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Study of local BGA for sustainable agriculture

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Abstract
Cyanobacteria are a morphologically diverse group of Gram-negative eubacteria. It is able to perform oxygenic photosynthesis and used as important food for other organisms. Moreover, it is widely found in various locations such as pond, soil, rock, bark, sea andfresh water. Cyanobacteria are several potential benefits to study on bioactive compounds from these organisms. Green Algae constitute an important group of nitrogen fixing Micro-organism. The BGA are unicellular, Colonial and filamentous form present in waterlogged soils. Solapur district is famous for Jowar cultivation and now sugarcane, Pomegranate, and Grapes are also cultivated in large scale and farmers use more fertilizers for same and soil become sterile. Rice is only crop which can cultivated in same land for long period without adding any manure. The present work was carried out to study cynobacteria from solapur region, isolation of bioactive compounds from cynobacteria, analysis of antimicrobial activity, biomass production & Effect of cyanobacterial strain on *Dolichos biflorus* L.

Keywords: Cynobacteria, biomass, antimicrobial, *Dolichos*

Introduction

Cyanobacteria are usually bluish green in color found in water. Cyanobacteria are a morphologically diverse group of Gram-negative eubacteria. It is able to perform oxygenic photosynthesis and used as important food for other organisms. Moreover, it is widely found in various locations such as pond, soil, rock, bark, sea andfresh water. Cyanobacteria are several potential benefits to study on bioactive compounds from these organisms. Cyanobacteria, belonging to the order Chroococcales and families Oscillatoriaceae and Nostocaceae occur ordinarily as planktonic forms. Cyanobacteria in freshwaters have been reported from sea level to high altitudes. They also occupy a variety of terrestrial environments. Soil is one of the most potential habitats for algal growth, particularly in moist or waterlogged conditions

Although antibacterial, antiviral, algaecide, antifungal and cytotoxic activities have been much researched in these organism.Blue Green Algae constitute an important group of nitrogen fixing Micro-organism. The BGA are unicellular, Colonial and filamentous form present in waterlogged soils. Most of the nitrogen fixing BGA belongs to Order Nostocales and Stigonematales. There are more than 100 species of these nitrogen fixing BGA genera.

Solapur district is famous for Jowar cultivation and now sugarcane, Pomegranate, and Grapes are also cultivated in large scale and farmers use more fertilizers for same and soil become sterile. Rice is only crop which can cultivated in same land for long period without adding any manure. Antibiotic resistance in bacteria and fungi is one of the major emerging health care related problems in the world; it became a greater problem of giving treatment against resistant pathogenic bacteria (Sieradzki, *et al*., 1999). One approach to antibiotic resistance is the discovery of novel antimicrobial compounds for clinical application (Desbois *et al*., 2008 and 2009) [1, 2]. Algal organisms are rich source of structurally novel and biologically active secondary and primary metabolites which may be potential bioactive compounds of interest in the pharmaceutical industry. The present work was carried out to study cynobacteria from solapur region, isolation of bioactive compounds from cynobacteria, analysis of antimicrobial activity, biomass production & Effect of cyanobacterial strain on *Dolichos biflorus* L.

Materials and methods

1. Collection of samples

Locality: College Campus, Sambhaji lake, Pakani lake, Hipparga lake, Sidhheshwar Lake.

2. Culturing of samples

The primary aim of studies was to prepare BG 11 medium and purification of Cyanobacteria from D.B.F. Dayanand college Solapur.

