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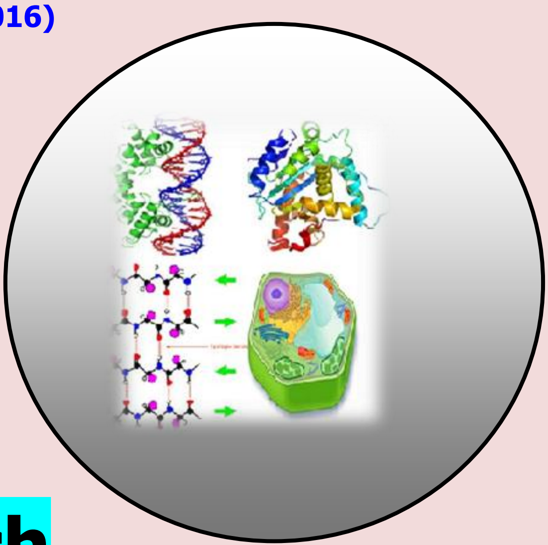

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RESEARCH PAPER

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Effect of Salinity on Growth and Lipid Content of Cyanobacterium *Calothrix marchica*

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ABSTRACT

Salinity stress, a principal abiotic stress, negatively impacts the crop productivity. Cyanobacteria are capable to survive and thrive in high salinity. In the present investigation, a heterocystous cyanobacterium Calothrix marchica, isolated from naturally saline alkali soils, was subjected to different salinity levels of EC 0.2, EC 5, EC 10 and EC 15 ds/m to study effect of salinity stress. The increase in salinity resulted in decreased growth in terms of biomass (dry weight) and absorbance. Photosynthetic pigments like chlorophyll, carotenoids and phycobilins also decreased with increasing stress. However, the lipid content increased at high salinity levels indicating that it may be one of the adaptive mechanisms under stress conditions in this cyanobacterium.
Keywords: Salinity, Cyanobacteria, Calothrix, Chlorophyll, Lipids, Carotenoids and Phycobilins.

INTRODUCTION

Soil salinization, a global problem and most important abiotic stress, negatively mitigate the growth & productivity in agriculture. Salinity, significantly affected 7% of total arable land area, is the most common form of desertification and land degradation (Wang et al., 2018).

Cyanobacteria (blue-green algae) are morphologically diverse, gram negative prokaryotic oxygenic photoautotrophs with ubiquitous distribution in almost all inevitable places of earth including deserts (Garcia – Pichel and Pringault, 2001). Cyanobacteria have the capability to fix atmospheric CO_2 to form carbohydrates and high value natural biochemicals of great commercial and industrial importance (Satyanarayana et al., 2011). Filamentous heterocystous forms have the cabability to fix atmospheric nitrogen and are a good source of global nitrogen economy specifically in tropical rice fields. The occurrence of cyanobacteria in saline-alkali soils have also been reported earlier by various workers (Langworthy, 1978; Arif, 1992; Rai, 2015) which indicates tolerance of cyanobacteria to stress environments. These may have the inherent capability to tolerate these conditions and can have the potential to be used for the bioreclamation of salt affected soils.

The present investigation was conducted to study the salt tolerance potential and biochemical responses of cyanobacterium, *Calothrix marchica* isolated from saline alkali soils, with an objective to check its suitability as potential biofertilizer for the saline soils.

MATERIALS AND METHODS

Calothrix marchica was isolated from saline-alkali soils of Rohtak district, Haryana state with EC ranging from 0.37 ds/m to 26 ds/m and pH from 8.0 to 8.6. Isolation of cyanobacterium was done by serial dilution, agar plating (Andersen, 2005) and streaking methods. Identification of cyanobacterium species was done by using standard keys given by Desikachary (1959).

