International Journal of Plant & Soil Science

34(22): 485-494, 2022; Article no.IJPSS.89239
ISSN: 2320-7035

Plant Growth-promoting and Biocontrol Potential of Selected Cyanobacteria from Stress Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2022/v34i2231400

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/89239

Original Research Article

Received 01 June 2022
Accepted 05 August 2022
Published 09 August 2022

ABSTRACT

Cyanobacteria are photosynthetic microorganisms and produce large number of diverse bioactive compounds. In the present study plant growth promotion and antifungal activities of fourteen cyanobacteria strains have been studied against selected phytopathogens. Among screened isolates highest production of IAA was found in *Chroococcidiopsis cubana* HC1 (102.9 µg/ml) when supplemented with 0.3 mg/mL tryptophan. Co-inoculation of *C. cubana* (HC1) and *Tolypothrix* sp. (RC1) were evaluated for plant growth promoting potential on rice (Rajendra Sweta) plant which showed enhanced root (20.7±1.0 cm) and shoot length (11.4±1.3 cm) as well as increased number of forks (36.0±1.0) as compared to control seedlings. Among fourteen isolates eight cyanobacterial isolates showed zone of inhibition against selected phytopathogens. *Tolypothrix* sp RC1 and *Nostoc* sp. SK1 showed highest rate of inhibition (50%) against *Ralstonia solanaceram* while *Pseudoanabaena* sp. RD1 showed highest inhibition rate (52%) against *Sclerotium rolfsi*. *Nostoc* sp. HC2 found to be highest inhibition (61%) against *Fusarium oxysporum*. The cyanobacteria reported in this study have immense potential for biocontrol against fungal plant pathogens and plant growth promoting ability which

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enhances crop growth. Cyanobacterial isolates obtained from the study could be worthy in sustainable agriculture due to dual advantages of biocontrol as well as plant growth promotion.

Keywords: Antifungal; cyanobacteria; plant growth promotion; biocontrol; phytopathogens; stress.

1. INTRODUCTION

"Use of chemical fertilizers and fungicides to control plant diseases increasing daily and need to think about climate resilient approach for mitigating these problems. Cyanobacteria are photosynthetic microorganisms involved in global oxygen supply and primary production of biomass in aquatic ecosystem. Cyanobacteria have the ability to fix atmospheric nitrogen. They are important components of rice ecosystem and known to fix 20-25 kg N /ha/season" [1] and important organisms which help in carbon dioxide (CO_2) sequestration [2]. "Cyanobacteria have a wider adaptability and found in diverse ecological niches". Bagul et al. [3] reported "different types of heterocystous and non heterocystous cyanobacteria from diverse ecological niches of India, including hot water spring of Odisha, cold regions of Leh and Uttarakhand, marine water from Odisha and arsenic contaminated field of Ballia, Uttar Pradesh. Over several years cyanobacteria have been utilized as biofertilizer in rice crop for nitrogen fixation along with plant growth promoting activities. In general, extraction of bioactive compounds from cyanobacteria is performed to discover new compounds for pharmaceutical, agricultural or biological application, also for the better understanding of the interactions of these organisms within ecosystem" [4]. "Phytohormones such as IAA, IBA, gibberellins, cytokinin, absisic acid and jasmonic acid have been reported to produce among cyanobacteria" [5]. "Many reports have shown production of plant growth promoting hormones such as IAA, IBA, cytokinin by cyanobacteria. Cyanobacteria could produce induced systemic resistance by producing diverse range of biologically active molecules in the rhizosphere which elicit the plant growth under different stresses" [6,7,8]. "Plant pathogens include fungi, nematodes, bacteria and viruses which can cause damages up to various levels. Among these pathogens plant pathogenic fungi are responsible for pre and post harvest diseases including yield losses in numerous economically important crops. *Ralstonia solanacearum* an economically important pathogen with a wide host range and distributed geographically in the large number of areas" [9]. "Cyanobacteria could protect plant by providing mechanical and physical strength of the cell wall. Physiochemical reactions are altered by producing defence related chemicals against the phytopathogens. Major defence enzymes involved in plant growth are chitinase, phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PO) phenolics and phytoalexins" [10]. Prasanna et al. [11] showed "enhanced production of defence enzymes 189% and 239% of PAL and PPO respectively, resulting in increasing of plant growth parameters and bioprotection against *Fusarium* wilt of tomato. Antifungal compounds heneicosane, palmitoleic acid, 2,4-di-tert-butylphenol, dibutyl phthalate, heptadecane, Tetradecane, 7-methylheptadecane, octadecanoic acid, nitrocyclohexane2-hexyl-1, boronic acid, cyclohexylethyl amine and methyl palmitate from cyanobacteria has been reported by various researchers" [9,12,13,4]. Spirulina extract showed antifungal activity by significantly reducing glucosamine production (56%) from *Aspergillus flavus* [14]. Essential metabolic reactions required for reproduction and growth of fungi has been shown to inhibit by changes in the enzyme nature inside the cell. Phenolic compound produced by cyanobacteria contains free hydroxy radical groups which forms hydrogen bonds with carbohydrates and proteins found in the fungal cell resulting in the inhibition of pathogen. Present study deals with the evaluation of cyanobacteria isolated from stressed environment for plant growth promoting potential and antifungal activity against selected phytopathogen.

