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The effect of culture filtrate of cyanobacterium (*Lyngbya*.sp) with coir pith on seed germination of *Vigna radiata* L.

Tharani.G[1], Sowmya.R[2], Malliga.P[3]

[1]Department of Life Sciences, Central University of TamilNadu, Thiruvarur, Tamil Nadu, India

[2,3]Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Abstract: **The aim of the present study was to investigate the effect of culture filtrate of cyanobacterium (*Lyngbya*.sp) with coir pith on seed germination of *Vigna radiata* L. in ASN III media. The impact of seed germination was analysed at different concs.(6.25, 12.5, 25, 50, 75 & 100%) and at various time intervals (24, 36 & 48 hrs) on 0th day and 30th day. Similarly the physicochemical parameters (TDS), biochemical parameters (chlorophyll *a*, protein, amino acid) and morphometric parameter (radicle length) were also performed on 0th and 30th day. The 30th day study revealed that the biochemical parameters (protein, amino acids and chlorophyll *a*) exhibited significant variations during germination in combined treatment when compared to control. The chlorophyll *a* content was high at 25% conc. while the protein levels were reduced and amino acid levels increased in combined treatment when compared to control. The TDS level was decreased with cyanobacterial treatment at 6.25% conc. when compared to control. The germination percentage was maximum in combined treatment at 36 hrs. and 48 hrs. in lower conc. when compared to other treatments while the radicle length was highly enhanced in combined treatment with cyanobacterium and coir pith when compared to other treatments. Thus, the outcome of this study results showed that the combination of cyanobacterium and coir pith in culture filtrate reduced the TDS content by converting the inorganic ions present in the filtrate into nutrients for growth which in turn promoted better seed germination.**

Keywords: ***Lyngbya*** **sp., Coir pith, ASN III media, seed germination,** ***Vigna radiata*** **L..**

I. INTRODUCTION

The high and changing salt concentrations represent a major abiotic factor limiting the growth of microorganisms. High salt concentrations in the medium challenge the cell with reduced water availability and high contents of inorganic ions. The basic mechanism of salt acclimation involves the active extrusion of toxic inorganic ions and the accumulation of compatible solutes, including sucrose, trehalose, glucosylglycerol and glycine betaine [1]. But many species are capable of not only surviving but thriving in conditions previously thought to be inhabitable, tolerating dessication, high temperatures, extreme pH and high salinity, illustrating their capacity to acclimate to extreme environments [2]. Cyanobacteria can exist in a broad range of salinities and therefore can be described as euryhaline. Marine cyanobacteria, although prevalent in brackish and estuarine systems, appear to have a salinity optimum in the high range of scale [3]. Cyanobacteria have been classified into 3 groups as relating to their salt tolerance as freshwater (or stenohaline), moderately halotolerant and extremely halotolerant. Example genera from each group include *Anabaena*, *Synechocytis and Aphanothece* respectively [4]. Moreover, they also play a crucial role in sustaining the productivity of ecosystems through photosynthesis and nitrogen fixation [5,6] since they have simple growth requirements and could be an attractive hosts for production of valuable organic products. They also play a vital role in the active transport of metals into the cells [7].

The coir pith a by-product of coconut industry is a spongy and dark brown material which due to its high lignocellulosic bonding results in slow degradation in the natural environment. The enzymatic machinery of degrading cellulose, hemicelluloses and lignin are preceded only by microorganism [8]. Biological treatments of organic contamination are based on the degradative abilities of the microorganisms [9]. Besides, various research on degradation of coir pith by fungi and bacteria already reported, recent research shows that coir pith can be partially decomposed through the action of cyanobacteria and can be used as biofertilizer for all varieties of food crops [10] . Cyanobacteria also have been shown to degrade naturally occurring coir pith [11]. A lignin degrading bacterium *Lyngbya* sp. was isolated through enrichment technique with lignin as a sole carbon source, for decaying coir wastes**.**

Hence, in the present study, the effect of culture filtrate using the marine cyanobacterium *Lyngbya* sp. with coir pith in ASN III media was investigated and thereby evaluating the effect of seed germination of *Vigna radiata*.L at various concentrations of the incubated culture on 0^{th} day and 30^{th} day at 24, 36 and 48 hrs..

