Phytology

Role of cyanobacteria in germination and growth of paddy seedlings

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Abstract

Cyanobacteria are first oxygen evolving prokaryotic photosynthetic organisms used as biofertilizer in several crop fields and play a crucial role to increase crop yields. They were isolated and screened for several plant growth promoting activities like indole acetic acid (IAA) production, phosphate solubilization, exopolysaccharide (EPS) production and their impact on seed germination, vigour index and root/shoot ratio (R/S ratio) of paddy plant. Isolated cyanobacteria *Nostoc calcicola*, *N. punctiforme*, *N. linckia* and *Anabaena oryzae* was identified using standard monographs. Paddy seeds were treated with different cyanofilterats in which *N. punctiforme* showed highest 97.33% seed germination while *N. calcicola* showed 90.66% and control (soaked in DDW) showed only 88% seed germination. Maximum vigour index was showed by *N. linkia* i.e., 988.26. IAA production was found 0.88 µg/ml in case of *N. calcicol*awhile phosphate solubilization 94.3 µm in diameter was maximum in *N. punctiforme* followed by 75 µm, 47.9 µm, 30.7µm in *N. calcicola*, *N. linckia* and *A. Oryzae*, respectively. EPS production was highest in *A. oryzae* i.e., 0.205 µl/ml and minimum in *N. linckia* which is 0.108 µl/ml. The data of our study showed that inoculation of these cyanobacterial species to paddy seedlings appear a potential candidate to promote seed germination, vigour index and R/S ratio of paddy plant. These results encourage use of *A. oryzae*, *N. calcicola*, *N. linckia* and *N. punctiforme* as biofertilizer for rice crop to enhancing growth without using harmful chemical fertilizers.

Keywords: cyanobacteria, rice, seed germination, phosphate solubilization, indole acetic acid, exopolysaccharide

Introduction

In order to fully satisfy the food requirement as a result of the rise in global population, we are relying on the use of dangerous chemical fertilizers, which is not suitable for the health of animals and humans. These chemical fertilizers clearly boost yield for a brief period of time, but they also have negative effects on the environment. Microorganisms including plant growth promoting rhizobacteria (PGPR) and cyanobacteria can help plants by improving vigour index, seed germination and enhancing yield production. It has also been demonstrated that adding PGPR and cyanobacteria, they protect plants against biotic and abiotic stresses, enabling farmers to enhance the production of food with minimal risk and reduces money expense [1]. Due to increased drought, degraded land and salinization, several crops are growing in less-than-ideal conditions in numerous countries of the world. Due to a variety of mechanisms like, phytohormone production, antioxidant defense, polyamines, dehydrins and osmolytes (e.g., sucrose, proline, trehalose etc.) production these microorganisms are not only able to increase the production of crop yield but it also enhances the tolerance level of the crop against these adverse conditions [2]. Chemical pesticides and fertilizers are used excessively, endangering both human health and ecosystem. In this regard, it is imperative to employ ecologically friendly techniques to lessen the negative impact of agro-chemicals, using microbe-based products [3, 4]. Numerous microorganisms are known to synthesize and secret growth-promoting chemicals that improve plant health through a variety of methods in addition to balancing mineral nutrition and naturally fertilizing the soils [5]. It is well known that in plants, pathological illnesses are brought by microbial pathogens or physiological disorders by abiotic stressors typically include damaging free radical-mediated oxidative degradation of biomolecules [6]. According to studies, plants protect themselves against these oxidative damages by altering their biochemical and physiological status and the presence of PGPRs and cyanobacteria in their roots help to fight against pathogens and abiotic stress-related losses [7, 8]. It is necessary to propose their role as PGPR because cyanobacteria naturally colonize the root surface of rice in saline soil, it is hypothesized that they promote plant growth in the soil having stress conditions. It can change the physical and chemical properties of soils together with nitrogen fixation and exopolysaccharide that are secreted from cyanobacteria may increase the organic carbon and nitrogen content and improve the water holding capacity of infertile soils [9, 10]. Release of miscellaneous metabolites by the growing cyanobacterial colonies in the soil may also assist in enhancing plant growth and yields in unfertile soils. However, due to the synthesis and production of phytohormones like auxin, gibberellin and cytokinin by genera like *Anabaenopsis*, *Anabaena* and *Calothrix* not only increase the acquisition of nutrients by plants, but they also actively promote their germination, growth and development [11, 12]. In addition to the direct interaction between cyanobacteria and plants, which contributes phytohormones, bio-stimulant chemicals can also be acquired through the commercial production of cyanobacteria. Cyanobacteria are emerging microorganism for the development of sustainable agriculture. In this article, we have made an attempt to discuss

how cyanobacterial colonization affects seed germination, vigour index, R/S ratio under control culture condition.

