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Allelopathic Effect of Cyanobacterial Strains on Phytopathogenic Bacteria.

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ABSTRACT

Allelochemicals are subsets of secondary metabolites that not required for metabolism of the allelopathic organism and their negative allelopathic effects are an important part of organism defense against antagonists. Allelopathic interactions involving Cyanobacterial flora are being explored for their pharmaceutical and environmental significance. Cyanobacterial allelopathy can be regarded as one of the significant factors influencing their dominance in diverse habitats and as unique producers of a variety of allelochemicals that can be utilized as eco-friendly bio-control agents. In present work detrimental (negative allelopathy) effects of locally isolated Cyanobacterial strains were evaluated against plant pathogenic three bacterial isolates (Bacillus sp., Pseudomonas sp., Xanthomonas sp.). It was observed that the crude extracts of four Cyanobacterial isolates (Microcystis aeruginosa, Oscilatoria boryana, Anabaena sphaerica and Nostoc calcicola), were capable of diminishing the growth and further development of phyto-pathogenic bacterial. Whereas M. aeruginosa showed more allelopathic activity compared to other cyanobacterial strains. Methanol crude extracts were more efficient against Pseudomonas sp. (ZOI- 19.9 ±0.23 mm.), Xanthomonas sp. (18.6 ±0.22 mm.) as compared to Bacillus sp. (17.6 ±0.17 mm.). Allelopathic potentiality of cyanobacteria have need to be further investigated that can offer promising solutions in bio-control against pathogenic microorganisms.
Keywords: Allelopathy, Cyanobacteria, Phyto-pathogens, Crude extracts, Bio-control agents.

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INTRODUCTION

An expanding world population and urgency of eradicating hunger and malnutrition call for determined policies and effective actions to ensure sustainable growth in agricultural productivity. India is a largest country in South Asia and contains 70 percent of the total regional population. After Green Revolution enormous progress with modern irrigation and fertilizer application has been made. The concern of pesticide use with respect to human health and environment has brought increasing interest in alternatives by avoiding negative effects on the environment. Secondary metabolites from cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects [1]. Recently algae are one of the chief biological agents that have been studied for the control of plant pathogenic fungi, particularly soil-borne disease [2]. Cyanobacteria and eukaryotic algal produce biologically active compounds that have antifungal and antibacterial activity [3] against plant pathogens. Allelopathy is a unique adaptation to achieve a competitive advantage over other organism inhabiting the same microbial community, Allelopathic impact of Cyanobacteria on pathogenic fungi was also studied [4]. Cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antialgal, antibacterial, antifungal and antiviral activity [5], [6], [7]. The antimicrobial substances involved may target various kinds of microorganisms. Secondary metabolites influence other organisms in the vicinity and are thought to be of phylogenetically important. [3] Stated a number of reasons, cyanobacteria and algae are suitable candidates for exploitation as bio-control agents of plant pathogenic fungi. The potential for using Cyanobacteria is the bio-control of plant pathogenic bacteria and fungi. Screening of cyanobacteria for antibiotics has opened a new horizon for discovering new drugs. Exploring allelopathic efficacy of cyanobacteria to control plant pathogenic microbes can prove to be an excellent option as they are easy to grow with minimum nutrients, cost effective, no side effects and environment friendly.

MATERIALS AND METHODS

Isolation and identification of microbes

For the selection of the experimental materials different species of bacteria and cyanobacteria were isolated applying proper media and methods. Identification of isolated microbes was completed by microscopic observation and biochemical tests.

Cyanobacteria

Soil samples were collected from various agro-fields of Bilaspur division. The samples were brought to laboratory in plastic vials /zipped polythene bag and washed with distilled water to prevent potential contaminants. Isolation of cyanobacterial strain was performed in specific nutrient media (Allen & Arnon, BG-110 and Chu-10) and was stored as suspension culture in growth medium under prescribed condition [8], [9]. Isolated strains were identified by microscopic observation with the help of Key standardized by [10] & [11].

