



## Comparative Physiological Studies of Dry-weight and Chlorophyll-a, Cellular protein and Nitrogenase activity of six different strains of the family Scytonemataceae (Cyanobacteria)

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### Abstract

The present research paper deals with the filamentous, heterocystous Cyanobacteria or Blue-green algae belonging to the genera *Scytonema*, *Tolypothrix*, *Camptylonemopsis* of the family Scytonemataceae, which were isolated from their natural materials and various rice growing fields in several districts of West Bengal and Uttar Pradesh. In the present study, we took six fast-growing strains, 1. *Scytonema chiasmum* Geitler., 2. *Scytonema tolypothricoides* Kuetzing ex Born. et Flah., 3. *Scytonema bewsii*, 4. *Tolypothrix distorta*, 5. *Tolypothrix byssoidea* (Brek) Kirchner and 6. *Camptylonemopsis lahorensis* (Ghose) Desikachary for the comparative study by evaluating their dry weight in nitrogenous and nitrogen-deficient medium, chlorophyll-a, cellular protein and nitrogenase activity as per chlorophyll-a and per-vial on 15<sup>th</sup> day.

Results indicate that all the strains have more dry weight in the nitrogenase medium than the nitrogen deficient medium but maximum increase (170%) due to the presence of normal recommended quality of nitrogen. But cellular protein ( $\mu\text{g/ml}$ ) was higher in such strains, those usually have higher dry weight under both conditions. Therefore, maximum cellular protein (0.195  $\mu\text{g/ml}$ ) was detected in *Scytonema tolypothricoides* and minimum (0.080  $\mu\text{g/ml}$ ) was in *Tolypothrix distorta*. Nitrogenase activity in terms of  $\mu\text{g/chl-a/h}$  was maximum (9.18 mole  $\text{C}_2\text{H}_4 / \mu\text{g chl.a/h}$ ) in *Tolypothrix distorta* and minimum (2.94 mole  $\text{C}_2\text{H}_4 / \mu\text{g chl.a/h}$ ) was in *Scytonema tolypothricoides*. However, it differs in its sequence in terms of n mole  $\text{C}_2\text{H}_3/\text{vial/h}$  and it is maximum (9.40 mole  $\text{C}_2\text{H}_4 / \text{vial/h}$ ) in *Tolypothrix byssoidea* and minimum (3.38 mole  $\text{C}_2\text{H}_4 / \text{vial/h}$ ) in *Tolypothrix distorta*.

**Keywords:** Cyanobacteria, Physiology, Dry-weight, Chlorophyll-a, Protein, Nitrogenase activity, Heterocysts, Scytonemataceae.

### 1. Introduction

Cyanobacteria or Blue green algae belong to a diversified group of photosynthetic prokaryotes. They were very common in most of the rice growing fields and were generally used as biofertilizers for their better role as diazotrophs, ameliorant for enrich in

soil health's better physical and chemical properties. Dhar DW, Prasanna R, Singh BV (2007) Comparative performance of three carrier based blue green algal biofertilizers for sustainable rice cultivation. The family Scytonemataceae Rabenhorst came into existence in 1865 while the genus *Scytonema* Agardh was described four decades ago by Agardh (1820), and

Bornet and Flahault described *Tolypothrix* in 1887. Komarek and Anagnostidis (1988) established genera like *Hassallia*, *Petalonema*, and *Camptylonemopsis*. According to them *Scytonema* is a member of Scytonemataceae and the rest viz. *Microchaete*, *Hassallia*, *Petalonema* and *Camptylonemopsis* are the members of Microchaetaceae. Desikachary (1948) studied *Camptylonema indicum* and *Camptylonema lahorensis* and found that *Camptylonema indicum* with true branching as well as false branching is a member of stigonemataceae. Anand and Gunaseeli (1978b) further studied both the genera *Scytonema* and *Tolypothrix* in different culture media. They found that *Tolypothrix* strain could be identified as *Tolypothrix distorta* or *Tolypothrix bouteillei* or *Tolypothrix tenuis* or *Tolypothrix limbata* depending on growth in different media and in the same way *Scytonema* strain could be identified as *Scytonema cincinnatum* or *Scytonema coactile*. Kaushik B;D; (2004) Use of blue-green algae and *Azolla* biofertilizers in rice cultivation and their influence on soil properties. Komarek and Anagnostidis (1988) referred to the International Association for Cyanophyte Research (IAC) and suggested the need for more intransitive study under various cultural study and not merely on the basis of natural materials.