Pure culture of *Calothrix marchica* was grown under continuous cool white fluorescent light of intensity 2000 lux at 27±3^{0}C in Fogg's nitrogen free medium (Fogg, 1949). For halotolerance studies, this isolate was grown in culture medium of four different salinity levels of EC 0.2, 5, 10, 15 ds/m, which were prepared by adding NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ in a ratio of 13:7:1:4 by weight, which is the general soil composition of salt in soils of this region (Sinha et. al., 1986) and maintained at pH 7.5. pH of medium was maintained by using 0.1 N NaOH and 0.1N HCl. Medium without salts was used as control (EC 0.2ds/m).

Growth was estimated by measuring the optical density of algal suspension at 665 nm on a spectrophotometer against a reference blank containing sterilized medium of same EC following Kaushik (1987).

For biomass estimation, 10 ml of thoroughly shaken algal suspension was centrifuged at 5000 r.p.m. for 10 minutes. Supernatant was discarded and the weight of the pellet was taken after repeatedly washing with distilled water and drying to constant weight at 80°C for 6 hours.

Photosynthetic pigments chlorophyll, phycobilins and carotenoids were estimated by following McKiney (1941), Bennet and Bogorad (1971) and Jensen (1978).

Lipid content was estimated following Dittmer and Wells (1969).

RESULTS AND DISCUSSION

Absorbance pattern of cell suspension of *Calothrix marchica* showed that this cyanobacterium was able to tolerate salt stress upto 15 ds/m, although the growth was maximum in non saline conditions (Figure. 1). Although initially the growth was slow but attained maximum values after 15 days. There was 40 to 50 % decrease in the absorbance at higher salinity levels of EC 10 & EC 15 on 25th and 30th day of observation. Similar type of reduction in growth has been earlier reported in cyanobacteria *Lyngbya confervoides* and *Nostoc commune*, with increasing salinity (Rai and Rajashekhar, 2014).

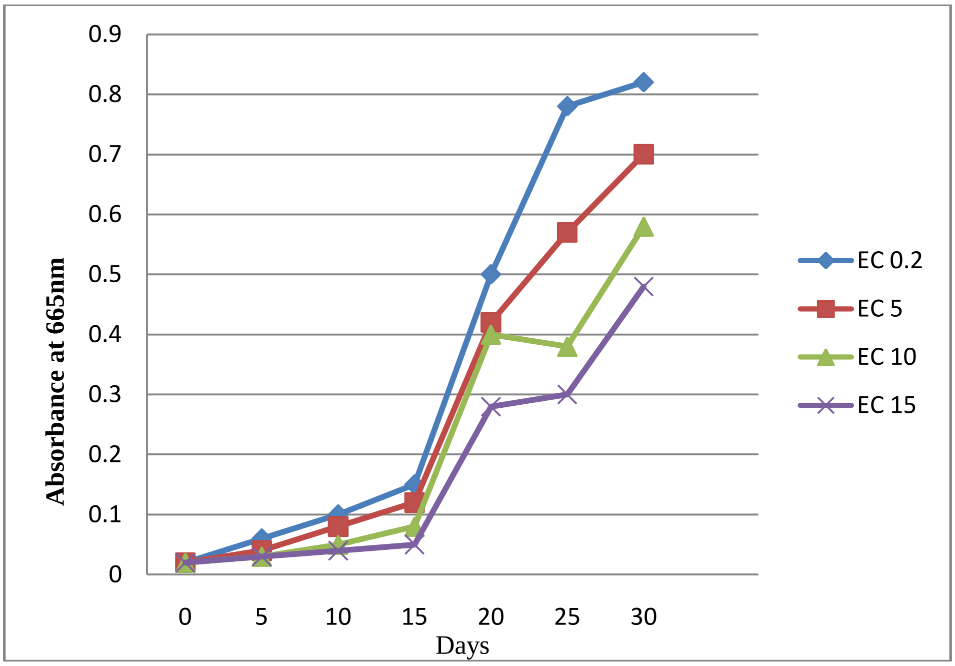

Figure 1. Temporal variation in absorbance of *Calothrix marchica* at varying salinity regimes.

