AN APPROACH TO UNDERSTAND AND CHARACTERIZE CYANOBACTERIA, CAUSING HARMFUL ALGAL BLOOM AND ITS EFFECT IN FRESH WATER POND AND CONTROL BY SOIL BACTERIUM

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ABSTRACT:

The widespread distribution of cyanobacteria in the aquatic environment is increasing the risk of water pollution caused by cyanotoxins. which possess a serious threat to human health.Biological methods of algal blooms control are environmentally friendly and may provide a cost-effective alternative strategy to physical and chemical method.Hence,in this present study the algicidal effect of soil bacterium was tested, initially the algal populated fresh water was collected then cyanobacterial causing HABs was identified microscopically and confirmed as *Anabaena* and *Microcystis*.Next the isolation and identification of algicidal bacteria from soil was done and it was identified as *Pseudomonas* sps.Then physical and chemical analysis of fresh water pond contaminated with HABs results indicated the changes when compared to control. Then isolated cyanobacterial species was grown in BG-11 selective medium and invitro control efficiency of soil bacterium was tested by inoculating the bacterial culture in the algal medium, along with KMnO4 and rice straw at different concentration and the result indicated the efficiency of soil bacterium in controlling algal growth. Then the effect of cyanotoxin in the fresh water fish *Oreochromis niloticus* (Tilapia) was studied by injecting cyanotoxin (100µl)and the total serum IgM and IgA was analyzed by ELISA test, and the result indicated the strong rise in the Igs level compared to control.

KEYWORDS: cyanobacteria, water pollution, cyanotoxins,biological methods, freshwater fish *Oreochromis niloticus* (Tilapia), *Pseudomonas sps*, rice straw and $KMnO_4$.

INTRODUCTION:

Cyanobacteria (blue-green algae) are a diverse group of bacteria that, in comparison to other bacterial communities, can uniquely perform photosynthesis and modulate the environmental oxygen content. Prolific growth under eutrophic conditions leads to the accumulation of cyanobacterial biomass and the formation of algal blooms in freshwater ecosystems, Freshwater algal blooms mainly comprise one or more of *Aphanizomenon, Cylindrospermopsis,*

Dolichospermum, *Microcystis*, *Nodularia*, *Planktothrix*, and *Trichodesmium*. Which have regulatory impacts on the ecological processes of aquatic ecosystems. The predominant density of cyanotoxigenic species in water bodies leads to the formation of harmful algal blooms (HABs). Microcystins, cylindrospermopsins, anatoxins, and saxitoxins are the most common cyanotoxins produced by harmful algal blooms, and doses as low as parts per billion can induce acute toxicity. Toxigenic health effects of HAB cyanotoxins can manifest directly through ingestion or indirectly through consuming contaminated food products, including fish, mollusks, and agricultural produce.A broad classification of harmful algal blooms distinguishes two groups of organisms: the toxin producers and the high-biomass producers, Some harmful algal blooms have characteristics of both. (Barathan Balaji-Prasath et.al 2022).Identification of blue-green algae When examined with the unaided eye, blue-green algae are non filamentous and often have the appearance of blue or green "scum of paint on or under the surface of the water. Numerous algal species, such as dinoflagellates, flagellates, chrysophytes, and diatoms, can produce toxins that are toxic to humans and other organisms and can result in harmful blooms.

Types of algal toxins

1. Hepatotoxins:

It is known that several strains of *Anabaena flos-aquae*, *Microcystis aeruginosa*, *Microcystis viridis*, and *Oscillatoria agardhii* produce microcystins. Hepatotoxins can enter the body through oral consumption, inhalation, or skin absorption just like other cyanotoxins do.

Types of hepatotoxins are: Microcystins and Nodularins.

