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Evaluation of Cyanobacterial toxicity against corresponding field-soil Bacteria

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Abstract

Toxic property of locally isolated Cyanobacterial strains were evaluated against soil bacteria, isolated from respective rice fields. Cyanobacterial samples, locating visually either conspicuous growth on soil surface or floated /submerged inlogged water, were collected, identified and isolated as an axenic culture. In the meantime soil samples from concerned fields were also collected for isolation of bacteria. Using recommended media, bacterial strains were isolated and characterized biochemically. Crude extracts of three Cyanobacterial strains (*Oscillatoria curviceps, Lyngbya shackletoni. and Anabaena unispora*) were taken for assessment of its antibacterial activity against Gram +ve and Gram –ve bacterial isolates i.e. *Staphylococcus sp. Xanthomonas sp. Bacillus sp., proteus sp. and Klebsiella sp*. respectively. Maximum growth-inhibition of bacterial isolates was found by crude extract of *Oscillatoria curviceps* rather *Lyngbya shackletoni* and *Anabaena unispora* correspondingly. Observation of present investigation reveals that exotoxins released by Cyanobacterial strains exhibit antibacterial property that may affect to microbial community of agriculture fields and ultimately to the nature/ quality of soil.

Key-Words: Cyanbacterial strains, Rice field's soil, Soil Bacteria, Antibacterial property

Introduction

Soil diversity reflects the variability among inhabiting microorganisms that constitute an important resource for sustainable agricultural systems. India has a large and diverse agriculture system with significant soil diversity. The newly formed and developing state of Chhattisgarh in central India is known for agriculture and forest recourses comprise variability in soils. Other than heterotrophic micro-flora, autotrophic microorganisms, i.e. Cyanobacteria are also an important micro-biotic component of soil mostly available in waterlogged or moist fields. Besides its popular properties of nitrogen fixation, most of the Cyanobacterial strains releases various forms of exotoxins, as secondary metabolites, generally quoted as Cyanotoxins, which have a considerable properties of inhibition the growth and development of soil bacteria.

As a chief component of microbial community in rice paddy fields, Cyanobacteria contribute to the fertility of agricultural ecosystems [1] and produce a potential source of biological active secondary metabolites, compounds that are not essential for cell metabolism [2].

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Secondary metabolites of cyanobacteria are associated with toxic, hormonal, antineoplastic and antimicrobial effects [3], [4]. Cyanobacteria have been reported as an important agent for control of various pathogenic microorganisms [5], [6], [7]. Some potential applications to consider for cyanobacteria are the production of antimicrobial compounds for the pharmaceutical industry and the agricultural sector as both bio-fertilizers and bio-control agents [8]. So many workers investigated Cyanobacteria as a source of antimicrobial Compounds [9], [10], [11], [12] and they have compared to antimicrobial drugs used against plant pathogens, produce antimicrobial metabolites which were safe for environment. Allelopathic effects of Cyanobacterial inoculums on soil characteristics and cereal growth have been investigated [13]. In order to find the potential of cyanobacteria for production of antibacterial compounds in rice-fields, cyanobacterial strains were isolated and their toxic potency were evaluated during present investigation and the findings are presented in this paper.

Material and Methods

Before For present investigation, the area of various agronomic fields was determined, that involved specially paddy fields. The Cyanobacterial samples showing visually conspicuous growth on soil surface were collected from August month to January

month regularly from various waterlogged paddy fields. Simultaneously soil samples were also collected from November to April month periodically. Samples were collected in sterilized zipped polythene bags. Cyanobacterial strains were identified after microscopic observation with the help of key given by [14], [15]. They were then cloned as laboratory culture intensively under aseptic condition at proper light & aeration using synthetic media i.e. BG-11 [16] by proper laboratory methods. Dense populated pure cultures of Cyanobacteria were taken for extraction and toxicity assessment, Cyanobacterial cells were concentrated by using continuous centrifugation.A portion of the concentrated samples were filtered through a 0.45 µm glass fiber filter (What men-41) and air dried in an oven at 600^0C. Dried cell mass - 100mg / 100ml (w/v) were extracted with methanol, for 3 to 4 hours; then centrifuged at 5000 rpm for 7min. The supernatant was separated in fresh glass vials and filtered with 45 µm pore size. Different Dilutions (25%, 50%, 75%, 100 %) were prepared for in vitro cyto-toxicity test.