Similarly for dry weight, nearly 35% decrease was observed at EC 15 ds/m as compared to control on the 30th day of observation (Figure 2). Similar types of results have been reported earlier in case of *Hapalosiphon welwitschii* (Manchanda, 2017). However, dry weight data when subjected to t-test, differences were not statistically significant, indicating greater salt tolerance in this species.

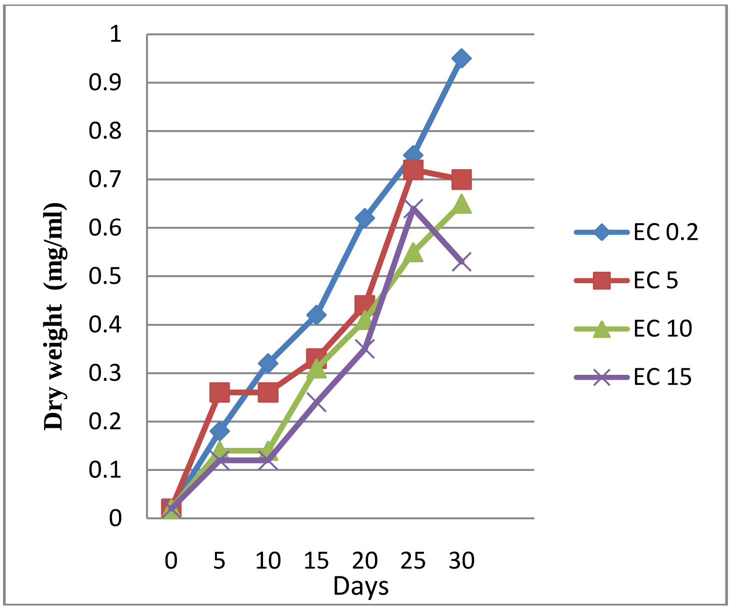

Figure 2. Temporal variation in biomass of *Calothrix marchica* under varying salinity regimes.

Chlorophyll content decreased with increasing salt stress with maximum value in control followed by EC 5, 10 and 15 ds/m (Figure 3). In *Calothrix marchica*, peak value of 3.5 µg/ml was observed on 25^{th} day in control whereas no significant effect of salinity was observed at low EC levels *i.e.* EC 5 but a decrease of 30 to 75% was observed in chlorophyll content at EC 10 and 15 showing values of 1.9 µg/ml and 0.88 µg/ml, thus showing a varying degree of tolerance. At higher EC levels, this strain continued to grow but with a decreased rate. Earlier reports in case of *Westiellopsis prolifica* have also shown a decreased growth at higher concentrations of NaCl (Shamina and Madusoodhanan, 2011). It has also been reported that primary target of increasing salinity is the chlorophyll, resulting in reduced photosynthesis and hence growth (Kirrollia et. al., 2011; Rai and Abraham, 1993).

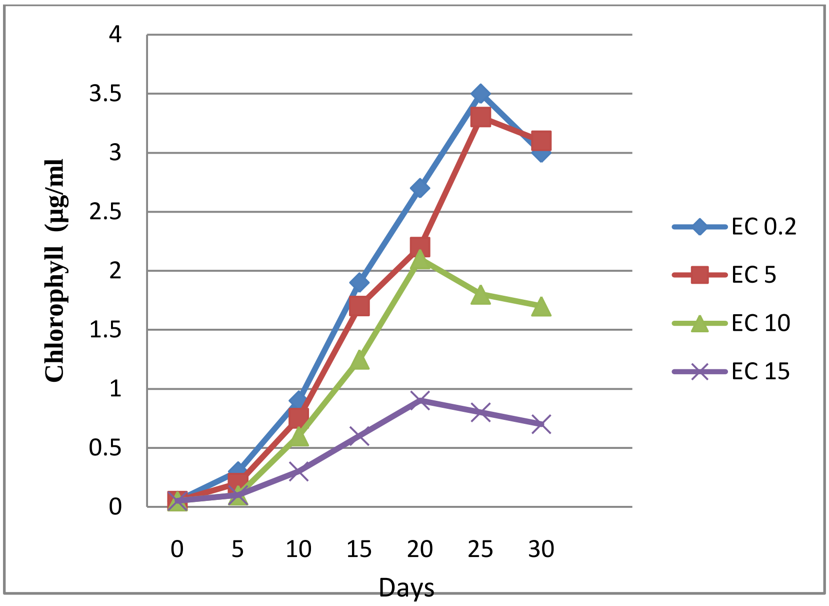

Figure. 3 Temporal variations in chlorophyll content of *Calothrix marchica* under varying salinity regimes.