The present study Scytonemataceae (Cyanobacteria) belong to a diversified group of photosynthetic prokaryotes. It serves an important role in increasing and maintaining soil fertility, results in increased growth rate of many grains, especially rice, making this organism a potent natural bio fertilizer. The environment of paddy field ecosystem is highly favourable for the growth of cyanobacteria as it matches all the requirements, such as light, water, temperature and nutrients for Cyanobacteria (Kumar et. al., 2010). In this study we surveyed and examined different paddy fields to know the biodiversity of Cyanobacteria. Comparative study on *Scytonema chiastum*, *Scytonema bewsii*, *Scytonema tolypothricoides*, *Tolypothrix byssoidea*, *Tolypothrix distorta*, and *Camptylonemopsis laehorensis* under the family Scytonemataceae through assessment of their dry weight in nitrogenous and nitrogen deficient medium, chlorophyll-a, cellular protein and nitrogenase activity (Yoon et. al., 2017) as per chlorophyll-a and per vial on 15th day. Quantitative assessment of nitrogen fixation by isolated blue-green algae from paddy-field was done by previous workers only through Micro-kjeldahl method (Kaushik, 2004).

## 2. Materials and Methods

### 2.1. Isolation and Maintain of the strains

In total 36 *Scytonema* (21 Strains) *Camptylonemopsis* (5 Strains) and *Tolypothrix* (10 Strains) of the family Scytonemataceae have been taken for the present study and finally, a few strains were selected as fast-growing strains after comparing their growth (chlorophyll-a and dry weight, Nitrogenase activity) with other strains of the order Scytonemataceae and Nostocaceae. A similar quantity of the inoculum was taken from their exponential growth phase of unialgal cultures grown in BG-11 nitrogen-free liquid medium (Stanier et. al., 1971). The streaking method carried out the isolation of these filamentous forms (Kaushik, 1987). Cyanobacterial samples were inoculated upto 15 days at  $32 \pm 2^\circ \text{C}$  and 4000-5000 Lux light intensity under 14/10 LD. The final pH of the sample was adjusted to physiological pH, which is 7.5. Required medium were prepared aseptically in 10 ml containing sterilized medium after making their stock solutions (Halder, 2010).

### 2.2. Morphological observation and identifications

Morphological observations were recorded with the help of Nikon and Motic microscopes with attaching photosystems.

### 2.3. Identification of these filamentous Cyanobacteria

The taxonomic identifications of isolated filamented strains were made by following the key given by Desikachary (1959), Komarek & Anagnostidis (1998, 2005), Tiwari (1972, 1975 and 1979) and and Tiwari et. al. (1979A) and also our present observations.

### 2.4. Physiological studies of Estimation of Cellular and Extracellular protein:

In 1965, estimated cellular and extracellular protein with Folin-Ciocalteu reagent by the spectrophotometer which was modified from Lowry et. al., (1951). After adding the equal volume of 10% (w/v) TCA in the sample, cell suspension was precipitated and subsequently removed by centrifugation at  $4^\circ \text{C}$  for 10 minutes. Following the

addition of the known volume of 1N NaOH and the pellet or algal filtrate (extracellular protein), it was boiled for 10 minutes. Then cooled and again centrifuged to eliminate light scattering materials.

#### Reagents: (1) Alkaline copper sulphate solution:

It was freshly prepared by making of 0.5 ml of 1%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 0.5 ml of 2% potassium sodium tartarate with 50.0 ml of 2% sodium carbonate in 0.1N NaOH.

#### (2) Folin and Ciocalteu's phenol reagent:

It was prepared freshly by diluting (1:1) folin and ciocalteu's reagent with the same volume of distilled water.

#### Procedure:

0.5 ml of aliquot was taken in 15.0 ml capacity borosil tubes and 5.0 ml of alkaline copper sulphate solution was added into it. Test-tube was allowed to incubate at room temperature for 15 minutes. After that, 0.05 ml of diluted folin phenol reagent was taken and mixed properly. Further this composition was again allowed to incubate for 30 minutes in the dark. The absorbance was taken in terms of optical density (O.D) at 660 nm wave length by spectrophotometer (SICO SPEC - 100). The reading were compared with standard curve of protein.

