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Effects of the decomposing liquid of *Cladophora oligoclona* on *Hydrilla verticillata* turion germination and seedling growth

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ABSTRACT

Excessive proliferation of filamentous green algae (FGA) has been considered an important factor resulting in the poor growth or even decline of submerged macrophytes. However, there is a lack of detailed information regarding the effect of decaying FGA on submerged macrophytes. This study aimed to investigate whether the decomposing liquid from *Cladophora oligoclona* negatively affects *Hydrilla verticillata* turion germination and seedling growth. The results showed that the highest concentrations of decomposing liquid treatments inhibited the turion germination rate, which was the lowest than other treatments, at only 84%. The chlorophyll *a* fluorescence (JIP test) and physiological indicators (chlorophyll *a* content, soluble sugars, Ca^{2+}/Mg^{2+}-ATPase and PAL activity) were also measured. The chlorophyll *a* content in the highest concentration (40% of original decomposing liquid) treatment group decreased by 43.53% than that of the control; however, soluble sugars, Ca^{2+}/Mg^{2+}-ATPase, and PAL activity increased by 172.46%, 271.19%, and 26.43% respectively. The overall results indicated that FGA decay has a considerable effect on submerged macrophyte turion germination and seedling growth, which could inhibit their expansion and reproduction. This study emphasized the need to focus on effects of FGA decomposition on the early growth stages of submerged macrophytes and offered technological guidance for submerged vegetation restoration in lakes and shallow waters.

1. Introduction

Submerged macrophytes are an important part of aquatic ecosystems, with functions of purifying water, improving water transparency, increasing dissolved oxygen (DO), and inhibiting algal growth (Hilt and Gross, 2008). Therefore, submerged macrophyte restoration as an important eutrophic lake ecological restoration technology has recently been widely promoted and applied. However, there has been a new phenomenon of excessive growth of filamentous green algae (FGA) in eutrophic lakes where submerged macrophytes have been successfully restored in recent years (Bakker et al., 2010). Excessive growth of FGA not only impedes the waterway traffic, reduces the value of entertainment, aesthetics, and commercial use of lakes (Verthouqstraete et al., 2010; Higgins et al., 2012), but also attaches to submerged macrophytes and affects the growth of submerged vegetation through mechanical damage and light competition. Furthermore, FGA decays because of self shading and nutrition competition when its biomass increases per unit area (Depew et al., 2011). In the process of FGA decomposition, the dissolved oxygen and visibility of the aquatic environment drastically decrease, and the decaying FGA could also release harmful substances such as phenolic acid and toxic aromatic compounds, that inhibit the growth of hydrophytes; the increased nutrients could also stimulate the growth of other primary producers such as microalgae and harmful algae, which cause blooms and negatively affect the health of aquatic animals and plants (Gubelit and Berezina, 2010; Ye et al., 2011). The biomass of submerged macrophytes generally decreased and their communities receded in lakes where FGA excessively decayed (Pieczyńska and Tarmanowska et al., 1996; Chen et al., 2007). However, there has been limited research on the effect of decaying FGA on submerged macrophytes (Wang et al., 2012). The mechanism by which decaying FGA affects the decline of submerged macrophytes is not yet clear; for example, what impact does the FGA decomposing liquid have on the growth and expansion of submerged macrophytes? What physiological and biochemical activities in submerged macrophytes occur with FGA decomposing liquid?

Previous studies have shown that when submerged macrophytes are grown under stress, photosynthesis is inhibited (Hussner et al., 2016; Xu et al., 2016). When the submerged macrophyte *Vallisneria natans* was cultured in an elevated carbon dioxide stress environment, its photosynthetic system was inhibited, as indicated by the JIP test results

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(Han et al., 2017). The JIP test was based on the theory of biofilm flow, a data analysis and processing method for a rapid chlorophyll *a* fluorescence induction curve (OJIP), which can reflect the actual physiological state of photosynthesis PSII (Clark et al., 2000). In response to stress, certain physiological and biochemical reactions occur in plants. Some plant organs store large amounts of soluble sugars to cope with changes in environmental conditions such as nutrition, light, and temperature; drastic changes in these conditions (low temperature, high light intensity, etc.) also lead to a change in the response of soluble sugar (Xie et al., 2015). Besides accumulated sugar, the activities of various enzymes in plant cells change with the emergence of stress change, e.g., a study found that plant Ca^{2+}-ATPase responded to some degree to adverse conditions (Ogunbayo et al., 2008). Ca^{2+}/Mg^{2+}-ATPase acts as an important Ca^{2+} regulator, which can regulate intracellular Ca^{2+} concentration, and maintain the cytoplasm Ca^{2+} steady-state equilibrium. Cytoplasmic Ca^{2+} steady-state balance is necessary for normal protein synthesis and cell growth to occur (Pande et al., 2005). In addition to changes in energy, it also affects the secondary metabolism of plants, phenylalanine ammonia-lyase (PAL) plays an important role in the formation of secondary substances, such as lignin and phytoalexin, which are important for plant growth, development, and disease resistance (Nadernejad et al., 2013; Cuéllar-Villarreal et al., 2016). These indicative parameters can reflect the plant's ability for survival and stress resistance in stressful environments.