Like chlorophyll, maximum carotenoid content was observed on 25^{th} day of observation with a value of 42 µg/ml in control to 21 µg/ml at EC 15 ds/m (Figure 4). In *Calothrix marchica*, concentration of carotenoids was exceptionally high. Carotenoids are generally found to be in less concentration as compared to chlorophyll.

But in this isolate carotenoids were formed in more quantity as compared to chlorophyll (Chlorophyll to carotenoid ratio was 1: 10 in control to 1:14 at EC 10). This seems that carotenoids may be playing a major role in decreasing the effect of salt stress as reported earlier in case of cyanobacterial consortium under high temperature condition (Preeti et al., 2015).

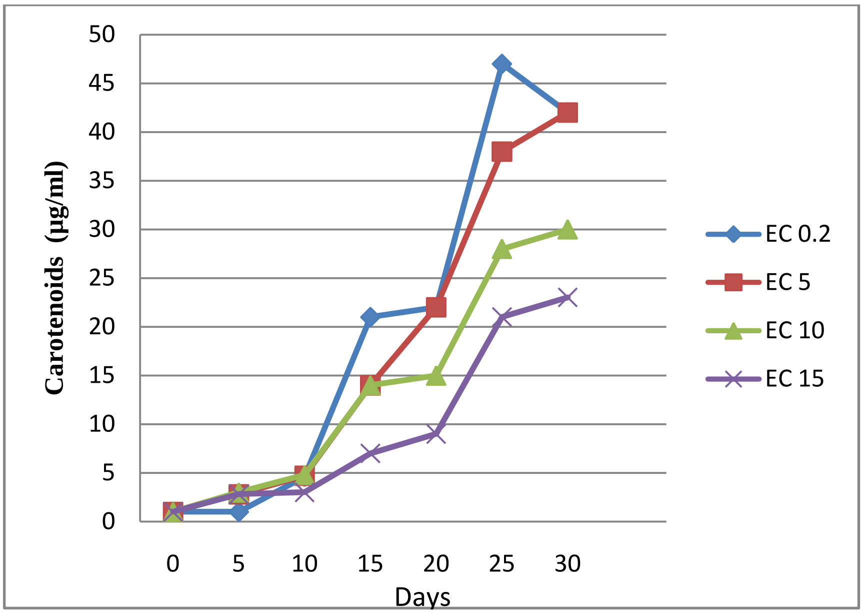

Figure 4. Temporal variation in carotenoid content of *Calothrix marchica* under varying salinity regimes.

Phycobilin content was low at the initial stage but continued to increase 5th day onward and a continuous exponential phase was observed after 15 days and continued to increase up to 30 days (Figure 5). The low salinity (EC 5) had no significant effect on phycobilin production but at EC 15, there was 71% reduction in phycobilin content as compared to control in compliance with the earlier studies (Sujatha and Nagarajan, 2014).

