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Research Article

EFFECT OF pH AND SALINITY ON GROWTH PERFORMANCE OF CYANOBACTERIUM *HAPALOSIPHON WELWITSCHII*

Harsh Manchanda*

Department of Botany, Post Graduate Govt. College for Girls Sector-11, Chandigarh

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A B S T R A C T

Salinity and pH tolerance of heterocystous, branched cyanobacterium *Hapalosiphon welwitschii,* isolated from semi-arid wasteland was studied in response to salinity levels ranging from EC 5 to 15 ds/m at pH 7.5 (neutral) and pH 8.5 (alkaline). Growth performance was studied in terms of absorbance, biomass, concentration of photosynthetic pigments like chlorophyll, carotenoids and phycobilins. A decrease in all these parameters was observed with increasing salinity, indicating that this may be regarded as halotolerant cyanobacterium in which growth was maximum in control (EC 0.2 ds / m) but it sustained well at higher salinity levels although with a reduced growth rate. When pH of medium was increased to 8.5 (alkaline), overall growth pattern did not change but the relative growth was more in alkaline conditions as compared to that at pH 7.5, indicating that this strain has the potential to be used as biofertilizer in saline – alkaline soils.

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INTRODUCTION

Cyanobacteria or blue-green algae are a diverse group of prokaryotic, photosynthetic organisms found in all conceivable places on earth (Garcia-Pichel and Pringault, 2001). They have the potential to fix atmospheric nitrogen and thus play an important role in agriculture (Vaishampayan *et. al.,* 2001). They are a good source of secondary metabolites like exopolysaccharides, vitamins and pharmaceutical products (Dutta *et. al*., 2005). Cyanobacteria have also been found to be useful in bioremediation of industrial effluents (Dubey *et. al.*, 2011). Moreover, in the present scenario, these are being used for the production of biofuels (Parmar *et. al.,* 2011). Some of the cyanobacterial species are cultivated for commercial purpose. Growth of the cyanobacteria is influenced by a number of factors like presence of salts, temperature and pH etc. and the conditions required for each of the cyanobacterial species may vary (Van Baalen, 1967). Among soil properties, pH is one of the factors affecting the growth of cyanobacterium (Kaushik, 1994). Therefore it becomes a necessity to optimize the growth conditions for their culture (Nagle *et. al.,* 2010).

There are reports that cyanobacteria are found to be abundant in alkaline environments (Langworthy, 1978). A close correlation is observed between the soil pH and density of blue-green algae (Anand *et. al.,* 1987).

Corresponding author:* **Harsh Manchanda
Department of Botany, Post Graduate Govt. College for Girls, Sector-11, Chandigarh

As reported by Rai (2015), cyanobacteria are capable to survive and thrive different conditions like extreme pH and high salinity and can be used for the reclamation of saline-alkali soils. There are reports of occurrence and isolation of heterocystous cyanobacteria from saline –sodic soils, which indicate their tolerance to stress environments (Arif, 1992). In India, approximately 7 million hectares of land is salt affected so there is a need to exploit and explore the halotolerant cyanobacterial strains (Kaushik and Jolley, 1992) which can proliferate in these soils and improve the nitrogen status and hence productivity. Generally, in most of the algalization programmes, cultured or foreign strains are used but the indigenous strains are likely to prove more effective due to their inherent salt tolerance capability.

In the present study, a heterocystous, branched cyanobacterium *Hapalosiphon welwitschii* was isolated from the semi-arid wasteland of Haryana. As the soil of the study site was saline-alkali in nature, therefore, the growth performance of the cyanobacterium was studied under varying salinity (EC 0.2, 5, 10 and 15 ds/m) and pH levels (7.5 & 8.5), with an objective to optimize the growth conditions of cyanobacterium before considering it to be used as biofertilizer in algalization programme for salt affected soils.

MATERIALS AND METHODS

Hapalosiphon welwitschii was isolated from the uncultivated, undisturbed semi-arid wasteland with a predominating vegetation of *Salsola baryosma*. Soil EC and pH were determined using 1: 2 soil water suspension using EC meter and pH meter. Soil was found to have salinity ranging from 0.37 ds/ m to 26 ds/ m whereas pH ranged from 8.0 to 8.6.

Isolation and Growth of Cyanobacerium

For isolation of *Hapalosiphon welwitschii* standard isolation and culturing technique was used. Identification of cyanobacterium was done by microscopic examination, following key given by Desikachary (1959). Pure strain was obtained by serial dilution, streaking and purification technique following Anderson and Kawachi (2005) using Fogg's nitrogen free medium (Fogg, 1949). Pure culture was maintained at 27±3°C under continuous illumination using cool white fluorescent light.