2. Neurotoxins:

Anabaena, *Aphanizomenon*, *Oscillatoria*, and *Trichodesmium species* strains are known to produce neurotoxins. When exposure to *Anabaena flos-aquae*, *Aphanizomenon flos-aquae* may cause acute neurotoxicoses, with respiratory failure being the most likely cause of death.It is known that cyanobacteria produce three chemically defined neurotoxins are:

(i). Anatoxin-a (ii). Anatoxin-a(s). (iii). Saxitoxin and Neo saxitoxins

of these, cyanobacteria are the only source of anatoxin-a and anatoxin-a(s), while marine dinoflagellates are the source of saxitoxin and neo saxitoxin.

3. Cytotoxins:

Cylindrospermopsins is a cyanobacterial cytotoxin that is highly toxic via both oral consumption and injection.

4.Irritant Toxins (Lipopolysaccharides):

Lipopolysaccharides are irritant toxins that are found in the outer membrane of Gram- negative bacteria's cell walls, including Cyanobacteria, where they form complexes with proteins and phospholipids.

5.Dermatotoxins:

Toxins causing severe dermatitis in swimmers who came into contact with benthic cyanobacteria. The organisms involved are *Lyngbya, Oscillatoria, and Schizothrix*.

Methods of controlling algal bloom:

Physical Method:

- Most physical methods are slow and expensive, making them difficult to use for massive blooms
- They are utilized mostly as algal bloom emergency measures rather than as a preventative strategy.

Biological Method;

- In Biological methods such as the use of algicidal bacteria have been widely studied in the control of HABs
- They are less harmful to the environment and may be a more cost-effective alternative to physical and chemical solutions.

Chemical Method

- In the chemical approach refers to use of foreign additives such as chemical Reagents.
- Chemical algaecides should be chosen with extreme careful regard for their potential toxicity

Hence, considering the critical impacts of algal blooms and cyanotoxins on fresh water ecosystem , in this present study an attempt has been made to isolate and characterise invitro control efficiency of algicidal bacterial from the soil was evaluated.

MATERIALS AND METHODS:

Collection of water sample:

For the present study, fresh water pond populated with cyanobacteria causing (HAB) was selected near the Tharuvaikulam (Thoothukudi). The water sample was collectedand brought to the lab for further analysis.

Water quality analysis:

The algal populated fresh water sample was given for the following analysis:

(i).**Physical Parameters**: Colour, Odour, pH, Electrical Conductivity, TDS, Total Hardness.

(ii).**ChemicalParameters**:Total Alkalinity, P-Alkalinity, Chloride, Calcium, Magnesium, Sulphate, Silica.

Cultivation of cyanobacteria in the lab:

To examine the growth pattern, morphological identification and controlling method, the cyanobacteria was collected from fresh water was cultivated in the lab under suitable environment.

Sample preparation:

An 50ml of water sample was inoculated in Erlenmeyer flask containing 250 ml of BG-11 algal culture medium and incubated at room temperature under continuous dark and sunlight period for 15-20 days.

Morphological identification of blue green algae:

To identify the cyanobacteria by morphology, microscopic observation was done by spreading isolated culture on glass slide using forceps, culture was covered with glass cover slips and observed under compound microscope.

Isolation of algicide bacteria from soil sample:

The soil sample was collected from the garden soiland serially diluted. The diluted soil sample was spreaded on nutrient agar mediumand the selected isolates were spreaded on cetrimide agar mediumand it was incubated for 48 hours.

Identification of soil bacteria:

The identification of bacteria was performed on the basis of microscopic, macroscopic examination and biochemical tests according to Bergey's manual of determinative bacteriology.

Preparation of bacterial crude bacterial broth for FTIR and GC-MS analysis:

Bacterial isolate was inoculated in an Erlenmeyer flask containing nutrient broth and incubated for 12 -16 hrs. After incubation the cultured containing flask was kept in the rotary shaker at room temperature for 2-3 days, and then broth was centrifuged and the supernatant was collected and added with equal volume of hexane (1:1), hexane added culture broth formed two layers; the solvent layer was separated using separating funnel and stored in sterile vials. Hexane added

culture broth was retained as aqueous extract. Then the organic phase containing the eluted compounds given for FTIR Spectroscopy and GCMS analysis. (Anantha Padmanabhan S et.al.,2016).

