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Allelopathic effects of *Ailanthus altissima* extracts on *Microcystis aeruginosa* growth, physiological changes and microcystins release

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HIGHLIGHTS

- *A. altissima* extract had a high potential ability to control algal blooms.
- *A. altissima* extract inhibited the growth of *M. aeruginosa*.
- The use of *A. altissima* decreased the amount of extracellular microcystins.
- *A. altissima* extracts destroyed the photosynthesis-related structure of cell.

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ABSTRACT

The use of allelochemicals has been proved an environmentally friendly and promising method to control harmful algal blooms. This study was conducted to explore the application potential of *Ailanthus altissima* (*A. altissima*) extracts in *Microcystis aeruginosa* (*M. aeruginosa*) control for the first time. Four treatments with *A. altissima* extractions (25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, and 200 mg L^{-1} respectively) and a control group were built to investigate the effects of *A. altissima* on the growth, cellular microstructure and cell viability, physiological changes, and release of extracellular matters. Results showed that the cell density of *M. aeruginosa* was effectively inhibited by *A. altissima* extract, and the inhibition rates were dose-dependent within 5 d. Especially for the treatment with 200 mg L^{-1} of extract, the inhibitory rates remains above 90% after 5 d exposure. In addition, *A. altissima* effectively decreased the amount of extracellular cyanotoxin microcystins and destroyed the photosynthesis-related structure of algae cell during the experimental period. The results demonstrated the *A. altissima* extracts can be used as an effective and safe algicide to control algal blooms. However, it must be noted that specific compounds responsible for algicidal effect should be isolated and identified to explore inhibition mechanism of *A. altissima* in future study.

1. Introduction

Cyanobacterial bloom in eutrophic aquatic ecosystems is becoming a major environmental problem worldwide (Xiao et al., 2010; Zhang et al., 2013; Ni et al., 2015). It significantly affects water quality and induces off-flavor problems (Laughinghouse et al., 2012). Moreover, cyanobacterial blooms usually break out along with release of cyanotoxins (Pei et al., 2014), which lead to a series of adverse effects such as the decreasing biodiversity, and illness in animals and humans. Therefore, it has become a significant issue to control and elimination of cyanobacterial blooms (Ni et al., 2012; Pei et al., 2014).

Chemical methods such as ozonation, addition of chemicals including potassium permanganate, sodium hypochlorite, copper sulfate, chlorine, hydrogen peroxide, and anti-algal flocculants are most widely adopted due to their quick and effective characteristics after application (Wu et al., 2010; Yan et al., 2011). However, the persistence of chemical compounds potentially induces secondary pollution and harm aquatic organisms even human (Zhang et al., 2013). In recent years, biological measures including using algaecidal bacteria, introducing fish, and biopond-wetland system have received much more attention as alternatives to chemical agents (Wu et al., 2010). Allelochemicals have been

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proved as environmentally benign chemical algaecide for their specificity and biodegradability (Park et al., 2009; Zhu et al., 2010; Zhang et al., 2013). Extracts of aquatic plants such as *Phragmites communis*, *Myriophyllum spicatum*, *Ceratophyllum demersum*, *Stratiotes aloides* and *Najas marina* spp. *Intermedia* reportedly inhibit the growth of cyanobacteria. However, in view of the limitation of resources, it seems impractical to control cyanobacteria using extracts of aquatic plants (Hong et al., 2010; Zhang et al., 2013). To explore the potential of terrestrial plants extracts for controlling cyanobacteria provide a new guide for developing algicide (Shao et al., 2013).

Ailanthus altissima, which belongs to the Simaroubaceae family, is commonly found in China, Europe, and the United States (Heisey and Heisey, 2003; Albouchi et al., 2013). It has been reported that *A. altissima* exhibits rapid establishment and growth, with a high reproduction rate (Kovarik and Säumel, 2007; Wang et al., 2013). The extracts of this plant possess variety of pharmacological effects such as antiviral, antitumor, antimalarial, and antimicrobial activities (Kundu and Laskar, 2010; Luis et al., 2012; Filippou et al., 2014). *A. altissima* have been long used as traditional Chinese herbal medicines for the treatment of bleeding, colds, and gastric diseases (Hong et al., 2013).

