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Impact of Cyanobacterial Filtrate on Seed Germination Behaviour of Wheat

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Abstract: **Cyanobacteria are a varied group of prokaryotes. These microorganisms are spread worldwide and improve the growth and development of the plants, with which they share the habitat, because they contribute to soil fertility in many ecosystems by many means, produce various biologically active substances. All four selected cyanobacterial species were identified as *Anabaena variabilis*, *Nostoc muscorum*, *Aulosira fertilissima* and *Tolypothrix tenuis*, were analyze for production of IAA, Phosphate solubilization, HCN production and effect of their filtrate on seed germination behavior of wheat late species var. DBW 550. The experiment conducted at STEP, Department of Biotechnology, Thapar University Patiala, Punjab. We investigated the effect of cyanobacterial filtrate used alone or in combination on seed germination behavior of wheat. The seed treated with cyanobacterial filtrate geminated earlier than non-treated seed. The proportion of seeds that germinated, and values of the Germination Velocity Index (GVI), Vigor Index (VI) and the seedling were higher in the seed treated with cyanobacterial filtrate compared to the control treatment.**

Keywords: **Prokaryotes, IAA, Phosphate solubilization, Biological active substances, Habitat.**

1. INTRODUCTION

The importance of agriculture in the socio-economic fabric of India can be realized from the fact that the livelihood of majority of the country population depends on agriculture Research. Collaborators throughout India have conducted work on various crops that include: legumes, row crops, vegetables, rice, ornamentals, forestry, spices etc. In India agriculture is ploughed many constraints the one and necessary, need of environmental friendly alternative of chemical fertilizers & second is gradually depletion of nutrient supply in Indian agriculture soil to increase the yield. The use of Cyanobacteria as PGPR can fulfill these criteria and to ensure that farming activity become more viable. Auxin the most important & diverse group of plant hormone used by plant as in the regulation of diverse biological processes including cell division, differentiation, root elongation. Indole acetic acid biosynthesis is also known through tryptophan dependent as well as tryptophan independent pathway in microorganism. Sergeeva et al. 2002 reported that cyanobacteria have the capability to accumulate IAA endogenously and to release the hormone [3].

Tryptophan boosts the production & accumulation of IAA and indicates tryptophan dependent production of this important plant hormone. These are also known to release a wide variety of extracellular substances e.g. plant growth regulators vitamin amino acid & polysaccharide which have direct and indirect effects on plant growth and yield [12]. However several strain of Anabaena exhibit the ability to excrete IAA (in low quantity) in the nitrogen free BG-11 media without added tryptophan [10], it show that tryptophan boost the production of IAA. Some cyanobacteria, particularly within the genus, Nostoc, still enter into intimate relationships with some eukaryotic organisms representing all plant divisions [11]. Therefore, the present investigation was made to assess the effect of the four cyanobacterial filtrates, *Nostoc muscorum*, *Anabaena variabilis*, *Tolypothrix tenuis* & *Aulosira fertilissima* separately or in combination on the seedling behavior of wheat.

2. MATERIAL AND METHOD

Pure algal culture of Anabaena variabilis, Aulosira fertilissima, Nostoc muscorum & Tolypothrix tenuis were grown in BG-11 media in the Biotechnology Department, Thapar University Patiala, Punjab under continuous fluorescent light at 2500 Lux and temperature 28 ± 2 °C & tested for their different plant growth promoting and regulating activity.

IAA Estimation. Four cyanobacterial species was inoculated in 250 ml conical flask containing 100 ml BG 11 media with Different concentration (10, 20, 30, 40, 50, 60, 70, 80, 90 & 100 µg/ml) of tryptophan & incubated at 28 ± 2 °C. 10 ml of each bacterial culture were centrifuged at 10000 rpm for 15 min at room temperature & the supernatant was collected. The supernatant (2 ml) was mixed with and 4 ml of the salkowski reagent (50 ml of 35% of perchloric acid and 1ml of 0.5M FeCl3 solution). The experiment was conducted in triplicate & OD was taken at 330 nm at the interval of 10, 20, 30 days.