2. MATERIALS AND METHODS

2.1 Organisms and Cultivation

Fourteen cyanobacterial cultures isolated and identified in previous study [3]. Among the cyanobacteria heterocystous and non heterocytous group have been selected for screening of IAA production and antifungal activity against plant pathogens. Pure cyanobacterial cultures were deposited at NAIMCC, Mau, Uttar Pradesh, India (Table 1)

and maintained under controlled light and temperature conditions in BG 11 medium with or without nitrogen source under 50-55 µmol photons m^{-2} s^{-1} light intensity, 27 ± 1°C temperature and 16:8 light and dark cycle [3].

2.2 Indol Acetic Acid Production

Cyanobacterial isolates were inoculated in BG11 medium supplemented with 300 µg/mL tryptophan. Cultures without tryptophan served as control. 15 days old cultures were harvested and centrifuged at 8000 g for 10 minutes. Supernatant was collected and filter sterilized with 0.45µm syringe filter (Axiva). Two mL of supernatant mixed with 4 mL of the Salkowski reagent (50 ml, 35% of perchloric acid, 1 mL 0.5 M $FeCl_3$ solution). The amount of IAA was quantified spectrophotometrically by recording the intensity of pink color at 530 nm using calibration curve of standard IAA (Himedia) stock solution (10–100 µg/mL) prepared in 50% ethanol [15].

2.3 Pot Experiment

Seeds of rice (Rajendra Sweta) were obtained from Indian Institute of Seed Science, Mau, Uttar Pradesh, India. Seeds were washed in tap water to remove any deceased seed. The seeds were surface sterilized with 2 % (v/v) sodium hypochlorite for 3 min, washed repeatedly with distilled water. Seeds were treated with 15 days old selected cyanobacteria inoculum. Treatments used for pot experiments were as T0=Control, T1=*Tolypothrix* sp., T2= *C. cubana*, T3=*Tolypothrix* sp. + *C*. *cubana*. The experiment was set up in Plant growth Chamber (Atlanta Drugs and Chemical, Kolkata, India). The pots (15 × 15 cm) used in experiment were sterilized by dipping them in 5% sodium hypochlorite solution for 20 min. Control and inoculated seeds (10 seeds per pot) were sown in sterilized pot containing autoclaved soil (approximately 500 g). Pots were observed and watered regularly during this period. After 15 days, plants were harvested and their growth parameters were analysed. All the root parameters were recorded with root scanner Expression 1200XL Scanner, with WIN RHIZO programme V. 2002 C software (Regent Instruments Inc. Ltd. Quebec, Canada). Shoot length was measured manually with scale [16].