Materials and Methods

Isolation and identification of cyanobacteria

Samples of cyanobacteria were collected from different sites of agriculture field of Banaras Hindu University. The collected samples were cultured in sterile petri plates using BG-11 medium that contains no nitrate [13]. The petri dishes were kept in a culture room at a temperature of 28±2ºC and a light-dark cycle of 14+10 hours with artificial light (55µ mol m^{-2} s^{-1}) for two weeks. Isolates were streaked on agar plates for purification after colonization. The vegetative and reproductive characteristics that were used to make the taxonomic characterization included the thallus shape, colour and size; the trichomes length and width; the vegetative cells, akinetes and heterocyst. Several taxa descriptions and images were provided by Shariatmadari *et al* [14]. Taxonomic determination was carried out by morphometric study of isolates with light microscopy [15].

Percentage of seed germination

Rice seeds were obtained from Rice Breeding Section, Agricultural Research Center, BHU, Varanasi, Uttar Pradesh, India. Seeds were washed with 0.2% $HgCl_2$ for 3 min and 70% ethanol for 4-6 seconds then rewashed with sterilized distilled water (5-6 times). The sterilized seeds were kept on blotting paper for 1 hour in laminar air flow for drying, then placed in the different cyanofiltrates for the seed germination whereas seeds were soaked in sterilized DDW (Double Distilled Water) taken as control. All the seeds which were suspended in cyanobacterial filtrate and those suspended in DDW were soaked overnight. Thirty fully soaked seeds were transferred to glass beaker containing 10 ml cyanobacterial filtrates and left for 2 days in dark. After 2-days seed germination % was determined by counting the number of germinated seeds by using following equation.

Germination % = Total No. of germinated seeds × 100/ Total No. of seeds sown .....Eq. 1

Effect of cyanobacterial cell suspension on rice seedlings

Germinated seeds were sown in Petri plates filled with different cyanobacterial cell suspension and kept in the culture room under the fluorescent light for the growth of seedlings, in control DDW was used. Petri plates were used in four replicates (5 seedlings per plates), which were supplied with a filter paper of the same diameter then that of Petri plate. The filter papers were saturated with different cyanobacterialcell suspension. Five rice seedlings were arranged in each petri-dish to which 20 ml of cyanobacterial cell suspension were inoculated and then incubated at 28± 2ºC for growth. After 3 and 10 days of growth, length of seedling roots and shoots were measured. Vigour index of paddy seedlings was calculated using following formula given in equation 2.

Vigour index = (Mean root length + mean shoot length) × germination (%) .... Eq. 2

Indole acetic acid (IAA)

To measure the amount of IAA produced by cyanobacterial isolates, 2 ml of culture broth was centrifuged at 10000 rpm for 10 minutes. 1 ml of the supernatant was mixed with 2 ml $FeCl_3$-$HClO_4$ reagent (Salkowski reagent) and kept under dark for 20 to 25 minutes. After 25 minutes the absorbance was recorded at 530 nm in UV-spectrophotometer. The amount of IAA produced per milliliter was estimated using a standard calibration curve [16].

Phosphate solubilization

Qualitatively phosphate solubilization activity of isolated cyanobacterial strains were conducted by precipitated tricalcium phosphate agar plating method. After 24 hours of incubation, the presence of clear zone around the cyanobacterial colonies indicated the phosphate solubilizing activity of selected cyanobacterial strain [17].

Phosphate solubilization= Colony diameter + Halozone diameter/ Colony Diameter

Exopolysaccharide (EPS)

The released EPS around the filaments was first identified by Alcian Blue staining [18] and their quantitative analysis was confirmed by Anthrone test [19]. In brief 2 ml of cyanobacterial culture was centrifuged for 10 minutes at 5000 rpm. 1 ml of each supernatant was placed into test tube for estimation of extracellular polysaccharide. Freshly prepared 4 ml Anthrone reagent (0.2 gm of Anthrone dissolved in 95% Sulfuric acid) was added to each test tube and kept in a boiling water bath for 15 min. The tubes were then cooled to ambient temperature, observed the turbidity at 620 nm.

