±0.012 µg mL^{-1}) and the result was similar to the findings of Prasanna *et al*. (2008). He reported that cyanobacteria strain no. RP 52, RP 53 and RP 70 belonging to genus *Anabaena* collected from Indian Fungal Type Culture Collection, Division of Plant Pathology, IARI have IAA production of 7.230 µg mL^{-1}, 7.980 µg mL^{-1} and 7.840 µg mL^{-1}. It was also reported that strain no. RP 1, RP 6, RP 7, RP 8, RP 16, RP 21 and RP 26 was shown to have IAA production of 1.920 µg mL^{-1}, 1.056 µg mL^{-1}, 1.573 µg mL^{-1}, 1.769 µg mL^{-1}, 1.948 µg mL^{-1}, 1.364 µg mL^{-1}, 1.900 µg mL^{-1}. Similar results was observed by Prasanna*et al*.(2009) in the cyanobacterial strains isolated from different locations like Aduthurai, Hazaribagh, Lucknow, Faizabad and Ghagraghat. He reported that IAA production was highest in strain number 66 and 47 within a range of 1 - 1.2µg mL^{-1}, strain number 53 and 13 within a range of 0.7 – 1 µg mL^{-1}. Sood et al.(2011) also reported that the culture filtrates of *Nostoc* PCC 9229 was shown to have within the range of 12 – 14 µg mL^{-1}.

Weighted average ranking of the ten cyanobacterial isolates was done to select the best cyanobacterial isolate.*Chroococcus* sp. VLY 6 was selected and plant growth promotion activity was tested by roll towel assay using rice seeds bioprimed with the isolate.

Seed germination and seedling growth are the critical stages of plant development and there are several ways in which cyanobateria can influence seed germination (Munoz-Rojas *et al*., 2018).Cyanobacteria play an important role in rice growth and soil fertility by delivering photosynthetically fixed carbon, nitrogen, phytohormones, and polysaccharides. Certain cyanobacterial species are capable of invading roots and providing growth promontory and defense responses in plants, and they have symbiotic/ associative relationship with different members of the plant (Priya *et al*., 2015). Apart from nitrogen fixation, cyanobacteria are widespread assemblages of photosynthesis prokaryotes that undertake oxygenic photosynthesis and excrete a vast number of organic and inorganic chemicals in the medium, which is acknowledged as a crucial element in plant growth promotion (Jaiswan*et al*., 2018). The current study revealed that the maximum germination (Figure 8.) was recorded inseedsbioprimed with 40 µL of cyanobacterial culture, *Chroococcus*sp. VLY 6 grown in medium amended with tryptophan (96%) and minimum in conrol (80%). Similar study done by Jaiswal *et al*.(2018) reported that cell free cyanobacterial cultures of *Anabaena variabilis* (CCC441), *Nostoc muscorum* (CCC442), *Tolypothrix tenuis* (CCC443), *Aulosirafertilissima* (CCC444) and *Westelliopsis prolific* (CCC128) showed germination percentage ranging from 93 to 99% as compared to control with 92%. The potential of cyanobacteria (*Anabaena laxa* and *Calothrix elenkinii*) as plant growth promoting agent was evaluated with spice seeds- coriander, cumin and fennel. The percentage germination (90.16%) in cumin was highest in seeds treated with *Anabaena laxa*(Kumar *et al*., 2013)

Besides seed germination, significant difference was observed between treatments in plant parameters like shoot length, root length, dry weight and shoot root ratio. Maximum shoot length (6.83cm) and root length (10.83 cm) was observed in seeds bioprimed with 40 µL of cyanobacterial culture, *Chroococcus*sp.VLY 6 grown in medium amended with tryptophan (Figure 9). Thiscan be linked to synthesis of hormones like IAA and IBA by cyanobacteria.Jaiswan*et al*. (2018) reported that the shoot length and root length of inoculated seedlings after 4 days of inoculation had significant difference as compared to the control with shoot length ranging from 4.1 - 5.25cm and root length ranging between 5.78 - 7.15 cm. Kumar *et al*.(2013) reported

that amendment of *Calothrix elenkinii* with fennel significantly enhanced the shoot length (14.00cm) and root length(14.83cm).

Similar study conducted by Ranjan *et al*.(2016) reported that the inoculation of *Calothrix elenkinii* (RPC1) strain led to higher total plant length (25.1cm) relative to uninoculated plants (30.6cm). Cyanobacterial inoculation resulted in a considerable increase in rice plant dry weight. The dry plant biomass of inoculated seedling was 31.6mg as compared to uninoculated with 23.2mg on 15 days after transplanting. The present study reported the highest shoot and root dry weight in seeds bioprimed with 40 µL of cyanobacterial culture,*Chroococcus*sp. VLY 6 grown in medium amended with tryptophan (Figure 10.).

Seedling vigour index was calculated using seed germination percentage, shoot length and root length. Seedling vigour index of the treated seedlings was higher as comared to control. The highest seedling vigour index was observed inseedsbioprimed with 40 µL of cyanobacterial culture,*Chroococcus*sp. VLY 6 grown in medium amended with tryptophan (1781) and lowest in control (sterile distilled water) with 1340. Vigour index of spice seeds inoculated with two cyanobacterial strains (*Calothrix elenkinii* and *Anabaena laxa*) was evaluated by Kumar *et al*., 2013 and reported the highest vigour index in coriander (578.33) and cumin (803.61) in seeds inoculated with *Calothrix elenkinii*. Highest vigour index in fennel was recorded in seeds inoculated with *Anabaena laxa* (1201.39).

Shoot root ratio was obtained from the ratio between shoot dry weight and root dry weight, the higher shoot root ratio observed in seeds bioprimed with 40 µL of cyanobacterial culture, *Chroococcus*sp. VLY 6 grown in medium amended with tryptophan (2.7). A study explained by Chittapun *et al*. (2017) reported the comparison of two cyanobacterial strains (*Nostoc carneum* TUBT04, *Nostoc commune* TUBT05) demonstrated that the supplement with *N. commune* showed greater potential in stimulating root length and dry weight than *N. carneum*. It was also reported that the treatments applied with the combination of cyanobacteria resulted in better rice production in terms of quality and quantity. The observed characteristics of rice seedling under *in vitro* plant growth promotion study increased

due to cyanobacterial inoculation. This may be due to increased IAA production which caused for increased plant growth.

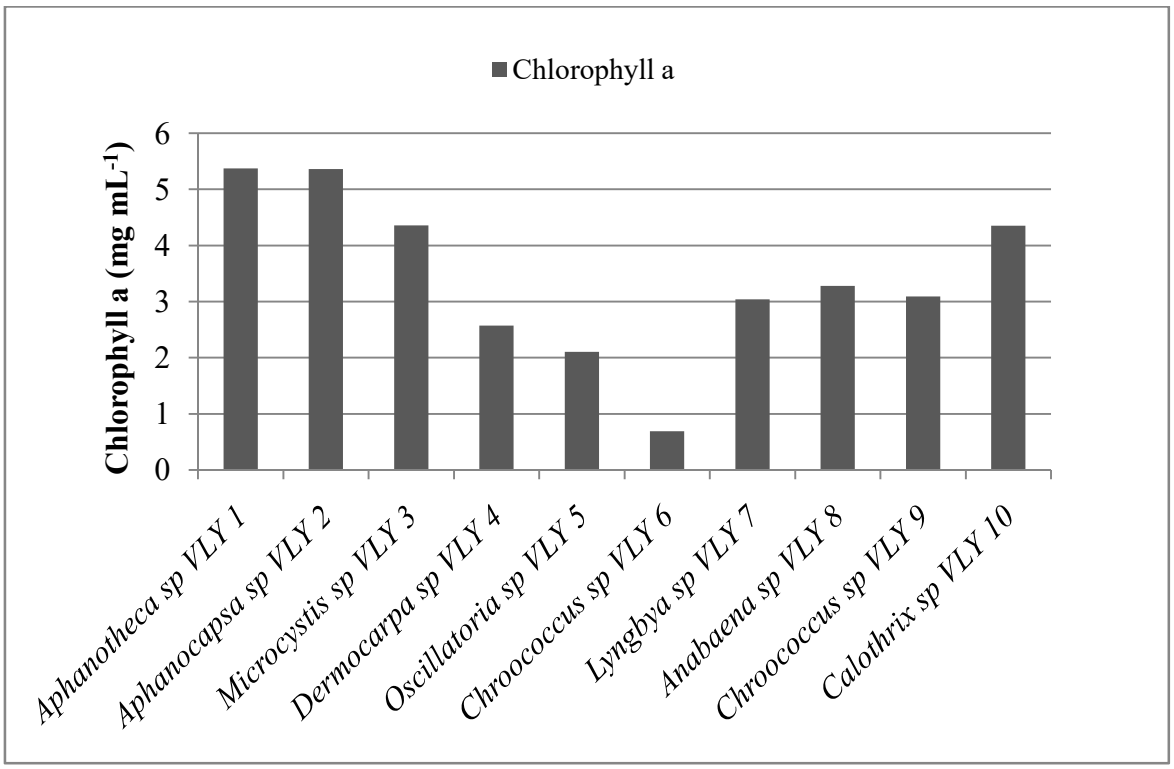


Figure 1. Chlorophyll a content in cyanobacterial isolates from wetland ecosystem of Vellayani

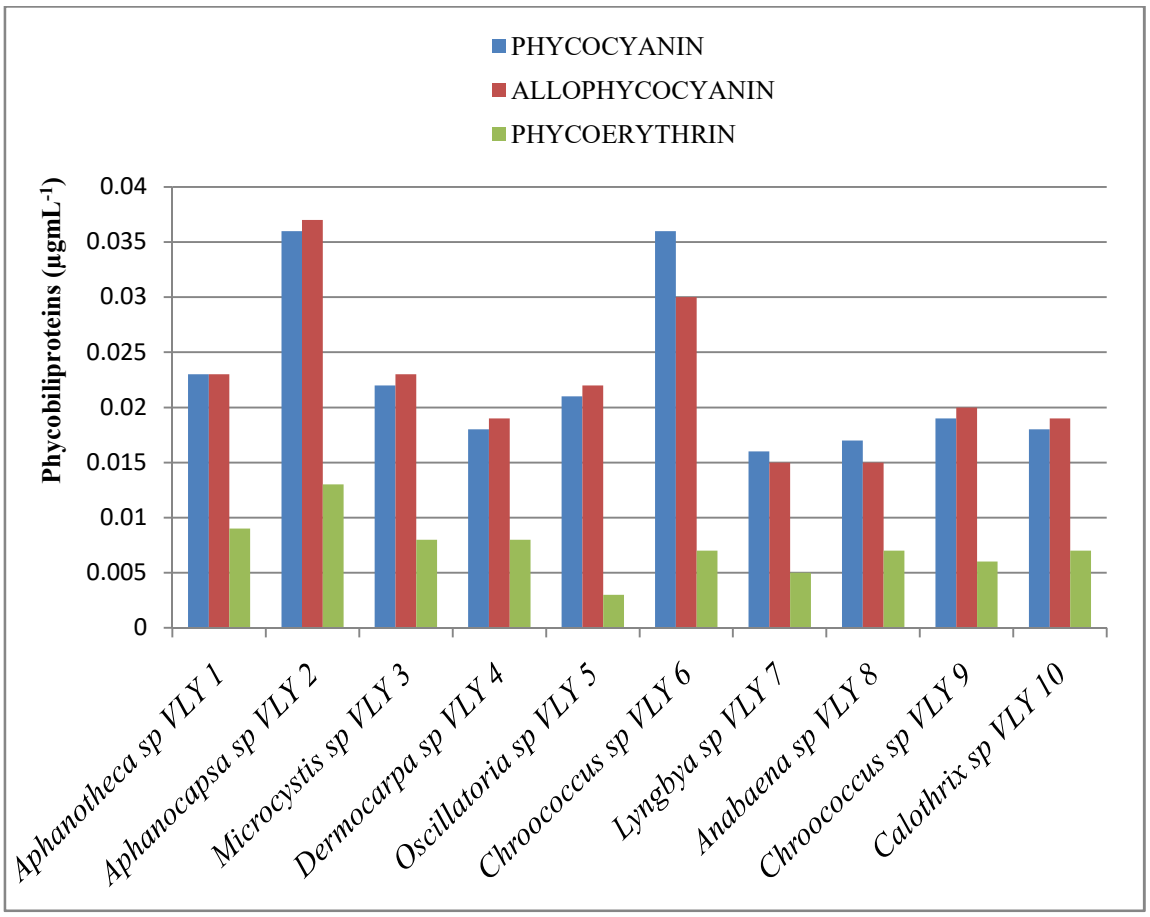


Figure 2. Phycobiliproteins content in cyanobacterial isolates from wetland ecosystem of Vellayani