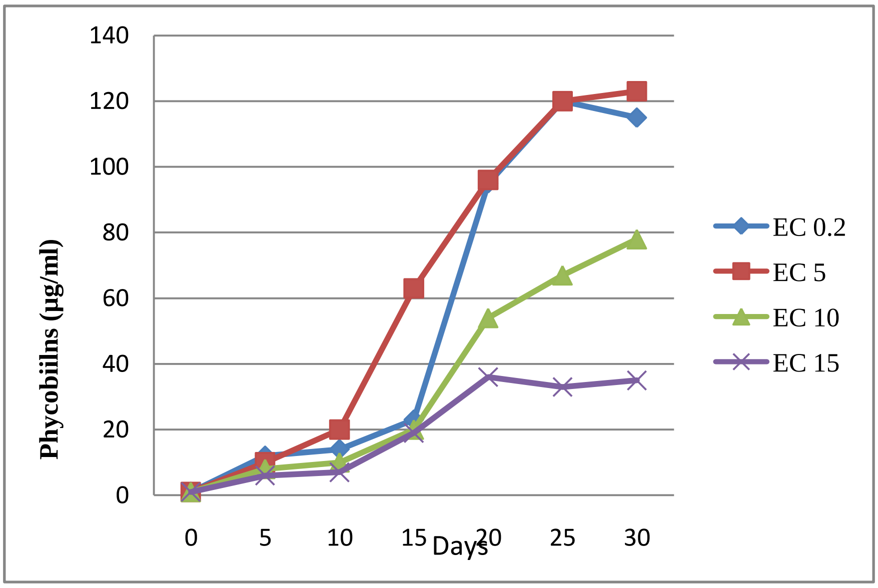

Figure 5. Temporal variation in phycobilin content of *Calothrix marchica* under varying salinity regimes.

Lipid content increased with increasing salt stress and the value ranged from 6.7 mg/gm biomass (EC 0.2) to 7.6 mg/gm (Figure 6). Increase in lipid content with increasing salinity has already been reported in *Chlorella sp.* (Rai et al, 2015). This increase in lipid content at higher salinity levels may be an adaptive mechanism for salt tolerance and involved in protection against salt stress, by acting as energy reserve during unfavourable or stress conditions (Asulabh et. al., 2012).

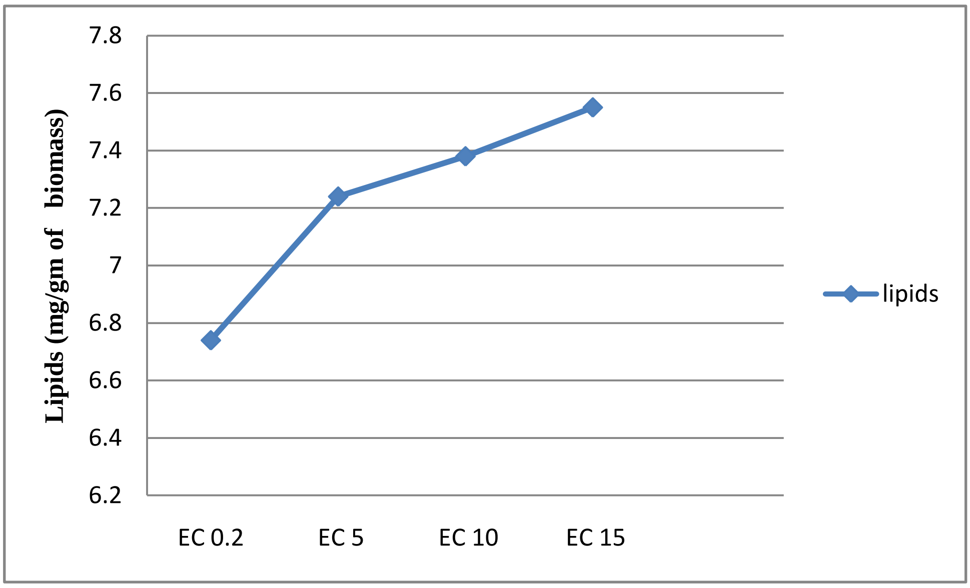

Figure 6. Effect of salt stress on lipid content of *Calothrix marchica.*

CONCLUSION

This study shows that salinity affects the growth and lipid content of *Calothrix marchica*. Overall growth of alga is reduced under high salinity but the lipid content increases and may be helping the alga to survive under stress although with a reduced growth rate, which makes this strain to be likely used for the reclamation of saline soils and needs to be explored further.

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