Statistical analysis

Statistical analysis was performed with one-way analysis of variance (ANOVA), using SPSS (Package for the Social Sciences, SPSS Inc., Chicago IL) version 16. Means were separated using the Tukey honestly significant difference (HSD) test at $P < 0.05$. Also, Microsoft Office Excel 2019 was used to draw graphs to study the effect of physicochemical parameters in different cyanobacterial strains.

Results

Identification of cyanobacteria

Organisms isolated from rice field habitat included 4 filamentous heterocystous cyanobacterial strains. The cyanobacterial strains were identified as *N. calcicola*, *N. linckia*, *N. punctiforme* and *A. oryzae* by observing the morphological features under the microscope and by matching the monograph of Desikachary [15] (Fig. 1).

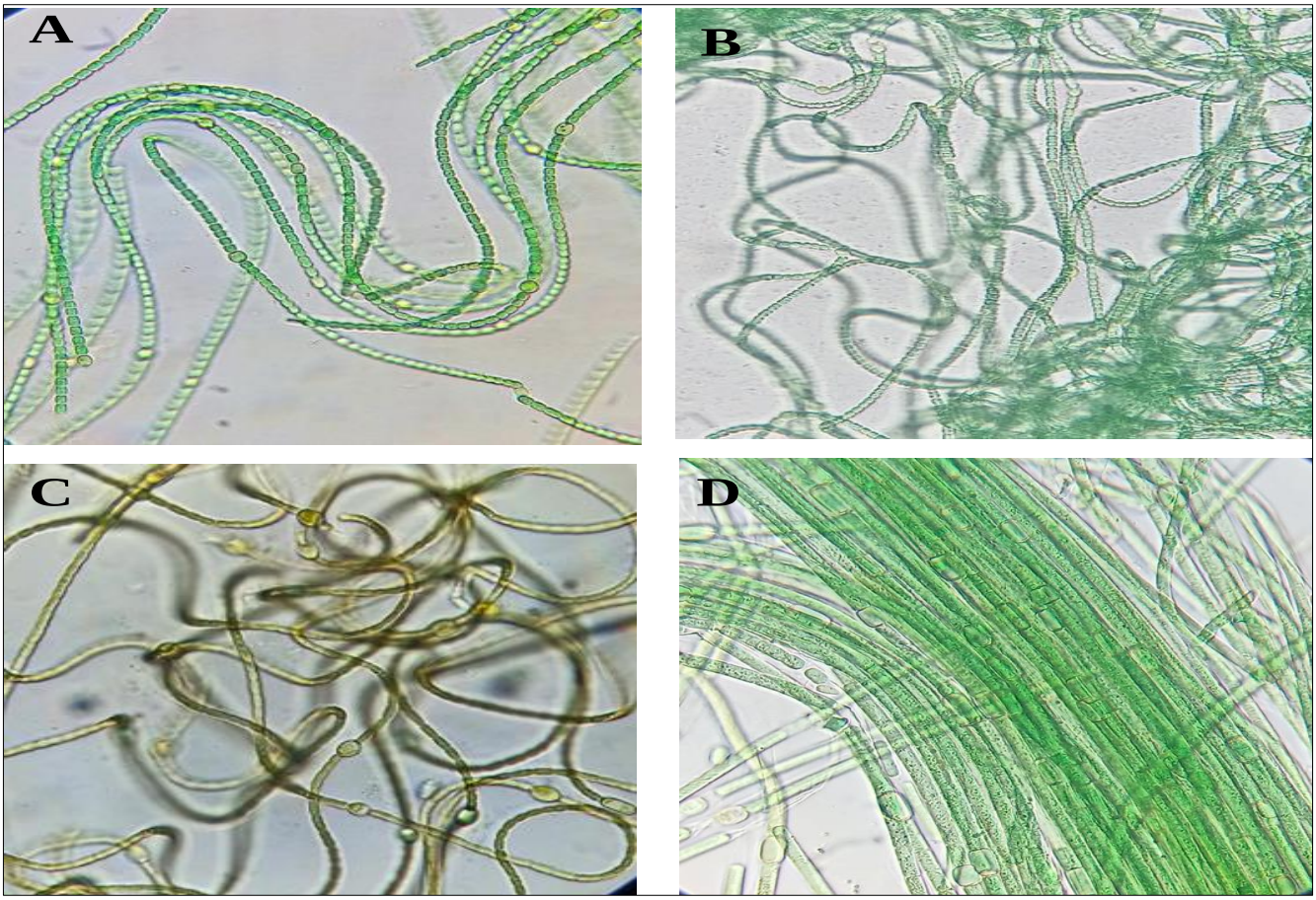

Fig 1: Microphotographs of (A) *Nostoc calcicola*, (B) *Nostoc linckia*, (C) *Nostoc punctiforme* and (D) *Anabaena oryzae*