II. MATERIALS AND METHODS

A. Organism and Source

A cyanobacterium strain, *Lyngbya* sp. was obtained from the germplasm of National Facility for Marine Cyanobacteria, Bharathidasan University, Tiruchirappalli, Tamilnadu, India.

B. Lignocellulosic Material

Coir pith was collected from coir industries near Srirangam, Tiruchirappalli, Tamil Nadu, India.

C. Media and growth condition

The marine cyanobacterium was grown in ASN III medium and maintained under white fluorescent light of 1,500 lux at 25+2ºC (laboratory condition, Rippka *et al*., 1979).

D. Treatments

S.No.	Medium preparation	Incubation Period	Filtrate collection
1.	**<u>Untreated ASN III medium</u>** 100ml of ASN III medium	30 days	Initial and incubated culture filtrate for seed germination.
2.	**<u>Treatment with cyanobacterial strain</u>** 100ml ASN III medium+1g *Lyngbya* sp.		
3.	**<u>Treatment with cyanobacterial strain and coir pith</u>** 100ml ASN III medium+1g *Lyngbya* sp.+ coir pith		

E. Seed germination study

The seeds of *Vigna radiata L* (Mung bean) purchased from local seed center were chosen for the test. The study was performed in 06 different concs. of test solution (viz., 6.25,12.5, 25, 50, 75 and 100%) using ASN III medium (media control), ASN III with *Lyngbya* sp., ASN III with *Lyngbya* sp. and coir pith (combined treatment) and control. The seed surface was sterilized with 0.1% $HgCl_2$ and washed thrice to remove the traces of unwanted particles. Germination and seedling growth were carried out on bilayered filter paper (125 mm in diameter, whatman No.1) placed above the glass petri dishes (20 mm x 120 mm) on top and a layer of cotton bed at the bottom. Each dish contained 5 ml of test solution and water as control along with 10 seeds for germination study.

The percentage of seed germination was calculated based on the results obtained through analysis of biochemical parameter (chlorophyll *a* by Arnon, 1941; protein by Lowery *et al.*, 1951; amino acid by Moore and Stein, 1948), physicochemical parameters (TDS by Valentine,1996) and morphometric parameters (radicle length).

The percentage of seed germination was calculated using the formula

$$\text{Germination \%} = \frac{\text{No.of seeds germinated X 100}}{\text{Total No.of seeds}}$$

F. Statistical Analysis

The experiments were performed in triplicates. All the results were expressed as mean ± S.D. The graphs were prepared using Microsoft Excel 2007.

III. RESULTS AND DISCUSSION

The objective of the study was to analyze the seed germination in culture filtrate along with cyanobacterium and coir pith. The obtained results showed that *Lyngbya* sp. was capable of growing photoautotrophically in low nutrient and required only nitrogen source. Treatment with *Lyngbya* sp. and coir pith showed better results for seed germination. The TDS value was high on 0^{th} day at different concs. and in all treatments when compared to control while a reduction was observed in all concs. and in all treatments on 30^{th} day. However, a maximum reduction was observed in treatment with *Lyngbya* sp. at lower conc., especially, 6.25% showed maximum reduction in the TDS level when compared to control **(Fig.1(a))**. The reduction maybe due to the combined action of *Lyngbya* sp. and coir pith which plays an important role in the adsorption and degradation of inorganic and organic matter present in the media. The TDS levels were almost reduced to half its orginal concentration after the cyanobacterial treatment (*Lyngbya* sp.) from 30 to 17g/l on 30^{th} day [12]. The high amounts of TDS were decreased after incubation [13]. Similar findings also showed that high level of TDS (3.2g/l) after treatment with consortium (*Chlorella vulgaris* and *Scenedesmus obliquus)* reduced to 1.0g/l on 21^{st} day [14].