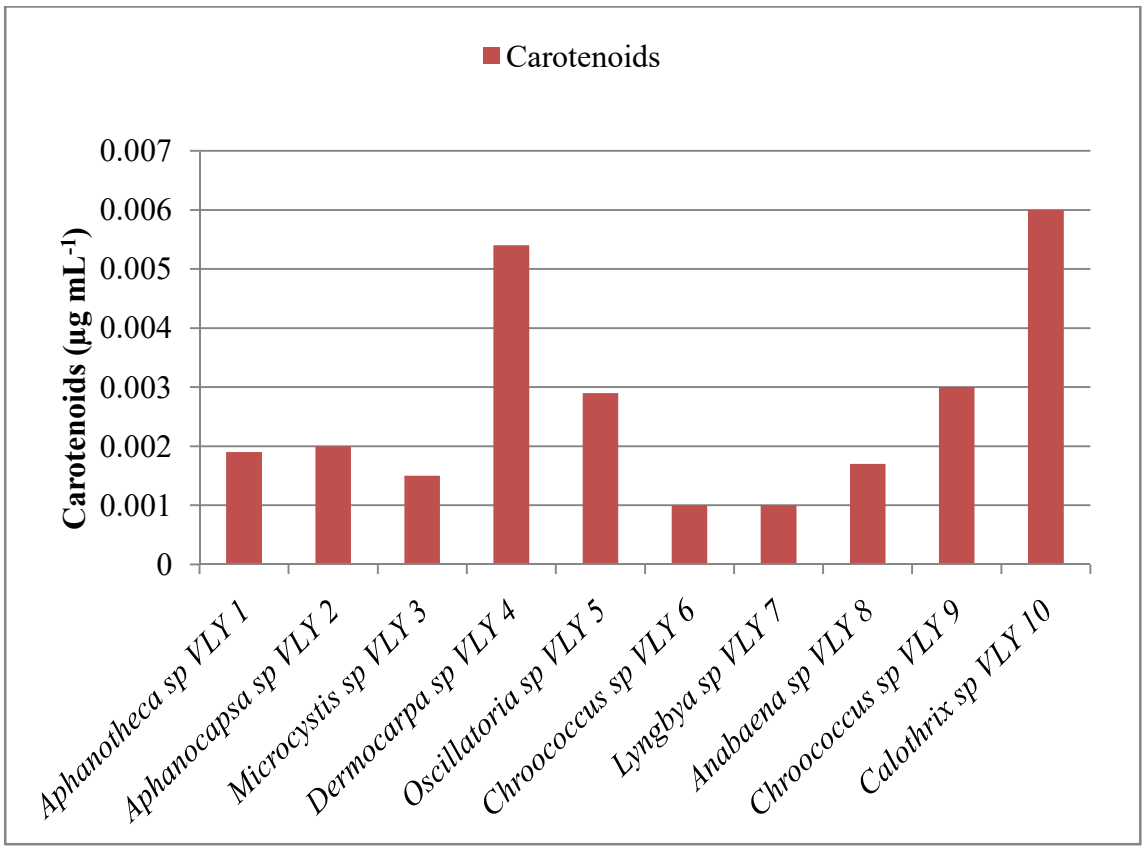


Figure 3. Carotenoids content in cyanobacterial isolates from wetland ecosystem of Vellayani

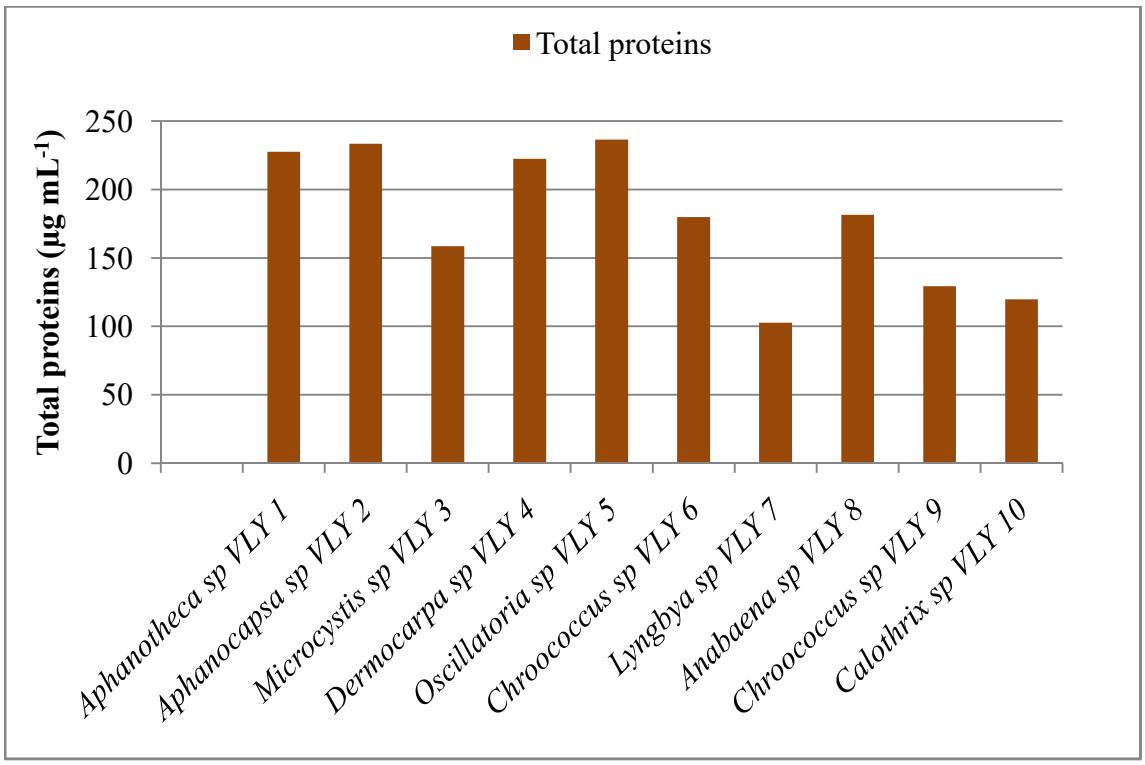


Figure 4. Total proteins content in cyanobacterial isolates from wetland ecosystem of Vellayani

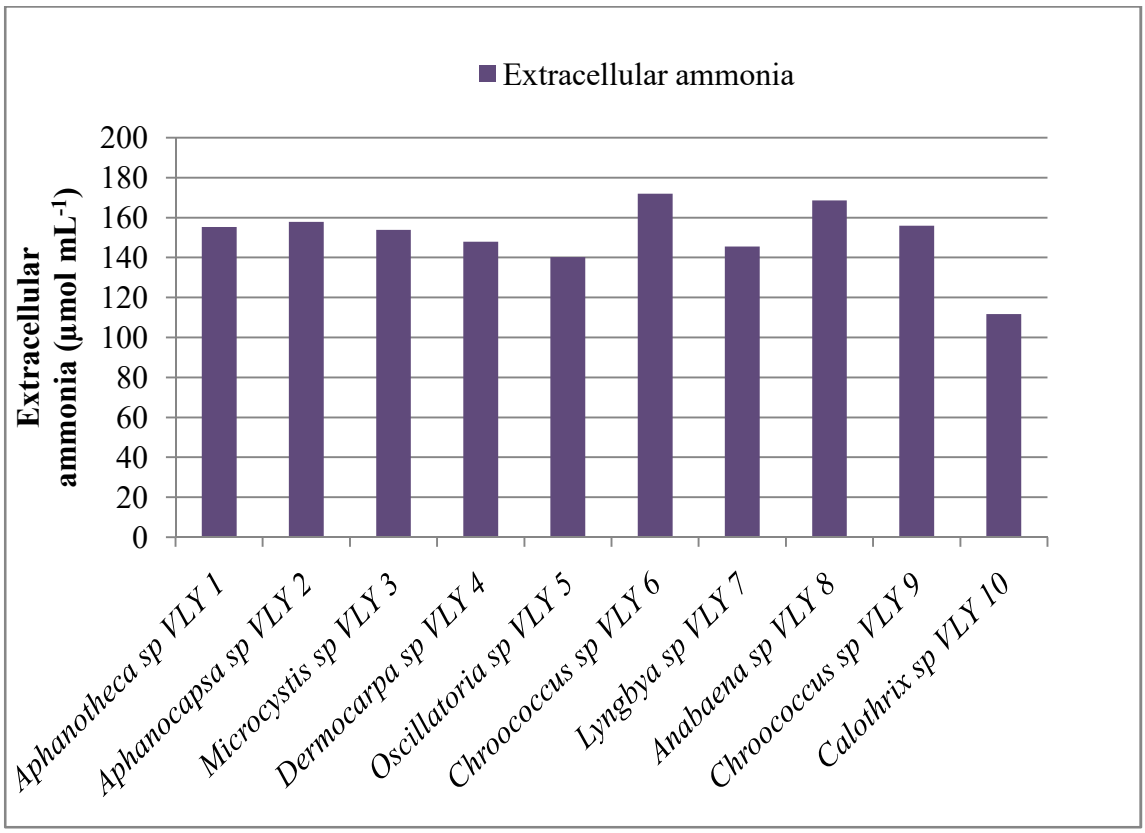


Figure 5.Extracellular ammonia production in cyanobacterial isolates from wetland ecosystem of Vellayani