Bacterial strains were isolated applying in vitro culture technique by using selective media. Pure culture was obtained by streak plate method. Isolated bacterial samples were identified and characterized biochemically. For assessment of toxicity of three cyanobacterial strains releasing exo-toxins against five bacterial isolates well diffusion method was applied and zone of inhibition in different concentrations (25%, 50%, 75% & 100%), were measured properly that was compared with the zone of inhibition in control set supplemented with antibiotics (Commercially standard and available in the market). Growth pattern of Bacterial isolates (*Staphylococcus sp., Xanthomonas sp., Bacillus sp., Proteus sp.* and *Klebsiella sp.*) treated with crude extracts of cyanobacterial strains (*Anabaena unispora, Lyngbya shackletoni, Oscillatoria curviceps*), were studied by spectrophotometric method, optical density of each bacteria were taken at 600nm half hourly.

Results and Discussion

During present investigation more than a dozen Cyanobacterial samples were collected and identified out of which three cyanobacterial strains **(*Anabaena unispora, Lyngbya shackletoni, Oscillatoria curviceps*)** have been used for toxicity assessment against five bacterial sp. isolated from the same agricultural fields. Bacterial isolates have been identified & characterized on the basis of biochemical characteristics as mentioned in table-1.toxic behaviour of three cyanobacterial strains against five bacterial isolates have been observed on culture plates and zone of inhibition was measured in triplicate set, which mean value with ± SD have been tabulated (Table-2), simultaneously the toxicity was also observed in broth of bacterial isolates, meanwhile the observed optical density at 600nm have been incorporated in Table-3 and presented graphically in Fig-2 (a - e).

Experimental result of present investigation clarifies the toxicity of each Cyanobacterium against all five bacterial isolates in both methods i. e. zone of inhibition and growth pattern observation. Zone of inhibition was found maximum in $Gram^{-ve}$ bacteria ***(Xanthomonas sp., Proteus sp.*** and ***Klebsiella sp.)*** that are more or less closer to standard antibiotics and minimum in $Gram^{+ve}$ bacteria **(*Staphylococcus sp*** and ***Bacillus sp.)*** that is lesser than standard antibiotics, whereas crude extract of ***Oscillatoria curviceps*** was observed more toxic rather ***Lyngbya shackletoni*** and ***Anabaena unispora*** correspondingly. More or less similar results have been observed in growth pattern of all five bacterial isolates (Fig.-2a - e).

Conclusion

Findings of present investigation reveals that secondary metabolites of Cyanobacteria can be used as biological material with potential application in agriculture. The result obtained in the present investigation was based on crude extracts, however suitable bacterial bioassays have been established to recognize and quantify antibacterial effect of Cyanobacterial extracts. During present course of investigation it was noticed that non heterocystous *Oscillatoria sp.* and *Lyngbya sp.* were more toxic than that of *Anabaena sp.* that was nitrogen fixing heterocystous strains. Cyanotoxins have the capacity to alter the native soil bacteria. It was concluded that there is a need to develop suitable cyanobacterial strains that improve crop growth and control disease in a cost effective, environmental-friendly manner further studies have to be made on fractionation and separation of crude extract in order to find out the principle antibacterial compounds.

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Table 1: Biochemical characterization of bacterial isolates

Biochemical Tests	*Staphylococcus sp.*	*Xanthomonas sp.*	*Bacillus sp.*	*Proteus sp.*	*Klebsiella sp.*
Catalase	+	-	+	+	+
Glucose	**A**	**A**	**AG**	**A**	**A**
Lactose	-	**A**	**A**	-	-
Mannitol	**A**	**A**	**AG**	-	**A**
Indole	-	-	-	-	-
MR	-	-	-	+	-
Vp	+	-	+	+	+
Citrate	-	-	-	-	+
Starch	-	+	-	-	+

A – Acid, G – Gas)

Table 2: Toxic behaviour of crude extracts of Cyanobacterial isolates against native soil bacterial isolates and its comparison with standard antibiotics