Effect of the cyanobacterial filtrate on seed germination and vigour index

Paddy seeds were treated by filtrates of four strains through Petri plate trial, seeds treated with all the four strains showed remarkable increase in seed germination percentage in comparison to control. The seed germination observed at 3 days differed considerably in which *N. punctiforme* supported 97% seed germination it was 1.10 folds greater than control while *N. linckia*, *A. oryzae*, and *N. calcicola*, showed 1.09, 1.07 and 1.03 folds greater then control respectively (Fig. 2 A). The seed germination was always high in all the treatment as compared to distilled water-soaked seeds it was only 88%. *N. linckia* showed the maximum vigour index of 988.26 followed by *N. calcicola* 882.08, *N. punctiforme* 861.33 and *Anabaena oryzae* 590.60 while the control was found to show only 595.6 vigour index represented in Fig. 2 B.

Effect of the cyanobacterial cell suspension on R/S ratio of rice seedlings

Germinated seeds were treated with stationary phase grown cultures of above-mentioned cyanobacteria (Fig. 3A) and after 3 and 10 days growth of seedlings, R/S ratio were determined. Results illustrated in Fig. 2 C and D showed that there is a highly significant difference between different cyanobacterial treatment and control. R/S ratio was maximum in the seedlings treated with *N. punctiforme* followed by *Anabaena oryzae*, *N. linckia*, *N. calcicola* and control. *N. punctiforme* showed 1.77 folds increase in R/S ratio while *N. calcicola* showed 1.04 folds increase. R/S ratio at 10 days was maximum in the seedlings treated with *N. calcicola* i.e., 1.82 folds followed by *N. linckia N. punctiforme A. oryzae* which is 1.36, 1.28, 1.05 folds greater than control respectively.

It is interesting to note that the roots of rice seedlings grown with the suspension of cyanobacteria either in the petri plates got colonized by the cyanobacteria (Fig. 3 B).