Bacterial strains

The bacterial strains were isolated from agronomic soil samples. They were grown in the laboratory in suitable media, Bacteria were identified on the basis of morphological and biochemical characteristics (i.e. IMViC tests, Catalase test, Glucose fermentation (Glucose, Lactose and Mannitol), Citrate test and starch hydrolysis tests).

Extraction

The bloom samples were collected from various zones of Bilaspur division. The bloom samples were collected using plankton net of 20µm mesh size. Samples were stored in sterile zipped polythene bags. Cyanobacterial cells were concentrated by using centrifugation. A portion of the concentrated samples was filtered through a 0.45 µm glass fiber filter (What's men-41) and air dried in an oven at 60 ^{0}C. Dried cell mass-2g/25ml (w/v) of Cyanobacteria were extracted with 75% methanol (HPLC grade), for one hour then centrifuged at 5000 rpm for 7 min. The supernatant was separated in fresh glass vials and filtered with 0.45 µm pore size. Different Dilutions were prepared for toxicity test *in-vitro*.

Toxicity assessment

For testing the allelopathic activities of crude extract of cyanobacteria against identified bacterial isolates with the help of two different methods and streptomycin and tetracycline as an antibiotic. The following methods had been employed to confer the toxicity of crude extract of different Cyanobacterial isolates on identified bacteria.

Disc diffusion method

Filter paper disk (6-mm) impregnated with a known concentration of an antimicrobial compound is placed on a Nutrient agar plate, immediately water is absorbed into the disk from the agar. The antimicrobial begins to diffuse into the surrounding agar. The size of the zone of inhibition of growth is influenced by the depth of the agar, since the antimicrobial diffuses in three dimensions, thus a shallow layer of agar will produce a larger zone of inhibition than a deeper layer.

Statistical analysis

All the experiments were done in replicates and only the mean data of the obtained results has been incorporated in the tables. Average or mean and SD was calculated with the help of Microsoft Excel software (version 2010).

RESULTS

Through examination of samples total 16 species of cyanobacteria were isolated, out of which nine heterocystous form and seven non-heterocystous forms were identified as nitrogen fixing and non nitrogen fixing strains respectively (TABLE -1, PLATE -1; Fig. – I to XVI).

Table 1: Morphologically dissimilar Cyanobacteria isolated during the survey.

Thallus organization	Non Nitrogen fixing	Nitrogen fixing
Unicellular	1. *Aphanocapsa elachista* 2. *Aphanothece saxicola*	NIL
Colonial	3. *Microcystis aeruginosa*	NIL
Filamentous	4. *Lyngbya birgei* 5. *Lyngbya shackletoni* 6. *Oscillatoria boryana* 7. *Oscillatoria cortiana*	8. *Anabaena oryzae* 9. *Anabaena sphaerica* 10. *Anabaena unispora* 11. *Aulosira prolifica* 12. *Calothrix elenkinii* 13. *Cylindrospermum indicum* 14. *Gloeotrichia echinulata* 15. *Nostoc calcicola* 16. *Nostoc ellipsosporum*

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PLATE - 1: Fig.- I – XVI

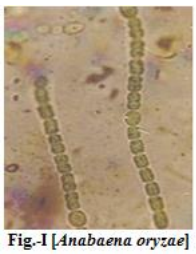
Fig.-I [*Anabaena oryzae*]


Fig.-II [*Aphaenocapsa elachista*]

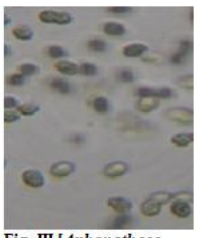
Fig.-III [*Aphanothece saxicola*]


Fig.-IV [*Lyngbya birgei*]

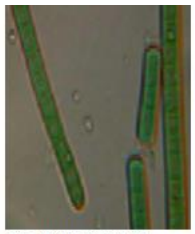
Fig.-V [*Oscillatoria boryana*]


Fig.-VI [*Anabaena sphaerica*]