Crude Extracts and Standard Antibiotics	Conc. of Extracts	Zone of inhibition in mm. (Mean ±SD)				
		Staphylococcus Sp.	*Xanthomonas Sp.*	*Bacillus Sp.*	*Proteus Sp.*	*KlebsiellaSp.*
Control	0%	00	00	00	00	00
CE	25%	00	00	1.2 ±0.53	00	00
Anabaena unispora	50%	2.7 ± 0.62	3.8 ±0.21	2.6 ± 0.37	3.9 ± 0.21	3.9 ± 0.37
	75%	4.2 ±0.33	4.5 ±0.38	4.0 ± 0.28	4.7± 0.33	4.8 ± 0.33
	100%	5.3 ±0.33	6.2 ±0.62	5.6 ± 0.33	5.9 ± 0.62	5.9 ± 0.42
Lyngbya shackletoni	25%	3.0 ±0.53	3.6 ±0.41	2.8 ±0.53	3. ±0.41	3.8 ±0.52
	50%	4.1 ±0.28	5.9 ±0.33	4.5 ±0.33	5.2 ± 0.33	5.8 ±0.21
	75%	5.1 ±0.39	6.4 ±0.26	4.7 ± 0.33	6.7 ± 0.26	6.8 ±0.38
	100%	6.2 ±0.62	7.2 ±0.46	6.5 ± 0.33	7.2 ± 0.46	8.6± 0.62
Oscillatoria Curviceps	25%	7.70 ±0.57	8.00 ±0.62	5.3 ± 0.43	8.20 ± 0.47	8.5± 0.33
	50%	10.3 ±0.46	12.50 ±0.29	9.7 ±0.36	12.8 ± 0.29	13.00 ±0.57
	75%	15.2 ±0.28	16.6 ±0.67	14.1 ± 2.9	16.6 ± 0.67	17.8 ± 0.28
	100%	18.4 ±0.13	20.0 ±0.35	17.5 ± 0.33	18.8 ± 0.83	19.6 ±0.47
AB Gentamycin	1mg/ml	19.0 ±0.37	23.40 ±0.35	16.0 ± 0.72	22.8 ± 0.37	24.6 ± 0.47
Chloremphenicol	1mg/ml	20.2 ±0.37	26.7 ±0.30	18.6 ± 0.47	26.7 ± 0.30	26.8 ± 0.37

Table 3: Growth of Bacterial isolates (Staphylococcus *sp.*, *Xanthomonas sp.*, *Bacillus sp.*, *Proteus sp.*, *Klebsiella* sp.) untreated and treated with crude extracts of Cyanobacterial strains (*Anabaena unispora, Lyngbya shackletoni , Oscillatoria curviceps*) and1mg/ml antibiotics

Growth Period (Hours)	Crude Extracts and Standard Antibiotics		Growth of Bacteria (Optical Density at 600nm)				
			Staphylococcus Sp.	*Xanthomonas Sp.*	*Bacillus Sp.*	*Proteus Sp.*	*KlebsiellaSp.*
0	**Initial OD**		0.042±0.023	0.060±0.189	0.053±0.045	0.045±0.023	0.058±0.067
0.5	**Control**	00	0.110±0.025	0.132±0.134	0.112±0.167	0.115±0.034	0.136±0.078
	Anabaena unispora	0.5ml/ml	0.063±0.065	0.068±0.023	0.075±0.064	0.067±0.056	0.074±0.056
	Lyngbya shackletoni	0.5ml/ml	0.060±0.053	0.063±0.087	0.056±0.042	0.062±0.023	0.060±0.034
	Oscillatoria curviceps	0.5ml/ml	0.058±0.042	0.054±0.056	0.049±0.027	0.050±0.087	0.052±0.065
	Gentamycin	1mg/ml	0.053±0.028	0.050±0.087	0.042±0.018	0.041±0.347	0.048±0.067
	Chloremphenicol	1mg/ml	0.054±0.034	0.048±0.037	0.045±0.025	0.043±0.430	0.050±0.985
01	**Control**	00	0.192±0.012	0.226±0.187	0.188±0.048	0.220±0.518	0.268±0.012
	Anabaena unispora	0.5ml/ml	0.123±0.143	0.141±0.084	0.135±0.087	0.145±0.452	0.167±0.054
	Lyngbya shackletoni	0.5ml/ml	0.112±0.031	0.135±0.021	0.128±0.056	0.137±0.234	0.135±0.034
	Oscillatoria curviceps	0.5ml/ml	0.088±0.028	0.110±0.026	0.097±0.065	0.112±0.546	0.086±0.037
	Gentamycin	1mg/ml	0.082±0.087	0.098±0.015	0.086±0.063	0.087±0.569	0.090±0.456
	Chloremphenicol	1mg/ml	0.083±0.045	0.092±0.049	0.078±0.081	0.080±0.167	0.092±0.764
1.5	**Control**	00	0.281±0.063	0.314±0.178	0.252±0.012	0.368±0.034	0.337±0.713
	Anabaena	0.5ml/ml	0.185±0.086	0.296±0.127	0.194±0.028	0.225±0.054	0.256±0.912