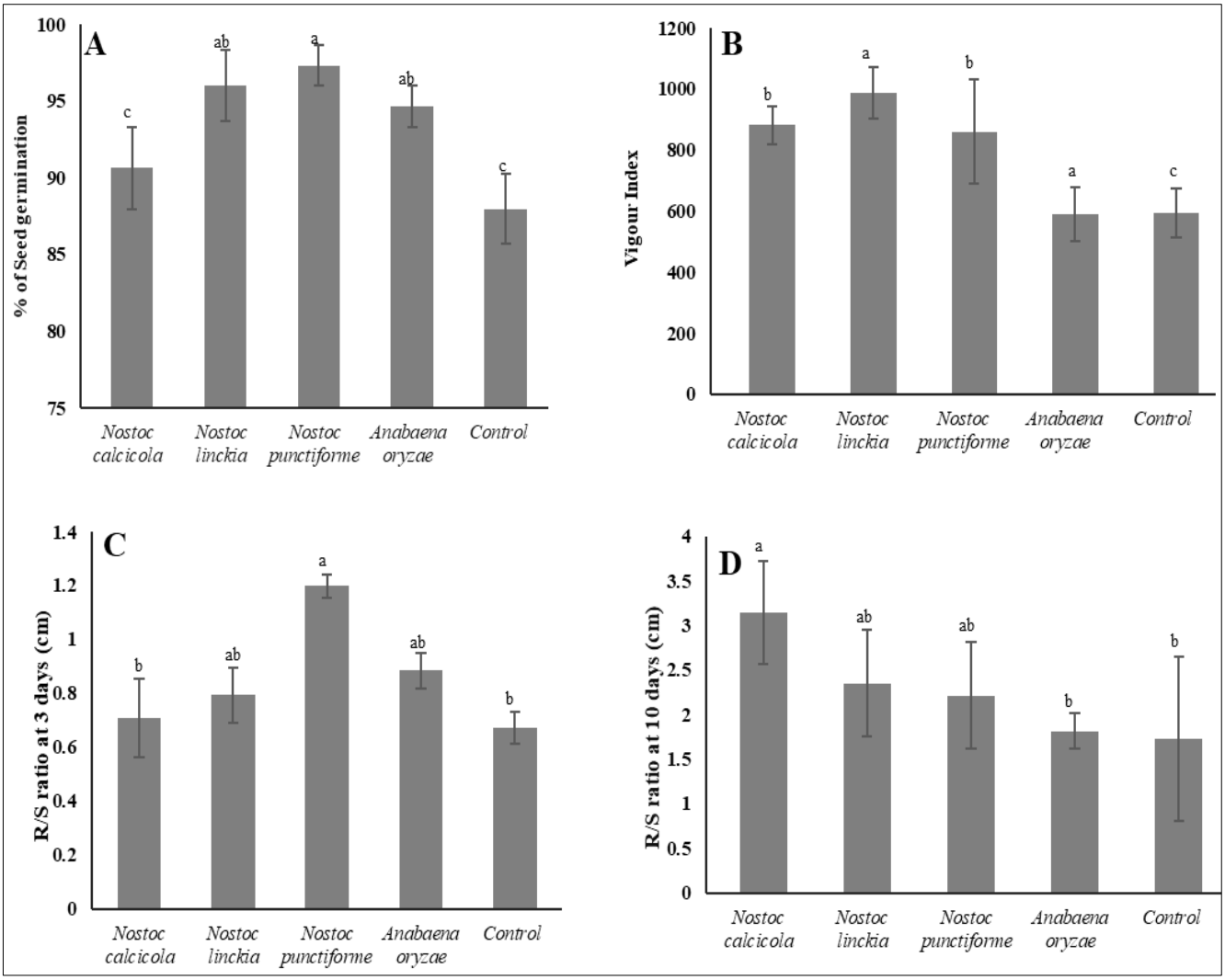

Fig 2: (A) Effect of different cyanobacterial filtrates on the percentage of seed germination, (B) Vigour index, (C) Impact of cyanobacterial cell suspension on the R/S ratio of rice germinated seeds after 3 days of planting and (D) R/S ratio of rice germinated seeds after 10 days of planting. Graphs were expressed as means of four replicates and vertical bars indicate standard error of the mean value. Bars affixed with different combinations of letters (a, b, c) are significantly different from each other ($P \leq 0.05$). Results taken according to Tukey's Post-hoc test at $P \leq 0.05$.

