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Evaluating Potential of *Spirulina* as Innoculant for Pulses

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Abstract: Many non-heterocystous cyanobacteria are also capable of nitrogen fixation and improve plant growth. *Spirulina platensis* and *S. maxima* at concentration of 500 mg and 1000 mg/kg soil were tested on two plants *Phaseolus aureus* and *P. mungo*. Growth was measured in terms of plant shoot lengths. The leaf chlorophyll content and protein content of the grains of both the plants were analyzed. Shoot length of both the plants were increased after 30 days. The leaf chlorophyll content and the protein content in the grains of the plants were not improved significantly. Conclusion was drawn that inoculation of *Spirulina* shows no beneficial effects in the crop plants.

Key words: Non-heterocystous · Nitrogen fixation · Shoot length · Chlorophyll content · Protein content

INTRODUCTION

Cyanobacteria are considered as an important group of microorganisms capable of fixing atmospheric nitrogen. They have a unique potential to contribute to productivity in a variety of agricultural and ecological situations. Most paddy soils have a natural population of cyanobacteria which provides a potential source of nitrogen fixation at no cost. Many cyanobacteria fix nitrogen under aerobic conditions in specialized cells called heterocyst which comprise 5-10% of cells in a filament [1]. Many cyanobacteria are also capable of using atmospheric dinitrogen (N_2) as the source of nitrogen and this is what most commonly termed nitrogen fixation. Non-heterocystous cyanobacteria are also able to promote plant growth and can also be used as biofertilizer.

Besides fixing atmospheric nitrogen, cyanobacteria play a major role in reducing soil erosion because of ability to secrete polysaccharides that bind soil [2]. Also they control soil run off and increase soil organic matter content and in producing certain substances which enhance the growth of plants [3]. Due to this important characteristic of nitrogen fixation, the utility of cyanobacteria in agriculture to enhance production is beyond doubt.

Spirulina contains a wide spectrum of nutrients that include proteins, carbohydrates, vitamins, minerals, β-carotene and super antioxidants apart from trace elements. The protein content is as high as 60-70% of its dry weight [4]. *Spirulina* has a high concentration of vitamins and lipid in the amount of 4-7% is also present in it. The essential fatty acids γ-linolenic acids, linolenic acid and also 13.6% of carbohydrates are present [5, 6].

The present study was undertaken on two plants *Phaseolus aureus* and *Phaseolus mungo* to study the effects of inoculation of *Spirulina* viz. *S. platensis and S. maxima* on the growth of these plants and protein content of their grains.

MATERIALS AND METHODS

Culturing of *Spirulina*: Pure culture of *S. platensis* and *S. maxima* were obtained from CCUBGA, IARI, New Delhi and was cultured in CFTRI media. The culture was harvested after 15 days by filtration and subsequent drying.

Inoculation of *Spirulina*: *S. platensis* and *S. maxima* were mixed with soil in quantities of 500 mg and 1000 mg/kg separately. These soils were taken into clay pots. Soil was pre sterilized to avoid contamination. All sets were prepared in triplicates. In each pot 5 seeds of each plant were sown and kept for germination in open atmosphere. Watering was done daily to keep the soil moist. Control plants were also sown in the experiment.

Growth Measurement: Growth was determined by measuring the shoot length of both the plants and leaf chlorophyll content by the method of Arnon [7]. The protein content of the grains of the plants was estimated by the method of Bradford [8].

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RESULTS AND DISCUSSION

Observations were made in every 10 days of each pot including control and growth of plant was measured up to 30 days. Plant shoot lengths were increased when *Spirulina* culture was inoculated in the soil. The shoot length of *P. aureus* and *P. mungo* was enhanced more in *S. platensis* inoculated soil as compared to *S. maxima* inoculated soil. The increase in growth of these plants can be compared with results of other workers, who also observed that after inoculation of some cyanobacteria in the soil, there was enhancement in the growth rate of some plants such as in oats, barley, rye, sugarcane, lettuce, chilli and other vegetables. This may reduce the need of using synthetic nitrogen fertilizers [9].

Also 1000 mg *Spirulina* inoculated plants showed characteristically more increase than 500 mg *Spirulina* inoculated plants. The inoculation of the soil with the nitrogen fixing blue green alga, *Calothrix anomala* had a positive effect on *Capsicum annum* and *Lactuca sativa* in terms of yield [10]. Furthermore, the height of cotton plants and their dry weights were increased by presoaking the seeds in *Nostoc muscorum* extract [11]. Rodgers *et. al*., [12] described in their report, inoculation of soils in pots containing radish or tomato plants with algal suspension and exudates resulted in increased growth rates of both plants and increased their overall yield. Nanda *et. al.,* [13] worked on soaking seeds of cucumber and pumpkin with an extract of *Westiellopsis prolifica*, a N_2 fixing cyanobacterium and concluded that it promoted germination and their subsequent growth and development pot experiments were conducted by Khan *et. al.,* [14]. They observed the effect of powdered dry filaments of a blue-green alga. *Microcoleus vaginatus* on tomato plants and they reported that it enhanced the growth of the plant. The growth of plants were observed in the study but the increase in shoot lengths of both the plants were not as much high as compared to heterocystous cyanobacteria which are already known to fix atmospheric nitrogen there by promoting plant growth.