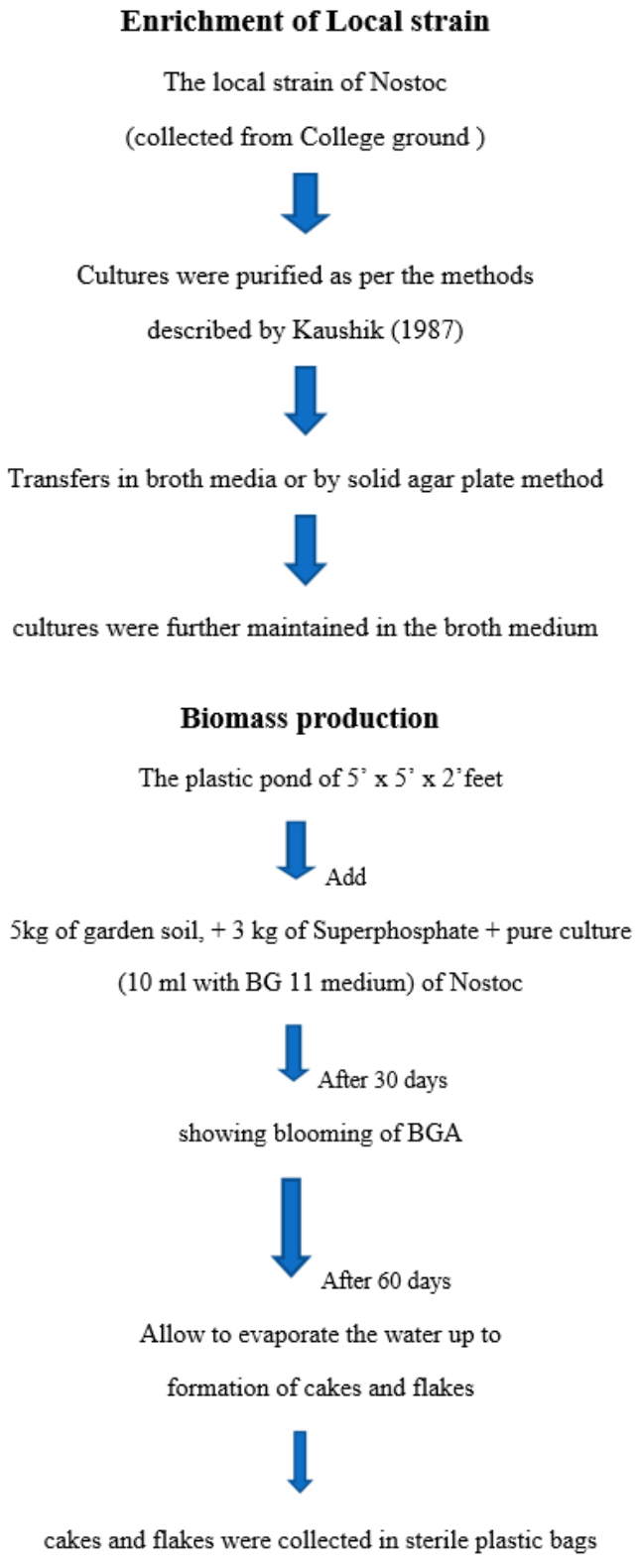
Antimicrobial activity

B. subtilis and *S. aureus* strains was collected from Department of Microbiology

1. Biomass production

1. Water samples were cultured by usual methods.
2. Cyanobacteria were grown in 250 ml conical flasks containing 100 ml of nutrient medium adjusted to pH 7.4.
3. The cultures were grown at 25 ± 2°C and under cool fluorescent lights of 12:12 L: D cycle.

On the basis of morphological characteristics Cyanobacterial cultures were identified at the level of genus and species, level as described by Rippka *et al*. (1979) and Desikachary (1959).



Extract Preparation for antimicrobial activity:

1. Dried powder of *Phormidium* isolated were used for extraction purpose.
2. 100 mg of *Phormidium* was dissolved in 10 ml Ethanol and 10 ml Methanol also.
3. We kept it for 24 hour.
4. In this way Algae Extract is prepared

Nutrient agar media was used

B. subtilis and *S. aureus* strains 10^{-6} dilution was used

Effect of Cyanobacteria on the growth of *Dolichos biflorus* L.

Root and Shoot length

1. The *Dolichos biflorus* plant seeds are treated with 10 ml of *Phormidium*, *Nostoc* and Distilled water.
2. It kept for 24 hours.
3. After 24 hrs these seeds are Sowned in three different pots filled with black soil.
4. After growth of plant we calculated root and shoot length.

Estimation of chlorophyll:-

1. The green leaves of 2 differently treated *Dolichos biflorus* plant is collected, weighted it 0.5gm each respectively.
2. Crushed the leaves by using morter and pestle.
3. We prepared the extract using 80% Acetone and also we added pinch of $MgCO_3$ powder for not loosing the structure of chlorophyll.
4. Then we filtered this extract using muslin cloth.
5. Finally we taken the absorbance reading using Spectrophotometer and measured absorbance at 663 and 645 nm.
6. By using formula we calculated the content of chlorophyll in each plant treated with *Phormidium* and *Nostoc* Cyanobacterial strain

Result & Discussion

1. Biomass production

The biomass production of local strain of Nostoc (BGA) was cultivated by pit method and the Physicochemical Characters of garden soil and BGA containing soil were compared (as shown in Table.)The biomass cultivation of local strain is possible and this method is easy and less expensive to operate by small farmer to increase the fertility of soil.