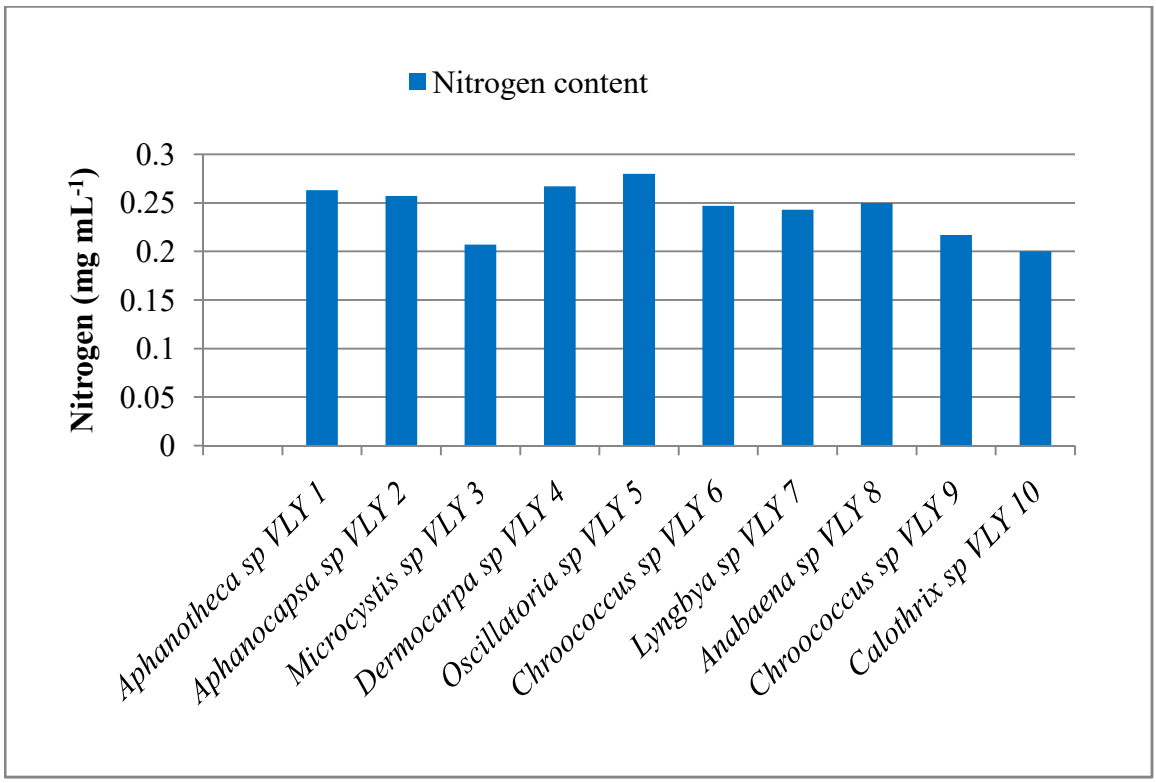


Figure 6. Nitrogen content in cyanobacterial isolates from wetland ecosystem of Vellayani

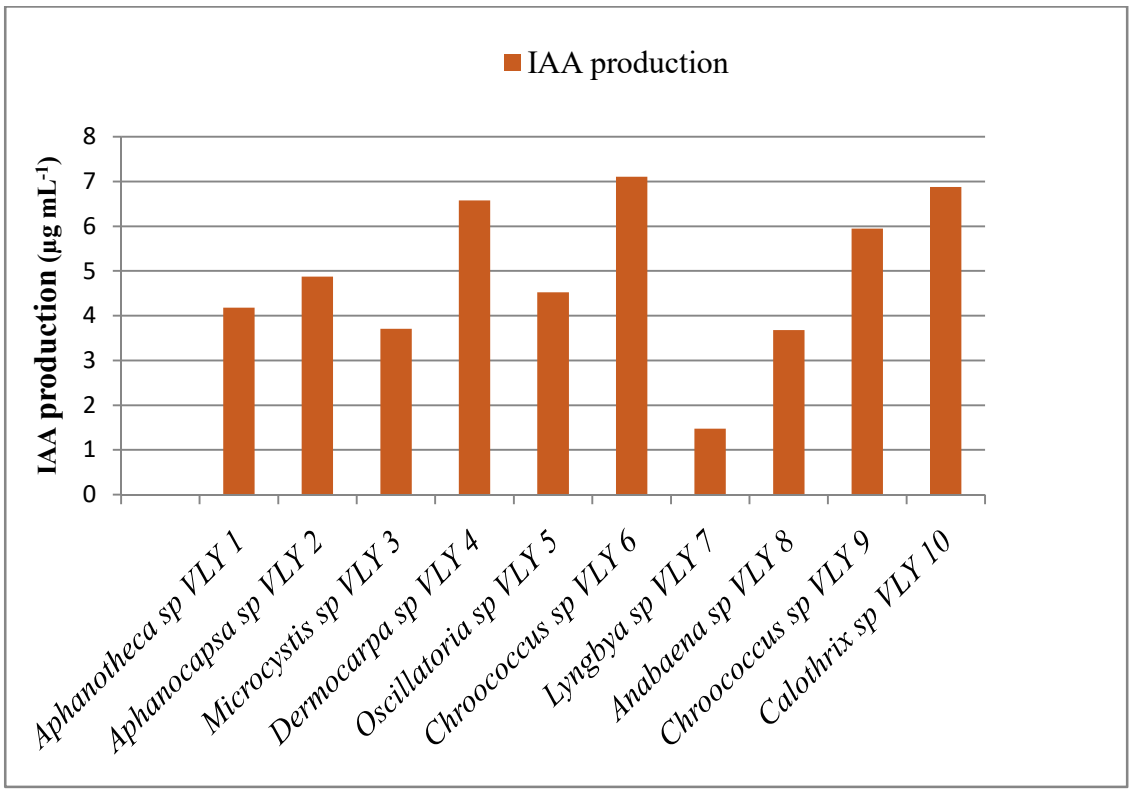


Figure 7. IAA production in cyanobacterial isolates from wetland ecosystem of Vellayani

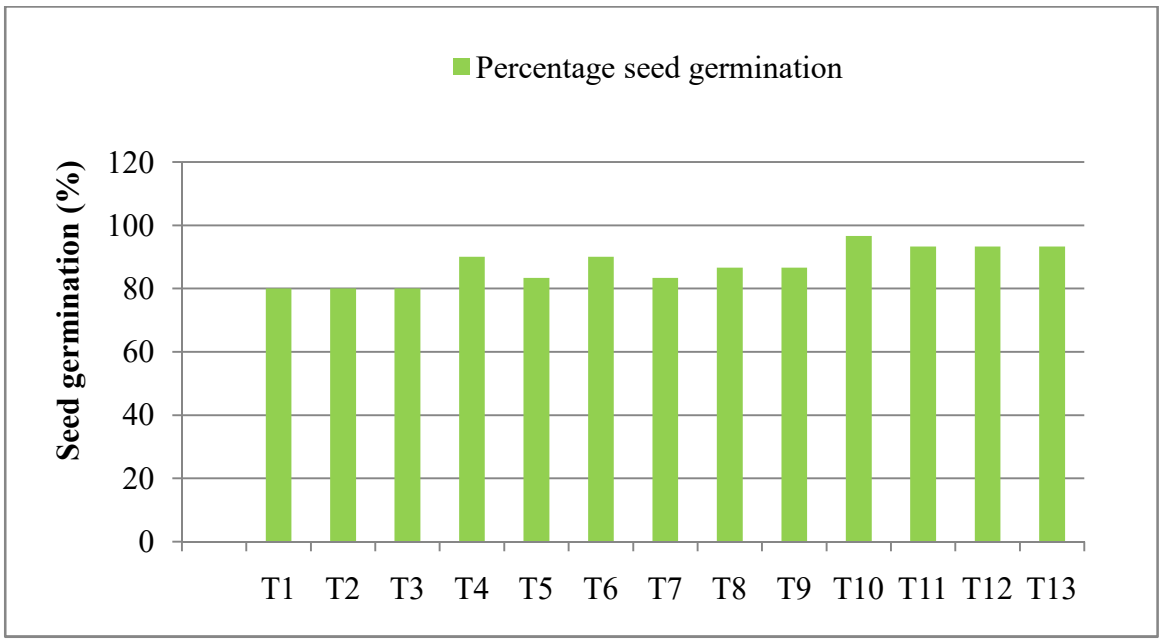


Figure 8.Effect of isolate *Chroococcus* sp. VLY 6 on germination percentage of bio-primed rice seeds

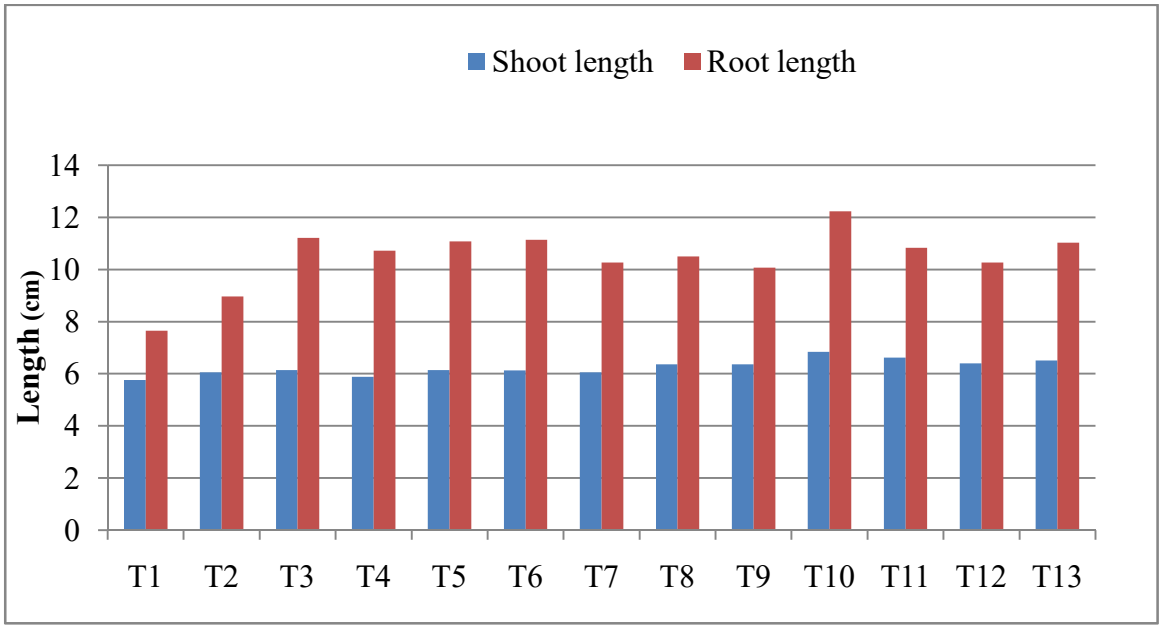


Figure 9.Effect of isolate *Chroococcus*sp. VLY 6 on shoot length and root length of bio-primed rice seeds

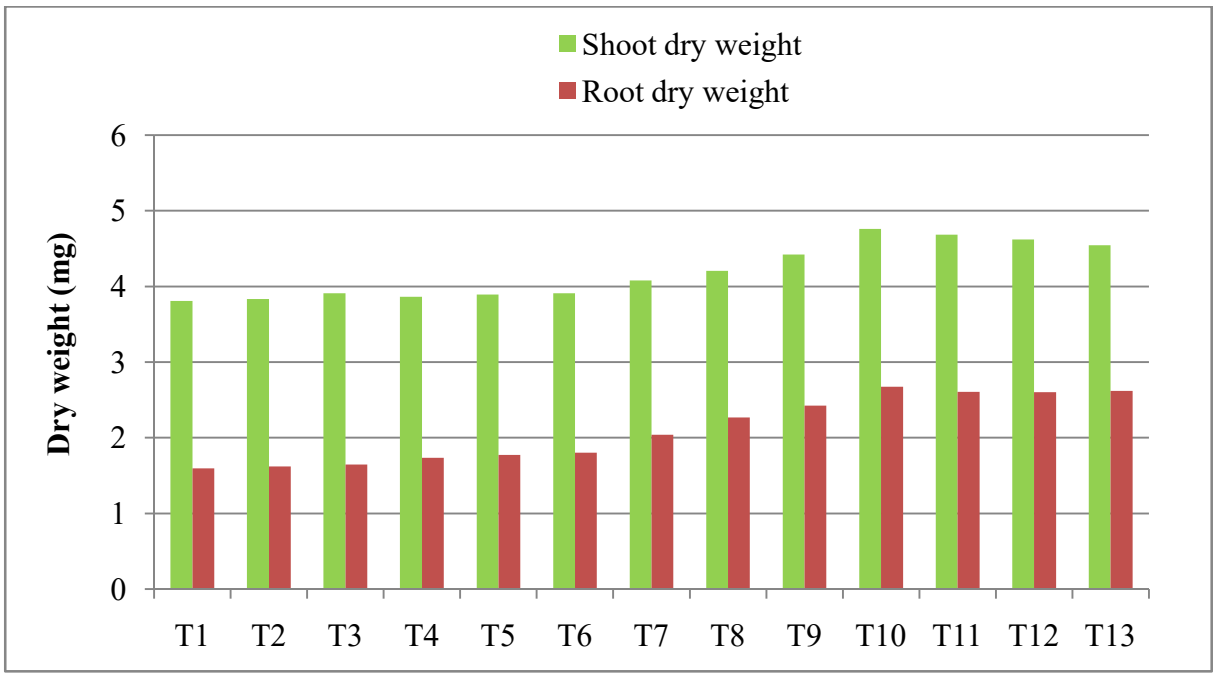


Figure 10.Effect of isolate *Chroococcus*sp. VLY 6 on shoot dry weight and root dry weight of bio-primed rice seeds