2.4 Preparation of Crude Extract

"For the extraction of metabolites 5 g each of the test cyanobacteria biomass was mixed in a glass flask with 100 ml of methanol: acetone: diethyl ether (5:2:1) and shaken for 3 days at 20 ºC after that mixture was separated from biomass by filtration with Whatman No. 1 filter paper. The extract was kept in the water bath at 40 ºC under the fume hood till the extract evaporated to dryness. The obtained residue was dissolved in 2 ml distilled water to get the final concentration of 50 mg/ml of the crude extract" [17].

2.5 Screening of Cyanobacterial Culture for Antifungal Bioassay

All fourteen cyanobacterial cultures have been evaluated against plant pathogens for antifungal activity by disc diffusion assay. Virulent strains of fungal plant pathogens (*Ralstonia solanceraum*, *Sclerocium rolfsii*, *Fusarium oxysporum*) were obtained from NAIMCC, Mau, Uttar Pradesh, India. Test organisms were inoculated on Potato Dextrose Agar (PDA (g/l); Potato 200, Glucose 20, and Agar 15). The plates were incubated at 28±2°C temperature. Sterile filter paper disc of 5 mm was soaked in 50 µl crude extract and placed on PDA. 5 mm diameter of 5 day old mycelial disc of test pathogen at the centre was also placed. Methanol (50 µl) and nystatin (100 U/disc) were used as negative and positive control respectively. The colony diameter and zone of inhibition was measured after incubation at 28 °C for 3-5 days. Percentage inhibition rate was calculated as (B-A)/(B-5) × 100%, where A represents the mean colony diameter in the Petri dishes with crude extract, B is the mean colony diameter in control Petri dishes.

3. RESULTS AND DISCUSSION

Phytohormones such as IAA, IBA, gibberellins, cytokinin, abscisic acid and jasmonic acid have been reported to produce in the cyanobacteria [5] (Manickavelu et al 2006). Plant Growth Promoting Rhizobacteria's (PGPRs/Biocontrol Agents (BAs)) mainly produce auxins, especially IAA, which enhance the development of the host plant root system and thus promote the growth of plants [18(28)]. Eighteen cyanobacteria were isolated from different ecological habitats of India (Table 1) in previous study [3] all tested isolates were characterized and grouped into hetreocytsuos (*Nostoc*, *Hapalosiphon*, *Tolypothrix*, *Scytonema*) and non hetrocystous (*Lyngbya*, *Leptolyngbya*, *Phormidium*, *Oscillatoria*, *Chroococcidiopsis*, *Aphanothece*) cyanobacteria. All the isolates have been deposited at National Agriculturally Important Microbial Culture Collection (NAIMCC), Mau, Uttar Pradesh, India.

Table 1. Details of cyanobacteria used in the study

Sr. No.	Strain name	NAIMCC accession No.	Habitat	Location	Longitude & latitude
1	N*ostoc* sp. HC2	NAIMCC--C-C-00239	Soil	Valley of Flower Uttarakhand, India	30.72 N and 79.60 E
2	*Nostoc sp*. BG1	*NA	Lake	Chilka, Odisha, India	19.84 N, 85.47 E
3	*Nostoc elongense* BG1	NAIMCC-C-C-00236	Lake	Chilka, Odisha, India	19.84 N, 85.47 E
4	*Arthronema africanum*. SB2	NAIMCC-*C*-C-00241	Hot spring	Atri, Odisha, India	20.15 N, 85.30 E
5	*Trichormus azollae. SB*1	NAIMCC-C-C-00234	Soil	Brahamagiri Odisha, India	19.78 N, 85.61 E
6	*Oscillatoria* sp. SK2	NAIMCC-C-C-00244	Lake	Pangong, Leh Jammu and Kashmir, India	33.75 N, 78.66E
7	*Hapalosiphon* s*p. BG2*	NAIMCC-C-C-00237	Soil	Bhitarkanika Odisha, India	20.71 N, 86.82 E
8	*C. cubana. HC1*	NAIMCC-C-C-00238	Soil	Valley of Flower Uttarakhand, India	30.72 N and 79.60 E
9	*Hapalosiphon sp. SB10*	NAIMCC-C-C-00235	Beach soil	Puri, Odisha, India	85.83 N, 19.81 E
10	*Chroococcus sp SB4*	NAIMCC-C-C- 00242	Hot spring	Atri, Odisha, India	20.15 N, 85.30 E
11	*Aphanothece sp. SB3*	NAIMCC-C-C-00243	Soil	Guwahati Assam, India	26.14 N, 91.73 E
12	*Nostoc sp. SK1*	*NA	Lake	Pangong, Leh Jammu and Kashmir, India	33.75 N, 78.66E
13	*Scytonema* sp. RC1	NAIMCC-C-C-00229	Arsenic contaminated soil	Phephna, Ballia, Uttar Pradesh, India	25.77 N; 84.03 E
14	*Pseudoanabaena* sp. RD1	NA/MCC-C-C-00231	Arsenic contaminated soil	Ekauna, Ballia, Uttar Pradesh, India	25.71 N, 84.27 E
15	*Leptoyngby*a *sp*. RH1	NAIMCC-C-C-00232	Arsenic contaminated soil	Phephna, Ballia, Uttar Pradesh, India	25.77 N; 84.03 E
16	*Leptolyngbya* sp. RC2	NAIMCC-C-C-00230	Arsenic contaminated soil	Phephna, Ballia, Uttar Pradesh, India	25.77 N; 84.03 E
17	*Phormidium sp. SB9*	NAIMCC-C-C-00233	Lake	Chilka, Odisha, India	19.84 N, 85.47 E
18	*Leptolyngbya* sp. SB6	NAIMCC-C-C-00240	Hot spring	Atri, Odisha, India	20.15 N, 85.30 E