#### 2.5. Determination of chlorophyll-a ( $\mu\text{g/ml}$ ):

The replicates were in the test tubes containing 10 ml of liquid medium. The growth of strains was measured after a definite period i.e., on 15<sup>th</sup> day of inoculation by an increase in chlorophyll-a  $\mu\text{g/ml}$ .

#### 2.6. Estimation of Nitrogenase activity:

Nitrogen-deficient medium was used to enumerate the nitrogenase activity in the cultures at the exponential growth stage. The activity was measured in terms of Acetylene Reduction Assay (ARA) (Kaushik and Venkataraman-1983 using Gas Chromatograph (Amil-Nucon model-5700) with para pack N and T columns (Stewart et al., 1967). Acetylene equivalent to 10% of the total air space was injected into a glass vial of 15 ml capacity. The vials were stopped with sub seals and incubated for 120 minutes at  $28 \pm 2^\circ\text{C}$  under 4000 – 5000 lux light intensity. The reaction was stopped by

injecting 0.1 ml of 50 % TCA (Trichloro acetic acid) and the gas phase was analyzed for ethylene and the activity was expressed as n mole  $\text{C}_2\text{H}_4$  /  $\mu\text{g}$  chl-a / h and it is also presented as n mole  $\text{C}_2\text{H}_4$  /  $\mu\text{g}$  /vial /h. Experiments were performed in three replicates.

#### Preparation of standard curve:

100 mg of pure bovine serum albumin was dissolved in 100 ml of glass distilled water. 1 ml of the solution was diluted into one litre, in which one ml of the diluted solution was contained 0.001 mg of protein/ml. The remaining procedure was adopted similarly as for sample and by a reading of different gradation of protein standard curve has been drawn.

### 3. Results

#### 3.1 *Scytonema chiasmum* Geitler.:

The present alga has 6 mg/100 ml and 9 mg/100 ml dry weight in nitrogen deficient and nitrogenous liquid medium respectively, Cellular protein was 0.165  $\mu\text{g}$  /ml in nitrogen deficient liquid medium on 15<sup>th</sup> day. It has 0.204  $\mu\text{g}$  /ml chlorophyll-a, Nitrogenase activity was 2.54 in terms of n mole  $\text{C}_2\text{H}_4$ /  $\mu\text{g}$  chl.a/h and nitrogenase activity was 6.88 in terms of n mole  $\text{C}_2\text{H}_4$ /  $\mu\text{g}$  /vial/h in nitrogen deficient medium on 15<sup>th</sup> day.

#### 3.2 *Scytonema tolypothricoides* kuetzing ex Born. et Flah:

According to the medium's composition, the growth algae varied distinctly; therefore its dry weight 9 mg/100 ml was nitrogen deficient and 13.5 mg/100 ml was in nitrogenous liquid medium, cellular protein was 0.195  $\mu\text{g/ml}$  on 15<sup>th</sup> day. On the same day 0.204  $\mu\text{g/ml}$  was chlorophyll- a, Nitrogenase activity was 2.94 in terms of n mole  $\text{C}_2\text{H}_4$ /  $\mu\text{g}$ /chl.a/h and nitrogenase activity was 5.67 in terms of n mole  $\text{C}_2\text{H}_4$ /vial/h in nitrogen deficient medium on 15<sup>th</sup> day.

#### 3.3 *Scytonema bewsii* Fritsch et Rich.:

Under optimum standard laboratory conditions, the alga showed dry weight 5 mg/100 ml in nitrogen deficient medium 13.5 mg/100 ml in nitrogenous medium and cellular protein was 0.155 mg/ml on 15<sup>th</sup> day. It appeared growth with 0.228  $\mu\text{g/ml}$  Chlorophyll-a and nitrogenase activity was 5.50 in terms of n mole  $\text{C}_2\text{H}_4$  /  $\mu\text{g}$  chl.a/h and nitrogenase activity was 3.37 in terms of n mole  $\text{C}_2\text{H}_4$ /vial/h in nitrogen deficient liquid medium on 15<sup>th</sup> day.