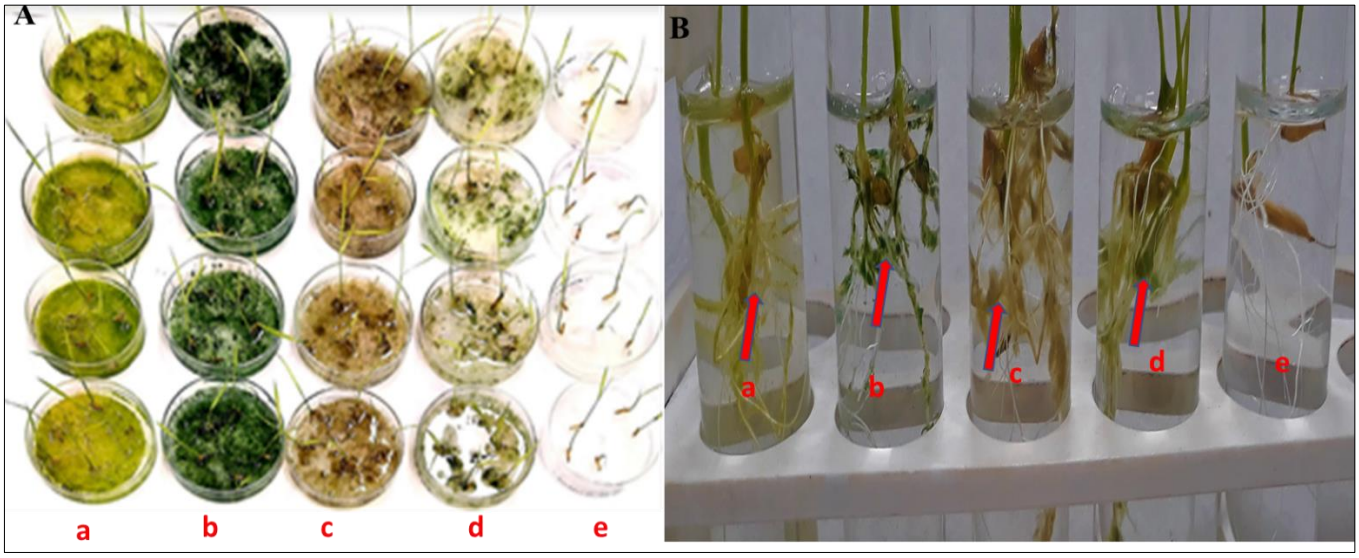

Fig 3: (A) Growth of rice seedlings in different cyanobacterial cell suspension and (B) Root colonization by different cyanobacteria, (a) *Nostoc linckia*, (b) *Nostoc calcicola*, (c) *Nostoc punctiforme* and (d) *Anabaena oryzae*, (e) Control.

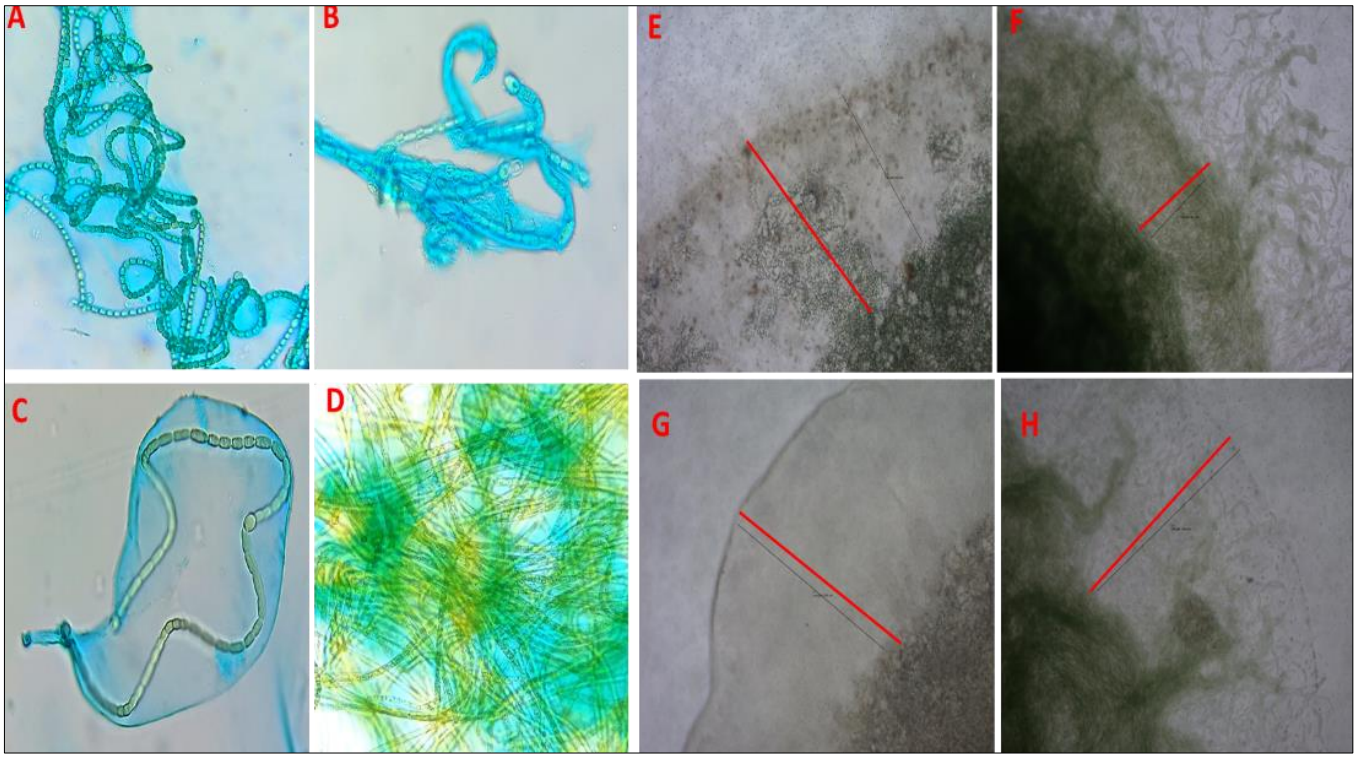

Fig 4: A-D is the photographs of Alcian blue stanning, stain released exopolysaccharides. (A) *Nostoc calcicola*, (B) *Nostoc linckia*, (C) *Nostoc punctiforme* and (D) *Anabaena oryzae*.
E-H is Zone of inhibition against precipitated tricalcium phosphate ((E) *Nostoc linckia*, (F) *Nostoc calcicola*, (G) *Nostoc punctiforme* and (H) *Anabaena oryzae*