NA= Not Assigned

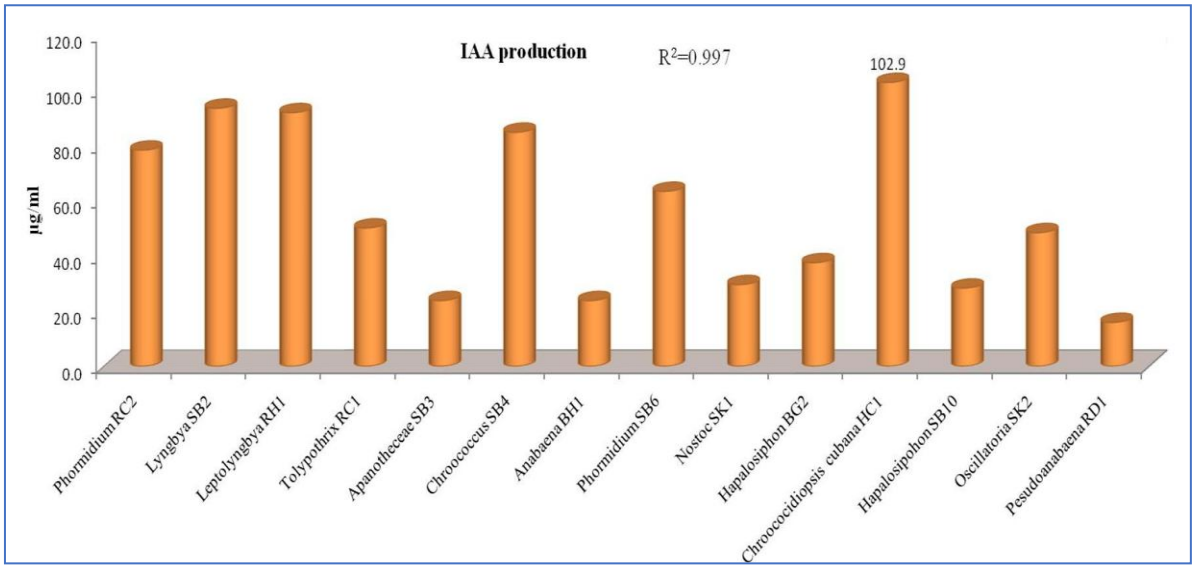

Fig. 1. Screening of IAA producing cyanobacteria from stressed environment

Cyanobacterial isolates were screened for their ability to Indole Acetic Acid production with or without tryptophan (Fig. 1). Among the all tested isolates, *C cubana* HC1 was found to produce highest IAA production (102.9 µg/ml) when supplemented with 300 µg/ml of tryptophan and lowest was recorded in *Pseudoanabeana* sp. RD1 (18 µg/ml).