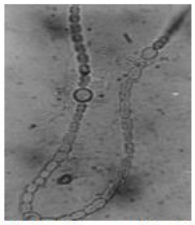
Fig.-VII [*Nostoc calcicola*]

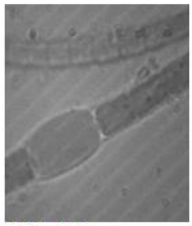
Fig.-VIII [*Cylindrospermum indicum*]


Fig.-IX [*Lyngbya shackletoni*]


Fig.-X [*Microcystis aeruginosa*]

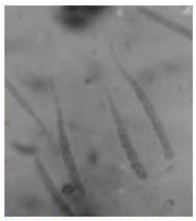
Fig.-XI [*Calothrix elenkinii*]


Fig.-XII [*Anabaena unispora*]

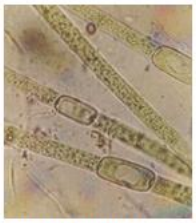
Fig.-XIII [*Aulosira prolifica*]

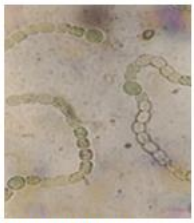
Fig.-XIV [*Nostoc ellipsosporum*]


Fig.-XV [*Oscillatoria cortiana*]


Fig.-XVI [*Gloeotrichia echinulata*]

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Simultaneously five species of bacteria (*Xanthomonas sp., Pseudomonas sp., Bacillus sp., Proteus sp.* and *Staphylococcus sp.)* were isolated that characterized by various biochemical tests (TABLE -2, PLATE -2; Fig. – I to IX) .