Optimization of Salinity and pH levels

For studying growth response of alga under varying salinity regimes of EC 0.2, 5, 10 and 15 ds/m, NaCl, Na_2SO_4, $MgCl_2$ and $CaSO_4$ were used in a proportion of 13:7:1:4 which is the general soil composition of this region and maintained at pH 7.5. Culture medium without salts served as control (EC 0.2 ds/m). Similarly, pH was adjusted by varying the concentration of sodium hydroxide in the medium at various salinity levels.

Growth Response

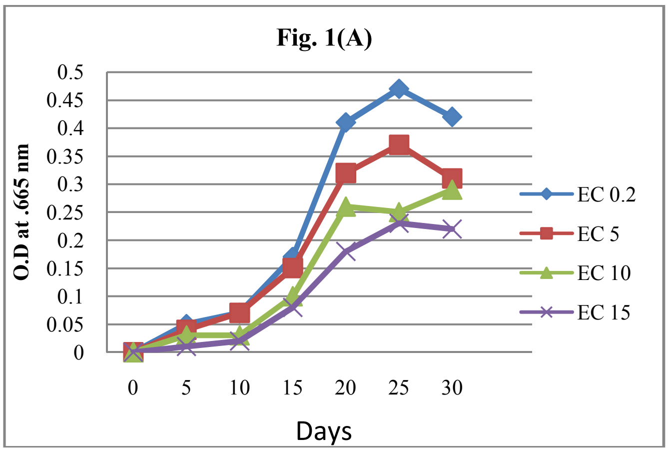
Growth in terms of optical density was studied by using turbidity technique (Kaushik, 1987). 10 ml of each of nutrient medium at EC 0.2, 5, 10 and 15 ds/m was taken in test tubes in triplicates. 1 ml. of algal inoculum was introduced in each of the test tube and incubated at 27±3°C in continuous light. For each parameter 96 test tubes were used, at each pH of 7.5 and 8.5.

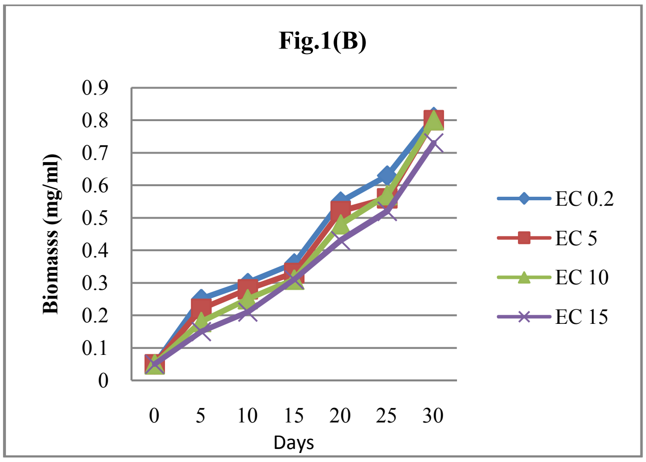
Similarly for biomass, 10 ml of thoroughly shaken algal suspension was taken and centrifuged at 5000 rpm for 10 minutes. Pellet was collected, washed thoroughly with distilled water and then dried to constant weight at 80°C for 6 hours and weighed.

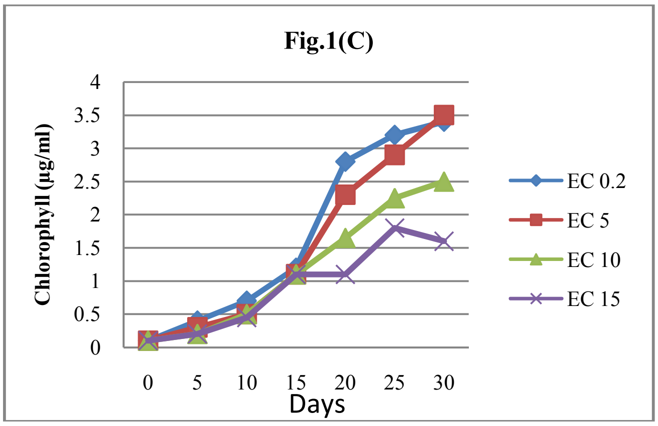
Chlorophyll, carotenoids and phycobilins were measured by following standard methods of McKiney (1941), Jensen (1978) and Bennet and Bogorad (1971).