On the basis of IAA screening and nitrogen fixation potential two strains were selected for pot trial on rice (*Oryza sativa*) plant. *C. cubana* HC1 and *Tolypothrix* sp. RC2 were evaluated for plant growth potential. Germination of rice seeds were recorded 100% in all the treatments. Coinoculation of *C. cubana* HC1 and *Tolypothrix* sp. RC2 recorded highest root (20.7 ± 1.0 cm) and shoot length (11.4±1.3 cm) control and individual treatments. Number of forks also recorded high in numbers (36.3 ± 3.2) as compared to control in which root and shoot length was recorded as (10.6 ± 1.1 cm) and (6.6 ± 1.3) respectively. Individual treatments were less effective than in combination of treatments, effect of different treatments on plant parameters have been shown in (Table 1). In present study unicellular nonhetrocystous cyanobacterial isolate *C.* cubana HC1 recorded highest IAA production (109 µg/ml) while lowest IAA was recorded in the strain *Pseudoanabaena* sp. RD1. A non-heterocyst *Chroococcidiopsis* sp. MMG-5 has been studied and showed significant amount of IAA (25 µg/ml) production and when co-treated with wheat, mung beans and pea crop showed significant increase in shoot and root length [19]. Although the strain found in this study was much higher IAA producer as compared to the strain MGG-5 reported by Ahmed et al. [20].

Arthrospira platensis MMG-9 with 194.3 (µg/ml) IAA production with enhanced root and shoot parameters [20]. *Anabaena vaginicola* reported to produce IBA and IAA, 2146.9 ng/g and 9.93 ng/g fresh weights respectively. Effect of these strains have been evaluated and found beneficial on several vegetable and herbaceous crops [21]. [11] investigated effect of *Anabaena* spp. (RPAN59/8) amended with compost and found enhanced growth parameters as well as quality of tomato fruit. Co- inoculation of plant growth promoting rhizobacteria along with cyanobacteria has also been reported to increase in plant growth and grain yield significantly [22]. Karthikeyan et al. [23] investigated potential of cyanobacteria on wheat along with different dose of chemical fertilizers, interestingly; all treatments showed enhanced plant growth and yield parameters. A study with *Anabaena* and *Trichoderma viride* biofilm showed 12-25% increase in yield of soybean as well as enhanced microbial activity. Kumar et al. [24] studied the germination behaviour of wheat seeds with cyanobacterial filtrate and found higher germination, vigor index and number of seedlings were higher as compared to untreated. Above mentioned all the studies indicate that cyanobacteria could be a potential component in integrated nutrient management. Experimental study on rice showed significant enhancement in root and shoot length as well as increased number of forks and links in T3 treatment. Plant

growth promoting rhizobacteria's are smart and sustainable strategy for biocontrol of soil borne pathogens of many important crops for sustainable agriculture [25]. Antifungal activities of all the cyanobacteria were tested against plant pathogens *S. rolfsii* and *R. solani*. Total eight cyanobacteria showed antifungal activity against these plant pathogens (Fig. 2 and Table 3). *Tolypothrix* sp. and *Leptolyngbya* sp. showed highest percent inhibition (50% each) against *R. solani* while *Nostoc* sp. SK1 showed highest inhibition against *S. rolfsii* (53%) while *Aphanothece* sp. showed highest inhibition against *F. oxysporum* (61%). No antifungal activity detected in the strains *Leptolyngbya* sp., SB2 and *Hapalosiphon* sp., BG1. Cyanobacterial strains RC1 and RC2 exhibited inhibition to all three tested phytopathogens. In the present study cyanobacteria from stressed environment such as *Nostoc* sp. HC2 (Valley of flower a cold region), *Leptolyngbya* sp RC2, *Tolypothrix* sp. RC1, *Peusoanabaena* sp. RD1, *Leptolyngbya* RH1 (Arsenic contaminated field), *Nostoc* sp. SK1 (cold region of Leh) showed significant