	unispora						
	Lyngbya shackletoni	0.5ml/ml	0.163±0.129	0.267±0.082	0.167±0.071	0.216±0.076	0.202±0.532
	Oscillatoria curviceps	0.5ml/ml	0.133±0.016	0.132±0.176	0.138±0.092	0.128±0.321	0.125±0.712
	Gentamycin	1mg/ml	0.111±0.086	0.125±0.066	0.120±0.022	0.089±0.065	0.076±0.654
	Chloremphenicol	1mg/ml	0.119±0.027	0.128±0.065	0.115±0.035	0.090±0.023	0.080±0.065
2.0	**Control**	00	0.367±0.085	0.398±0.138	0.300±0.074	0.402±0.034	0.444±0.087
	Anabaena unispora	0.5ml/ml	0.214±0.022	0.372±0.063	0.219±0.036	0.378±0.065	0.378±0.034
	Lyngbya shackletoni	0.5ml/ml	0.192±0.053	0.203±0.045	0.200±0.046	0.213±0.065	0.225±0.027
	Oscillatoria curviceps	0.5ml/ml	0.154±0.028	0.156±0.092	0.132±0.028	0.149±0.098	0.138±0.086
	Gentamycin	1mg/ml	0.122±0.189	0.098±0.058	0.110±0.037	0.116±0.034	0.092±0.076
	Chloremphenicol	1mg/ml	0.130±0.097	0.085±0.035	0.089±0.048	0.094±0.233	0.101±0.087
2.5	**Control**	00	0.423±0.076	0.488±0.128	0.458±0.154	0.517±0.760	0.623±0.048
	Anabaena unispora	0.5ml/ml	0.221±0.027	0.378±0.067	0.267±0.176	0.421±0271	0.487±0.043
	Lyngbya shackletoni	0.5ml/ml	0.213±0.043	0.225±0.054	0.214±0.312	0.267±0.023	0.256±0.021
	Oscillatoria curviceps	0.5ml/ml	0.135±0.038	0.189±0.044	0.128±0.654	0.118±0.016	0.121±0.035
	Gentamycin	1mg/ml	0.125±0.093	0.110±0.072	0.126±0.096	0.088±0.049	0.121±0.036
	Chloremphenicol	1mg/ml	0.128±0.081	0.112±0.027	0.127±0.074	0.085±0.028	0.112±0.065
3.0	**Control**	00	0.425±0.032	0.378±0.065	0.460±0.035	0.520±0.068	0.623±0.033
	Anabaena unispora	0.5ml/ml	0.224±0.028	0.218±0.054	0.255±0.096	0.426±0.015	0.438±0.022
	Lyngbya shackletoni	0.5ml/ml	0.211±0.029	0.165±0.028	0.199±0.56	0.235±0.026	0.242±0.096
	Oscillatoria curviceps	0.5ml/ml	0.150±0.035	0.109±0.026	0.111±0.065	0.123±0.054	0.120±0.032
	Gentamycin	1mg/ml	0.079±0.038	0.098±0.056	0.065±0.027	0.086±0.028	0.112±0.067
	Chloremphenicol	1mg/ml	0.056±0.054	0.063±0.037	0.060±0.042	0.079±0.065	0.099±0.042