Phosphate Solubilization. For quantitative assay of phosphate solubilization the experimental flasks were inoculated with 5% inoculums of P-starved, 15 day old actively growing cultures of both the algae for 35 days. The P-starved cultures were grown in presence of TCP at concentrations 20 mg by replacing K2HPO4 in the usual BG-11 medium. Potassium chloride of equivalent amount (34.2 mg/l) was added in the medium to maintain the availability of potassium. The experiments were conducted in triplicates by taking 100 ml medium in 250 ml conical flask. The available 'P' in cell free supernatant was determined by vanado-molybdphosphoric yellow complex method [4, 5].

HCN Production. HCN production was evaluated by the qualitative method of Kremer and Souissi [6].

Germination Test. Late phase (21 days) of the cyanobacterial strains culture were centrifuged and the cell free filtrate were used for seed germination studies. The healthy seeds of wheat were surface sterilized using 0.1% HgCl2 solution for 5 min followed by several washing with distilled water for about 1 h. selected no. of seeds (20) was then distributed on water agarized Petri plates (0.5% agar) and 10 ml of the following different treatment were added, control 1, A1, A2, A3, A4, A1+A2, A1+A3, A1+A4, A2+A3, A2+A4, A3+A4, A1+A2+A3, A1+A2+A4, A1+A3+A4, A2+A3+A4, and A1+A2+A3+A4.

A1: Aulosira fertilissima
A2: Anabaena variabilis
A3: Nostoc muscorum
A4: Tolypothrix tenuis

In control Petri dishes cyanobacterial filtrate were replaced by water for evaluation. Percentage radical emergence and seed germination speed was recorded at 25°C after every 24 h time interval. Time for initial signs of radical emergence and maximum emergence was recorded up to three days.

Germination Velocity Index. The germination speed index (GVI) was calculated as described in the Association of Official Seed Analysts [7] by following formula:

$$G = \frac{N1}{1} + \frac{N2}{2} + \frac{N3}{3} + \cdots \text{so on}$$

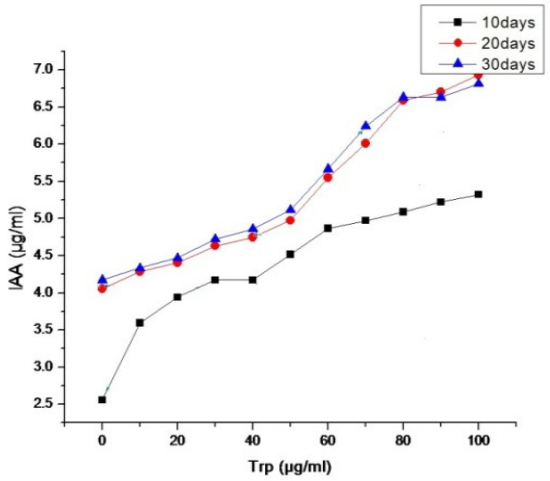
Here **N1 N2 N3** etc. are the no. of new germinant on day 1, 2, 3 etc. following the start of germination test. Since the no. of new germinant on a particular days divided by the serial number of that days the GVI is higher if more seeds germinate in the fewest number of days.

Vigor Index. Seed vigor is the sum total of those properties of seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Although differences in physiological attributes of seed lots can be demonstrated in the laboratory, it was suggested that the term should be used to illustrate the performance of seed were shown in the field. The seed lot showing the higher seed vigor index is considered to be more vigorous [8]. Seedling vigor index was calculated following modified method of Abdul-Baki & Anderson [8]:

$$VI = \text{Seedling lenth (mm)} \times \text{Germination \%}$$

Statistical Analysis

The data of the various parameters was analyzed in triplicates for the growth and biochemical parameters and subjected to one way ANOVA (Analysis of variance) in accordance with the experimental design (completely randomized block design) using OriginPro 8 statistical package to quantify and evaluate the source of variation. It is a procedure used for testing the differences among the means of two or more treatments. The treatment means were compared at a significance level of 0.05.