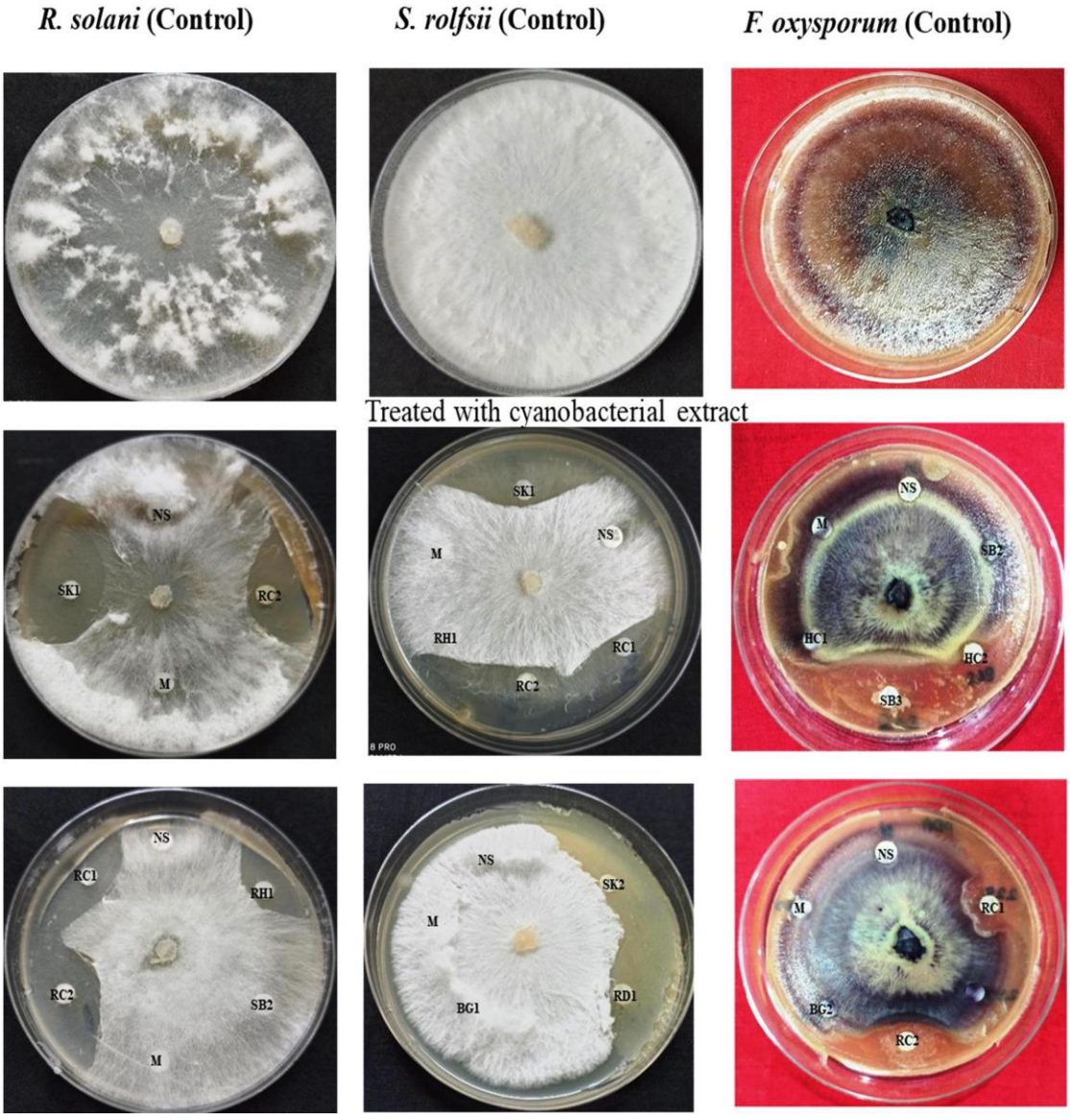

Fig. 2. Antifungal activity of selected cyanobacteria against *R. solani, S. rolfsii and F. oxysporum.* SK1-*Nostoc* sp., RC2-*Leptolyngbya* sp, RC1- *Leptolyngbya* sp., RD1-*Psuedoanabaena* sp., SK2-*Oscillatoria* sp., Chroooccus sp SB3 *Aphanothece* sp., HC2 *Nostoc* sp. M-Methanol (Negative control), N-Nystatin (Positive control)