Table 1

Plant Parameters	Control	Nostoc	Phormidium
Biomass	3.0	4.2	3.8

2. Antimicrobial activity

The methanolic extracts of the five algal species (Nostoc sp., Phormidium sp., Spirulina sp., an Microcystis sp.,) were tested for their antimicrobial activity against one Gram positive (*Bacillus*) and one Gram- negative (*E. colli*). In addition to Ethenolic and Merhenolic respectively. Scendesmus inhibited only B. subtilis and S. aureus with inhibition zones 15 and 16.25 mm, respectively. Nostoc showed the highest inhibition zone (24 mm) against Phormidium sp, followed by 18 and 17 mm inhibition zone with Microcystis sp and Spirulina sp, respectively. Oscillatoria inhibited only S. aureus with 17.5mm inhibition zone and K. Pneumonia with 16 mm. Microcystis affected on S. aureus, and B. subtilis with inhibition zones 18 and 15 mm, respectively. On the other hand acetone extract of Chlorella sp. inhibited the growth of, B. subtilis, S. aureus and, with inhibition zone diameters 20, 18mm respectively, Scenedesmus, Oscillatoria and Nostoc have antibacterial effect on *S. aureus* only with 16, 15 and 14 mm inhibition zone, respectively.

Effect of cyanobacterial strain on *Dolichos biflorus* plant

Pot experiment in laboratory were conducted to evaluate the phytostimulatoery effect of cyanobacterial strains on *Dolichos biflorus*. Seeds, were surface sterilized with 0.1% HgCl2 for 5-7 min. with continuous shaking. The seeds were then rinsed 5 times with sterile water to remove any traces of HgCl2. Seeds were incubated in two strains of cyanobacterial suspension. Water treated seeds were used as control. The pots used in experiment were sterilized by dipping them in 5% Sodium Hypochloriode solution for 20 min, control and inoculated seeds were sown in sterilized pot containing autoclaved soil. The pots kept at 25 C, 60% relative humidity, for 15 days. After 45 days plants were harvested and there growth parameters like Root length, Shoot length and chlorophyll content

Table A: Root and shoot length-

Plant material	Control (D.W)		Phormidium		Nostoc	
	RL	SL	RL	SL	RL	SL
Dolichos biflorus	0.11	0.05	0.9	0.03	0.12	0.09

Table B: Chlorophyll Content

Sr No.	Plant material	Chl.mg/100gm of plant material
1.	Control	6.45
2.	*Dolichos biflorus* (phormidium)	8.47
3.	*Dolichos biflorus* (nostoc)	7.86