SUMMARY

6. SUMMARY

Cyanobacteria are photosynthetic Gram-negative prokaryotes andoccur naturally or in symbiotic relationships with a wide range of lower and higher plants, as well as in microbial mats. They consist of more than 2000 species ofunicellular, colonial, filamentous, and branching forms of cyanobacteria which are included in 150 genera.They are divided into five subgroups *viz.* Chroococcales, Pleurocapsales, Oscillatoriales, Nostocales, and Stigonematales.These microorganisms have a wide range of physiological qualities and tolerance to environmental challenges, as evidenced by their widespread distribution.

Cyanobacteria's economic importance in agriculture stems from its agronomic value as biofertilizers due to their nitrogen-fixing capabilities. They also play important role in production of plant growth promoters, soil conditioners, phosphorous uptake, green manuring, degradation of agrochemicals, bio control agents etc. From the 1960s onwards, researchers have been researching cyanobacterial biofertilizers for paddy and efforts are continuously being made to exploit this property over a large area. Therefore, isolating and characterizing the efficient nitrogen fixing strains of cyanobacteria from Vellayani area have significance. The present study entitled “Development of inoculant cultures of nitrogen fixing cyanobacteria from wetland ecosystem of Vellayani”, was conducted in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram.

The primary goal of the current study was to isolate and characterize cyanobacterial strains with nitrogen fixing capacity from wetland ecosystem of Vellayaniand to select an efficient isolate based on plant growth promotion activitiesandtestfor*in vitro* plant growth promotion in rice using roll towel assay. The most important findings of the study aresummarized below.

Ten isolates of cyanobacteria capable of fixing atmospheric nitrogen wasisolated from rice fields andwetland ecosystem nearVellayanilakeusingN free BG-11 liquid medium. The isolates were tentatively identified based on morphology. The isolates were tested for their pigment concentration like chlorophyll *a*, phycobiliproteins,

carotenoids. The chlorophyll *a* production by the ten isolates showed a wide variation ranging from 0.68 mg mL^{-1} to 5.37 mg mL^{-1}. The isolate *Aphanotheca*sp. VLY 1 showed maximum chlorophyll *a* production of 5.37 mg mL^{-1}. Estimation of phycobiliproteins like phycocyanin, allophycocyanin and phycoerythrin was calculated for all the isolates. Maximum phycocyanin (0.03µgmL^{-1}), allophycocyanin (0.03 µgmL^{-1}), phycocyanin (0.01 µgmL^{-1}).

Carotenoid concentration of all the isolates were estimated and ranged between 0.001µgmL^{-1} to 0.006 µgmL^{-1}. Maximum concentration was recorded by *Calothrix*sp. VLY 10 with 0.006 µgmL^{-1}. Total proteins of all the isolates were estimated and it ranged between 119.75 µg mL^{-1} to 236.32 µg mL^{-1}. Maximum total protein was recorded in *Oscillatoria*sp. VLY 5.Antibioticsensitivity of the ten isolates were assessed using seven antibiotics with different mode of action. Among the isolates, *Aphanotheca*sp. VLY 1 showed sensitivity against rifampicin, streptomycin, tetracycline with a zone of 14mm, 12mm, 8mm respectively. *Oscillatoria*sp. VLY 5 was sensitive against rifampicin(5 µg) and tetracycline(10 µg) with a zone of 10mm and 13mm. *Lyngbya* sp. VLY 7 and *Chroococcus*sp. VLY 9 was sensitive against rifampicin(5 µg) with a zone of 9mm and 11mm.

The extracellular ammonia of the cyanobacterial isolates ranged between 111.74 µmol mL^{-1} to 171.98 µmol mL^{-1}. The nitrogen content of the isolates ranged between 0.280 mg mL^{-1} to 0.200 mg mL^{-1}. *Oscillatoria*sp. VLY 5 recorded maximum nitrogen content of 0.280 mg mL^{-1}. The antagonistic effect of cyanobacterial isolates wastestedagainst bacterial pathogen*Xanthomonasoryzae* and fungal pathogens *Rhizoctonia solani*, *Pythiumultimum*, *Phytophthoracapsisci*, *Fusariumoxysporum*f.sp. *solani.*The Indole Acetic Acid(IAA) production by all the ten isolates showed a wide variation ranging 1.48 µg mL^{-1} to 7.11 µg mL^{-1}. The maximum IAA production was recorded by *Chroococcus*sp. VLY 6.

The isolate *Chroococcus*sp. VLY 6 was selected as the superior nitrogenfixingcyanobacteriafromthe ten isolates based on the weighted average ranking of plant growth promoting characters. Effects of biopriming with *Chroococcus*sp. VLY 6 were assessed by *in vitro* plant growth promotion study in rice

seeds using roll towel assay. The study showed a significant increase in germination percentage, days taken for germination, plant height, plant biomass, seedling vigour index and root shoot ratio over control.

Maximum germination percentage was recordedby seeds treated with 40 µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan with 96.6%. Under the roll towel assay the treatment, seeds treated with 40 µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan recorded maximum shoot length with 3.31±0.11 cm and root length with 7.76±0.70 cm. Maximum dry weight of the shoot was observedfor seeds treated with 40 µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophanwith 4.76 mg and root dry weight of 2.67 mg. Shoot root ratio was obtained by the ratio between shoot dry weight and dry root mass, the higher shoot root ratio was reported by seeds treated with sterile distilled water (control) with 2.38. Highest seedling vigour index-I and seedling vigour index-II was reported by seeds treated with 40 µL of cyanobacterial culture grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan with 1781 and 713.82.

In the present study, the seeds treated with 40 µL of cyanobacterial culture of *Chroococcus* sp. VLY 6 grown in BG-11 media amended with 5 mg mL^{-1} of tryptophan showed increased seedlinggrowth in roll towel assay, which was due to the production of IAA. The same isolate showed highest extracellular ammonia secretion. Among the isolates, *Oscillatoria* sp. VLY 5 reported highest nitrogen content. *Aphanotheca* sp. VLY 1 and *Microcystis* sp. VLY 3 exhibited antibacterial activity and can be exploited as a biocontrol agent.

Further studies on the cyanobacterial isolates may be done to assess the response of these isolates under *in vivo* conditions in plant growth promotion and disease suppression activity.

REFERENCES

Abdul-Baki, A. A. and Anderson, J. D. 1973. Vigor determination in soybean seed by multiple criteria. Crop Sci. 13: 630-633Anand, N. and Kumar Hopper, R.S., 1987. Blue-green algae from rice fields in Kerala State, India. *Hydrobiologia*. 144(3): 223-232.

Adams, D.G. and Duggan, P.S., 1999. Tansley Review No. 107. Heterocyst and akinete differentiation in cyanobacteria. *New Phytologist*. 144(1): 3-33.

Anand, N., Thajuddin, N., and Dadheech, P. K. 2019. Cyanobacterial taxonomy: Morphometry to molecular studies. In *Cyanobacteria*. pp. 43-64.

Anand, N. and Kumar Hopper, R.S., 1987. Blue-green algae from rice fields in Kerala State, India. *Hydrobiologia*. 144(3): 223-232.

Arnold W., Rump A., and Klipp, W. 1988. Nucleotide sequence of a 24,206 base-pair DNA fragment carrying the entire nitrogen fixation gene cluster of Klebsiella pneumonia. J Mol Biol 203: 715–738.

Ashraf, M.A., Asif, M., Zaheer, A., Malik, A., Ali, Q. and Rasool, M., 2013. Plant growth promoting rhizobacteria and sustainable agriculture: a review. *African Journal of Microbiology Research*. 7(9): 704-709.

Bennett, A. and Bogorad, L., 1973. Complementary chromatic adaptation in a filamentous blue-green alga. *The J. of cell biology*. 58(2): 419-435.

Biondi, N., Piccardi, R., Margheri, M. C., Rodolfi, L., Smith, G. D., and Tredici, M. R. 2004. Evaluation of *Nostoc* strain ATCC 53789 as a potential source of natural pesticides. *Appl. Environ. Biotechnol.* 70: 3313–3320.

Bonjouklian, R., Smitka, T.A., Doolin, L.E., Molloy, R.M., Debono, M., Shaffer, S.A., Moore, R.E., Stewart, J.B. and Patterson, G.M., 1991. Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrixtjipanasensis*. *Tetrahedron*. 47(37): 7739-7750.

Bordowitz, J.R. and Montgomery, B.L., 2008. Photoregulation of cellular morphology during complementary chromatic adaptation requires sensor-kinase-class

protein RcaE in Fremyella diplosiphon. *J. of bacteriology*. 190(11): 4069-4074.

Burja, A. M., Banaigs, E. B., Abou-Mansour, A., Burgess, J. G., and Wright, P. C. 2001. Marine cyanobacteria – a prolific source of natural products. *Tetrahedron.* 57: 9347–9377.

Cappuccino, J. G. and Sherman, N. 1999. Microbiology-a laboratory manual. Harlow, England, 458 p.

Carpenter, E.J. and Romans, K., 1991. Major role of the cyanobacterium *Trichodesmium* in nutrient cycling in the North Atlantic Ocean. *Science*. 254(5036): 1356-1358.

Chakraborty, T., 2014. Chemical characterization of exopolysaccharide from cyanobacteria with special reference to stress induced changes. 146p.

Chittapun, S., Limbipichai, S., Amnuaysin, N., Boonkerd, R. and Charoensook, M., 2018. Effects of using cyanobacteria and fertilizer on growth and yield of rice, Pathum Thani I: a pot experiment. *J. of Applied Phycology*. 30(1): 79-85.

De Caire, G.Z., De Cano, M.S., De Mule, M.C.Z. and De Halperin, D.R., 1990. Antimycotic products from the cyanobacterium *Nostoc muscorum* against *Rhizoctonia solani*. *Phyton, Buenos Aires*. 51(1):1-4.

De Fátima Fiore, M., Neilan, B.A., Copp, J.N., Rodrigues, J.L., Tsai, S.M., Lee, H. and Trevors, J.T., 2005. Characterization of nitrogen-fixing cyanobacteria in the Brazilian Amazon floodplain. *Water research*. 39(20): 5017-5026.