Table 2. Pot trial data of selected cyanobacterial isolates on rice cultivar after 15 days of growth

Treatments	Root length (cm)	Shoot length (cm)	Projected area (cm^2)	Surface area (cm^3)	Average Diameter (cm)	Links	Forks	% Germination
T0	10.6±1.1	6.6±1.3	0.6±0.1	2.4±0.2	0.4±0.0	62.7±5.0	28.7±9.3	100
T1	18.2±1.0	10.7±0.6	0.8±0.1	2.4±0.2	0.5±0.1	69.7±1.5	30.7±6.7	100
T2	16.3±0.4	10.0±1.0	0.8±0.1	2.6±0.7	0.5±0.1	71.0±5.0	33.0±1.0	100
T3	20.7±1.0	11.4±1.3	0.9±0.0	2.8±0.1	0.5±0.1	79.0±5.1	36.3±3.2	100

Values are mean of three replicates ± SE, T0 = Uninoculated; T1 = C. cubana; T2 = Tolypothrix sp., T3 = C. cubana + Tolypothrix sp.

Table 3. Inhibitory activity of selected cyanobacteria against plant pathogens

Strain code	Treatment/Strain name	% Inhibition		
		R. solani	***S. rolfsii***	***F. oxysporum***
Nystatin (NS)	Positive control	5	10	0
Methanol (M)	Negative control	0	0	0
RC1	*Tolypothrix* sp.	50	46	57
RC2	*Leptolyngbya* sp.	38	43	51
RD1	*Pesudoanabaena* sp.	46	52	0
RH1	*Leptolyngbya* sp.	43	0	0
BG1	*Hapalosiphon* sp.	0	0	0
SB2	*Leptolyngbya* sp.	0	0	0
SK2	*Oscillatoria* sp.	41	43	0
SK1	*Nostoc* sp.	50	53	0
SB3	*Aphanothece* sp.	0	0	61
HC2	*Nostoc* sp.	0	0	40

antifungal activity against phytopathogens (*S. rolfsii*, *R. solani* and *F. oxysporum*). Radhakrishnan et al. [26] studied the effect of cultural filtrate of *Calothrix elenkinii* on fungicidal and algicidal activity which showed promising result. The strain was also evaluated for plant growth promotion and dual advantage of plant growth promotion and biocontrol potential make the strain more suitable candidate for agricultural use.

Biondi et al. [27(37)] found insecticidal and nematicidal activities of *Nostoc* ATCC53789 on *Helicoverpa armigera* and *Caenorhabditis elegans*. Prasanna et al. [28,(38)] showed biocidal activity against phytopathogenic fungi with Anabaena strain. The world is looking for organic farming, certainly cytanobacteria are one of the important catalyst that could play a vital role. Extracellular metabolites of *N*. *muscorum* and *Oscillatoria* sp. significantly reduced the linear growth of *Alternaria porri* [29(39)]. Roberti et al. [30(40)] found that a water extract of *Anabaena* sp. induced systemic defence response and reduced symptoms (25%) caused by *Podosphaera xanthii* in zucchini (*Cucurbita pepo*) leaves. The selected strains showed both antifungal as well as plant growth promoting potential which could be suitable candidates for sustainable agriculture due to the dual advantages they possess.

4. CONCLUSIONS

Cyanobacteria from stressed environment showed promising plant growth promoting activity and were able to suppress growth of plant pathogens. *Leptolyngbya* sp. RC2 and *Tolypothrix* sp. RC1 showed inhibitory potential in all tested phytopathogens. *C. cubana* HC1 exhibited higher indole acetic acid production along with antifungal activity. Certainly cyanobacteria are one of the important catalyst that could play a vital role in control of fungal phytopathgens. Cyanobacteria evaluated in the present study found suitable for sustainable agriculture due to the dual advantages they possess.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper

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