On analyzing the biochemical parameters, chlorophyll *a* content was high in control when compared to all treatments and various concs. on 0^{th} day. But on 30^{th} day, a gradual reduction in the level of chlorophyll *a* was observed as the conc. of ASN III media increased in all the treatments when compared to control. The reduction maybe probably due to the inhibitory effect of the accumulated ions of various salts at different concs.. But it was also observed that the chlorophyll *a* content was very high at 25% conc. of combined treatment when compared to all other concs. on 30^{th} day **(Fig.1(b))**. Fundamentally, chlorophyll has been regarded as an indicator to determine the biomass of cyanobacteria [15, 16]**.** Therefore, increase in chlorophyll *a* content maybe due to the presence of organic compounds which acts as a nutrient source and stimulates the growth of *Lyngbya* sp. in the media. Moreover, the increased chlorophyll *a* content in the test sample indicated that the cyanobacterium degraded the coir pith and utilized the coir pith as a source of nutrient for its growth and other metabolic activities. The observation was in concurrence with the findings of [17]. According to [18], chlorophyll *a* is an important pigment present in cyanobacteria and plays a significant role in electron transport system which is an important factor for growth analysis. Another study showed that the variation in chlorophyll *a* content was performed to determine the growth response of *Azolla pinnata* to various nitrogen sources like nitrogen free medium, sodium nitrate, ammonium chloride and ammonium nitrate [19].

The protein content was high on 0^{th} day in all treatments but comparitively very high at concs. starting from 50 % to 100%. But a gradual decrease in protein content was observed in all treatments including control after seed germination. The higher conc. of combined treatment showed more protein content when compared to lower conc. on 30^{th} day. But a significant reduction in protein level was observed at 25% conc. in combined treatment on 30^{th} day when compared to control **(Fig.1(c))**. This maybe due to the breakdown of proteins into amino acids for the seedling growth during germination. There was a drastic decrease in the level of protein on 7^{th} day of treatment when the cultures were grown in 1:100 and 1:1000 dilutions of effluent [20]. The supporting evidence also showed that the protein content was lowest at lower dilution of 12.5% in combined treatment and highest at 100% conc. due to poor germination [21]. The reports are in concurrence with the results of [22] in germinating *A.tristis* where lower conc.of bijamrita, cyanospray combination showed less protein content when compared to control.

A significant reduction was observed in the amino acid content in all the treatments when compared to control on 0^{th} day. However, on 30^{th} day, the level of amino acid showed significant variation on treatment with *Lyngbya* sp. and combined treatment, especially, the level of amino acid was high at 25% conc. in combined treatment when compared to control which maybe due to the breakdown of proteins during the seed germination. Further, the loss of proteins from cotyledons

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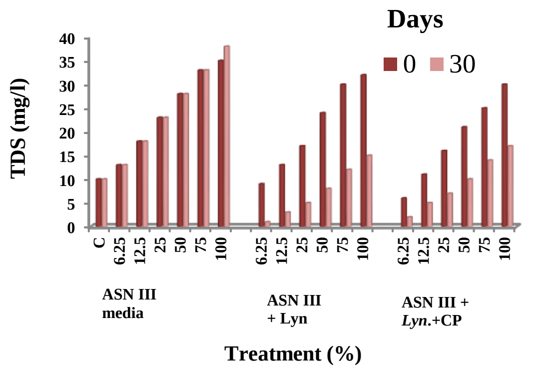
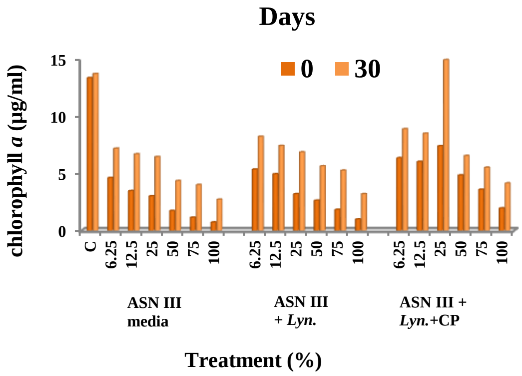
could be due to the transport of amino acids to the growing axis or for respiratory loss or it maybe due to the accumulation of free amino acids in the cotyledons **(Fig.1(d)).** The study by [23] observed that there was an increase in amino acid level during germination in Ceiba pentandra seeds. The findings were in concurrence with the reports of [24] which showed that amino acid content increased during germination process in the seeds of broccoli and the increase in amino acid content could be due to rapid hydrolysis of proteins, which resulted in release of free amino acids. The growth promoting substance from cyanobacteria influences amino acids and sugars in paddy field was reported by [25].

