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ORIGINAL ARTICLE

Growth Response of Floating Water-Moss *(Salvinia natans)* to Eutrophic Waters under Controlled Environment

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ABSTRACT

Salvinia natans *is a free floating pteridophyte, which has a potential to remove different ionic forms of nutrients from aquatic systems. In present experiment growth responses of* Salvinia *were studied at varying temperatures and pH levels in terms of dry weight, chlorophyll-*a*, nitrogen, phosphorus, potassium, peroxidase (POD), catalase (CAT). Optimum growth of* Salvinia *was recorded between 20 to 30˚C as the plants absorb, accumulate and utilize all ionic forms of the nutrients. Growth was suppressed at 10˚ and 40˚C as there was some symptoms of oxidative damage to plant tissues. Plants grow well between pH 5.5 to 6.5. A sustainable phytoremediation system of eutrophic water would develop using* Salvinia *under controlled environmental conditions to restore affected eutrophic water bodies.*

Key words: *Eutrophic water, growth, pH, phytoremediation,* Salvinia*, temperature*

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INTRODUCTION

Eutrophication is one of the major threats to aquatic ecosystems and is becoming an increasingly prevalent problem worldwide (Andersen *et al.* 2006). An aquatic system takes thousands of years to become eutrophic which is a natural process. However, a high rate of nutrients inputs due to anthropogenic activities significantly enhances the condition in a very short period of time (Ansari and Khan 2002, 2006a, b, 2007). Nutrient inputs from different sources cause eutrophication and are responsible for degradation of aquatic ecosystems and loss of biodiversity (Ansari and Khan 2009b). Changes in climatic factors like seasonality, species diversity, nutrients loading, hydraulic regimes, plant harvesting, light intensity, dissolved oxygen, carbon dioxide and particularly pH and temperature, affects the sustainability of phytoremediation systems (Feuchtmayr *et al.* 2009, El-Shafai *et al.* 2007, Ansari and Khan 2009a, Lu *et al.* 2010, Olive *et al.* 2009, Lau and Lane 2002, Shen 2002, Khan and Ansari 2005).

The pH and temperature significantly control the bioremoval of pollutants from water using aquatic plants (Uysal and Fadime 2009). Temperature is an important environmental factor which influences functioning of an aquatic ecosystem (Ansari *et al.* 2011a). pH controls absorption of nutrients and bichemical reactions taking place in living organisms (Ansari *et al.* 2011b, c). Aquatic plants are highly efficient to remove upto 80-90% of nutrients from waters (Fox *et al.* 2008, Zhou *et al.* 2007, Xia and Xiangjuan 2006, Mishra *et al.* 2007). In the present study the growth responses of *Salvinia natans* at

various temperature and pH levels was investigated for its possible application to develop a sustainable phytoremediation system for eutrophic waters.

MATERIAL AND METHOD

Plants of *Salvinia* collected from waste water bodies, washed thoroughly in lab and cultured for two weeks in large earthen pots of size 40 x 25 cm (diam x depth) containing 15 liters of distilled water with macronutrients 1ml L^{-1}. Stock solution of macronutrients was prepared using following compounds $NH_4H_2PO_4$, KNO_3, Ca (NO_3), $MgSO_4 .7H_2O$ in a ratio of 0.23, 1.02, 0.492, 0.49 g L^{-1}, respectively. For the experiments beakers of 1 liter were used as experimental pots containing distilled water with macronutrients (1ml L^{-1}). The plants were disinfected by immersing them in NaClO (1%v/v) and then rinsed with distilled water. The final volume (1 liter) of the growing medium in the experimental pots was maintained using distilled water. Five *Salvinia* plants (approx. 2 grams) were transferred from the maintained stock to each experimental pot. Beakers of each treatment were maintained in triplicate. Plant growth at 10, 15, 20, 25, 30, 35 and 40˚C was measured by placing pots inoculated with *Salvinia* in a growth chamber maintained at each specific temperature in a light of 36 µ mol m^{-2} s^{-1}. For all temperature treatments the sets were maintained at pH 7.0. In the experiment with various pH levels (5, 5.5, 6, 6.5, 7, 7.5 and 8) the sample water was measured regularly with a pH meter (Elico Limited, Hyderabad) and NaOH or HCl were added to the growth medium to maintain the specific pH. All the experimental pots with various pH levels were placed in a growth chamber that was maintained at 25˚C in 36 µ mol m^{-2} s^{-1} light condition.