De, P.K. and Mandal, L.N., 1956. Fixation of nitrogen by algae in rice soils. *Soil Science*. 81(6): 453-458.

Department of Agriculture, Cooperation and Farmers Welfare. 2018. *Annual Report* 2018- 2019. 224p.

Desikachary, T.V., 1959. *Cyanophyta* (Vol. 2). New Delhi: Indian Council of Agricultural Research.

Dias, E., Oliveira, M., Jones-Dias, D., Vasconcelos, V., Ferreira, E., Manageiro, V. and Caniça, M., 2015. Assessing the antibiotic susceptibility of freshwater Cyanobacteria spp. *Frontiers in microbiology*. 6. p.799.

Doan, N.T., Stewart, P.R. and Smith, G.D., 2001. Inhibition of bacterial RNA polymerase by the cyanobacterial metabolites 12-epi-hapalindole E isonitrile and calothrixin A. *FEMS microbiology letters*. 196(2):135-139.

El-Habbasha, S.F., Hozayn, M. and Khalafallah, M.A., 2007. Integration effect between phosphorus levels and biofertilizers on quality and quantity yield of faba bean (Vicia faba L.) in newly cultivated sandy soils. *Research J. of Agriculture and Biological Sciences*. 3(6): 966-971.

Folia Microbiol (Praha). 2010 Sep; 55(5):474-80.

Galavi, M., Yosefi, K., Ramrodi, M. and Mousavi, S.R., 2011. Effect of bio-phosphate and chemical phosphorus fertilizer accompanied with foliar application of micronutrients on yield, quality and phosphorus and zinc concentration of maize. *Journal of Agricultural Science*, *3*(4), p.22.

Gantar, M., Berry, J.P., Thomas, S., Wang, M., Perez, R. and Rein, K.S., 2008. Allelopathic activity among cyanobacteria and microalgae isolated from Florida freshwater habitats. *FEMS microbiology ecology*, *64*(1), pp.55-64.

Ghosh, T.K. and Saha, K.C., 1993. Effects of inoculation with N_2fixing cyanobacteria on the nitrogenase activity in soil and rhizosphere of wetland rice (*Oryza sativa* L.). *Biology and fertility of soils.*16(1): 16-20.

Gol'din, E., 2012. Biologically active microalgae and cyanobacteria in nature and marine biotechnology. *Turkish Journal of Fisheries and Aquatic Sciences*, *12*(5), pp.423-427.

Golakoti, T., Yoshida, W.Y., Chaganty, S. and Moore, R.E., 2000. Isolation and structures of nostopeptolides A1, A2 and A3 from the cyanobacterium *Nostoc* sp. GSV224. *Tetrahedron*. 56(46): 9093-9102.

Gopinath, P. P, Parsad, R., Joseph, B., and Adarsh, V. S. 2020. GRAPES: General Rshiny Based Analysis Platform Empowered by Statistics. https://www.kaugrapes.com/home. version 1.0.0. DOI: 10.5281/zenodo.4923220.

Gordon S. A. and Weber R. P. 1951. Colorimetric estimation of indole acetic acid. *Plant Physiol*. 26: 192–195.

Grimm, N.B. and Petrone, K.C., 1997. Nitrogen fixation in a desert stream ecosystem. *Biogeochemistry*, *37*(1), pp.33-61.

Grizeau, D., Bui, L.A., Dupré, C. and Legrand, J., 2016. Ammonium photo-production by heterocytous cyanobacteria: potentials and constraints. *Critical reviews in biotechnology*. 36(4): 607-618.

Gurung, S. and Prasad, B.N., 2005. Azolla and Cyanobacteria (BGA): Potential biofertilizer for rice. *Scientific World*. 3(3): 85-89.

Hagmann, L. and Juttner, F. 1996. Fischerellin-A, a novel photosystem-ll inhibiting allelochemical of the cyanobacterium *Fischerellamuscicola* with antifungal and herbicidal activity. *Tetrahedron Lett.* 37: 6539–6542.

Hashem, M.A., 2001. Problems and prospects of cyanobacterial biofertilizer for rice cultivation. *Functional Plant Biology*. 28(9): 881-888.

Hashtoudi, M.S., Ghassempour, A., Riahi, H., Shariatmadari, Z. and Khanjir, M., 2013. Endogenous auxins in plant growth-promoting Cyanobacteria-*Anabaena vaginicola* and *Nostoc calcicola*. *Journal of applied phycology*. 25(2): 379-386.

Holajjer, P., Kamra, A., Gaur, H. S., and Dhar, D. W. 2012. *In vitro*nematicidal activity of a terrestrial cyanobacterium, Synechococcusnidulans, towards plant-parasitic nematodes. *Nematol.* 14: 85–92.

Hussain, A. and Hasnain, S., 2011. Phytostimulation and biofertilization in wheat by cyanobacteria. *Journal of Industrial Microbiology and Biotechnology*. 38(1): 85-92.

Issa, A.A., Abd-Alla, M.H. and Ohyama, T., 2014. Nitrogen fixing cyanobacteria: future prospect. *Advances in biology and ecology of nitrogen fixation*. *2*: 24-48.

J Biol Chem. 2008 Nov 21; 283(47):32516-26.

Jaiswal, A., Das, K., Koli, D.K. and Pabbi, S., 2018. Characterization of cyanobacteria for IAA and siderophore production and their effect on rice seed germination. *Int. J. Curr.Microbiol. App. Sci. 5*. 212-222.

Jeevanantham, G., Vinoth, M., Hussain, J.M., Muruganantham, P. and Ahamed, A.K.K., 2019. Biochemical characterization of five marine cyanobacteria species for their biotechnological applications. *Journal of pharmacognosy and phytochemistry*, *8*(2), pp.635-640.

Jeevanantham, G., Vinoth, M., Hussain, J.M., Muruganantham, P., and Ahamed, A.K.K. 2019. Biochemical characterization of five marine cyanobacteria species for their biotechnological applications. *J. Pharmacognosy and Phytochem*. 8: 635-640.

Jensen, A., 1978. Chlorophylls and carotenoids. *Handbook of phycological methods, physiological and biochemical methods.* Cambridge University Press, Cambridge, pp.59-70.

Juttner, F., Todorova, A. K., Walch, N., Von Philipsborn, W., and Nostocyclamide, M. 2001. A cyanobacterial cyclic peptide with allelopathic activity from Nostoc 31. *Phytochem.* 57: 613–619

Kawaguchi, T. and Decho, A.W., 2000. Biochemical characterization of cyanobacterial extracellular polymers (EPS) from modern marine stromatolites (Bahamas). *Preparative Biochemistry and Biotechnology*. 30(4): 321-330

Kerby, N.W., Rowell, P. and Stewart, W.D.P., 1989. The transport, assimilation and production of nitrogenous compounds by cyanobacteria and microalgae. *Algal and cyanobacterial biotechnology*. pp.50-90.

Khairy, H.M. and El-Kassas, H.Y., 2010. Active substance from some blue green algal species used as antimicrobial agents. *African J. of Biotechnology*. *9*(19): 2789-2800.

Khan, Z.U.M., Begum, Z.T., Mandal, R. and Hossain, M.Z., 1994. Cyanobacteria in rice soils. *World J. of Microbiology and Biotechnology*. 10(3): 296-298.

Kleiner, D., 1985. Bacterial ammonium transport. *FEMS Microbiology Letters*. 32(2): 87-100.

Kulik, M. M. 1995. The potential for using cyanobacteria (blue green algae) and algae in the biological control of plant pathogenic bacteria and fungi. *Eur. J. Plant Pathol.* 101: 585–599.

Kumar, A.R.U.N. and Singh, J.S., 2016. Microalgae and cyanobacteria biofuels: a sustainable alternate to crop-based fuels. *Microbes and environmental management.* Studium Press Pvt. Ltd., New Delhi, pp.1-20.

Kumar, M., Prasanna, R., Bidyarani, N., Babu, S., Mishra, B.K., Kumar, A., Adak, A., Jauhari, S., Yadav, K., Singh, R. and Saxena, A.K., 2013. Evaluating the plant growth promoting ability of thermotolerant bacteria and cyanobacteria and their interactions with seed spice crops. *Scientia Horticulturae*, *164*. pp.94-101.

Kundu, D.K. and Ladha, J.K., 1995. Efficient management of soil and biologically fixed N2 in intensively-cultivated rice fields. *Soil Biology and Biochemistry*. 27(4-5): 431-439.

Ladha, J.K. and Reddy, P.M., 2003. Nitrogen fixation in rice systems: state of knowledge and future prospects. *Plant and soil*. 252(1): 151-167.

Lopes, V.R., Ramos, V., Martins, A., Sousa, M., Welker, M., Antunes, A. and Vasconcelos, V.M., 2012. Phylogenetic, chemical and morphological diversity of cyanobacteria from Portuguese temperate estuaries. *Marine environmental research*. 73. pp.7-16.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. of biological chemistry*. 193. pp.265.-275.

Mackinney, G., 1941. Absorption of light by chlorophyll solutions. *J. of biological chemistry*. 140(2): 315-322.

Malik F.R., Ahmed S., Rizki Y.M. (2001). Utilization of lignocellulosic waste for the preparation of nitrogenous biofertilizer. *Pakistan J. of Biological Sciences.* 4.1217–1220

Malliga, P., Uma, L. and Subramanian, G., 1996. Lignolytic activity of the cyanobacterium Anabaena azollae ML2 and the value of coir waste as a carrier for BGA biofertilizer. *Microbios*. 86(348): 175-183.

Maqubela, M.P., Mnkeni, P.N.S., Issa, O.M., Pardo, M.T. and D'acqui, L.P., 2009. Nostoc cyanobacterial inoculation in South African agricultural soils enhances soil structure, fertility, and maize growth. *Plant and Soil*, *315*(1), pp.79-92.

Mishra, A.K., Shukla, E. and Singh, S.S., 2013. Phylogenetic comparison among the heterocystous cyanobacteria based on a polyphasic approach. *Protoplasma*. 250(1): 77-94.

Mishra, U. and Pabbi, S., 2004. Cyanobacteria: a potential biofertilizer for rice. *Resonance*. 9(6): 6-10.

Misra, S. and Kaushik, B.D., 1989. Growth promoting substances of cyanobacteria. I: vitamins and their influence on rice plant. *Proceedings of the Indian National Sci. Academy. Part B Biological sciences*. *55*(4): 295-300.

Mitsui, A. and Cao, S., 1988. [7] Isolation and culture of marine nitrogen-fixing unicellular cyanobacteria Synechococcus. *Methods in enzymology*. 167. pp.105-113.

Mundt, S., Kreitlow, S., Nowotny, A. and Effmert, U., 2001. Biochemical and pharmacological investigations of selected cyanobacteria. *International journal of hygiene and environmental health*. 203(4): 327-334.

Muñoz-Rojas, M., Chilton, A., Liyanage, G.S., Erickson, T.E., Merritt, D.J., Neilan, B.A. and 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(1): 91-100.

Nascimento, S.S., Santos, V.S.V., Watanabe, E.O. and de Souza Ferreira, J., 2020. Assessment of the purification of phycobiliproteins in cyanobacteria through aqueous two-phase systems with different proportions of PEG/salt. *Food and Bioproducts Processing*, *119*, pp.345-349.

Okmen, G., Bozanta, E., Aysel, U.G.U.R. and Ceyhan, N., 2011. Zinc effect on chlorophyll a, total carbohydrate, total protein contents and biomass of cyanobacterial species. *J. of Applied Biological Sci*. 5(2): 67-73.