**3.4 *Tolypothrix distorta* Kuetz. ex Born. et Flah. :**

Physiological characterization:

The alga produced 8.50 mg/100 ml and 5.00 mg/100 ml dry weight in nitrogen-deficient and nitrogenous liquid medium respectively, and cellular protein was 0.594 µg/ml. However, the Chlorophyll-a, 0.8 µg/ml in nitrogen-deficient liquid medium respectively. The nitrogenase activity was 9.18 in terms of n mole C<sub>2</sub>H<sub>4</sub>/µg/chl.a/h and nitrogenase activity was 3.38 in terms of n mole C<sub>2</sub>H<sub>4</sub>/vial/h in nitrogen deficient medium on 15th day.

**3.5 *Tolypothrix byssoidea* (Brek. Kirchner):**

There appeared about more bio-mass i.e. dry weight in nitrogenous medium than nitrogen deficient medium. Therefore, it has 6.50 mg/ 100 ml dry weight in nitrogen deficient liquid medium and was 9.50 mg/ 100 ml dry weight in nitrogenous medium respectively and cellular protein was 0.461 µg /ml in nitrogenous liquid medium on 15th day. The

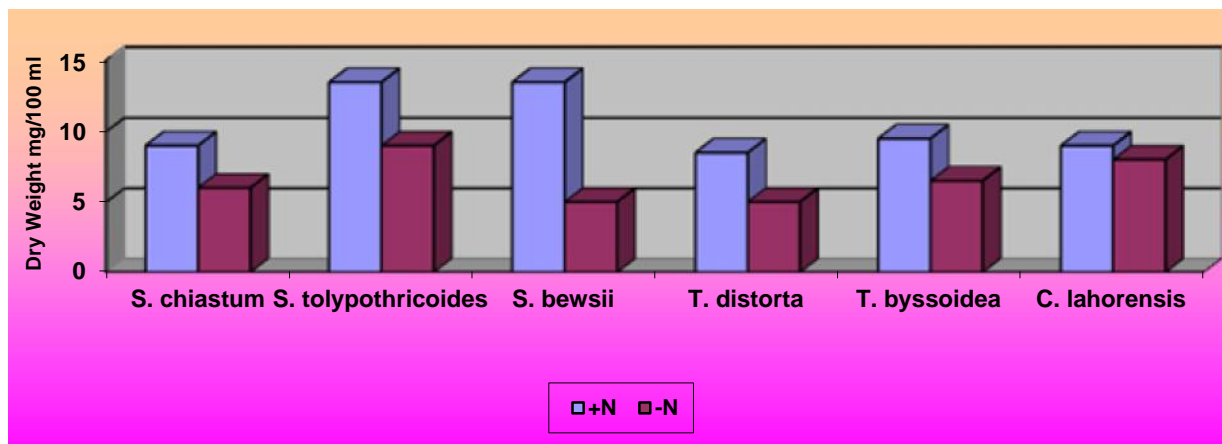
Chlorophyll-a was 0.94 µg /ml in nitrogen deficient medium. The nitrogenase activity were 6.40 in terms of n mole C<sub>2</sub>H<sub>4</sub>/µg/chl.a/h and nitrogenase activity 9.61 in terms of n mole C<sub>2</sub>H<sub>4</sub>/vial/h in nitrogen deficient medium on 15th day.

**3.6 *Camptylonemopsis lahorensis* (Ghose) Desikachary.:**

The present alga showed maximum growth in rich nitrogenous medium than nitrogen deficient liquid medium on 15th day. However, it has dry weight 8mg/100ml and 9mg/100ml in nitrogen deficient and nitrogenous medium respectively. The cellular protein was 0.569 µg /ml and The Chlorophyll-a was 0.115 µg /ml both in nitrogen deficient medium respectively. The nitrogenase activity were 3.76 in terms of n mole C<sub>2</sub>H<sub>4</sub>/µg chl.a/h and nitrogenase activity 8.90 in terms of n mole C<sub>2</sub>H<sub>4</sub>/ µg/ vial/h in nitrogen deficient medium on 15<sup>th</sup> day.