Hydrilla verticillata is a dominant submerged macrophyte in many shallow lakes and considered a pioneer plant in the restoration of aquatic plants for controlling eutrophication (Ye et al., 2009). The germination and normal growth of its turions and seedlings are the key factors to ensure its expansion and biomass enhancement. *Cladophora oligoclona* is a typical FGA, often found in shallow lakes, and its excessive growth often leads to environmental problems in many shallow freshwater environments (Higgins et al., 2008). We hypothesized that the decomposing liquid of FGA negatively affects turion germination and seedling growth of submerged macrophytes, and could contribute to submerged vegetation loss and lake eutrophication. In the present study, we determined the effect of the decomposing liquid of FGA on *Hydrilla verticillata* using the JIP test, and analyzed the chlorophyll *a* content, soluble sugar content, and Ca^{2+}/Mg^{2+}-ATPase and PAL activities. Some water quality parameter changes were monitored during the same period. The aims of the study were to determine how the growth and expansion of submerged macrophytes were affected by FGA, and provide technical guidance for the restoration of submerged macrophytes in lakes and shallow waters.

2. Materials and methods

2.1. Preparation of the decomposing liquid of C. oligoclona

Cladophora oligoclona was collected from the East Lake, Wuhan, China (114°36' N, 30°55' E) in February 2017. After being washed free of impurities using deionized water, *C. oligoclona* was stored at −20 °C for 24 h. Then, 50 g of *C. oligoclona* (fresh weight) was thawed and added to a 2 L flask with 1 L deionized water, all flasks were covered with opaque tin foil (to create darkness and prevent oxygen) and placed in a chamber at 25 °C for 15 d.

Decomposing liquid from the preparation described in the above steps was filtered (Whatman filters 102, 15–20 μm) and centrifuged for 20 min at 5000 × *g*, and the supernatant was filtered with Whatman filters GF/C, pore size 1.2 μm and membrane filters, pore size 0.45 μm, 0.22 μm successively, and stored at 4 °C to prepare for subsequent experiments. The degree of decomposition of *C. oligoclona* was approximately 50% biomass loss of initial weight (modified from Pieczyńska and Tarmanowska, 1996).

2.2. Turion germination test

The turions of *H. verticillata* were purchased in February 2017 from Tianfu Aquaculture Cultivation Cooperatives in Nanjing, China (local growth environmental conditions, pH: 7.6–8.6; COD: 23–30 mg L^{-1}; DO: 8.8–9.7 mg·L^{-1}). The turion germination test was performed in laboratory conditions during February 2017. Uniformly sized, full and healthy turions were sterilized in 70% alcohol for 30 s and 2% NaClO for 8 min, respectively, and then rinsed with deionized water three times.

The original decomposing liquid was diluted with deionized water to different concentrations. The four treatments were: CG (control group, no decomposing liquid), T1 (10% of original decomposing liquid treatment group), T2 (20% of original decomposing liquid treatment group), and T3 (40% of original decomposing liquid treatment group). A total of twelve sterilized glass dishes (Φ14.5 × 7.5 cm) with a layer of sponge seedbed (1 cm thick) and 150 mL decomposing liquid were prepared, and then 25 turions were planted in each glass dish. Each treatment was performed in triplicate. All glass dishes were sealed with parafilm to minimize water loss and then incubated in a germination chamber (PGX, Ningbo Laifu Technology Co. Ltd, China) at 25 ± 1 °C, an irradiance of 2000 lx, and a photoperiod of 12:12 (L: D).

Germinated turions were counted daily during the germination test. Duration for the test was 7 days. The germination vigor (the percentage of normal germinated turions in all tested turions) was calculated on the 3rd day and the germination rate (the percentage of normal germinated turions in all tested turions at the end of the full test) was calculated on the 7th day and included turions with a bud length > 3 mm (modified from Guan et al., 2013).

2.3. Physiological and biochemical analyses of seedlings

To obtain the tested seedlings, *H. verticillata* turions from the same source as above were cultured and germinated in the outdoor expansion from February 2017. After 60 d, the healthy seedlings with no branches were selected for the experiment. A total of 12 1-L flasks with 1-L culture solution, which included decomposing liquid (concentrations same as in Sections 2.2) and 1/1000 (v/v) concentrated nutrition of 1/2 MIII medium (Nicklisch, 1992) were prepared, and six seedlings were cultivated in each flask. Each treatment was performed in triplicate, and in all treatments, the pH was modified to 8.0 before adding in the decomposing liquid. The growth conditions were the same as that in the turion germination test. The chlorophyll *a* content, soluble sugars, Ca^{2+}/Mg^{2+}-ATPase, and PAL activity, and the water quality were measured on days 1, 3, and 5. Chlorophyll *a* fluorescence and water quality were analyzed in situ. Three seedlings from each treatment were randomly selected to measure chlorophyll *a* fluorescence.

The top 2–3 cm of the seedlings were selected to measure chlorophyll *a* fluorescence using a Handy PEA (Hansatech, England) in conditions of 25 ± 1 °C and 10 min dark adaptation. The excitation light at the time of measurement was provided by a 650-nm LED to a measured area (4 mm in diameter), and the light intensity was 3,000 μmol photons m^{-2} s^{-1}. The fluorescence signal was detected by a highly efficient PIN photodetector, with data acquisition every 10 μs before 2 ms and every 1 ms thereafter. Three seedlings were selected in parallel for each treatment, and each reading was repeated three times. The OJIP fluorescence induction curve was analyzed by a JIP test according to Schansker et al. (2005). The following JIP test parameters could be obtained directly from the rapid chlorophyll fluorescence induction kinetic curve: F_0, the initial fluorescence, measured at 50 μs (O-step); F_J, the fluorescence intensity at 2 ms (J-step); F_I, the fluorescence intensity at 30 ms (I-step); F_M was equal to F_p, the maximal fluorescence intensity at P step; PI_{abs}, performance index on absorption basis. The fluorescence parameters were listed in Table 1 (Pan et al., 2011).

The chlorophyll *a* content of fresh leaves of seedlings was determined using an ethanol extraction method (Wang, 2006). Fresh

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