Separation of cyanotoxin by column chromatography:

A dry burette was clamped vertically, with a small bit of cotton pressed down to the bottom as a pad. A thick slurry of adsorbent (silica) in a suitable solvent (ethanol) was poured into the open and allowed to settle under gravity until a column of the desired height was formed. The mixture was put into the column with a pipette. The burette stopper was gently opened to allow the solvent to elute through the column until a solution of the mixture remained in the column, covering the top of the packing material. The eluting solvents were then injected and allowed to easily flow through the column. When the eluent percolated through the column, distinct substances in the sample were separated.

Toxin study by HPLC:

To understand and identify the nature of cyanotoxin present in the water sample, the HPLC method was done as follows: (Priscila S. Corrêa et.al., 2021).

study on effect of cyanotoxin in freshwater fish *Oreochromis niloticus* (Tilapia):

The fresh water fish *Oreochromis niloticus* (Tilapia) was caught from fresh water pond in a trap and brought to the lab and maintained in fish tank. After 5 days of acclimatization, all fishes were injected intraperitoneally, with 0.1-0.2ml of previously isolated cyanotoxin sample and kept for observation (1-2 days). Subsequent to this period 2 fishes per treatment wereanesthetized in benzocaine (0.1 g L–1) and blood was drawn for serum isolation. (BillerTakahashi, JD et.al 2014).

Serum collection:

The blood was allowed to coagulate for overnight at 40C. Next the blood was centrifuged by centrifuge tubes at 7000 rpm for 15 mins. The serum (upper clear supernatant layer) was collected and then the serum was transferred to a new sterile tube and it was stored at –20°C for future analysis.

Isolation and purification of fish Igs:

Serum immunoglobulins (Igs) were precipitated with 50% saturated ammonium sulphate (SAS) for 6 hours at 4°C. The precipitate was centrifuged at 9000g for 15 min at 4°C. Then, the

precipitate was suspended in 1 ml of PBS (1x) and dialyzed for 24 hours at 4°C.(Azadeh Yektaseresht et.al.,2018).

Quantitative assay of IgM and IgA by ELISA:

To evaluate the total IgM and IgA in fish serum after the injection of cyanotoxin the sandwich ELISA method was done using (Biomad – Fish serum IgM and IgA ELISA kit).

Study on invitro control of cyanobacteria

Invitro control of cyanobacterial growth by potassium permanganate (kmno4)

In 250ml of cyanobacterial culture flasks 1 and 2 mg/L concentrations of KMNO4 were added separately. Further, an un-inoculated control containing 250 mL of cyanobacteria was also maintained. Then the flasks were placed on shelves for incubation.The control of growth was measured by changing in the colour of the medium and turbidity (S. Dia et.al.,2016).

Invitro control of cyanobacterial growth with kmno4 and pseudomonas culture

In the 250 mL of the cyanobacterial culture flasks 50mL of *pseudomonas* culture and 1mg, 2mg /L of KMNO4 were added separately and the total volume was adjusted to 300 mL and incubated at 260C. Further, an un-inoculated control containing 250 mL of cyanobacteria was also maintained. The control of growth was measured by changing in the colour of the medium and turbidity. (Xiulin Wang et.al., 2005).

Invitro control of cyanobacterial growth with rice straw and pseudomonas culture:

1kg of rice straw was collected and pre sterilized then sprayed with 300ml of *pseudomonas* broth culture and then dried on hot sun for 2-3 days. Then rice straw treated with broth culture was introduced in a conical flask containing cyanobacterial culture. The control of algal growth was measured by changing in the colour of the medium and turbidity. (Rubeena et.al.,2014)

RESULT:

Collection of cyanobacteria from fresh water pond:

The cyanobacterial populated (HABs) water sample was collected from fresh water pond and stored in the lab until further analysis.