Fig 1: Collection sites

Culturing of sample Biomass Production

Fig 2

Fig 3

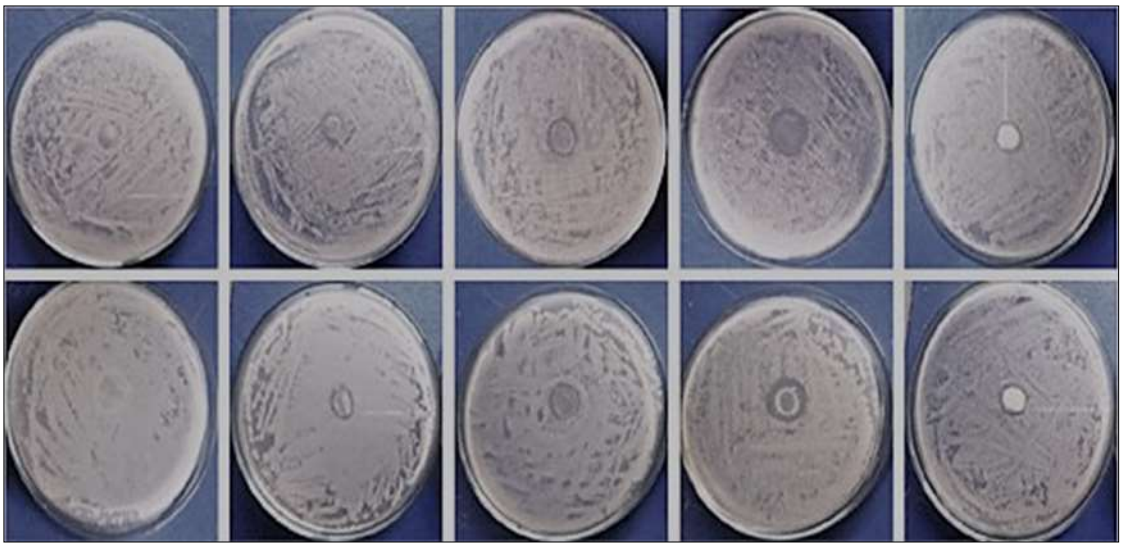


Fig 4: Zone of Inhibition in ehanol & methanol in *B. substilis*

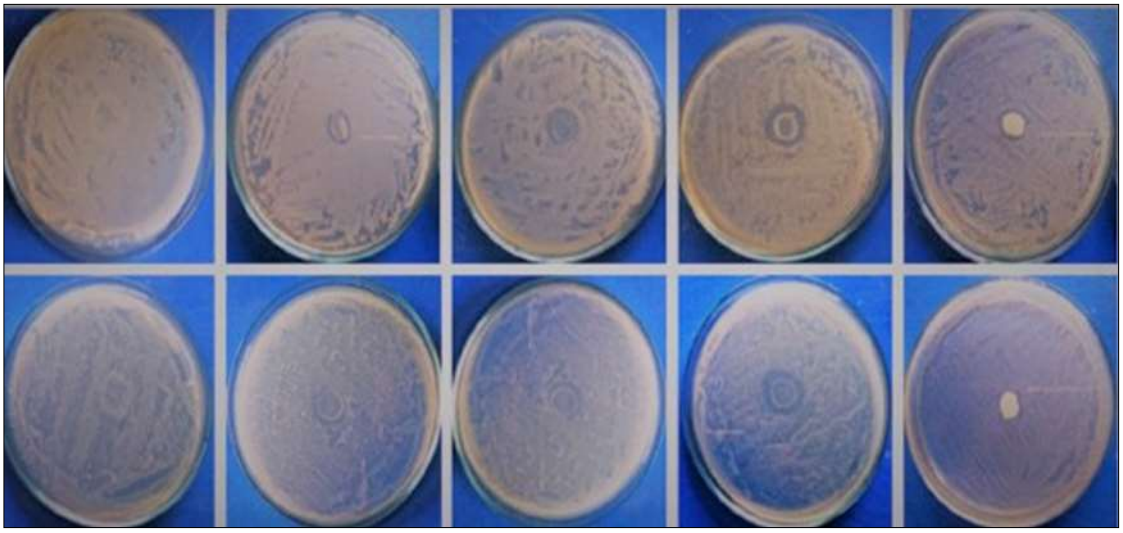


Fig 5: Zone of Inhibition in ethanol & methanol in *S. aurous*

Effect of cyanobacterial strainon *Dolichos biflorus* L. plant

Fig 6: Nostoc treated seedlings

Fig 7: *Phormidium* treated seedlings

Table 2: Physicochemical characters of garden soil and BGA

Parameters	Garden soil	BGA containing soil
pH	5.0	6.0
E.C.	0.7	1.0
W.H.C. %	38	47
Texture	Sandy loam	Sandy loam

Discussion

The present study indicated that the isolation and biomass production of cynobaterial species basically *Nostoc*, *Phormidium*, *Spirulina*, and *Microcystis*. After biomass production antibacterial properties of the five algal species against the selected strains of bacteria vary depending upon the species and the organic solvent used for extraction was evaluated. The data of methanol extract showed that the most sensitive bacteria were *S. aureus* and *B. subtilis*, which inhibited by 4 algal species. The methanol extract showed that S. aureus was the most affected bacteria, inhibited by all algal species, From the previous results we can say that the methanolic extract was more effective against the studied strains, where they inhibited *B. subtilis*, and *S. aureus*. *Dolichous* plant shows more shoot and root growth when treated along with *phormidium* species as well as chlorophyll content of phormidium treated *Dolichous* was more than nostoc treated species. From above paper it was concluded that the cynobacterial species have antimicrobial activity, and found to be effective as a biofertilizers to promote growth & development in plants.

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