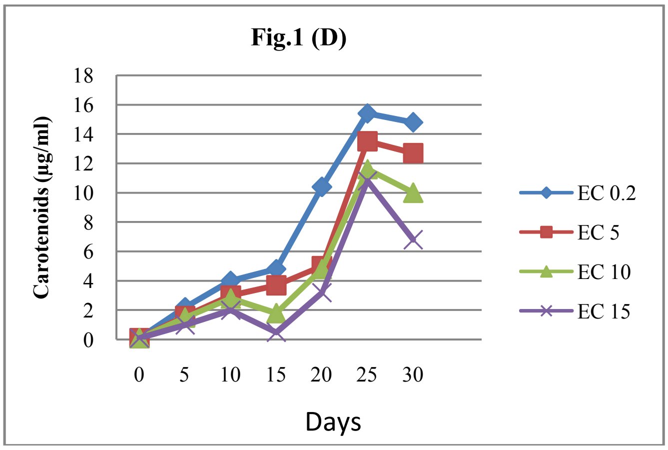
RESULTS AND DISCUSSION

Growth response in terms of absorbance at pH 7.5 showed that difference in growth at varying EC levels became distinct after 10 days (Fig. 1 (A)). However, the growth of *Hapalosiphon welwitschii* was found to be reduced at higher EC levels particularly at EC 15 ds/m and was nearly half (0.23) of that at control (0.47).









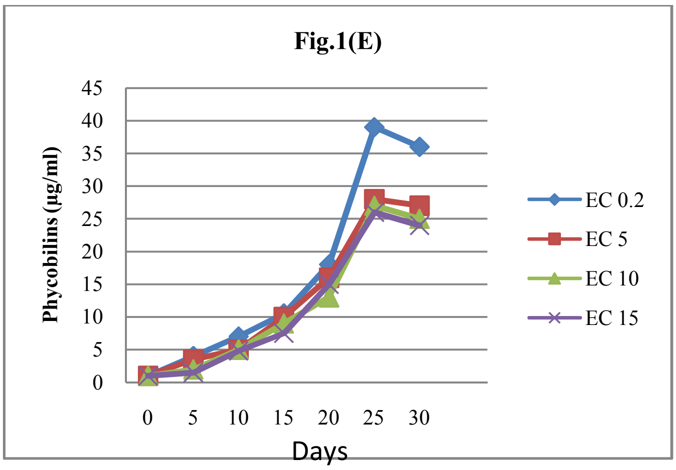


Fig 1 Variation in Optical density (A), Biomass (B), Chlorophyll (C), Carotenoids (D) and Phycobilins (E) of *Hapalosiphon welwitschii* under varying salinity levels at pH 7.5

Similarly, dry weight of alga was maximum at EC 0.2 (control) and reduced with increasing salinity stress (Fig. 1(B)). Although the dry weight was maximum in control but after 15 days, response of *H. welwitschii* to salinity was stabilized and there was just 10% decrease in dry weight at EC 15 ds/m as compared to control on 30th day of observation. Similar observations of reduced algal growth under increasing salinity stress have been reported for *Chlorella vulgaris* (El-Sheekh and Omar, 2002).

The effect of salt stress was less on chlorophyll initially (upto 15 days) but in later stages about 20 to 30% reduction in chlorophyll was found at EC 10 and 40 to 60% at EC 15 (Fig. 1 (C)). When tested for significance of difference using t-test, showed that the decrease was significant ($p<0.05$) only at EC 15 ds/ m.

Peak carotenoid levels were observed on 25th day at all the salt concentrations (Fig.1 (D)). Carotenoids concentration was 15.6 µg/ ml in control whereas under salt stress it ranged from 10.7 to 13.5 µg/ ml.

Like all other growth parameters, phycobilin synthesis was more in absence of salts and decreased with progressively increasing salinity. Maximum reduction in phycobilins due to salt stress was 40% as compared to control (Fig.1 (E)).

On the basis of these results, *Hapalosiphon welwitschii* can be regarded as halotolerant as it grew well at low salinity but can sustain at higher salinity levels with reduced growth rate. Similar type of results are obtained for *Nodularia,* which can grow in salinity range of 0 to 20 g/l but with a reduced growth rate (Moisander *et. al*., 2002)). There are also reports showing that many freshwater cyanobacteria can withstand higher salinities (Carr and Wyman, 1986).

Effect of pH

The relative values of absorbance of cell suspension and dry weight showed a distinct improvement in the growth of these algae in response to salinity stress when the medium had a pH of 8.5. Absorbance of algal suspension at pH 8.5 was more as compared to absorbance at pH 7.5 and values of absorbance ranged from 0.45 to 0.79 under salt stress at pH 8.5 (Fig. 2 (A)) as compared to a value of 0.25 to 0.47 at pH 7.5.