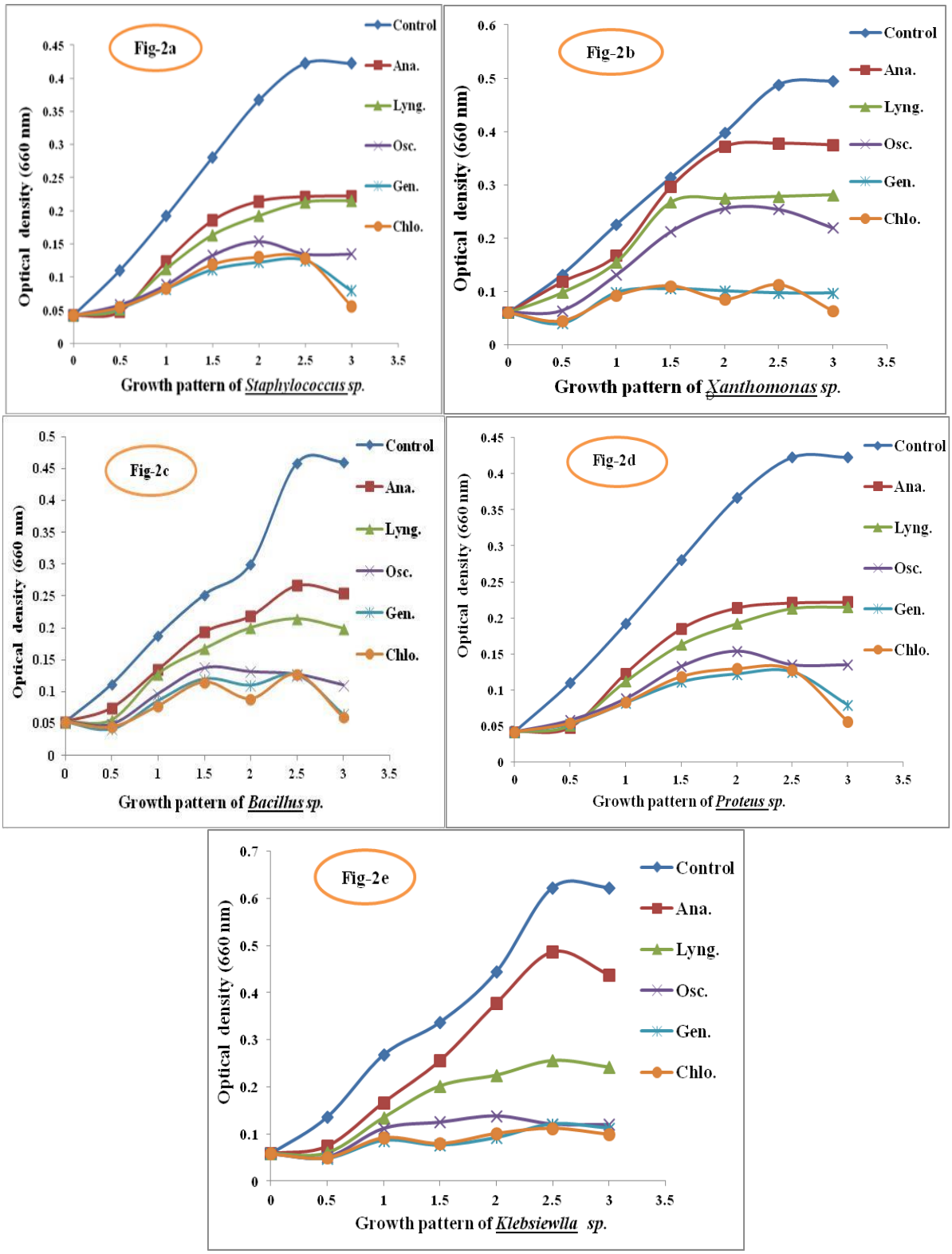

Fig. 2(a- e): Bacterial growth inhibitory effect of Cyanobacterial crude extracts

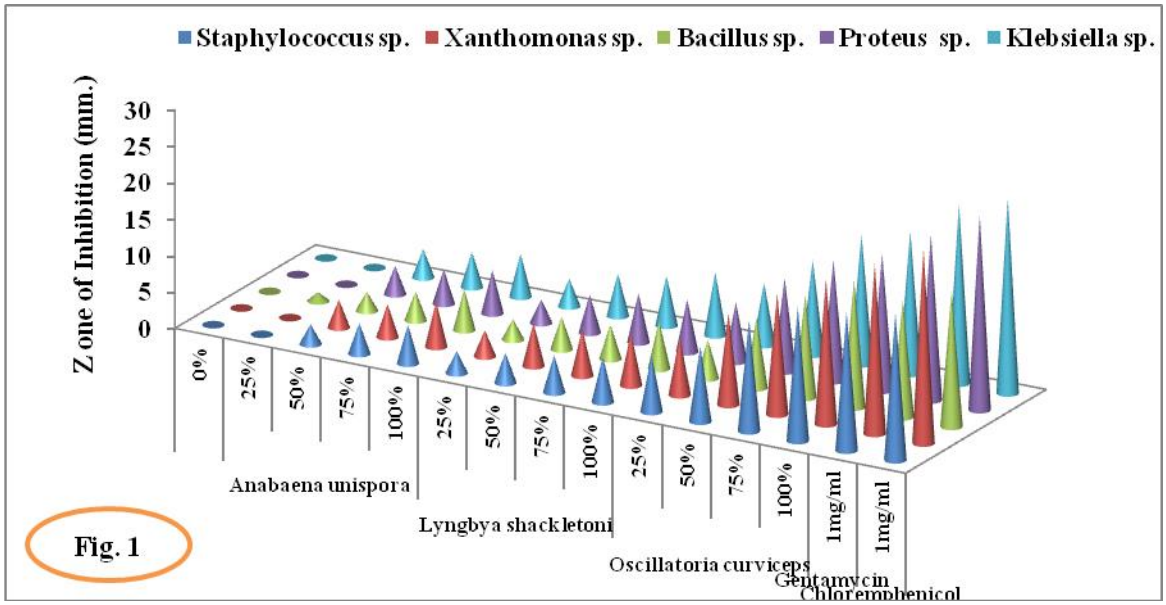

Fig. 1: Toxic behavior of crude extracts of Cyanobacterial isolates against native soil bacterial isolates and its comparison with standard antibiotics

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