**Table 1:** Comparison of Physiological work of 6 fast growing strains under the family of Scytonemataceae their dry-weight and in presence and absence of nitrogen and determination of Cellular Protein in absence of Nitrogen

Sl.Nos.	Name of the strains	Dry weight mg/100 ml		Protein µg/ml
		+N	-N	
1..	<i>Scytonema chiasmum</i>	9.0	6.0	0.165
2.	<i>Scytonema tolypothricoides</i>	13.5	9.0	0.195
3.	<i>Scytonema bewsii</i>	13.5	5.00	0.155
4.	<i>Tolypothrix distorta</i>	8.5	5.00	0.080
5.	<i>Tolypothrix byssoidea</i>	9.50	6.50	0.94
6.	<i>Camptylonemopsis lahorensis</i>	9.00	8.00	0.115



**Figure 1A.** Comparison of Dry Weight and in presence and absence of nitrogen.

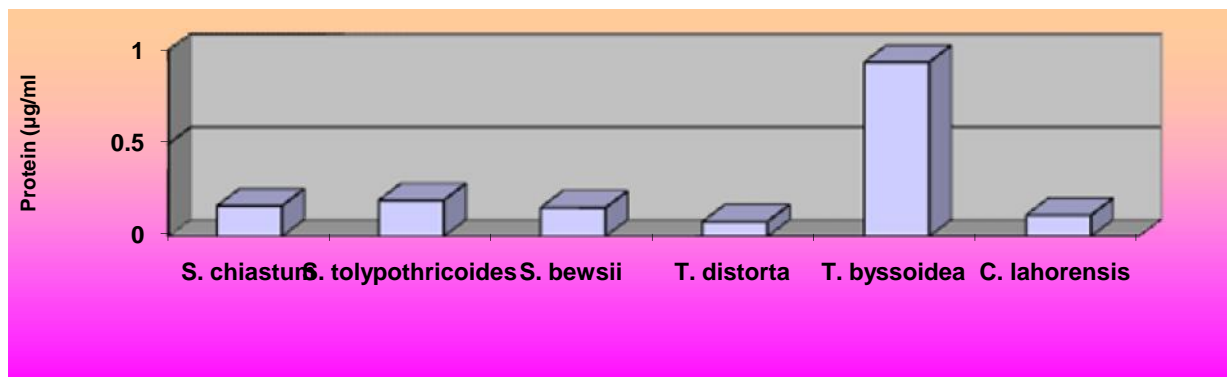


Figure 1B. Determination of Intracellular protein ( $\mu\text{g/ml}$ ) in absence of Nitrogen under the family of Scytonemataceae.

Table 2. Comparison and Determination of on Nitrogenase activity ( $\text{n mole C}_2\text{H}_4/\mu\text{g chl.a/h}$ ) and ( $\text{n mole C}_2\text{H}_4/\mu\text{g/ vial/h}$ ) in six different strains of the family Scytonemataceae on 15<sup>th</sup> day.

Sl.Nos	Name of the strains	Chl-a ( $\mu\text{g/ml}$ )	n mole $\text{C}_2\text{H}_4$ $\mu\text{g /chl.a/h}$	nmole $\text{C}_2\text{H}_4/\mu\text{g Chl.a/h vial/h}$
1..	<i>Scytonema chiastum</i>	0.204	2.54	6.88
2.	<i>Scytonema tolypothricoides</i>	0.492	2.94	5.675
3.	<i>Scytonema bewsii</i>	0.228	5.50	3.73
4.	<i>Tolypothrix distorta</i>	0.594	9.18	3.38
5.	<i>Tolypothrix byssoidea</i>	0.461	6.50	0.94
6.	<i>Camptylonemopsis lahorensis</i>	0.569	3.76	8.90

