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ORIGINAL ARTICLE

Growth Responses of *Wolffia arrhyza* to Eutrophic Water under Controlled Environment

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ABSTRACT

Wolffia arrhyza *is a free floating aquatic plant and capable for morphological and physiological adaptations to the aquatic environment. It has a potential to take up and accumulate different ionic forms of nutrients from water. In this experiment growth responses of* Wolffia *plants were studied at various temperatures and pH levels to investigate its possible application to develop a sustainable nutrients phytoremediation system. Dry weights, chlorophyll-a, nitrogen, phosphorus, potassium, peroxidase (POD), catalase (CAT) were estimated. At different applied levels of temperatures, growth of* Wolffia *plants was optimum between 20 to 30˚C. Growth of plants was suppressed at 10 and 35-40˚C may be due to the oxidative tissue damages. Among the different tested pH levels of growth medium, plants grow well between pH 6.5 to 7.5. To develop a sustainable phytoremediation system of eutrophic water,* Wolffia *plants would be an effective tool when applied at pH 6.5-7.5 and temperature between 20 to 30˚C. Under these suitable environmental conditions and when* Wolffia *plants harvested regularly, application of this phytoremediation system could restore eutrophic water bodies.*

Key words: *eutrophication, growth, pH, phytoremediation, temperature*

INTRODUCTION

Eutrophication is one of the major threats to aquatic ecosystems through out the world. The European Union (EU) Water Framework Directive (WFD) given directions to prevent deterioration, protect aquatic systems and to promote the sustainable use of water (Andersen *et al.* 2006). An aquatic system takes thousands of years to become eutrophic which are 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). The nutrient input to waters from various sources causes eutrophication and is responsible for degradation of aquatic ecosystems and plant biodiversity (Ansari and Khan 2009b). Phytoremediation systems depend on many factors, including dissolved oxygen, carbon dioxide, retention time, season, temperature, pH, diversity of species, nutrients loading, hydraulic regimes, plant harvesting, light intensity (Khan and Ansari, 2005, El-Shafai *et al.* 2007, Ansari and Khan 2009a, Ansari *et al.* 2011a, b, c, Feuchtmayr *et al.* 2009, Olive *et al.* 2009, Lu *et al.* 2010). Aquatic plants are reported for their efficiency to remove about 60-90% of different ionic forms of nutrients from water (Xia and Xiangjuan 2006, Mishra *et al.* 2007, Zhou *et al.* 2007, Fox *et al.* 2008). The pH and temperature significantly control the bio-removal of pollutants from water using aquatic plants (Uysal and Fadime 2009). In the present study the growth responses of *Wolffia* at various temperature and pH levels were investigated for its possible use and application to develop a sustainable phytoremediation system for eutrophic waters.

MATERIAL AND METHOD

Plants of *Wolffia* collected from waste water bodies washed thoroughly in the 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. One gram of *Wolffia* plants were transferred from the maintained stock to each experimental pot. Beakers of each treatment were maintained in triplicate. The plant growth at different temperatures *viz.,* 10, 15, 20, 25, 30, 35 and 40˚C was measured by placing pots inoculated with *Wolffia* 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 (2000). 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). The data were analyzed statistically following Dospekhov (1984).

RESULTS AND DISCUSSION

Wolffia plants were found very sensitive to any change in environmental factors (*viz.* pH and temperature) within an aquatic ecosystem. Growth responses of *Wolffia* were studied under various temperature and pH levels to investigate the best suitable environmental condition to develop a sustainable phytoremediation system for eutrophic water. The optimum uptake of nitrogen, phosphorus and potassium was noted when *Wolffia* plants were grown in nutrient media at 25˚C (Fig 1A). Plant analysis of tested plants showed that the dry matter and chlorophyll-*a* accumulation were significantly higher between 20 to 30˚C (Fig.1C & E). Growth patterns of *Wolffia* plants and dry weight accumulation in nutrient media with different concentration were studied as the primary productivity is a strong indicator of eutrophication (Ansari and Khan 2006a, Smith 2007, Ansari and Khan 2013, Ansari and Gill 2013).

POD and CAT activity in *Wolffia* plants were significantly higher at temperature 10, 35 and 40˚C (1E). Temperatures 10, 35 and 40˚C significantly suppressed the nutrient uptake, dry matter, soluble protein and chlorophyll-*a* concentration. Overall growth of plants was optimum between temperatures 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 regulates cell division, translocation of food and photosynthesis in plants. A temperature effect pronounced when duration of treatment exceeded (Devlin and Witham 1986).

Growth responses of *Wolffia* at varying pH levels are summarized in figure 1B, D & F. A variation in pH of nutrient media did not affect chlorophyll-*a* and potassium contents in *Wolffia* plants (Fig. 1B & D). 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 (Fig. 1F), but greater pH changes affects plant metabolism (Huang *et al.* 2000). *Wolffia* 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 6.5-7.5 of nutrient media (Fig. 1B, D & F). The pH controls origin, mobility and accumulation of different ionic forms of nutrients in aquatic ecosystems (Huang *et al.* 2000). 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 *Wolffia* plants showed a direct dependence upon pH of the medium (Ansari and Khan 2008).