Padmapriya, V. and Anand, N., 2010. Evaluation of some industrially important enzymes in filamentous cyanobacteria. *ARPN J. Agric. Biol. Sci*. 5(5): 86-97.

Pandey, S.N., Verma, I. and Kumar, M., 2020. Cyanobacteria: potential source of biofertilizer and synthesizer of metallic nanoparticles. *In Advances in Cyanobacterial Biology*. Academic Press.pp. 351-367.

Pandey, U. and Pandey, J., 2002. Antibacterial properties of cyanobacteria: A cost-effective and eco-friendly approach to control bacterial leaf spot disease of chilli. *Current Sci*. 82(3): 262-264.

Pereira, I., Ortega, R., Barrientos, L., Moya, M., Reyes, G. and Kramm, V., 2009. Development of a biofertilizer based on filamentous nitrogen-fixing

cyanobacteria for rice crops in Chile. *J. of applied phycology*. *21*(1): 135-144.

Prabaharan, D., Sumathi, M. and Subramanian, G., 1994. Ability to use ampicillin as a nitrogen source by the marine cyanobacterium *Phormidium valderianum* BDU 30501. *Current Microbiology*. 28(6): 315-320.

Prasad R C and Prasad B N(2001). Cyanobacteria as a source Biofertilizer for sustainable agriculture in Nepal. Journal in Plant Science Botanica Orientalis 127-133.

Prasad, R.C. and Prasad, B.N., 2001. Cyanobacteria as a source biofertilizer for sustainable agriculture in Nepal. *J. Plant Sci. Bot. Orientalis*. 1. pp.127-133.

Prasanna, R., Babu, S., Rana, A., Kabi, S.R., Chaudhary, V., Gupta, V., Kumar, A., Shivay, Y.S., Nain, L. and Pal, R.K., 2012a. 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. and Nayak, S., 2007. Influence of diverse rice soil ecologies on cyanobacterial diversity and abundance. *Wetlands Ecology and Management*. *15*(2): 127-134.

Prasanna, R., Jaiswal, P., Nayak, S., Sood, A. and Kaushik, B.D., 2009. Cyanobacterial diversity in the rhizosphere of rice and its ecological significance. *Indian J. of Microbiology*. 49(1): 89-97.

Prasanna, R., Madhan, K., Singh, R.N., Chauhan, A.K. and Nain, L., 2010. Developing biochemical and molecular markers for cyanobacterial inoculants. *Folia microbiologica*. 55(5): 474-480.

Prasanna, R., Nain, L., Tripathi, R., Gupta, V., Chaudhary, V., Middha, S., Joshi, M., Ancha, R. and Kaushik, B.D., 2008. Evaluation of fungicidal activity of extracellular filtrates of cyanobacteria–possible role of hydrolytic enzymes. *Journal of basic microbiology*, *48*(3), pp.186-194.

Prasanna, R., Sharma, E., Sharma, P., Kumar, A., Kumar, R., Gupta, V., Pal, R.K., Shivay, Y.S. and Nain, L., 2013. Soil fertility and establishment potential of inoculated cyanobacteria in rice crop grown under non-flooded conditions. *Paddy and Water Environment*. 11(1): 175-183

Prasanna, S.N.R., 2007. Soil pH and its role in cyanobacterial abundance and diversity in rice field soils. *Applied ecology and environmental Research*, *5*(2), pp.103-113.

Priya, H., Prasanna, R., Ramakrishnan, B., Bidyarani, N., Babu, S., Thapa, S. and Renuka, N., 2015. Influence of cyanobacterial inoculation on the culturable microbiome and growth of rice. *Microbiological research*, *171*, pp.78-89.

Priya, T.R., Nelson, A.R.L.E., Ravichandran, K. and Antony, U., 2019. Nutritional and functional properties of coloured rice varieties of South India: a review. *J. of Ethnic Foods*. 6(1): 1-11.

Rana, Y.S.S. and Nain, L., 2012. Radha Prasanna, Monica Joshi, Anuj. *World J MicrobiolBiotechnol*, *28*, pp.1223-1235.

Ranjan, K., Priya, H., Ramakrishnan, B., Prasanna, R., Venkatachalam, S., Thapa, S., Tiwari, R., Nain, L., Singh, R. and Shivay, Y.S., 2016. Cyanobacterial inoculation modifies the rhizosphere microbiome of rice planted to a tropical alluvial soil. *Applied Soil Ecology*. 108. pp.195-203.

Reddy, K.J., Haskell, J.B., Sherman, D.M. and Sherman, L.A., 1993. Unicellular, aerobic nitrogen-fixing cyanobacteria of the genus Cyanothece. *J. of bacteriology*. 175(5): 1284-1292.

Reynaud, P.A. and Franche, C., 1986. Isolation and characterization of nonheterocystous tropical cyanobacteria growing on nitrogen-free medium. *MIRCEN journal of applied microbiology and biotechnology*. 2(4): 427-443.

Richmond, A. ed., 1986. *CRC Handbook of microalgal mass culture*. Boca Raton, Florida: CRC press.pp. 148-244.

Rodríguez, A.A., Stella, A.M., Storni, M.M., Zulpa, G. and Zaccaro, M.C., 2006. Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in Oryza sativa L. *Saline systems*. *2*(1): 1-4.

Roger, P.A. and Ladha, J.K., 1992. Biological N_2 fixation in wetland rice fields: Estimation and contribution to nitrogen balance. *Biological nitrogen fixation for sustainable agriculture*. pp.41-55.

Roger, P.A. and Reynaud, P.A., 1982. Free-Living blue-green algae in tropical soils. *In Microbiology of tropical soils and plant productivity*. Springer, Dordrecht.pp. 147-168.

Roger, P.A., 1996. *Biology and management of the floodwater ecosystem in rice fields*. Int. Rice Res. Inst., Philippines, 250p.

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

Sabin, M., Susann, K., and Jansen, R. 2003. Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei* HUB 051. *J. App. Phycol.* 15: 263–267.

Sahu, D., Priyadarshani, I. and Rath, B., 2012. Cyanobacteria–as potential biofertilizer. *CIBTech Journal of Microbiology*. 1(2-3): 20-26.

Sathiyamoorthy, P. and Shanmugasundaram, S. 1996. Preparation of cyanobacterial peptide toxin as a biopesticide against cotton pests. *Appl. Microbiol. Biotechnol.* 46: 511–513.

Scott, J.T. and Marcarelli, A.M., 2012. Cyanobacteria in freshwater benthic environments. In *Ecology of cyanobacteria II*. Springer, Dordrecht. 271-289.

Sharma, H.K. and Gaur, H.S., 2008. Hatch inhibition of Meloidogyne incognita by aqueous extracts and exudates of five species of cyanobacteria. *Nemat. Medit*. 36.99-102

Sharma, H.K., Gaur, H.S., Singh, B.V. and Dhar, D.W., 2007. Bionematicidal potential of ten species of cyanobacteria against the root-knot nematode, Meloidogyne incognita. *International Journal of Nematology*. 17(1): 35.

Sharma, N.K., Rai, A.K. and Stal, L.J., 2013. *Cyanobacteria: an economic perspective*. John Wiley & Sons.

Singh, J.S., Kumar, A., Rai,A.N. and Singh, D.P., 2016. Cyanobacteria: a precious bio-resource in agriculture, ecosystem, and environmental sustainability. *Frontiers in microbiology*. 7. p.529

Singh, J.S., Singh, D.P. and Dixit, S., 2011b. Cyanobacteria: an agent of heavy metal removal. *Bioremediation of pollutants. IK International Publisher,* New Delhi, pp.223-243

Singh, A.L. and Singh, P.K., 1987. Comparative study on Azolla and blue-green algae dual culture with rice. *Israel Journal of Plant Sciences*. 36(2): 53-61.

Singh, H., Khattar, J.S. and Ahluwalia, A.S., 2014. Cyanobacteria and agricultural crops. *Vegetos*. 27(1): 37-44.

Singh, J.S., 2014. Cyanobacteria: a vital bio-agent in eco-restoration of degraded lands and sustainable agriculture. *Climate Change and Environmental Sustainability*. 2(2): 133-137.

Singh, N.K., Dhar, D.W. and Tabassum, R., 2016. Role of cyanobacteria in crop protection. *Proceedings of the National Academy of Sciences, India Section B: Biological Sci*. 86(1):1-8.

Singh, P.K. and Bisoyi, R.N., 1989. Blue-green algae in rice fields. *Cyanobacterial Biofertilizers 457 for Rice*.Phykos. 28: 181-195.

Singh, R.N., 1961. Role of blue-green algae in nitrogen economy of Indian agriculture. *Role of blue-green algae in nitrogen economy of Indian agriculture*. pp. 175

Singh, Y.V., 2010. Role of blue green algae in rice production. *Algal biotechnology: new vistas*. pp.145-158.

Sinha, R.P. and Hader, D.P., 1996. Invited Review Photobiology and Ecophysiology of Rice Field Cyanobacteria. *Photochemistry and photobiology*. 64(6): 887-896.

Sinha, R.P. and Kumar, A., 1992. Screening of blue-green algae for biofertilizer. *Proceedings of the National Seminar on Organic Farming* pp. Pune, India. pp. 18-19.

Smith, G. D. and Doan, N. T. 1999. Cyanobacterial metabolites with bioactivity against photosynthesis in cyanobacteria, algae and higher plants. *J. Appl. Phycol.* 11: 337–344.

Song, T., Mårtensson, L., Eriksson, T., Zheng, W. and Rasmussen, U., 2005. Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *FEMS Microbiology Ecology*. 54(1): 131-140.

Sood, A., Singh, P.K., Kumar, A., Singh, R. and Prasanna, R., 2011. Growth and biochemical characterization of associations between cyanobionts and wheat seedlings in co-culturing experiments. *Biologia*. 66(1): 104-110.

Subhashini, R., Kumar, K. and Kannaiyan, S., 2003. Intrinsic antibiotic resistance and biochemical characteristics of Anabaena azollae isolated from Azolla-cultures. *Indian J. of Microbiology*. 43(3): 165-169.

Sundaram, S. and Soumya, K.K., 2011. Study of physiological and biochemical alterations in cyanobacterium under organic stress. *Am J. Plant Physiol*. 6(1): 1-16.

Swarnalakshmi, K., Prasanna, R., Kumar, A., Pattnaik, S., Chakravarty, K., Shivay, Y.S., Singh, R. and Saxena, A.K., 2013. Evaluating the influence of novel cyanobacterial biofilmed biofertilizers on soil fertility and plant nutrition in wheat. *European Journal of Soil Biology*. 55. pp.107-116.

Tantawy, S.T., 2011. Biological potential of cyanobacterial metabolites against some soil pathogenic fungi. *J. of food, agriculture & environment*. 9(1): 663-666.

Thilak, T.S., Madhusoodanan, P.V., Pradeep, N.S. and 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.

Thomas, J.J., 2011. Paddy cultivation in Kerala. *Review of Agrarian Studies*, *1*(2369-2021-121).

Tomlinson, M.C., Stumpf, R.P., Wynne, T.T., Dupuy, D., Burks, R., Hendrickson, J. and Fulton III, R.S., 2016. Relating chlorophyll from cyanobacteria-dominated inland waters to a MERIS bloom index. *Remote Sensing Letters*. 7(2):141-149.

Urbach, C., Fastrez, J. and Soumillion, P., 2008. A new family of cyanobacterial penicillin-binding proteins: a missing link in the evolution of class A β-lactamases. *J. of Biological Chemistry*. 283(47): 32516-32526.