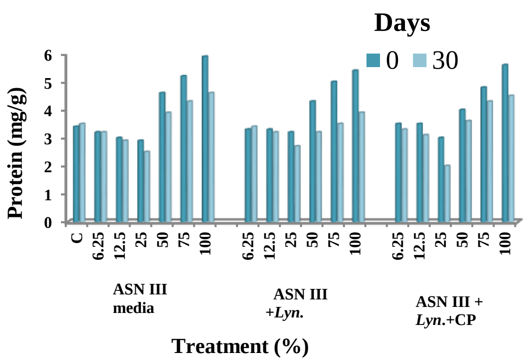
(a)



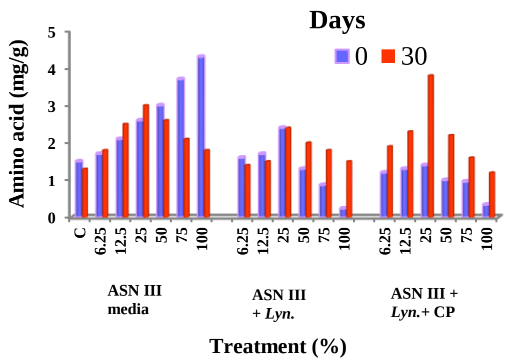
(b)



(c)



(d)

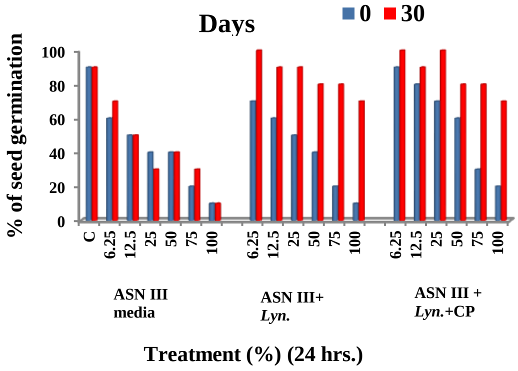


Lyn-Lyngbya sp., CP – coir pith

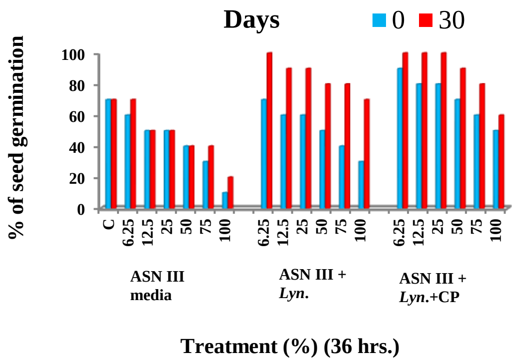
Fig 1: Effect of different concs. of culture filtrate and various treatments on a) TDS b) Chlorophyll *a* c) Protein d) Amino acid

The seeds started germinating on 0th day at 24 hrs.. At 36 hrs., on 0th day , the lower concs. (6.25% to 25%) showed significant variation in seed germination of all treatments when compared to control. However, 50-100% conc. showed less germination due to high conc. of media. But on 30th day, increased germination percentage was observed in treatment with cyanobacterium at all concs. and almost maximum germination was observed in combined treatment in all conc. when compared to control . At 48 hrs., the germination percentage was similar to 36 hrs. germination study of 0th day. The 30th day results showed that the lower concs. (6.25% to 25%) of all treatments exhibited maximum growth when compared to higher concs. in treatment with cyanobateria and in combined treatment. **(Fig 2. (a)(b)(c))**. Reports by [26] showed positive effect of algal extract on germination percentage of *Vinca* seeds at various incubation periods and all the treatments with *Anabaena*, *Scytonema*, *Oscillatoria* and *Lyngbya* sp. extracts at 25% level showed enhanced growth over the control with time. The seedling growth of groundnut was increased at 10% and 25% concs. of various cyanobacterial treatments. The presence of inorganic nutrients in the diluted effluent induced the seed germination, growth and development at 25% conc. in red gram plant [28].