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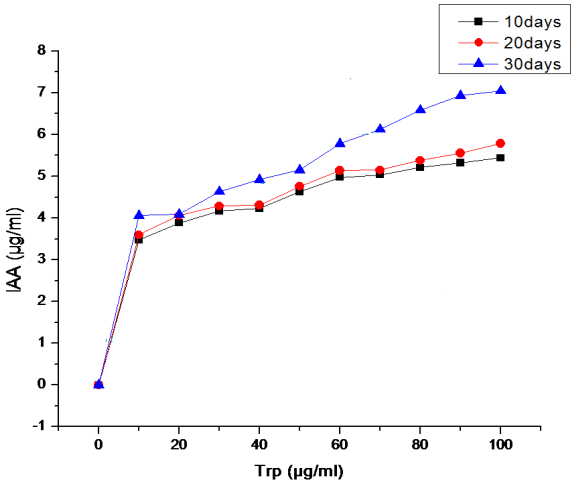

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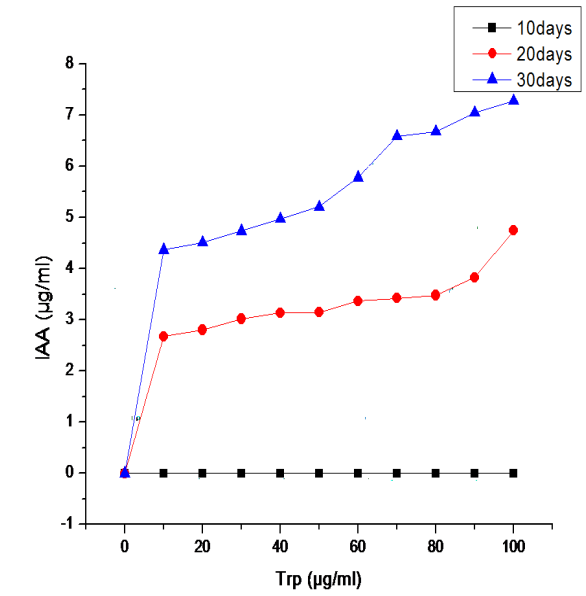

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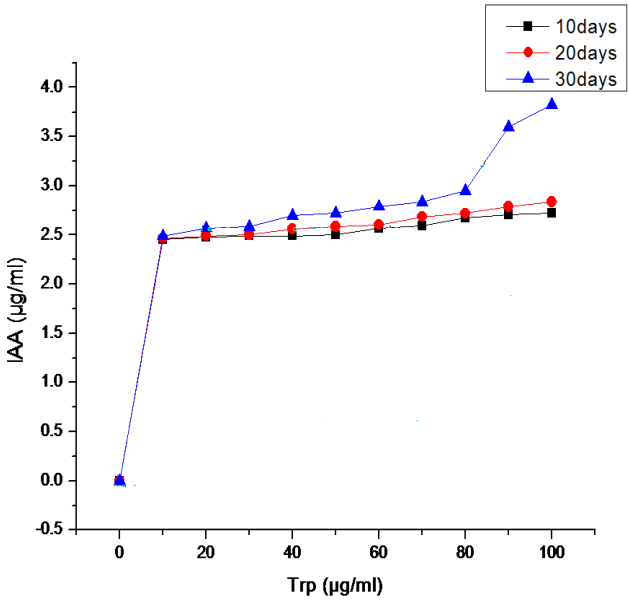


(D)

Fig.1. IAA production profile of (a) Anabaena variabilis (b) Aulosira fertilissima (C) Nostoc muscorum (d) Tolypothrix tenuis