PLATE – 2: Fig.- I-IX

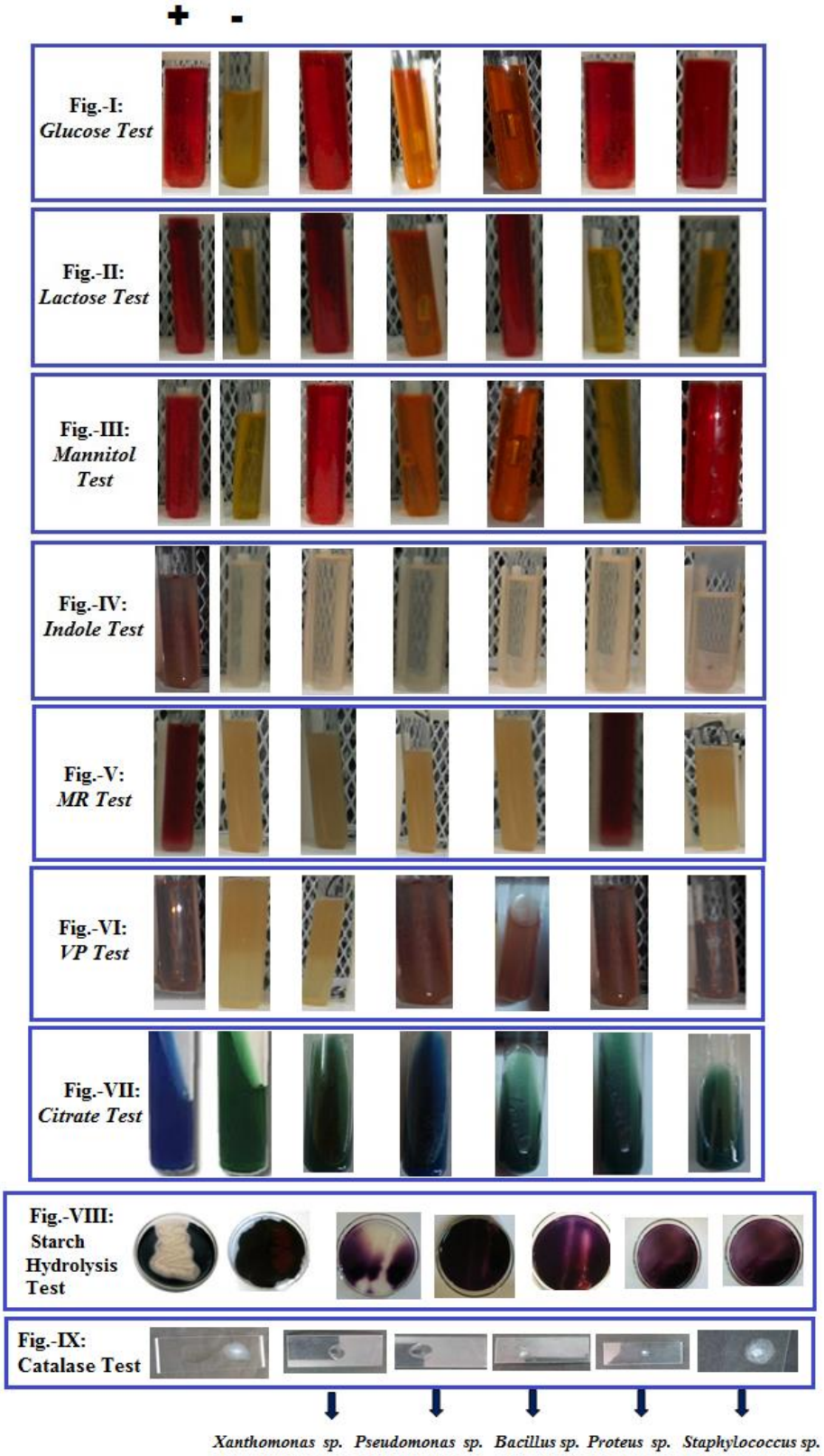

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Table 2: Biochemical characterization of bacterial isolates.

Biochemical tests	*Xanthomonas sp.*	*Pseudomonas sp.*	*Bacillus Sp.*	*Proteus sp.*	*Staphylococcus sp.*
Glucose	**A**	**AG**	**A/G**	**A**	**A**
Lactose	**A**	**AG**	**A**	**-**	**-**
Mannitol	**A**	**AG**	**A/G**	**-**	**A**
Indole	-	-	-	-	-
MR	-	-	-	+	-
VP	-	+	+	+	+
Citrate	-	+	-	-	-
Starch hydrolysis	+	-	-	-	-
Catalase	-	-	+	+	+

The efficacy of Cyanobacterial allelopathy was screened against the growth of *E. coli* (ATCC-25922).The effect of crude extracts of all Cyanobacterial isolates was examined on growth pattern of test strain by measurement of optical density (600nm). And observed data have been mentioned in TABLE–3 and PLATE -3.

Table 3: Screening of allelopathic effects of Cyanobacterial isolates against Bacterial strain *E. coli (ATCC 25922)*

