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Impacts of *Eichhornia crassipes* (Mart.) Solms stress on the physiological characteristics, microcystin production and release of *Microcystis aeruginosa*

Qing Zhou, Shiqun Han, Shaohua Yan*, Junyao Guo, Wei Song, Guofeng Liu

Institute of Agricultural Resources and Environment, Jiangsu Academy of Agricultural Sciences, People's Republic of China

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ABSTRACT

Eichhornia crassipes (Mart.) Solms is effective in assimilating nutrients from eutrophic waters. However, it is not clear whether *E. crassipes* has an adverse impact on the waters in which heavy blooms of *Microcystis aeruginosa* occur. The objective of this study was to understand the interactions of *E. crassipes* with toxigenic *M. aeruginosa* and the consequences on environmental safety. Thus, the growth, physiological characteristics, microcystin production and release of *M. aeruginosa* influenced by *E. crassipes* were investigated using a co-existence experiment. The risk of microcystin-LR (MC-LR) accumulation in *E. crassipes* was also evaluated. Our results indicated that the cell death of *M. aeruginosa* occurred at a quicker pace due to the presence of *E. crassipe*. But the caspase-3 activity of *M. aeruginosa*, as the proxy for programmed cell death, was suppressed significantly by *E. crassipes*. Photosystem (PS) II-Hill reaction in *M. aeruginosa* was not significantly interrupted by *E. crassipes*, but a direct positive relationship between phycocyanin (PC) and algae biomass ($R^2 = 0.661$, $P < 0.01$) and a consistent relationship between phycocyanin/allophycocyanin (PC/APC) ratio in *M. aeruginosa* and algae biomass ($R^2 = 0.598$, $P < 0.01$) were found to be statistically significant. These results suggested that the energy harvest and electron transfer processes in the photosystem of *M. aeruginosa* might be disturbed by *E. crassipes* due to its damage of PC and a change in the PC/APC ratio. After this 12-day experiment, the level of extracellular MC-LR was significantly eliminated from 212.68 ± 25.05 μg L^{-1} to 18.98 ± 0.35 μg L^{-1} and the MC-LR production in *M. aeruginosa* was not stimulated by the influence of *E. crassipes*. The MC-LR level in the whole plants of *E. crassipes* was 3.88 ± 0.49 ng g^{-1} FW.

1. Introduction

Water eutrophication is a worldwide environmental problem. Nutrient enrichment, especially nitrogen (N) and phosphorus (P), has been a major threat to water systems in China (SEPAC, 2001). Various strategies, such as sediment dredging (Murphy et al., 1999), sediment curing (Kuang and Zhang, 2011) and phytoremediation were adopted to control nitrogen and

* Corresponding author. No. 50 Zhongling Street, Nanjing, Jiangsu Province 210014, People's Republic of China. Tel.: +86 (0)25 84390241; fax: +86 (0)25 84390248.

E-mail addresses: qqzhouqing@hotmail.com (Q. Zhou), shqunh@126.com (S. Han), gaotzy@hotmail.com (S. Yan), jjimguo@gmail.com (J. Guo), njweisong@163.com (W. Song), njliugf@yahoo.con (G. Liu).

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phosphorus pollutants. Phytoremediation technology is more attractive and widely used because of its low-cost (Sun et al., 2009). *Eichhornia crassipes* (Mart.) Solms, previously considered as one of the world's worst water weeds, is now widely applied in the bioremediation of eutrophic waters, due to its ability to grow in heavily polluted waters and to effectively assimilate nutrients (Malik, 2007). A technical system for bioremediation of eutrophic waters using *E. crassipes* was established in China, this includes confining its growth for safety reasons (Song et al., 2011), mechanized harvesting (Yan et al., 2011), large-scale disposing and resource utilization (Ye et al., 2010). In 2012, the area in which *E. crassipes* could be grown was expanded to 12 km^2 in Dianchi Lake and 16.7 km^2 in Taihu Lake.