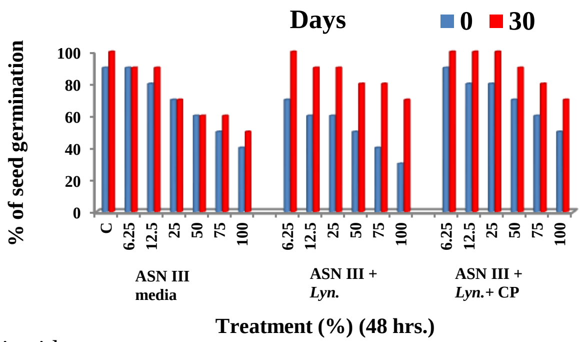
(a)



(b)



(c)

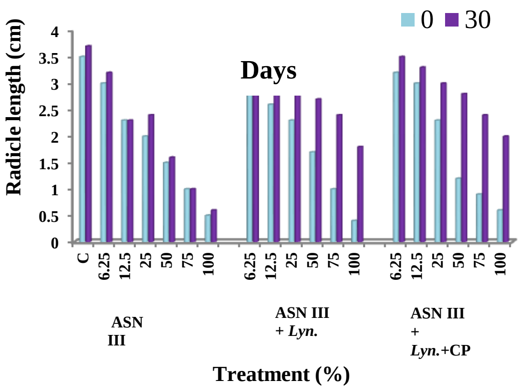


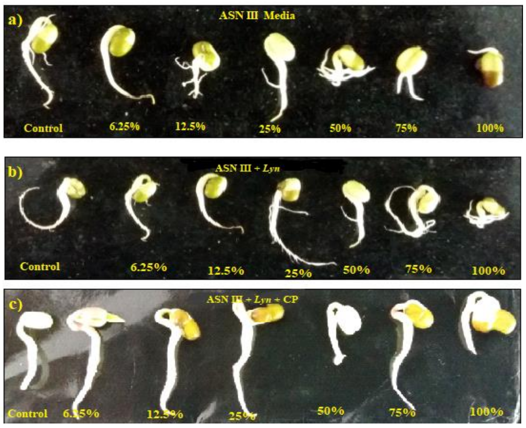
Lyn-Lyngbya sp., CP – coir pith

Fig 2: Effect of percentage of seed germination on *Vigna radiata* L. at a) 24 hrs. b) 36 hrs. c) 48hrs.

The radicle length of the seeds decreased as the conc. increased. On 0th day, the radicle length was high in lower concs. in treatment with ASN III media when compared to higher concs.. However, the radicle length increased in combined treatment when compared to treatment with *Lyngbya* sp. and control. On 30th day, the radicle length was reduced at higher concs. in treatment with *Lyngbya* sp. and control when compared to combined treatment **(Fig 3. (1) and (2)).** The findings were in concurrence with [29] which stated that at 25% conc. and 72 hrs. the root and shoot length was high in combined treatment but showed deleterious effects as the conc. increased. The findings of [30] reported increased germination percentage, shoot and root length in the seedlings of *Helianthus annus L*. grown in effluent blended with cyanobacteria.

1) 2)





Lyn-Lyngbya sp., CP – coir pith

Fig 3: 1) Effect of radicle length at different concs. of culture filtrate and various treatments (graphical representation) 2) Effect of radicle length at different concs. of culture filtrate and various treatments (30th day - Plate representation)

IV. CONCLUSION

The results of the study revealed that the cyanobacterium (*Lyngbya* sp.) which constitue a vast potential resource in varied applications (like food, fuel, medicine, industrial products and combating pollution) can reduce the salt content in ASN III media through photosynthesis process thereby inducing the growth promoting substance present in cyanobacterial extract and can support plant growth. Moreover, *Lyngbya* sp. also showed degradation of coir pith in the culture filtrate which was observed in the seed germination percentage. The combination of both cyanobacterium and coir pith culture filterate showed better biodegradable activity which promotes plant growth. The analysis of biochemical, morphological and phyiscochemical parameters showed maximum seed germination percentage at lower concs. in combined treatment indicating that the *Lyngbya* sp. along with coir pith can reduce the salinity and thereby promote seed germination in the culture filtrate.

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