



# Response of paddy field cyanobacterium, *Westiellopsis prolifica* Janet to the inorganic nitrogenous fertilizers

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## ABSTRACT

Cyanobacteria are prokaryotic nitrogen fixing microorganism which played a significant role in the nitrogen fixation and contributed much to the submerged rice cropping system of the world. It can luxuriantly flourish in paddy fields and providing nitrogen as well as various growth promoting substances that influence the growth of paddy plants. India, being an important rice producing country of the world, depends upon various inorganic fertilizers for rice cultivation. The heterocystous cyanobacterium, *Westiellopsis prolifica* is a common inhabitant in most of the paddy fields of Kerala. The growth and other biochemical parameters upon the growth of cyanobacterium were assayed. The nitrogenous fertilizer ammonium sulphate influences the chlorophyll-a synthesis, protein content and growth rate. But the ammonia excretion was drastically reduced in cyanobacterial cultures upon the treatment with inorganic fertilizers.

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## INTRODUCTION

Cyanobacteria are oxygen evolving, nitrogen fixing prokaryotes occur in every conceivable habitats but abundantly in water logged rice fields in several rice growing countries. Nitrogen fixing cyanobacteria play a vital role in the maintenance of soil fertility and sustainability in rice field ecosystems (Roger & Reynaud, 1979). Cyanobacterial inoculation to rice crop was found to be effective in different agroclimatic conditions like tropical or temperate climates and soil types such as saline soils, phosphorous rich soils, alkaline or acidic soils etc. (Singh *et al.*, 2017). The paddy field ecosystem provides an environment favourable for the growth of cyanobacteria with respect to their requirement for light, water, temperature, humidity and nutrient availability. In submerged soil system, biological nitrogen fixation contributes 25 to 30 kg nitrogen per hectre for one cropping season (Saexena *et al.*, 2007; Kaushik, 2001). They are cosmopolitan in distribution and occur in every conceivable habitat where life is possible. They are reported to occur in extreme climatic conditions such as hot springs, polar deserts and Antarctic regions (Halder, 2015, 2016). Even though they are ubiquitous, they prefer to grow in rice fields because the soil pH, temperature, humidity, crop canopy and soil moisture are favourable for its growth in paddy fields.

It releases a large number of secondary metabolites into the paddy fields which also influences the growth of paddy (Wilson, 2006). The beneficial effect of cyanobacterial biofertilizer for paddy such as increase in number of tillers, seeds, length of the plant and leaf, yield etc. has been well documented (Karthikeyan *et al.*, 2009; Sao & Samual, 2018; Radhakrishnan & Venkitaraman, 2005). Cyanobacterial biomass is also used for the production of various bioactive compounds, food items and biofuels (Hall *et al.*, 1995; Malik *et al.*, 2001; Paumann *et al.*, 2005). They can control the deficiency of nitrogen in the soil and also improves its properties. Apart from nitrogen fixation, it also influences the overall growth of paddy including the grain yield (Roger *et al.*, 1980; Singh, 1981; Alam *et al.*, 2014). Since the urea and ammonium sulphate are the easily available and commonly used inorganic nitrogen fertilizer in the paddy fields of India, this made the the author to study how much these synthetic nitrogen fertilizers such as urea and ammonium sulphate influences the growth of paddy field cyanobacterium, *Westiellopsis prolifica*.

## MATERIALS AND METHODS

The cyanobacterium, *Westiellopsis prolifica* Janet was collected from the paddy fields of Kerala. The organism was made into

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unialgal culture by dilution culture followed by culturing in BG-11 nitrogen free medium (Rippka *et al.*, 1979) containing 0.8% (w/v) agar. Pure colonies were transferred to liquid medium and incubated. The cultures were maintained in culture racks at a temperature of  $25 \pm 2$  °C under continuous illumination of 1500-2000 lux for 12 hours light and 12 hours darkness. The growth was assayed in terms of chlorophyll-a. For this, the culture suspension of cyanobacterium was homogenized and centrifuged at 5000 rpm for 10 minutes. The supernatant was discarded and the pellet was suspended in 80% acetone in dark refrigerator for 20-24 hours. The absorbance of the acetone extract was measured using a spectrophotometer. The proteins were estimated (Lowry *et al.*, 1951) and for this the cyanobacterial pellet was suspended in 10% TCA. It was then treated with alkaline reagent and also with 1N Folin-Ciocalteu reagent and incubated in dark for 30 minutes and the absorbance was measured at a wavelength of 750 nm using spectrophotometer. The release of ammonia was measured by phenol- hypochlorite method with a modification in pH of the reagent (Solorzano, 1969).

## RESULTS

For studying the effect of inorganic nitrogen fertilizers such as urea and ammonium sulphate, the stock solutions were prepared in distilled water and aseptically added to the culture medium to obtain the concentrations of 1, 5, 10 and 20 ppm respectively. Equal amount of exponentially growing cultures were inoculated into the medium and harvested after 28 days. *W. Prolifica* grown in ammonium sulphate showed better growth rate than the cultures grown in urea under all the concentrations such as 1, 5, 10, and 20 ppm measured at an absorbance of 760 nm after 28 days of inoculation. But the production of protein was almost same in both urea and ammonium sulphate grown cultures. The chlorophyll- content was higher in the cyanobacterial cultures grown in ammonium sulphate than that of grown under urea treated cultures. Ammonia excretion was more in control than in nitrogenous fertilizers treated samples. However, *W. Prolifica* could with stand more in urea than in ammonium sulphate in all the treated concentrations. The results are given in Table 1 and 2.