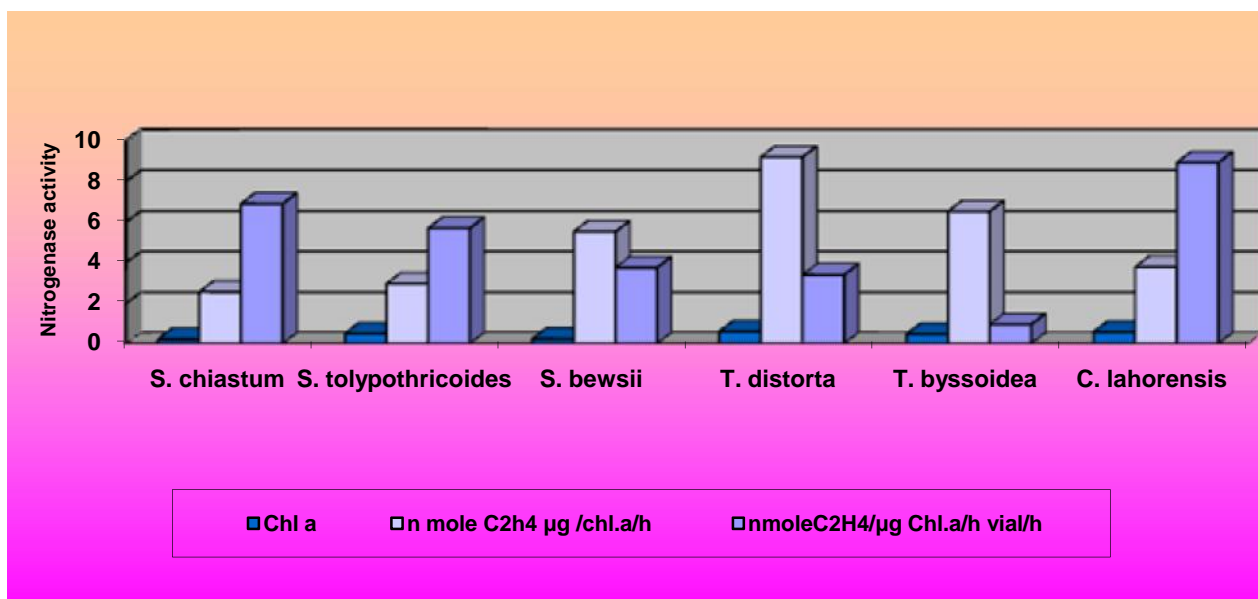


Figure 2. Comparison and Determination of on Nitrogenase activity ( $\text{n mole C}_2\text{H}_4/\mu\text{g chl.a/h}$ ) and ( $\text{n mole C}_2\text{H}_4/\mu\text{g/ vial/h}$ ) in six different strains of the family Scytonemataceae on 15<sup>th</sup> day.



## 4. Discussion

### Dry weight:

Results indicate that all the strains have more dry weight in the nitrogenase medium than the nitrogen deficient medium but maximum increase (107%) due to the presence of normal recommended quality of nitrogen. It was found in *Scytonema bewsii* and minimum (12.5%) in *Camptylonemopsis lahorensis*. On comparison of their biomass in the same medium *Scytonema tolypothricoides* and *Scytonema bewsii* show maximum dry weight (13.5 mg/100 ml) under normal nitrogenous conditions but under nitrogen deficient conditions only. *S. tolypothricoides* has maximum dry weight (9.0 mg/100 ml) in comparison to all other strains. However, *Tolypothrix distorta* has minimum dry weight i.e. 8.5 mg/ 100ml and 5.0 mg/100 ml under normal nitrogenous and nitrogen deficient medium respectively.

### Chlorophyll-a:

The results of chlorophyll-a ( $\mu\text{g/ml}$ ) indicate that strains which have more dry weight, not necessary to have more Chlorophyll-a. On the similar way, only. *Tolypothrix distorta* shows maximum Chlorophyll-a (0.594  $\mu\text{g/ml}$ ) and *Scytonema chiastum* shows minimum chlorophyll-a (0.204  $\mu\text{g/ml}$ ).

### Cellular protein:

The results of cellular protein ( $\mu\text{g/ml}$ ) was higher in such strains, those usually have higher dry weight under both the conditions. Therefore, maximum cellular protein (0.195  $\mu\text{g/ml}$ ) was observed in *Scytonema tolypothricoides* and least (0.80  $\mu\text{g/ml}$ ) was in *Tolypothrix distorta*.

### Nitrogenase activity:

Nitrogenase activity in terms of  $\mu\text{g/chl.a/h}$  was highest (9.18 mole  $\text{C}_2\text{H}_4 / \mu\text{g chl.a/h}$ ) in *Tolypothrix distorta* and lowest (2.09 mole  $\text{C}_2\text{H}_4 / \mu\text{g chl.a/h}$ ) was in *Scytonema tolypothricoides*. However, it differs in its sequence in terms of n mole  $\text{C}_2\text{H}_4 / \mu\text{g/vial/h}$  and it is maximum (9.61 mole  $\text{C}_2\text{H}_4 / \text{vial/h}$ ) in *Tolypothrix byssoidea* and minimum (3.38 mole  $\text{C}_2\text{H}_4 / \mu\text{g/vial/h}$ ) in *Tolypothrix distorta*.