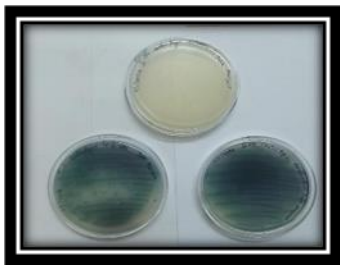
Water quality analysis:

Then physical and chemical analysis of fresh water pond contaminated with HABs was done and results indicated the changes in physical and chemical profile like pH , Odour, Taste, TDS, Calcium, chloride etc compared to control.

Cultivation of cyanobacteria in bg-11 medium and morphological identification:

The cyanobacteria causing HABs were collected from fresh water pond and cultivated in the lab by using BG-11 medium. Next the morphological examination of cultivated cyanobacteria was conducted through microscopic observation which confirmed the presence of *Anabaena sps* and *Microcystis sp.*

Microcystis sp



Isolation and identification of algicidal bacteria from soil sample:

The isolation of algicidal bacteria from garden soil was done using spread plate method, and then based on Bergey's manual classification, microscopic observation, biochemical characterization and cultural characteristics were done and the suspected organism confirmed as *Pseudomonas sp*.

Preparation of crude extract for bacterial metabolite analysis:

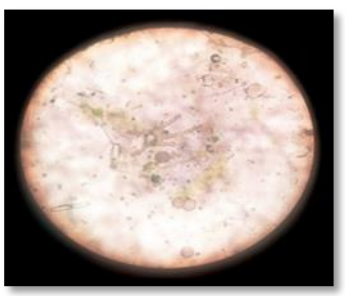
In order to analyze the bacterial metabolites in the crude broth the sample was centrifuged and the supernatant was treated with equal amount of hexane and the organic phase containing eluted compounds were separated.

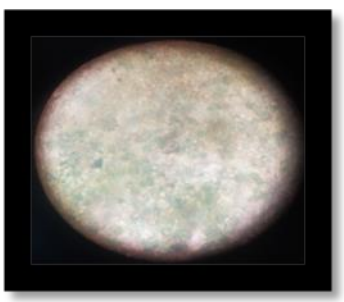
Analysis of bacterial metabolites by FTIR & GC-MS analysis

From the GC-MS and FTIR analysis. The results of analytical technique clearly proved the presence of different compounds.

Cyanotoxin separation by column chromatography and characterization by hplc:

- The separation of cyanotoxin from the water sample was done by column chromatography.
- In (High Performance Liquid Chromatography- HPLC)the results of chromatography confirmed the presence of toxin compounds from the water sample.





Study on effect of cyanotoxin in freshwater fish *Oreochromis niloticus* (Tilapia):

The effect of cyanotoxin in fresh water fish. (*Oreochromis niloticus*) Tilapia was studied, by injecting toxin in to the fish peritoneally and the serum was collected to examine immunoglobulin profiles.

Collection of blood through peritoneal cavitySerum

Quantitative assay of IgM and IgA by ELISA:

Based on ELISA result there was a clear increase in the IgM and IgA antibodies compared to normal one.

ELISA plate with fish serum antibodies (cyanotoxin injected)

Study on invitro control of cyanobacteria:

Finally, the invitro control study of cyanobacteria causing HABs was done using KMNO4 at a different concentration, Rice straw and *Pseudomonas* bacterial broth, the invitro control efficiency was found to be higher in Pseudomonas culture broth followed by KMNO4 and rice straw.

***Pseudomonas* broth and $KMnO_4$ $KMnO_4$rice straw &*Pseudomonas* culture**

CONCLUSION:

Based on the results of above study, it is clearly concluded that metabolite of bacterial culture (*Pseudomonas sps*) isolated from the garden soil, have direct impact in controlling cyanobacteria that causing algal bloom in fresh water, and also it might be used with rice straw and organic chemical like KMNO4 to control the growth in short period of time.So high importance must be given to monitor and control HABs through physical and biological methods which will keep the fresh water bodies useful and problem free.

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