The experiments were terminated after 15 days. The plants removed from the flask, fresh material was taken for chlorophyll estimation and rest of the plant dried at 80˚C in order to obtain dry weight. Chlorophyll-*a* content in the plants was estimated following the method of Zhao (2000b). The nitrogen and phosphorus contents were determined using the method of Lindner (1944) and Fiske and Subba Row (1925) respectively. Potassium was determined with a Flame photometer (AIMIL). Soluble protein was extracted following the methods of Lazan et al (1983) and determined using the folin-phenol reagent method of Lowery *et al.* (1951). For peroxidase (POD) assay, the crude enzyme was extracted in a phosphate buffer (0.1 M, pH 7.0) following the technique of Kar and Mishra (1976) and the activity was determined as per Putter (1974) method. CAT activity was estimated following Lu (2002). MDA contents were estimated according to the method of Zhao (2000a). The data were analyzed statistically following Dospekhov (1984).

RESTULTS AND DISCUSSION

Salvinia plants were found very sensitive to any change in environmental factors (viz. pH and temperature) within the aquatic ecosystem. They are also found sensitive to nutrient concentration in the growing media. Growth responses of *Salvinia* were studied under various temperature and pH conditions to investigate the best suitable environmental condition to develop a sustainable phytoremediation system for eutrophic water. Plant analysis of tested plants showed that the dry matter and chlorophyll-*a* accumulation were significantly higher between 20 to 30˚C (Fig 1 C & D). The optimum uptake of nitrogen, phosphorus and potassium was noted when *Salvinia* plants were grown in nutrient media at 25˚C (Fig 1A).

Growth patterns of *Salvinia* plants and dry weight accumulation in nutrient media with different concentration were studied as the primary productivity is a strong indicator of eutrophication (Smith 2007; Ansari and Khan, 2006a, Ansari and Khan, 2013, Ansari and Gill, 2013). Temperatures of 10 and 40˚C increases POD and CAT activity in *Salvinia* plants (Fig 1A) and causes significant suppression in nutrient uptake, dry matter and chlorophyll-*a* concentration (Fig 1A, C & E). Overall growth of plants was optimum between temperature treatments of 20 to 30˚C (Fig 1A, C & E). Higher temperature

retards absorption of nutrients by aquatic plants from water (Ansari and Khan, 2006b; 2008). Temperature is an important limiting factor for enzymatic activities and metabolism of plants also regulates cell division, translocation of food and photosynthesis in plants. A temperature of 30˚C is optimal for most biochemical processes (Devlin and Witham, 1986) in plants. Effect of temperature also pronounced when duration of treatment exceeded (Li *et al.* , 1995).