Vaishampayan, A., Sinha, R.P., Hader, D.P., Dey, T., Gupta, A.K., Bhan, U. and Rao, A.L., 2001. Cyanobacterial biofertilizers in rice agriculture. *The Botanical Review*. *67*(4): 453-516.

Vanlalveni, C. and Lalfakzuala, R., 2021. Isolation and Genetic Diversity Studies of Nitrogen-Fixing Cyanobacteria from Wetland Paddy Field of Mizoram, India. *Geomicrobiology J*. *38*(10): 946-954.

Venkataraman, G.S., 1975.The role of blue-green algae in tropical rice cultivation. *Nitrogen fixation by free-living micro-organisms*. *6*:207-217

Venkataraman, G.S., 1981. All India-Co-ordinated project on algae. *Annual report (1980-81). Division of Microbiology,(Indian Agricultural Research Institute), New Delhi, India*.

Venkataraman, G.S., 1993. Blue-green algae (cyanobacteria). *Biological nitrogen fixation*. pp.45-76.

Vessey, J.K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil*. 255(2): 571-586.

Vijayan, D. and Ray, J.G., 2015. Ecology and diversity of cyanobacteria in Kuttanadu paddy wetlands, Kerala, India. *American J. of plant sciences.* 6(18): 2924.

Volk, R.B. and 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.

Wilson, L.T., 2006. Cyanobacteria: a potential nitrogen source in rice fields. *Texas Rice*. 6(9): 10.

Yanni, Y.G. and Abdallah, F.E., 1990. Role of algalization in rice growth, yield and incidence of infestation with the stem borer ChiloagamemnonBles. and the leaf miner Hydrelliaprosternalis Deeming in the Nile Delta. *World journal of Microbiology and Biotechnology*, *6*(4), pp.383-389.

Yibeltie, G., Sahile, S. and Box, P. 2018. The Role of cyanobacteria on agriculture.*J. of Natural Sci. Research*. 8(23): 1-5.

Zaidan, M.R., Noor Rain, A., Badrul, A.R., Adlin, A., Norazah, A. and Zakiah, I., 2005. In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop biomed*. 22(2): 165-170

APPENDICES

APPENDIX-I

COMPOSITION OF MEDIA

1. BG -11 Media

K_2HPO_4	-	0.04g
$MgSO_4.7H_2O$	-	0.075 g
$CaCl_2.2H_2O$	-	0.036 g
Citric acid	-	0.006 g
Ferric ammonium citrate	-	0.006 g
EDTA	-	0.001g
Na_2Co_3	-	0.002 g
Agar-agar	-	20 g
Trace metal mix	-	1 mL
Distilled water	-	1000 mL
pH	-	7.5

Trace metal mixture (g L^{-1})

H_3BO_4	-	2.84 g
$MnCl_2.4H_2O$	-	1.81 g
$ZnSO_4.4H_2O$	-	0.22 g
$Na_2MoO_4.2H_2O$	-	0.39 g
$CuSO_4.H_2O$	-	0.07 g
$Co(NO_3)_2.6H_2O$	-	0.04 g

K_2HPO_4 , $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, Citric acid, Ferric ammonium citrate, EDTA and Na_2Co_3 were dissolved in 500 mL distilled water. 1 mL of trace metal mix was added and volume made upto 1000 mL. It was then distributed to 250 mL flask of 100 mL each and pH was adjusted. Two grams of agar-agar was added to each flask and autoclaved at 15 lbs pressure at 121^{o}C for 15 minutes.

2. Potato Dextrose Agar

Peeled and sliced potatoes	-	200.0 g
Dextrose	-	20.0 g
Agar-agar	-	20.0 g
Distilled water	-	1000 mL

Potatoes were boiled in 500 mL of distilled water and the extracts was collected by filtering through a muslin cloth. Agar-agar was dissolved separately in 500 mL of ditilled water. The potato extract was mixed in the molten agar and 20 g of dextrose was dissolved in the mixture. The volume was made up to 1000 mL with distilled water and medium was sterilized at 15 lbs pressure and 121^{o}C for 15 minutes

3. Nutrient agar

Peptone	-	6.00 g
Agar-agar	-	20.0 g
Distilled water	-	1000 mL

Peptone were dissolved in 500 mL distilled water and volume made upto 1000 mL. It was then distributed to 250 mL conical flask of 100 mL each. Two grams agar-agar was added to each flask and autoclaved at 15 lbs pressure at 121^{o}C for 15 minutes.

4. Reagents for the spectrophotometric estimation of Total proteins
   A. 2% Na_2Co_3 in 0.1 N NaOH
   B. 1% NaKTartarate in H_2O
   C. 0.5% $CuSo_4.5H_2O$ in H_2O
   1. Reagent I: 48 mL of A, 1mL of B, 1 mL of C
   2. Reagent II: 1 partFolin-Phenol [2 N]: 1 part water
   3. BSA (Bovine Serum Albumin) standard: 1mg/mL

Procedure:

0.2 mL of BSA working standard was taken in 5 test tubes and made upto 1 mL using distilled water. The test tube with 1 mL distilled water serve as blank. 4.5 mL of reagent I was added to all the test tubes and incubated for 10 minutes. After incubation, 0.5 mL of reagent II was added and incubated for 30 minutes. Absorbance of the standards was measured at 660 nm and standard graph was plotted.

5. Reagent for the spectrophotometric estimation of Indole acetic acid

Standard curve: were made in culture medium at 0, 5, 10, 20, 50 and 100 µg mL^{-1} (ppm).

1. To a glass beaker of 10 mL acetone in the fume hood, 10 mg of IAA was added. It was stirred with metal spatula until it is completely dissolved. This was the 1000 µg mL^{-1}stock.

2. The series of amber vials was labeled with dilution series.

3. 100 µg mL^{-1} standard was prepared by adding 1 mL of the 1000 µg mL^{-1}stock and 9 mL of culture medium into the first vial. It was mixed well by inversion.

4. 5 mL of the 100 µgmL^{-1}standard and 5 mL of culture media was transferred into another vial to make 50 µg mL^{-1}standard.

5. 1 mL of the 100 µgmL^{-1}standard and 9 mL of culture media was transferred into another vial to make 10 µg mL^{-1}standard and it was mixed well by inversion.

6. 2 mL of the 100µgmL^{-1}standard and 8 mL of culture media was transferred into another vial to make 20 µg mL^{-1}standard and it was mixed well by inversion.

7. 1 mL of 50 µg mL^{-1}standard and 9 mL of culture media was transferred into another vial to make 50 µg mL^{-1}standard.

8. 2 mL of Salkowski reagent was added into 6 test tubes labelled with each standard.

9. 1 mL of each standard and a no-IAA control of pure media was transferred into test tubes and incubated at room temperature and optical density was measured at 530 nm.

DEVELOPMENT OF INOCULANT CULTURES OF NITROGEN FIXING CYANOBACTERIA FROM WETLAND ECOSYSTEM OF VELLAYANI

by

ARYA S.

(2019-11-224)

ABSTRACT

Submitted in partial fulfilment of the

requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

COLLEGE OF AGRICULTURE

VELLAYANI, THIRUVANANTHAPURAM-695522

KERALA, INDIA

2022

ABSTRACT

The study entitled “Development of inoculant cultures of nitrogen fixing cyanobacteria from wetland ecosystem of Vellayani” was conducted during the year 2019-2021 in the Department of Agricultural Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram with the objective to isolate and characterize cyanobacterial strains with efficient nitrogen fixing capacity from wetland ecosystem of Vellayani and assess the plant growth promotion and biocontrol activity through *in vitro* screening process.

Ten nitrogen fixing cyanobacteria were isolated from wetland ecosystem of Vellayani region and they were designated as VLY 1 - VLY 10. Tentative taxonomic identification of the isolates were done based on their morphological and biochemical characters. The isolates were tested for their pigment concentration like chlorophyll *a*, phycobiliproteins, carotenoids. The chlorophyll *a* concentration varied from 0.688 mg mL^{-1} to 5.372 mg mL^{-1}. The phycocyanin, allophycocyanin and phycoerythrin concentrations ranged between 0.016 mg mL^{-1} to 0.036 mg mL^{-1},0.015 mg mL^{-1} to 0.037 mg mL^{-1} and 0.005 mg mL^{-1} to 0.017 mg mL^{-1} respectively. Maximum chlorophyll a and phycobiliproteins was recorded in *Aphanocapsa* sp.VLY 2**.** Carotenoid concentration ranged between 0.001 µg mL^{-1} to 0.006 µg mL^{-1} and the maximum was reported in *Calothrix*sp.VLY 10**.** The concentration of total proteins ranged between 102.57 µg mL^{-1} to 236.32 µg mL^{-1} and the maximum was reported in *Oscillatoria*sp.VLY 5. Antibiotic sensitivity profiling of the isolates was done against streptomycin (25 µg), tetracycline (10 µg), nalidixic acid (30 µg), penicillin (10 µg), erythromycin (15 µg), rifampicin (5 µg) and chloramphenicol (30 µg). The isolates like *Aphanotheca*sp.VLY 1, *Oscillatoria*sp.VLY 5, *Lyngbya*sp.VLY 7, *Chrococcus*sp.VLY 9 showed sensitivity against rifampicin with a sensitivity zone of 14mm, 10mm, 9mm, 11mm respectively. The isolate *Aphanotheca*sp.VLY 1 showed sensitivity against streptomycin and tetracycline with a zone of 12mm and 8mm respectively. *Oscillatoria*sp.VLY 5 was sensitive against tetracycline with a zone of 13mm.

The cyanobacterial isolates were tested for their plant growth promotion activity studies. Extracellular ammonia production ranged between 111.74 µmol mL^{-1} to 171

µmol mL^{-1} and maximum was reported by *Chrococcus*sp.VLY 6. Nitrogen content of the isolates ranged between 0.200 mg $mL^{-1}$to 0.280 mg mL^{-1} and the isolate *Oscillatoria*sp.VLY 5 reported the maximum nitrogen content. The Indole Acetic Acid production ranged between 1.47 µg mL^{-1} to 7.10 µg mL^{-1}. Maximum IAA production was recorded in *Chrococcus*sp.VLY 6. The antagonistic activity of the isolates against plant pathogenic bacteria (*Xanthomonas oryzae*) and fungi (*Pythium ultimum*, *Phytophthora capsici*, *Fusarium oxysporum*f.sp. *solani*, *Rhizoctonia solani*) was investigated using disc diffusion method. The isolate *Aphanotheca*sp.VLY 1 and *Microcystis*sp.VLY 3 showed antagonistic activity against *Xanthomonas oryzae*with an inhibition zone of 7.12 mm amd 8.20 mm respectively. The cyanobacterial isolates were screened based on weighted average ranking and *Chrococcus*sp.VLY 6 was selected and evaluated for growth promotion under *in vitro* condition in rice. The rice seeds were subjected to thirteen treatments such as T_1 – 10 mL sterile distilled water, T_2 – 10 mL sterile BG-11 medium(without tryptophan). T_3 – 10 mL sterile BG-11 medium amended with 5 mg mL^{-1} tryptophan, T_4 to T_8– 10mL sterile distilled water mixed with 20 µL, 40 µL, 60 µL, 80 µL, 100 µL of cyanobacterial extract (culture medium amended without tryptophan), T_9 to T_{13} – 10 mL sterile distilled water mixed with 20 µL, 40 µL, 60 µL, 80 µL, 100 µL of cyanobacterial extract (culture medium amended with 5 mg mL^{-1} tryptophan). Maximum germination percentage was observed in seeds treated with 10 mL sterile distilled water and 40 µL cyanobacterial culture in BG 11 amended with 5mg$mL^{-1}$of tryptophan.Also this treatment recorded the maximum shoot length (6.83 cm), root length (12.23 cm), shoot dry weight (4.62 mg), root dry weight (2.91 mg), seedling vigour index (1781) and shoot root ratio (2.7).