Cyanobacterial Isolates	Growth of *E. coli (ATCC 25922)* (Optical Density at 600 nm)				
	Initial O D	30 min.	60 min.	90 min.	120 min.
Anabaena oryzae	**0.14 ±0.46**	**0.205±0.34**	**0.532 ±0.28**	**0.708 ±0.26**	**0.788 ±0.35**
Aphanocapsa elachista	**0.14 ±0.38**	**0.212 ±0.22**	**0.465 ±0.21**	**0.648±0.21**	**0.753 ±0.36**
Aphanothece saxicola	**0.14 ±0.21**	**0.208 ±0.22**	**0.493 ±0.35**	**0.648 ±0.28**	**0.698 ±0.19**
Lyngbya birgei	**0.14 ±0.36**	**0.211 ±0.36**	**0.402 ±0.35**	**0.532 ±0.42**	**0.618 ±0.35**
Oscillatoria boryana	**0.14 ±0.43**	**0.183 ±0.41**	**0.292 ±0.42**	**0.248 ±0.37**	**0.209 ±0.25**
Anabaena sphaerica	**0.14 ±0.48**	**0.205±0.24**	**0.291 ±0.43**	**0.356 ±0.36**	**0.328 ±0.29**
Nostoc calcicola	**0.14 ±0.35**	**0. 196±0.79**	**0.271 ±0.33**	**0.305 ±0.19**	**0.265 ±0.32**
Cylendrospermum indicum	**0.14 ±0.43**	**0.215 ±0.49**	**0.498 ±0.35**	**0.693 ±0.43**	**0.735 ±0.32**
Lyngbya shackletoni	**0.14 ±0.46**	**0.221 ±0.21**	**0.346 ±0.36**	**0.505 ±0.46**	**0.559 ±0.45**
Microcystis aeruginosa	**0.14 ±0.38**	**0.186 ±0.33**	**0.258 ±0.26**	**0.227±0.23**	**0.198 ±0.34**
Calothrix elenkinii	**0.14 ±0.21**	**0.218 ±0.29**	**0.494 ±0.25**	**0.659 ±0.23**	**0.702 ±0.33**
Anabaena unispora	**0.14 ±0.43**	**0.215 ±0.41**	**0.477 ±0.42**	**0.665 ±0.32**	**0.763 ±0.55**
Aulosira prolific	**0.14 ±0.48**	**0.201 ±0.29**	**0.487 ±0.25**	**0.656 ±0.23**	**0.692 ±0.33**
Nostoc ellipsosporum	**0.14 ±0.38**	**0.212 ±0.41**	**0.453 ±0.42**	**0.652 ±0.32**	**0.736 ±0.55**
Oscillatoria cortiana	**0.14 ±0.21**	**0.203 ±0.42**	**0.324 ±0.55**	**0.415 ±0.41**	**0.365 ±0.35**
Gleotrichia echimulata	**0.14 ±0.23**	**0.208 ±0.23**	**0.442 ±0.38**	**0.667±0.26**	**0.722±0.25**
Without crude extract	**0.14 ±0.18**	**0.252 ± 0.24**	**0.562 ±0.37**	**0.712 ±0.21**	**0.834 ±0.31**

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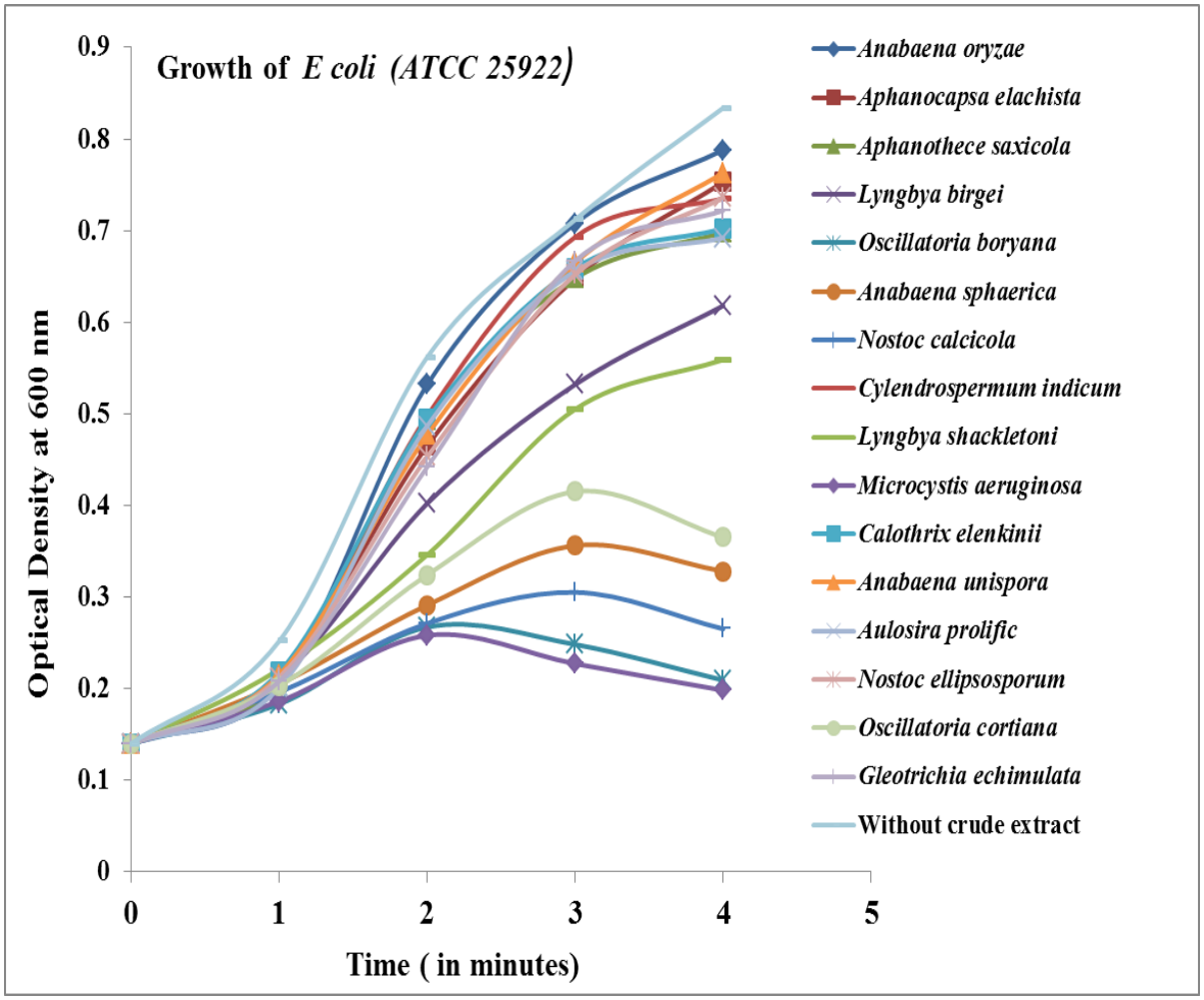