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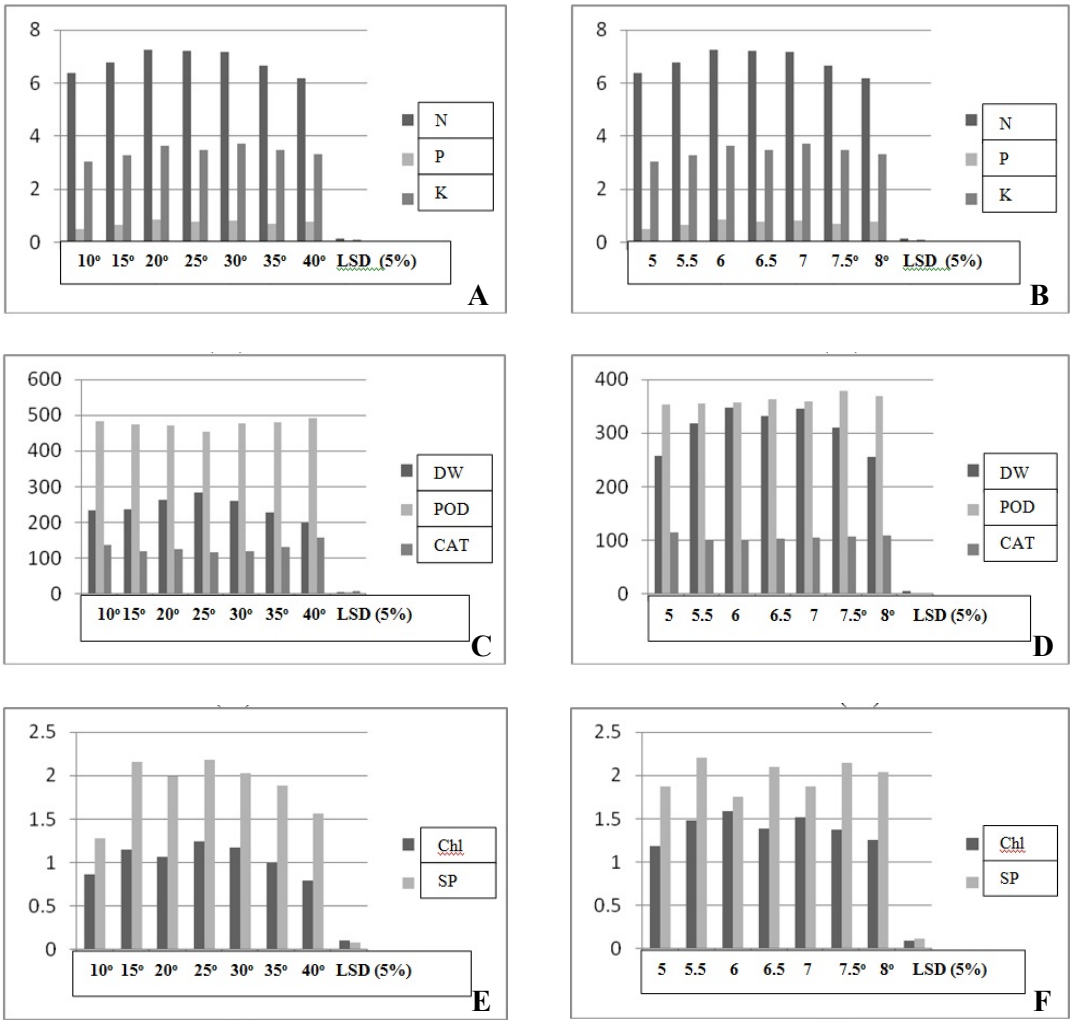

Fig. 1: NPK contents (% of dry weight) in *Salvinia* plants in response to various temperatures **(A)** and pH levels **(B);** Dry weight (DW) (mg g^{-1} of fresh weight), POD & CAT activity (mg^{-1}protein min^{-1}) in *Salvinia* plants in response to various temperatures **(C)** and pH levels **(D)**, Chlorophyll-a (Chl) and Soluble Proteins (SP) (mg g^{-1} of fresh weight) in *Salvinia* plants in response to various temperatures **(E)** and pH levels **(F).**

The Growth responses of *Salvinia* at varying pH levels are summarized in (Fig 1B, D, F). A variation in pH of nutrient media did not affect chlorophyll-*a*, potassium and MDA contents in *Salvinia* plants. No significant change in activities of POD and CAT enzymes was recorded at different pH levels of nutrient media. Enzymatic activities of aquatic plants were not sensitive to small changes in pH levels, but greater pH changes affects plant metabolism (Li *et al.* , 1995). *Salvinia* plants grew well at all the pH levels, however, dry matter accumulation; nitrogen, phosphorus and protein contents of plants were significantly higher in a pH range of 5.5-6.5 of nutrient media (Fig 1B, D, F). The pH

controls origin, mobility and availability of ions and their different forms in aquatic ecosystems (Huang *et al.*, 2005). The availability of ions to plants is directly related with hydrogen ion concentration of nutrient media (Devlin and Witham, 1986, Ansari and Khan, 2009a). Uptake and accumulation of Nitrogen and Phosphorus by *Salvinia* plants showed a direct dependence upon pH of the medium (Cordes *et al.*, 2000). The rate of biochemical reaction in plants depends upon the temperatures, pH, substrate concentration, and incubation period (Abraham and Kurup, 1997).

CONCLUSION

This study indicated that, if the pH of eutrophic water is in between 5.5 to 6.5 and a temperature range between 20 to 30˚C, *Salvinia* plants can be used to develop a sustainable phytoremediation system for eutrophic water. By removing the rapidly growing *Salvinia* plants, absorbing high nutrient contents specially nitrogen and phosphorus from the growing medium, and replacing old *Salvinia* with fresh plants at a regular intervals, the eutrophic aquatic ecosystem can be restored.

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