Based on the results of present study, *Oscillatoria*sp.VLY 5 and *Chrococcus*sp.VLY 6 are the superior isolates for plant growth promotion. Also, *Microcystis*sp.VLY 3 is the best among the isolates which showed antibacterial activity.

സംഗ്രഹം

"വെള്ളായണിയിലെ തണ്ണീർത്തട ആവാസവ്യവസ്ഥയിൽ നിന്നും നൈട്രജൻ ഫിക്സ് ചെയ്യാൻ ശേഷിയുള്ള സയനോബാക്ടീരിയകളുടെ ഇനോക്കലെന്റ് കൾച്ചറുകളുടെ വികസനം" എന്ന വിഷയത്തെപറ്റിയുള്ള പഠനം 2019- 2021 കാലയളവിൽ വെള്ളായണി കാർഷിക കോളേജിലെ, അഗ്രികൾച്ചറൽ മൈക്രോബയോളജി വിഭാഗത്തിൽ നടത്തി. നൈട്രജൻ ഫിക്സ് ചെയ്യാൻ ശേഷിയുള്ള സയനോബാക്ടീരിയൽ ഐസൊലേറ്റുകളുടെ സ്വഭാവ സവിശേഷതകളും, സസ്യങ്ങളുടെ വളർച്ച ത്വരിതപ്പെടുത്താനുള്ള ശേഷിയും, ജൈവ നിയന്ത്രണ സാധ്യതകളും പരിശോധിക്കുക എന്ന ഉദ്ദേശത്തോടെയാണ് ഈ പഠനം നടത്തിയത്.

വെള്ളായണി മേഖലയിലെ തണ്ണീർത്തട ആവാസവ്യവസ്ഥയിൽ നിന്നും പത്ത് നൈട്രജൻ ഫിക്സ് ചെയ്യാൻ ശേഷിയുള്ള സയനോബാക്ടീരിയൽ ഐസൊലേറ്റുകളെ വേർതിരിച്ച് അവയെ VLY 1- VLY 10 എന്നിങ്ങനെ നാമകരണം ചെയ്തു. അവയുടെ സ്വഭാവ സവിശേഷതകളും, ബിയോക്കെമിക്കൽ സവിശേഷതകളും അടിസ്ഥാനമാക്കി താൽക്കാലിക നാമകരണം നടത്തി. ക്ലോറോഫിൽഎ, ഫൈകോബിലിപ്രോട്ടീൻ, കരോറ്റെനോയിഡ് പോലെയുള്ള പിഗ്മെന്റ് സാന്ദ്രധക്കായി ഐസൊലേറ്റുകളെ പരിശോധിച്ചു. ഇവയിൽ 0.68 mg mL^{-1} മുതൽ 5.37 mg mL^{-1} വരെ ക്ലോറോഫിൽ എ യുടെ അളവ് ലഭിച്ചു. ഫൈകോസയാനിൻ, അലോഫൈകോസയാനിൻ, ഫൈകോറിത്രിൻ എന്നിവയുടെ അളവ് 0.02 mg mL^{-1} മുതൽ 0.04 mg mL^{-1}, 0.02 mg mL^{-1} മുതൽ 0.04 mg mL^{-1}, 0.01 mg mL^{-1} മുതൽ 0.02 mg mL^{-1} വരെയും ആയിരുന്നു.

പരമാവധി ക്ലോറോഫിൽ എ യും ഫൈകോബിലിപ്രോട്ടീനും *അഫനോക്യാപ്സ* sp. VLY 2 -ൽ ആണ് കാണപ്പെട്ടത്. *കാലോത്രിക്സ്* sp. VLY 10 (0.01 µg mL^{-1})-നു ആണ് പരമാവധി കരോട്ടിനോയിഡ് സാന്ദ്രത കാണപ്പെട്ടത്. പ്രോട്ടീനുകളുടെ സാന്ദ്രത 102.57 µg mL^{-1} മുതൽ 236.32 µg mL^{-1} വരെ ആയിരുന്നു. പരമാവധി പ്രോട്ടീനുകളുടെ അളവ് *ഓസ്സിലാറ്റോറിയ* sp. VLY 5- ൽ ആണ് കാണപ്പെട്ടത്. സ്ട്രെപ്റ്റോമൈസിൻ (25 µg), ടെട്രാസൈക്ലിൻ (10 µg), നാലിഡിക്സിക്ആസിഡ് (30 µg), പെൻസിലിൻ (10 µg), എറിത്രോമൈസിൻ (15 µg), റിഫാംപിസിൻ (5 µg), ക്ലോറാംഫെനിക്കോൾ (30 µg) എന്നീ ആന്റിബയോട്ടിക്കുകൾക്ക് എതിരെയുള്ള ഐസൊലേറ്റുകളുടെ സംവേദനക്ഷമത പരിശോധിച്ചു. 14 mm, 10 mm, 9 mm, 11 mm യഥാക്രമത്തിൽ സെൻസിറ്റിവിറ്റി സോൺ കാണിച്ചു. *അഫനോത്തീക്ക* sp. VLY 1, *ഓസ്സിലാറ്റോറിയ* sp. VLY 5, *ലിൻഗ്ബ്യാ* sp. VLY 7, *ക്രൂകോക്കസ്* sp. VLY 9 ഐസൊലേറ്റുകൾ റിഫാംപിസിന് എതിരെ സംവേദനക്ഷമത കാണിച്ചു. സ്ട്രെപ്റ്റോമൈസിനും, ടെട്രാസൈക്ലിനുമെതിരെ *അഫനോത്തീക്ക* sp. VLY 1 എന്ന ഐസൊലേറ്റ് 12 mm, 8 mm സെൻസിറ്റിവിറ്റി സോണോടുകൂടി സംവേദനക്ഷമത പ്രകടിപ്പിച്ചു. 13 mm സെൻസിറ്റിവിറ്റി സോണുമായി *ഓസ്സിലാറ്റോറിയ* sp. VLY 5 ടെട്രാസൈക്ലിനെതിരെ സംവേദനക്ഷമത കാണിച്ചു.

സയനോബാക്ടീരിയൽ ഐസൊലേറ്റുകളുടെ സസ്യ വളർച്ച ത്വരിതപ്പെടുത്താനുള്ള ശേഷി പഠിച്ചു. ഐസൊലേറ്റുകളിലെ ബാഹ്യകോശ അമോണിയ ഉല്പാദനം 111.74 µmol mL^{-1} മുതൽ 171 µmol mL^{-1} വരെ ആയിരുന്നു. നൈട്രജന്റെ അളവു 0.200 mg mL^{-1} മുതൽ 0.280 mg mL^{-1} വരെ ആയിരുന്നു. ഇതിൽ *ഓസ്*

സിലാറ്റോറിയ sp. VLY 5 എന്ന ഐസൊലേറ്റ് ആണ് പരമാവധി നൈട്രജന് ഉല്പാദിപ്പിച്ചത്. ഐസൊലേറ്റുകളിലെ ഇന്ഡോള് അസെറ്റിക് ആസിഡ്ഡിന്റെ ഉല്പാദനം 1.47 µg mL^{-1} മുതല് 7.10 µg mL^{-1} വരെ ആയിരുന്നു. ഇതിൽ *ക്രൂകോക്കസ്* sp. VLY 6 എന്ന ഐസൊലേറ്റ് ആണ് പരമാവധി ഇന്ഡോള് അസെറ്റിക് ആസിഡ് ഉല്പാദിപ്പിച്ചത്. രോഗകാരികളായ ബാക്റ്റീരിയയെയും (*സാന്തോമൊണാസ് ഒറൈസെ*), കുമിളുകളെയും (*പിത്തിയം ആൾടിമം*, *ഫ്യ്റ്റോഫ്ത്തോറ കാഷ്സിസി*, *ഫ്യൂസേറിയം ഓക്സിസ്പോറം* f.sp. *സൊളാനി*, *റൈസാക്റ്റോണിയ സൊളാനി*) പ്രതിരോധിക്കാനുള്ള ഐസൊലേറ്റുകളുടെ കഴിവ് പരിശോധിച്ചു. *അഫനോത്തീക്ക* sp. VLY 1 (7.12 mm), *മൈക്രോസിസ്റ്റിസ്* sp. VLY 3 (8.20 mm) എന്നീ ഐസൊലേറ്റുകൾ *സാന്തോമൊണാസ് ഒറൈസെ* എന്ന ബാക്റ്റീരിയക്കെതിരെ പരമാവധി നിരോധന ശേഷി പ്രകടിപ്പിച്ചു. സയനോബാക്ടീരിയൽ ഐസൊലേറ്റുകൾ വെയ്റ്റഡ് ആവറേജ് റാങ്കിംഗിനെ അടിസ്ഥാനമാക്കി *ക്രൂകോക്കസ്* sp. VLY 6 എന്ന ഐസൊലേറ്റിനെ തുടർ പഠനത്തിനായി തിരഞ്ഞെടുത്തു. സസ്യങ്ങളുടെ വളർച്ച ത്വരിതപ്പെടുത്താനുള്ള ഈ ഐസൊലേറ്റിന്റെ കഴിവ് നെൽവിത്തുകളിൽ റോൾ ടവൽ മെത്തേഡ് ഉപയോഗിച്ച് വിലയിരുത്തി. 10 മില്ലി അണുവിമുക്തമായ വെള്ളവും ,5 mg mL^{-1} ട്രിപ്റ്റോഫാൻ ഉപയോഗിച്ച് ഭേദഗതി ചെയ്ത BG-11 മീഡിയയിൽ വളർത്തിയ സയനോബാക്ടീരിയ (40 µL) ഉപയോഗിച്ച് പരിചരിച്ച വിത്തുകൾ കൂടുതൽ മുളച്ചതായി കാണപ്പെട്ടു (96.66 %). ഇതേ പരിചരണത്തിൽ മുളച്ച വിത്തുകളിൽ തണ്ടിന്റെയും (6.83 cm) വേരിന്റെയും (12.23 cm) നീളം, തണ്ടിന്റെ ഈർപ്പരഹിത ഭാരവും (4.62 mg), വേരിന്റെ ഈർപ്പരഹിത ഭാരവും (2.91 mg) ഏറ്റവും

കൂടുതലായി കാണപ്പെട്ടത്. അണുവിമുക്തമായ വെള്ളത്തിൽ പരിചരിച്ച വിത്തുകളിൽ ആണ് തണ്ട് വേര് അനുപാതം കൂടുതലായി കണ്ടു.

ഈ പഠനത്തിനെ ആസ്പദമാക്കി സയനോബാക്ടീരിയൽ ഐസൊലേറ്റുകളിൽ *ഓസ്സിലാറ്റോറിയ* sp. VLY 5 ഉം *ക്രൂകോക്കസ്* sp. VLY 6 ഉം നെൽച്ചെടികളിൽ വളർച്ച ത്വരിതപ്പെടുത്താൻ ഏറ്റവും ഫലപ്രദമാണെന്നു കണ്ടെത്തി. *മൈക്രോസിസ്റ്റിസ്* sp. VLY 3 രോഗഹേതുവായ *സാന്തോമൊണാസ് ഒറൈസെ* എന്ന ബാക്റ്റീരിയയെ നിയന്ത്രിക്കാൻ സഹായിക്കുന്നതായും കണ്ടെത്തി.