PLATE- 3

On the basis of toxic potentiality of crude extracts two non- heterocystous (*Microcystis aeruginosa* & *Oscillatoria boryana*) and two heterocystous (*Nostoc calcicola & Anabaena sphaerica*) cyanobacterial isolates were selected for further experiment in view of the assessment of the allelopathic effect of cyanobacterial isolates on phytopathogenic Bacterial isolates. To assess the effect of cyanobacterial crude extracts on the growth of phytopathogenic bacteria, three bacterial isolates (*Xanthomonas sp.,* and *Bacillus sp. and Pseudomonas sp.)* were taken as test organisms. Allelopathic effect of cyanobacterial was evaluated through growth inhibition of bacteria by disc diffusion method. The effect of different concentrations (25%, 50%, 75% and 100%) of both Ethanol and Methanol crude extracts of all four cyanobacterial isolates on three bacterial isolates were observed by the measurement of zone of inhibition including the ZOI caused by standard antibiotics and solvent supplemented control plates. Observed inhibition zone in mm (Mean ±SD) have been computed in **TABLE – 4, PLATE -4; Fig. I - IV.**

Table 4: Allelopathic effect of crude extracts of Cyanobacterial isolates on phytopathogenic bacterial isolates (ZOI = Mean ±SD).

Cyanobacterial Crude Extracts and Standard Antibiotics		Concentration	Zone of inhibition in mm (Mean ±SD)		
			Xanthomonas sp.	*Bacillus sp.*	*Pseudomonas sp.*
Anabaena sphaerica	Ethanol	25%	2.8 ±0.18	2.2 ±0.15	3.5 ±0.19
		50%	6.3 ± 0.11	5.3 ±0.20	6.8 ±0.23
		75%	8.7 ±0.20	6.6 ±0.32	9.1 ±0.30