Indole acetic acid (IAA) and phosphate solubilization

It is apparent that the cyanobacteria have promoted the seed germination and seedling growth and the effect was strain specific. The results (Fig. 5 A) were further supported by the phytohormone IAA production in the culture filtrates of cyanobacteria which differed significantly among the species. It was found to be 0.88 µg/ml in *N. calcicola* which was maximum and minimum in *Anabaena oryzae.* Exogenous phosphate solubilization was 94.3 µm in diameter in *N. punctiforme* which was maximum followed by *N. calcicola*, *Anabaena oryzae* and *N. linckia* given in Fig. 5 B.

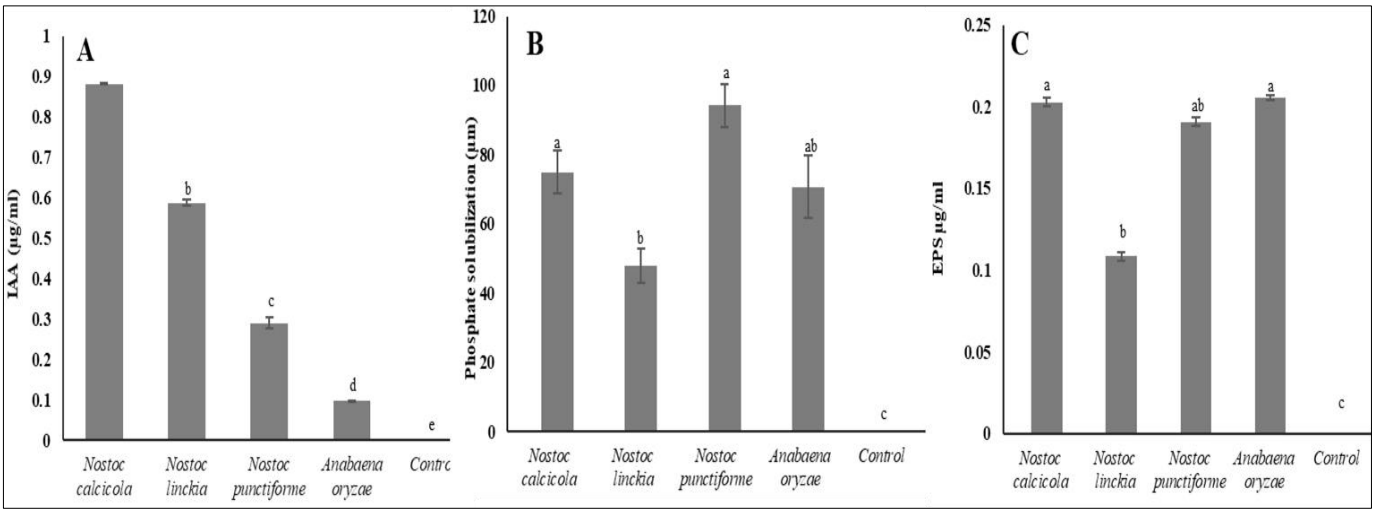

Fig 5: (A) IAA production, (B) P-solubilization activity and (C) EPS production. Graphs were expressed as means of four replicate and vertical bars indicate standard error of the mean value. Bars affixed with different combinations of letters (a, b, c, d, e) are significantly different from each other (P ≤ 0.05). Results taken according to Tukey's Post-hoc test at P ≤ 0.05

Exopolysaccharide secretion

Isolated cyanobacterial strain synthesizes and secret extracellular polysaccharide and it was maximum in *Anabaena oryzae* 0.205 µg/ml followed by *N. calcicola* *N. punctiforme* and *N. linckia* i.e., 0.202, 0.190, 0.108 µg/ml (Fig. 5 C). This result was also supported by Alcian blue stanning (Fig. 4 A-D).