Outbreaks of toxic cyanobacterial blooms appear frequent in China. *Microcystis aeruginosa* is the most common bloom-forming cyanobacteria in Chinese eutrophic freshwaters (Wu and Kong, 2008). The microcystins released from *M. aeruginosa* have become hazardous to aquatic environments (Papadimitriou et al., 2012). Although *E. crassipes* contributes to the bioremediation of eutrophic waters by removing nutrients (Wang et al., 2012), cyanobacterial blooms continue. This is because of the existence of high pollutant loading from nonpoint sources. In China, the regions in which *E. crassipes* could be grown are mainly confined near the bay, in order to avoid *E. crassipes* being damaged by violent waves. But in summer, thick bloom scum of cyanobacteria accumulate easily at the water surface of bay by the influence of wind. As a result, a mass of toxigenic *M. aeruginosa* co-exist with the large-scale confined *E. crassipes* in the surface waters.

Many macrophytes had been reported to reduce cyanobacteria growth by allelopathy, such as *Stratiotes aloides*, *Elodea canadensis*, *Ceratophyllum demersum* and *Myriophyllum verticillatum* (Gross et al., 2007).

Although the previous reports have been made about the isolation of algaecidal compounds from *E. crassipes* and the influence of the algaecidal compounds on different algae (Chen et al., 2005), there is little information regarding how *E. crassipes* influences the survival of toxigenic *M. aeruginosa* and whether the cell death of *M. aeruginosa* induced by *E. crassipes* has effects on water environment. In this study, we report the effects of *E. crassipes* on physiological characteristics and MC-LR production of *M. aeruginosa*. Furthermore, the risk of MC-LR releasing from *M. aeruginosa* under stress of *E. crassipes* and the risk of MC-LR accumulation in *E. crassipes* are described.

2. Materials and methods

2.1. Strains and culturing conditions

The microcystin-producing strain *M. aeruginosa* (FACHB-912) was purchased from Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). This strain was cultivated in batch cultures using 1/10 modified Hoagland's medium (pH 7.0, adjusted with NaOH) and was then used in co-existence experiments when at exponential growth phase (OD_{650} = 0.3956). *E. crassipes* was collected from a concrete breeding pool at Jiangsu Academy of Agricultural Sciences (Nanjing, China), cleaned of debris, washed several times with tap-water and distilled water. The plants of similar height (about 30 cm) were selected and pre-incubated in 1/10 modified Hoagland's medium for 7 days before being used in co-existence experiments. 1/10 modified Hoagland's medium was sterilized at 121 °C for 20 min. The incubator was maintained at 28 °C with a constant relative humidity of 75% and illuminated by cool-white fluorescent lamps (40 μmol photons m^{-2} s^{-1}) in 12-hour diurnal cycles.

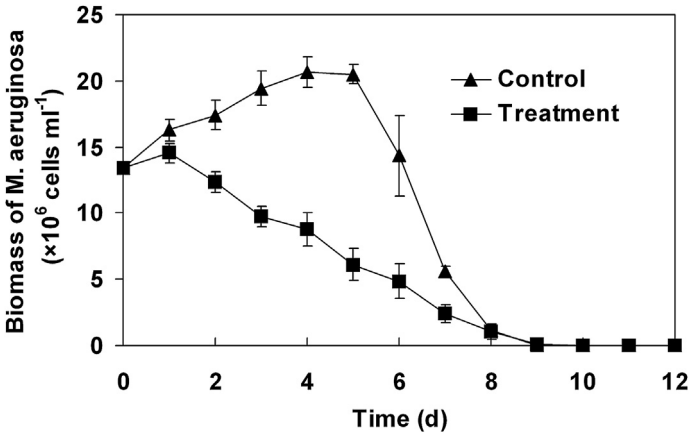

Fig. 1. Change in biomass of *M. aeruginosa* under the impact of *E. crassipes*. Control means *M. aeruginosa* samples without influence of *E. crassipes*. Treatment means *M. aeruginosa* samples under the influence of *E. crassipes*. Values are averages ± standard deviations (n = 3). Initial density of *E. crassipes* was 16.41 ± 0.71 g L^{-1}.