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		100%	11.3 ±0.21	9.8 ±0.28	12.2 ±0.24
	Methanol	25%	4.1 ±0.23	2.5 ±0.27	5.3 ±0.25
		50%	7.2 ±0.19	6.3 ±0.16	7.5 ±0.18
		75%	9.3 ±0.22	7.8 ±0.26	9.8 ±0.30
		100%	12.2 ±0.25	10.2 ± 0.21	13.5 ±0.19
Microcystis aeruginosa	Ethanol	25%	8.2 ±0.23	7.6 ±0.15	8.9 ±0.24
		50%	10.3 ±0.22	8.8 ±0.31	11.5 ±0.30
		75%	12.3 ±0.21	10.9 ±0.12	14.2 ±0.23
		100%	17.2 ±0.27	15.2 ±0.22	18.5 ±0.25
	Methanol	25%	9.6 ±0.23	8.2 ±0.22	10.4 ±0.21
		50%	11.8 ±0.26	9.8 ±0.23	12.7 ±0.25
		75%	13.5 ±0.21	12.6 ±0.25	14.3 ±0.22
		100%	18.6±0.22	17.6 ±0.17	19.9 ±0.23
Nostoc calcicola	Ethanol	25%	3.7 ±0.33	2.8 ±0.15	4.8 ±0.23
		50%	4.9 ±0.28	4.1 ±0.16	6.3 ± 0.12
		75%	7.1 ±0.17	6.8 ±0.12	8.7 ±0.15
		100%	12.5 ±0.23	8.3 ±0.18	13.8 ±0.27
	Methanol	25%	6.9 ±0.25	5.2 ±0.23	7.3 ±0.25
		50%	8.8 ±0.15	7.7 ±0.26	9.8 ±0.33
		75%	10.9 ±0.17	9.5 ±0.22	11.8 ±0.15
		100%	13.7 ±0.22	11.4 ±0.18	14.5 ±0.22
Oscillatoria boryana	Ethanol	25%	7.8 ±0.22	7.1 ±0.18	8.2 ±0.25
		50%	9.9 ±0.23	8.3 ±0.33	10.8 ±0.34
		75%	11.4 ±0.26	9.9 ±0.22	12.8 ±0.25
		100%	15.5 ±0.27	12.8 ±0.22	16.2 ± 0.25
	Methanol	25%	9.2 ±0.26	7.8 ±0.23	9.9 ±0.22
		50%	11.8 ±0.24	9.3 ±0.26	12.2 ±0.23
		75%	12.9 ±0.23	11.3 ±0.27	13.8 ±0.26
		100%	16.7±0.24	14.7 ±0.17	17.8 ±0.22
Solvents	Ethanol	100%	00	00	00
	Methanol	100%	00	00	00
Antibiotics	Streptomycin	30mcg/disc	22.4 ±0.52	21.8 ±0.52	23.4 ±0.35
	Tetracycline	30mcg/disc	26.2 ±0.24	25.7 ±0.73	27.5 ±0.51