Discussion

Cyanobacteria are one of the most important candidates for their use as biofertilizer in crop fields. It requires minimum nutrient for growth and widely used as biofertilizers for rice crops [20-22]. These heterocystous cyanobacteria have the ability to fix-N_2, which can increase the amount of readily available N_2 or ammonium in the soil. According to the results of the current experiment nitrogenase, IAA and P-solubilization may be responsible for the favorable influence on plant development metrics. The findings demonstrated that heterocystous cyanobacteria contain IAA and P-solubilization, which may be the cause of the increased seed germination percentage and growth of the plants [23-25]. IAA can enhance root development and ion as well as water uptake in rice seedlings, similar effect of heterocystous cyanobacteria on nutrient uptake and plant growth was also reported by Obana *et al* [26]. We have also found that the cyanobacteria increase the % of seed germination and R/S ratio in paddy seedlings. Katoh *et al*., observed that application of *Nostoc* sp. increased the nitrogen and carbon element of soil and promote ion uptake and plant growth [27]. They considered that the macro and micronutrients are essential for plant growth and these nutrients are being supplied by cyanobacteria. In addition, exopolysaccharides secreted by cyanobacterial cells improve the structural and physical stability of the soil, increase water holding capacity and promote soil texture [28]. Phytohormone production from cyanobacteria had been reported mostly isolated from crop fields but Boopathi *et al*., demonstrated, mangrove root-connected cyanobacterium, *Phormidium* sp. to synthesize and produce exogenous IAA [29]. The cell suspension of cyanobacteria was tested on seed germination of tobacco seeds and was found to enhance it not only promotes seed germination but also showed positive result in callus differentiation and extract-treated callus showed multiple root formation, a signature character of IAA. There are several findings resulting that exogenous IAA producers enhance root length and biomass which helps in nutrient uptake and plant growth [30-32]. IAA like substance was also marked by Misra and Kaushik, in *Haplosiphon* and *Nostoc* by Salkowski's reagent [33]. Hussain *et al*., quantified IAA and cytokinin in *Anabaena* sp. Ck1, *Chroococcidiopsis* sp. Ck4, *Oscillatoria* sp. Ck2, *Phormidium* sp. Ck3 and *Synechosystis* sp. Ck5 by using cucumber cotyledon bioassay in *Arabidopsis* ARR5 [34]. IAA and cytokinin have been also detected in unicellular and filamentous cyanobacterial strains of *Synechocystis, Anabaena, Chroococcidiopsis, Oscillatoria* and *Phormidium* [34, 35], endogenous IAA production has also been present in cyanobacteria [36]. HPLC (High performance liquid chromatography) analysis of the extracts of several cyanobacteria like *N. carneum and Wollea vaginicola* showed their ability to produce Auxin, a positive and significant correlation was observed between auxin and growth of *Matricaria chamomilla* plants [37]. However, cyanobacteria are not only able to promote the acquisition of nutrients by plants but they also enhance their germination percentage, growth and ultimately yield [38, 12]. Phosphatase enzyme present in cyanobacteria and PGPRs help to solubilize and mobilize the insoluble organic phosphate and to improve the bioavailability of phosphorus to the plants.

Conclusion

Cyanobacteria play an advantageous role in seed biopriming technique as our result exhibits potential effect on promoting seed germination, vigour index, IAA and EPS production phosphate solubilization and seedling growth. They are the precious microorganisms existing in our ecosystem, having no or very little side effects on the environment, act as natural biofertilizers able to grow at a very low cost. Isolated cyanobacterial strains such as *N. calcicola, N. linckia, N. punctiforme* and *A. oryzae* were screened for several plant growth promoting activities, after treatment of these cyanobacterial isolates on paddy seeds, showed positive impact in plants by numerous plant growth promoting attributes. As per review there are very little studies done on the impact of above cyanobacterial isolates on % seed germination, vigour index and R/S ratio of paddy plant. Thus, treatment of paddy seedlings with cyanobacterial strains are eco-friendly, cost effective and sustainable approach to cooperate with the farmers and enhance the agricultural fertility and productivity.

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Conflicts of interest

The authors declare no conflict of interest.

Authors contribution

PY and RS conceived the research, wrote the manuscript, analyzed the data, acquire the funding. PY performed the research. RKG supervised the experiment. All authors have read and agreed to the published version of the manuscript.

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Data availability

All datasets analyzed during this experiment are included in the manuscript.

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