Hapalosiphon welwitschii showed peak values of dry weight in non-saline medium (EC 0.2 ds/m) followed by that at EC 5, 10 and 15 ds/m (Fig.2 (B)). Biomass increased with time in all the salinity levels upto 30th day with a value of 0.72 mg/ml (EC 15) to 0.8 mg/ml (EC 5) at pH 7.5 and 0.89 (EC 15) to 0.93 mg/ml (EC 5ds/m) at pH 8.5. Sethi and Kaushik (1993) had also reported that high pH is conducive for the growth of cyanobacterium *Anabaena oryzae* while its growth is reduced under salinity stress.

The chlorophyll content of *Hapalosiphon welwitschii* showed an increase at pH 8.5 (Fig.2 (C)) as compared to that at pH 7.5 (Fig. 1(C)) at all the salinity levels and in non-saline medium. From the very beginning, chlorophyll content was found to increase and on 5th day of observation, chlorophyll content was almost double at all the salinity levels at pH 8.5 (0.3 to 0.8 µg/ ml) than at pH 7.5 (0.2 to 0.45 µg/ml). Peak concentration of chlorophyll was observed on 25th day of observation with a maximum of 1.8 (pH 7.5) and 3.2 (pH 8.5) at highest salinity level (EC 15).

Fig.2(A)

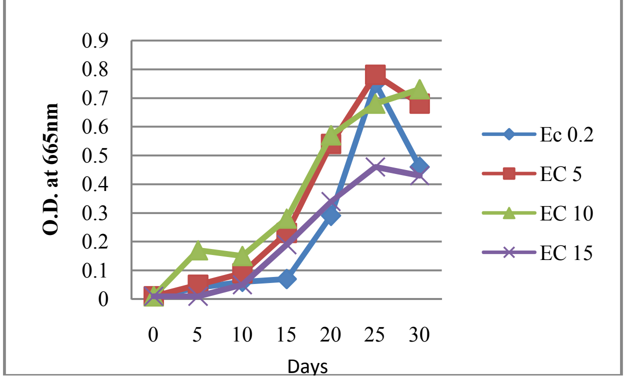


Fig. 2(B)

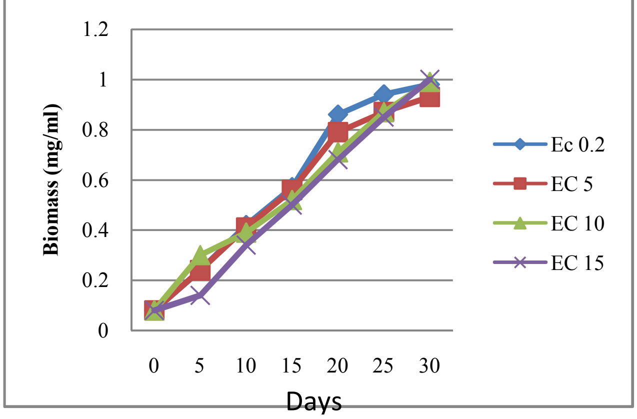


Fig.2(C)

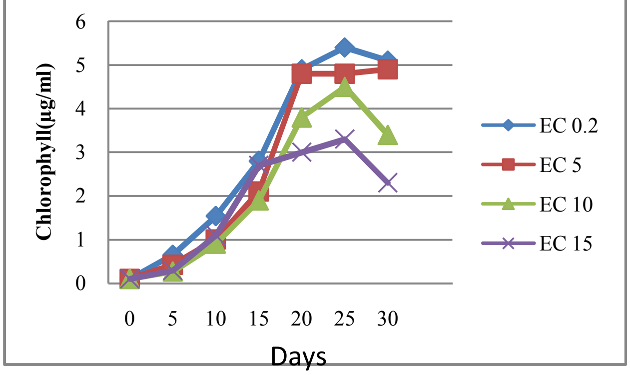
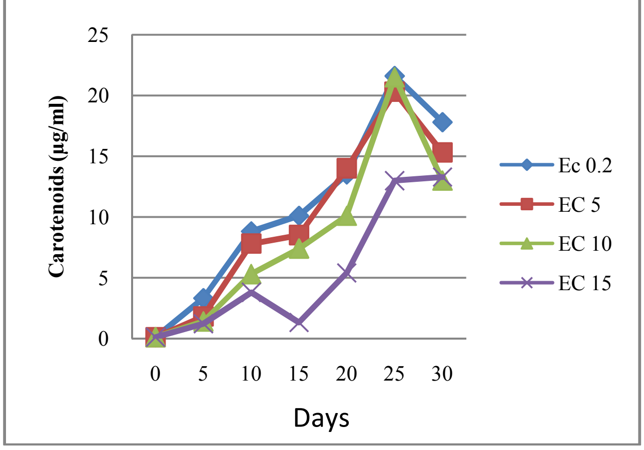