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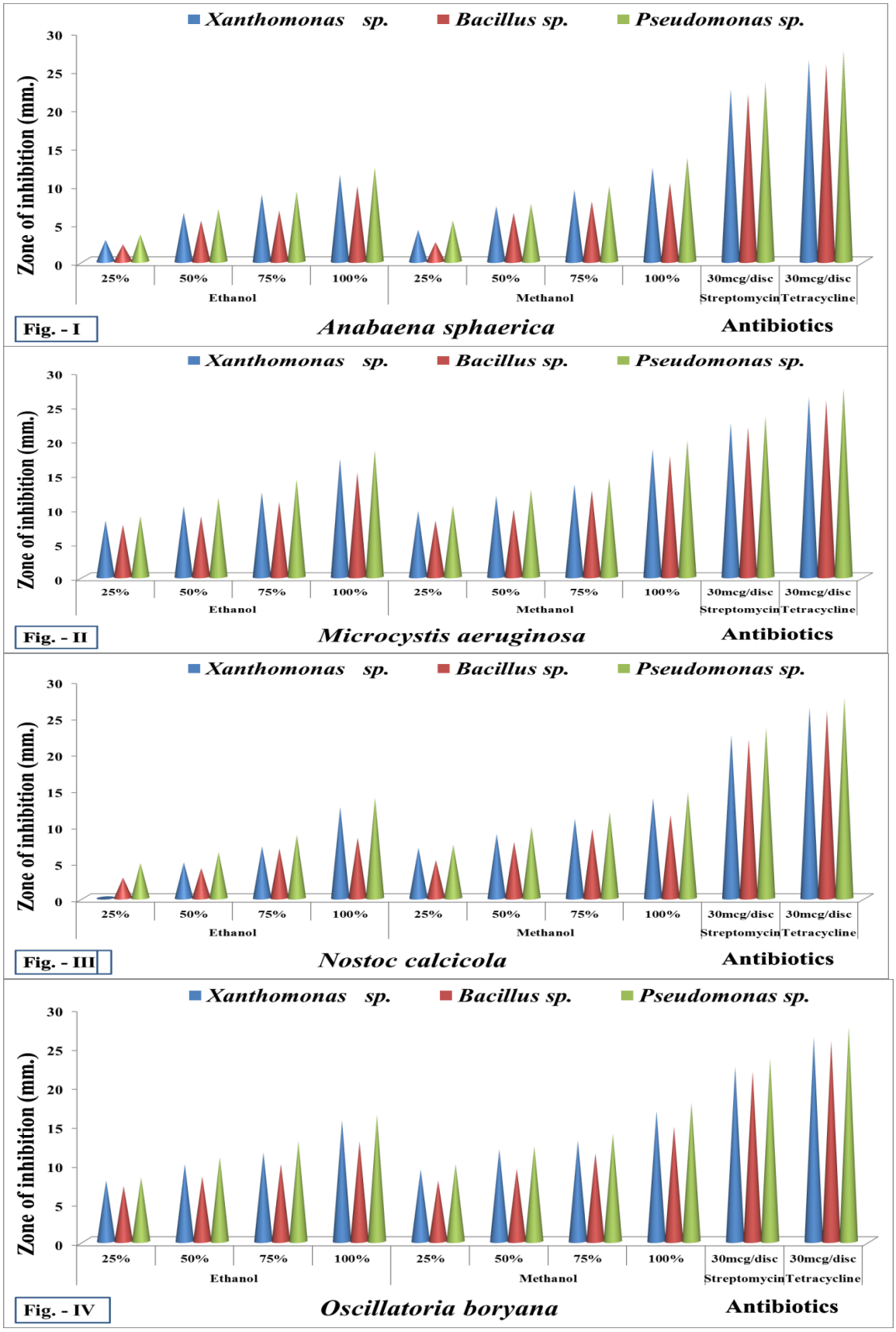

PLATE- 4: Fig I – IV

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DISCUSSION

Regarding Cyanobacterial toxicity, the results obtained during present course of investigation is revealed that the growth of bacterial isolates were inhibited whenever bacteria were subjected to crude extracts (Ethanol or Methanol) on solid nutrient media. Growth inhibition potency of crude extracts of cyanobacterial isolates have also been compared with standard antibiotic against bacterial growth. Findings of allelopathic study against pathogenic bacteria revealed that they had considerable and significant impact on phytopathogenic bacteria. Cyanobacteria produce biologically active compounds that have allelopathic and toxic activity against plant pathogens [12], [13]. [14] Reported the inhibition of *F. oxysporum* f. *sp*. *lycopersici* by extracts of *N. commune* FA-103. [5] Investigated that the suppression effect of cyanobacterial species- *Nostoc endophytum* and *Nostoc muscurum* against, the causal agent of soyabean root rot *Rhizoctonia solani.*

The present research work correlates the findings of various workers as reported earlier regarding concerned research work. In this study Cynobacteria showed significant allelopathic activities. This kind of investigation produce a much generalized view that cynobacteria are capable of inhibiting phytopathogens. This work focuses on the ability of Cyanobacteria to be used as allelopathic biocontrol agent further studies on Cyanobacterial metabolites are essential for scientific workers to identify the toxic impact on phytopathogenic bacteria which may lead to the formulation of significant bio-active compounds of biological origin.

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