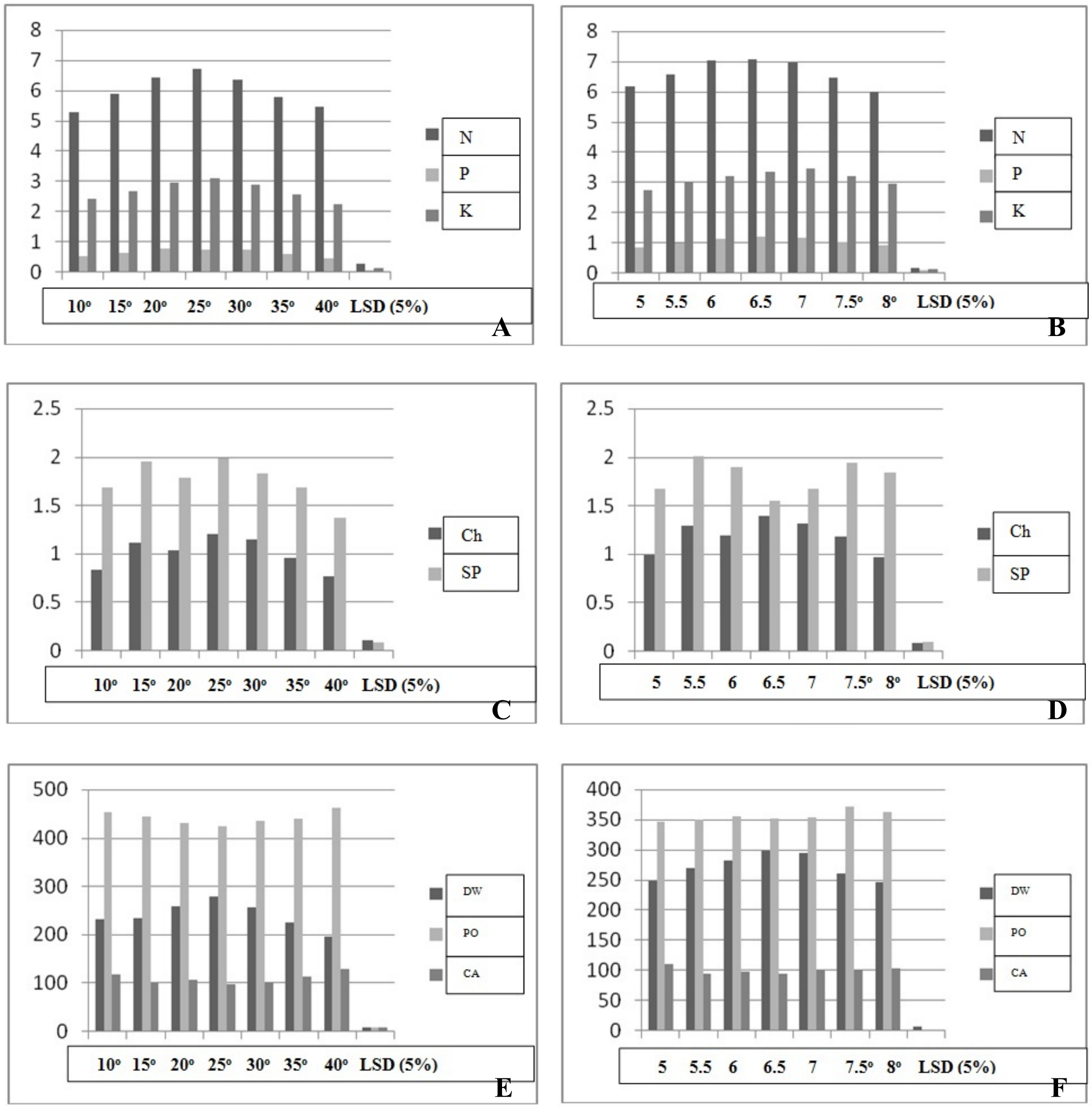

Fig.1: NPK contents (% of dry weight) in *Wolffia* plants in response to various temperatures **(A)** and pH levels **(B);** Chlorophyll-a (Ch) and Soluble Proteins (SP) (mg g^{-1} of fresh weight) in *Wolffia* plants in response to various temperatures **(C)** and pH levels **(D);** Dry weight (DW)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

CONCLUSION

This study indicated that, if the pH of eutrophic water is in between 6.5 to 7.5 and a temperature range between 20 to 30˚C, *Wolffia* plants can be used to develop a sustainable phytoremediation system for eutrophic water. By removing the rapidly growing *Wolffia* plants, absorbing high nutrient contents specially nitrogen and phosphorus from the growing medium, and replacing old *Wolffia* with fresh plants at a regular intervals, the eutrophic aquatic ecosystem can be restored.

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