3. RESULT & DISCUSSION

Plant Growth Promoting Activity. All cyanobacterial strains showed positive results for auxin production, phosphate solubilization. All strains showed growth in nitrogen free media. Phosphorus is very crucial nutrients for plant growth and inoculation with phosphate solubilizing microorganism has been shown to improve plant growth by mounting the availability of phosphate [13]. Moreover, cyanobacteria are analyzed to produce different growth regulators such as auxin-like substances Indole acetic acid. Salisbury stated that indole-3-acetic acid is a phytohormone which is known to be involved in root initiation, cell division, and cell enlargement. Cyanobacterial strains may protect plants from phytopathogens due to hydrogen cyanide production [14]. Phytohormone producing cyanobacteria are also involved in the promotion of plant growth [1, 2]. We checked the auxin production of cyanobacteria with familiar colorimetric method.

Table 2. Phosphate solubilization and HCN production by test cyanobacteria

Cyanobacteria	Solubilized P (µg/ml)	HCN Production
Af	4.02	–
Av	3.94	–
Tt	3.72	–
Nm	3.61	–

(*Af* = *Aulosira fertilissima*, *Av* = *Anabaena variabilis*, *Tt* = *Tolypothrix tenuis*, *Nm* = *Nostoc muscorum*, Negative sign (–) = No HCN production)

Colorimetric analysis of cyanobacterial cultures showed variable amount of auxin production in the absence and presence of different concentration of L-tryptophan. *Anabaena variabilis* produced highest amount of auxin in the media supplemented with 100 µg/ml tryptophan after 20 days of inoculation. There was maximum production of IAA from 10 to 20 days for all concentration of L- tryptophan.

The result also showed that there was a production of IAA without L-tryptophan, 2.56 µg/ml after 10 days, 4.05 µg/ml after 20 days and 4.17 µg/ml after 30 days of inoculation without L-tryptophan, whereas in case of *Aulosira fertilissima*, *Tolypothrix tenuis*, *Nostoc muscorum*, there was no production of IAA without tryptophan. *Aulosira fertilissima* produced highest amount of IAA (7.1 µg/ml) after 30 days of inoculation with 100 µg/ml of tryptophan.

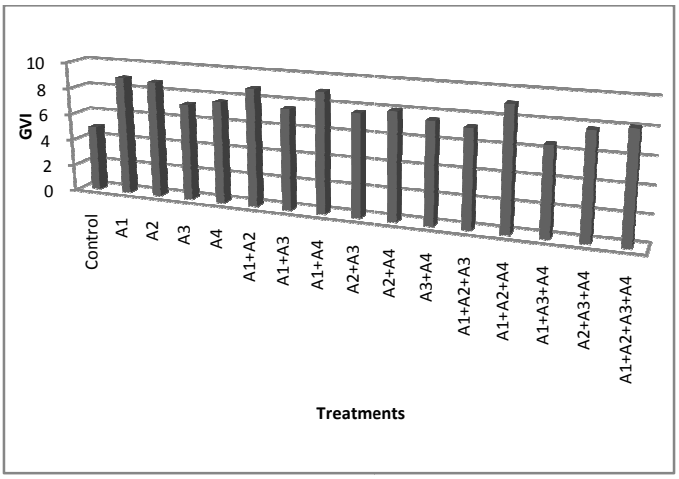
In cause of *Nostoc muscorum* high amount of auxin (7.42 µg/ml) production was observed after 30 days of inoculation and there was no sign of IAA production till 10 days. Out of four cyanobacterial species *Tolypothrix tenuis* produced least amount of IAA (3.82 µg/ml). The data presented in table. 2 shows that out of four cyanobacteria tested *Aulosira fertilissima* shown maximum amount of pho-sphate solubilization (4.02 µg/ml). Production of HCN was not shown by these tested Cyanobacteria.