The chlorophyll content of the plants during our study was increased very less after addition of *Spirulina*. It was also observed that both the species of *Spirulina* equally affect the chlorophyll content in the leaves of the plants and that quantity of *Spirulina* have not shown any significant changes in the pigment content. The results were in harmony with those obtained by Haroun and Hussein [15] who found that the chlorophyll a and total pigments were not increased significantly after the treatment with filtrate of *Anabaena fertilissima*.

Table 1.1: Shoot Length of Plants (in cm) after inoculation of *Spirulina*

	S. platensis						*S. maxima*					
	P. aureus			*P. mungo*			*P. aureus*			*P. mungo*		
Growth in days	Control	500 mg	1000 mg	Control	500 mg	1000 mg	Control	500 mg	1000 mg	Control	500 mg	1000 mg
10	9.65±0.80	10.38^ns^±0.80	10.90^ns^±0.90	10.38±0.60	12.60**±0.65	13.30**±0.45	9.47±0.83	9.81^ns^±0.81	10.65^ns^±0.90	10.46±0.64	11.69^ns^±0.69	12.30*±0.45
20	15.65±0.65	17.80*±0.70	18.90**±0.95	18.04±0.84	20.90**±0.65	22.85**±0.60	15.35±0.70	17.69*±0.64	18.78**±0.90	18.46±0.64	20.63*±0.70	22.60**±0.55
30	20.03±0.60	22.98**±0.70	25.48**±0.88	23.08±0.70	26.18**±0.78	27.25**±0.60	20.16±0.65	22.26*±0.70	24.56**±0.80	23.43±0.70	25.73*±0.80	26.85**±0.80

Values are given as mean ± S.D for three set in each group.

Experimental groups were compared with control, ns p> 0.05, *p< 0.05, **p<0.001

Table 1.2: Leaf Chlorophyll Content (mg/g) of Plants after inoculation *Spirulina*

	P. aureus			*P. mungo*		
Species	Control	500 mg	1000 mg	Control	500 mg	1000 mg
S. platensis	0.20±0.02	0.21^ns^±0.01	0.23^ns^±0.03	0.14±0.02	0.15^ns^±0.02	0.16^ns^±0.03
S. maxima	0.21±0.01	0.22ns±0.02	0.23ns±0.03	0.14±0.02	0.16^ns^±0.01	0.16^ns^±0.03

Values are given as mean ± S.D for three set in each group.

Experimental groups were compared with control, ns p> 0.05, *p< 0.05, **p<0.001

Table 1.3: Grain Protein Content (mg/g) of plants after inoculation of *Spirulina*

	P. aureus			*P. mungo*		
Species	Control	500 mg	1000 mg	Control	500 mg	1000 mg
S. platensis	22.00±0.06	23.65**±0.05	24.76**±0.08	18.20±0.03	18.65**±0.04	19.18**±0.03
S. maxima	22.06±0.05	23.50**±0.03	24.70**±0.08	18.28±0.02	18.60**±0.02	19.05**±0.05

Values are given as mean ± S.D for three set in each group.

Experimental groups were compared with control, ns p> 0.05, *p< 0.05, **p<0.001

The growth stimulative effect of the cyanobacteria on plants may be attributed to elevated levels of GA_3 which is known to inhibit chlorophyllase activity [16]. Lakshmi and Annamalai [17] emphasized the effective response of the plant to culture filtrates. Higher the volume, greater was the output by the plant. The total chlorophyll, total nitrogen and total carbohydrates analyzed showed manifold increase in plants treated with culture filtrates compared to control plants.

The protein content of the grains of *P. aureus* and *P. mungo* were analyzed. The protein content was also not improved much as compared to control plants. Ghallab and Salem[18] stated that, in wheat plant, growth characters and nutrients, sugar, amino acids and growth regulators (1AA, GA_3 and kinetin) as well as crude protein contents of the tested plant, increased by using biofertilizer; Cerealin (*Azospirillum* spp.) and Nemales (*Serratia spp.*). They concluded that the growth promotion of plant by cyanobacteria may not necessarily affect the protein content or pigment content but may be attributed to the secretion of plant growth regulators by the organism.

Recently, it has been demonstrated that high yielding varieties which need high levels of nitrogenous fertilizers also respond to algal inoculation by increasing yield upto 10-15 percent, which has been attributed to the growth promoting substances secreted by blue green algae. It is however possible that for various reasons, the introduced cyanobacteria may be poor [19-21].

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