## DISCUSSION

The growth and abundance of cyanobacterial flora is chiefly influenced by the type and mode of application of inorganic fertilizers such as nitrogen, phosphorous and potassium (Kaushik, 1987). Among the various nutrients supplemented as fertilizers in the paddy fields, nitrogen plays an important role in sustaining crop productivity because it is an essential element required for the growth of paddy. According to the present study, the ammonia excretion was drastically reduced in cultures grown in nitrogen sources and it agrees with the findings of Anand and Parameswaran (1990). But enhancement of nitrogenase activity in the presence of exogenous ammonium source in *Anabaena* sp. has been reported (Bottomley & Stewart, 1976). The cyanobacterium *Cylindrospermum* showed nitrogenase activity in presence of nitrogenous fertilizers (Anand, 1990).

However, the cyanobacteria utilizes all available nitrogen present in the soil as organic and inorganic salts besides the elemental nitrogen. In addition, cyanobacteria can grow well in ammonia based commercial fertilizers. The cyanobacterial cultures grown in ammonium sulphate showed better growth rate than the cultures grown in urea. The cyanobacteria can use organic nitrogen to fulfil their nitrogen requirements but at the same time it can effectively assimilate the inorganic fertilizers such as urea and ammonium sulphate. In the present study, excretion of ammonia was greater in urea treated cyanobacterial cultures than ammonium sulphate treated cultures. There are reports that ammonium present in the growing medium does not allow the developments of heterocyst (Anand & Karuppusamy, 1987) and it also inhibit the synthesis of nitrogenase in cultures that bear fully developed heterocyst. On the other hand, urea is a good nitrogen source for many cyanobacteria and its application will prevent various losses like ammonia volatilization, denitrification, run off and leaching. The chlorophyll-a and protein contents were comparably high in inorganic fertilizers treated samples than in control. It shows that the growth of *W. Prolifica* was stimulated by these inorganic fertilizers. The present study confirms that the cyanobacterium *W. Prolifica* can thrive well in presence of inorganic fertilizers and they are not degraded by the application of synthetic fertilizers. But, ammonia excretion was reduced in inorganic fertilizer treated cultures shows that these synthetic fertilizers affect the nitrogen fixation. Moreover, the nitrogen fixation rate will always reduce if excess nitrogen is present in the culture medium. The excess

**Table 1: Effect of nitrogen sources on the protein and chlorophyll-a content after 28 days of inoculation**

Treatments	Protein content in $\mu\text{g ml}^{-1}$		Chlorophyll a content in $\mu\text{g ml}^{-1}$	
	Urea	Ammonium sulphate	Urea	Ammonium sulphate
Control				
1 ppm	94.71 $\pm$ 0.11	94.71 $\pm$ 0.11	8.94 $\pm$ 0.03	8.94 $\pm$ 0.03
5 ppm	99.37 $\pm$ 0.05	108.71 $\pm$ 0.11	8.84 $\pm$ 0.05	9.21 $\pm$ 0.02
10 ppm	110.81 $\pm$ 0.11	133.46 $\pm$ 0.05	9.35 $\pm$ 0.05	10.13 $\pm$ 0.03
20 ppm	115.43 $\pm$ 0.11	116.27 $\pm$ 0.09	9.64 $\pm$ 0.08	10.39 $\pm$ 0.03
	139.27 $\pm$ 0.09	141.11 $\pm$ 0.08	11.14 $\pm$ 0.04	12.11 $\pm$ 0.03

(Values represent mean of three replicates  $\pm$  S.D.)

**Table 2: Effect of nitrogen sources on ammonia excretion (n moles  $\text{ml}^{-1}$ )**

Concentration	Days after inoculation			
	7	14	21	28
Control	30.12 $\pm$ 0.15	96.45 $\pm$ 0.10	78.37 $\pm$ 0.08	31.06 $\pm$ 0.09
1 ppm urea	22.38 $\pm$ 0.08	72.11 $\pm$ 0.011	49.24 $\pm$ 0.05	26.72 $\pm$ 0.06
5 ppm urea	18.78 $\pm$ 0.06	54.67 $\pm$ 0.08	36.44 $\pm$ 0.08	17.19 $\pm$ 0.11
10 ppm urea	15.34 $\pm$ 0.10	45.47 $\pm$ 0.05	21.33 $\pm$ 0.07	14.41 $\pm$ 0.10
20 ppm urea	13.22 $\pm$ 0.09	19.83 $\pm$ 0.06	15.72 $\pm$ 0.04	7.65 $\pm$ 0.07
1 ppm (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	19.37 $\pm$ 0.08	54.32 $\pm$ 0.09	36.78 $\pm$ 0.07	18.91 $\pm$ 0.06
5 ppm (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	16.71 $\pm$ 0.10	25.36 $\pm$ 0.11	23.11 $\pm$ 0.06	17.03 $\pm$ 0.10
10 ppm (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	14.09 $\pm$ 0.09	20.31 $\pm$ 0.07	16.12 $\pm$ 0.05	11.23 $\pm$ 0.13
20 ppm (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	12.93 $\pm$ 0.12	19.18 $\pm$ 0.05	14.36 $\pm$ 0.09	10.24 $\pm$ 0.10

(Values represent mean of three replicates  $\pm$  S.D.)

nitrogen also reduced the frequency of production of heterocyst which in turn affects the nitrogen fixating potential of this microorganism. So it is recommendable to use smaller amount of inorganic nitrogenous fertilizers in paddy fields along with the cyanobacterial biofertilizers for getting better yield from paddy. Thus, *W. Prolifica* is a promising biofertilizer which can be effectively utilized for paddy cultivation along with inorganic fertilizers.

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