Comparative study on *Scytonema chiastum*, *Scytonema bewsii*, *Scytonema tolypothricoides*, *Tolypothrix byssoidea*, *Tolypothrix distorta*, and

*Camptylonemopsis lahorensis* and compare among these six experimental strains of the family Scytonemataceae through assessment of their dry weight (in nitrogenous and nitrogen deficient medium), chlorophyll-a, cellular protein and nitrogenase activity (as per chlorophyll- a and per vial) on 15th day. Quantitative assessment of nitrogen fixation by isolated blue-green algae (Dhar et. al., 2007) from paddy-field was done by previous workers only through Micro-kjeldahl method. For the first time, in India, Roy choudhury et al., (1986) estimated the nitrogen fixation efficiency of isolated strains of a genus Nostoc by Gas Chromatograph which is not comparable with present results. After that, Roy Choudhury and Kaushik (1989) analysed nitrogenase activity of 63 species of blue-green aglae and observed that strains of the genera *Calothrix* (*C. bharadwajae* and *C. membranacea*), *Scytonema* and *Tolypothrix* (*Tolypothrix tenuis*) had been showing the best performance in values of acetylene reduction assays. Further, Tripathi et al., (1990) also recorded both biomass and nitrogenase activity of 17 isolated unialgal forms of six genera (i.e. *Nostoe*, *Anabaena*, *Calothrix*, *Cylindrospermum*, *Seytonema* and *Hapalosiphon* and they found only superior genus *Calothrix* than others. They agreed with results of Kolte and Goyal (1986) who estimated the total fixed amount of nitrogen of 62 strains belonging to 9 genera by Microkjeldahl method. Among them they reported the best nitrogen fixing forms of the genera *Tolypothrix*, *Calothrix* and *Nostoc*. Singh (1993) studied 167 strains of 12 heterocytous forms (*Nostoe*, *Anabaena*, *cylindrospermum*, *Aulosira*, *Scytonema*, *Tolypothrix*, *Calothrix*, *Gloetrichia*, *Microchaete*, *Chlorogloeopsis*, *Hapalosiphon* and *Westiellopsis*) and found vigorous growth of *Nostoc*, *Cylindrospermum*, *Anabaena*, *Aulosira*, *Scytonema*, *Microchaete* and *Gloetrichia*. He observed that in comparison to *Scytonema* strains, *Tolypotrix* strains have higher chlorophyll-a and nitrogenase activity values. We took 3 strains of *Scytonema*, two strains of *Tolypothrix* and one strain of *Camptylonemopsis* for their characterization on the basis of chlorophyll-a and nitrogenase activity.

The present results also indicate that both the two species of *Tolypothrix* have higher values of chlorophyll-a and nitrogenase activity in terms of Chl-a  $\mu\text{g/ml}$  than the other two selected fast growing strains of *Scytonema*. This type of research work really very few and most of the workers result more or less similar but they did not observe all these present parameters of the present genera.

## 5. Conclusion

The analysis and their overall contribution indicate that only *Scytonema tolypothricoides* shows more values than an average in four parameters dry weight in normal nitrogenous and nitrogen deficient medium, Chlorophyll-a and cellular protein of certain strains viz. *Scytonema bewsii*, *Tolypothrix byssoidea* and, *Camptylonemopsis lahorensis* have more value than their respective average in these parameters *S. bewsii* in dry weight under normal nitrogenous medium, cellular protein and nitrogenase activity in terms of  $\mu\text{g chl.a/ml}$ ; *Tolypothrix byssoidea* in chlorophyll-a, and nitrogenase activity in both terms and *Camptylonemopsis lahorensis* in dry weight under nitrogen deficient medium, chlorophyll-a and nitrogenase activity (in terms of per vial/h). Remaining two strains i.e. *Scytonema chiastum* and *Tolypothrix distorta* have more value than average only in two parameters. Thus, on the basis of their comparative performance. *Scytonema tolypothricoides* is the best strain and *Scytonema bewsii*, is better in comparison to remaining two i.e. *Tolypothrix* is poor performance showing algae.

The present results also indicated that both the two species of *Tolypothrix* have higher values of chlorophyll-a and nitrogenase activity in terms of per  $\mu\text{g Chl-a } \mu\text{g /ml}$  than the other two selected fast growing strains of *Scytonema*. The results of other workers are very limited and more or less similar but they did not observe all these present parameters of these present genera.

## Conflict of Interest

The authors of this paper have no conflict of interest.

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