Effect on Seed Germination Behavior of Wheat. The results of the present investigation elucidated that the presoaking of the plant seeds in culture filtrates of the tested cyanobacterial species alone or in combination stimulated their germination and germination metabolism. A highly significant increase in percentage germination was reached 95% with the combination of the four treatments (A1 + A2 + B1 + B2) (Table 1). The percentage increase in germination over control reached 62.9% and in cause of single cyanobacterial suspension, *Aulosira fertilissima* and *Anabaena variabilis* were shown higher percentage increase (78.33%). Investigations showed that germination was increased significantly over control. Cyanobacterial filtrate hastened seedling recruitment. Seeds treated with cyanobacterial filtrate either in alone or in combination germinate faster, while in control seeds germinate slowly, so cyanobacterial filtrate led to a significantly higher value of the germination velocity index compared to seeds in under controlled treatment.

Table 1. Effect of cyanobacterial filtrate on germination percentage.

Treatments	Germination % of total seeds
Control	58.33 ± 3.33
A1	78.33 ± 7.26
A2	78.33 ± 1.67
A3	70 ± 0
A4	81.66 ± 1.67
A1+A2	91.66 ± 1.67
A1+A3	80 ± 2.88
A1+A4	90 ± 2.88
A2+A3	80 ± 2.88
A2+A4	83.33 ± 3.33
A3+A4	80 ± 2.88
A1+A2+A3	70±2.88
A1+A2+A4	68.33 ± 1.67
A1+A3+A4	90 ± 5
A2+A3+A4	78.33 ± 1.67
A1+A2+A3+A4	95 ± 0
F value	9.5047

Alone *Aulosira fertilissima* and *Anabaena variabilis* shows higher value of mean difference in germination velocity index (A1, control = 4), (A2, control = 3.83) respectively, over control and in combination of two Aulosira fertilissima + Nostoc muscorum & Aulosira fertilissima + Anabaena variabilis show (A1+A4, control = 4.05) & (A1+A2, Control = 3.89) respectively. Despite cyanobacteria used alone or in combination shows higher germination velocity index over control (fig. 2a). Further, the seedlings recruited from cyanobacterial filtrate treated seeds also had signific-antly higher values of the vigor index (fig. 2b).



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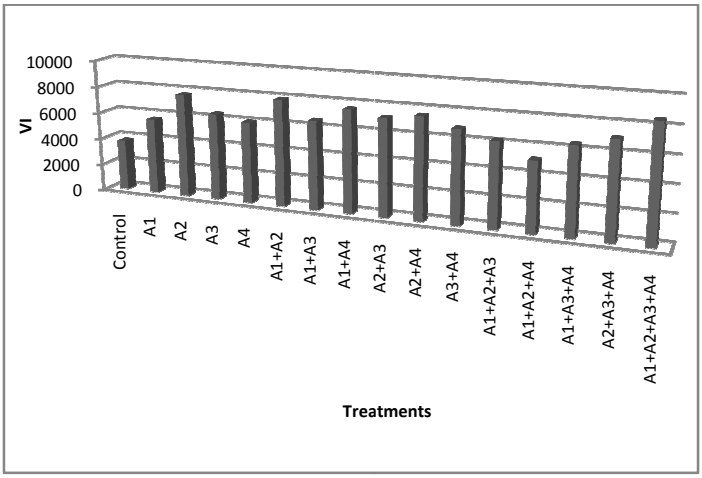

(b)

Fig. 2. (a) Effect of Cyanobacterial filtrate alone or in combination on Germination Velocity Index (GVI). (b) Effect of Cyanobacterial filtrate alone or in combination on Velocity Index (VI)

4. CONCLUSIONS

It is concluded that the cyanobacterial filtrates studied effect significantly the germination behavior of investigated wheat as compared to that of control either used alone or in combination. The germ- ination percentage, germination velocity index, vigor index were notably enhance. The result also shows, in combination cyanobacteria were more effective.

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