Fig.2(D)



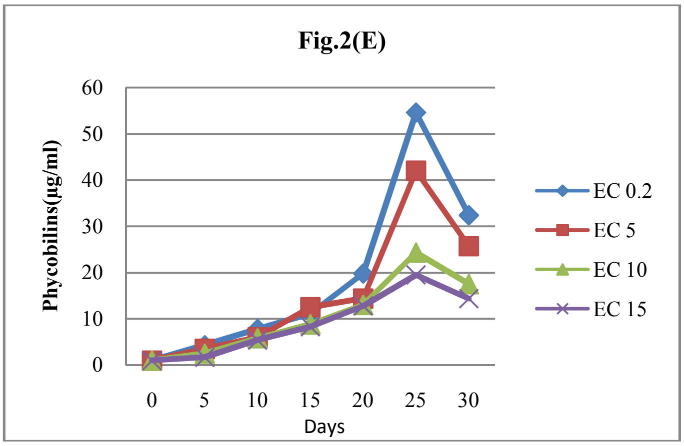


Fig 2 Variation in Optical density (A), Biomass (B), Chlorophyll (C), Carotenoids (D) and Phycobilins (E) of *Hapalosiphon welwitschii* under varying salinity levels at pH 8.5

Similar type of observations were found for carotenoids (Fig. 2(D)) and phycobilins (Fig. 2(E)) at pH 8.5. Values of carotenoids at pH 8.5 showed a marked increase at EC 5, 10 and 15 ds/m as compared with that at pH 7.5. On 25th day of observation, in saline media amount of carotenoids varied from 11µg (EC 15) to 13.5µg/ml (EC 5) at pH 7.5 and 13µg (EC 15) to 20 µg/ml (EC 5) at pH 7.5.

Phycobilins concentration increased in control and at EC 5 upto 25th day but at higher salinity levels (EC 10 and 15 ds/m) increase in phycobilins occurred upto 15 days. Thus in this alga, at low salinity levels increase was more pronounced due to altered pH and all the photosynthetic pigments chlorophyll, carotenoids and phycobilins were favoured at pH 8.5. Increased concentration of these photosynthetic pigments at pH 8.5 indicate that the energy requirements for biosynthesis and reorganization under salt stress are met by enhanced photosynthetic activity and consequently high accumulation of sugars in the algae. There are reports indicating increased accumulation of sugars in response to alkaline pH (Saxena and Kaushik, 1992).

From all these observations, it can be concluded that an increase in pH of medium results in increased growth rate of this cyanobacterium with maximum growth in non-saline medium followed by that at higher salinity levels. It indicate that salinity stress was relieved to some extent, may be due to the reason that pH effects the solubility of carbon-dioxide and various minerals in the medium which in turn affect the metabolism of a species (Markl, 1977).

These observations are also in accordance with the earlier reported studies. According to Vonshak *et. al*., (1988) protein rich cyanobacterium *Spirulina* and *Arthospira* have been reported to tolerate a pH of 8.0 to 11.0. Kaushik (1985) had also shown that blue green algae are alkali loving in nature. At very low pH of 4.5 (acidic) cyanobacteria are found to be totally absent (Rippka *et. al.,* 1979). But with increasing pH, growth of cyanobacterium *Spirulina major* was found to increase with highest growth at pH 8.0, indicating that this cyanobacterium preferred higher pH for better growth (Bano and Siddiqui, 2004). Alkaline salts also have been reported to have a stimulating effect on cyanobacterial growth (Roychoudhary *et. al*, 1983). Thus, soil pH regulates the distribution and predominance of algae.

CONCLUSION

Since the soil from which the present strain was isolated was not only saline in nature but having an alkaline pH, it is quite probable that this alga has been exposed to both high EC and pH for years together and thus have adapted accordingly. Selection pressure in saline-alkali habitat of the cyanobacterium seems to be responsible for developing tolerance to combined soluble and alkaline salts. That is why the algal growth is improved at pH 8.5 than pH 7.5. From this, it may be predicted that *Hapalosiphon welwitschii* has the ability to grow at an increased rate preferentially at high pH under salt stress and has the potential to be used as biofertilizer in saline alkali soils, which needs to be explored further.

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