In recent years, it has attracted increasing attention due to their strong competitive effects on neighboring plants such as *Lepidium sativum*, *Acer rubrum, A. saccharum* and *Quercus rubra* (Heisey and Heisey, 2003; Carter and Fredericksen, 2007; Gómez-Aparicio1 and Canham, 2008; Constán-Nava et al., 2010). Phytochemical investigation indicated that the main constituents of the title plant are b-carboline alkaloids, quassinoids, lipids, and sterols (Luis et al., 2012), which belong to the common allelopathic compounds. However, little literature focuses on describing the potential of *A. altissima* extracts for alga blooms control.

Hence, the goals of this study were to: (1) document the effects of *A. altissima* extracts on the growth of *M. aeruginosa*; (2) assess the effect of *A. altissima* extracts on microcystins production of alga; and (3) illustrate the potential influence mechanism through changes in cellular microstructure and cell viability, chlorophyll a (Chl-a), total protein, malondialdehyde (MDA), superoxide dismutase (SOD) activity, and extracellular organic matters (EOM) release.

2. Materials and methods

2.1. Preparation of A. altissima extracts

The tree bark of *A. altissima* was collected in Taian, Shandong province, October 2013. The plant materials were dried at 70 °C to constant weight, and then ground to powder by a micro pulverize (FW100, Tianjin Taisite Instrument Co., LTD). The powdered materials were selected by a 40 mesh sieve for use. Each 20 g of sample was extracted by Soxhlet method with 1 L ethyl alcohol. The solvent was stored in a refrigerator at 4 °C.

2.2. Algal culturing

M. aeruginosa (FACHB-905) was provided by Freshwater Algae Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The algae were grown in BG11 medium at 25 °C under 2800 lx in laboratory. The tested organisms were cultivated to exponential growth phase (10^7) for use.

2.3. Algal bioassay

Small amount of *A. altissima* extractions were added into a 5 L sterilized flask to obtain 4 concentration groups (25 mg L^{-1}, 50 mg L^{-1}, 100 mg L^{-1}, and 200 mg L^{-1} respectively). When the solvent was completely evaporated at room temperature under aseptic conditions, *M. aeruginosa* (2 L) with initial algal density of 1.0×10^6 cells mL^{-1} was inoculated into the flask. There were four replicates for each concentration and control groups. All flasks were cultivated at 25 °C under 2800 lx conditions. The algal cells were harvested and counted each day using microscopy a hemocytometer (OLYMPUS CX31, Japan).

Inhibitory rate (IR) was determined by the following formula:

$$\mathrm{IR}\ (\%) = (1 - N/N_0) \times 100$$

where N and N_0 are the cell numbers in the treatment and control cultures respectively.

After 5 d exposure to the *A. altissima* extracts, the changes in cell microstructure of *M. aeruginosa* were photographed using a scanning electron microscope (SEM, S-570, HITACHI, Japan) at 100 kV. Cell viability was determined by a fluorescence microscope (Nikon TE2000, Japan) on the 0, 2, and 5 d.

2.4. Effect on physiological indicators

Chl-a was extracted with acetone and then determined using a UV–vis spectrophotometer (UV-2450, Shimadzu Instrument Co. Ltd., Japan) at 630, 645, 663 and 750 nm. Chl-a content was calculated according to the method described by Strickland and Parsons (1968).

The samples were centrifuged at 8000*g* for 10 min to collect algal cells. Cells were then re-suspended with phosphate buffer and then homogenized by an ultrasonic cell pulverizer (JY92-2D, Xinzhi Co., China) at 600 W. The ultrasound was processed under ice-bath cooling with total time of 10 min (ultrasonic time: 2 s; rest time: 8 s). Then the homogenate was centrifuged at 12,000*g* for 15 min. The cell-free enzyme supernatant was maintained at −20 °C for further use.

MDA content was measured by a colorimetric method (Shiu and Lee, 2005). Total protein content was determined by Bradford method (1976) using bovine serum albumin as standard. Superoxide dismutase (SOD) activity was measured according to the method of Beauchamp and Fridovich (1971).

2.5. EOM and microcystins analysis

Each of the samples was filtered through a 0.45 μm filter membrane and then subjected to EOM and microcystins analysis. Fluorescence excitation–emission matrix (EEM) was used to distinguishing the EOM by a fluorescence spectrophotometer (model F-4600, Hitachi, Japan). Excitation wavelengths were incrementally increased from 220 to 450 nm in 5 nm steps, and the emission spectra ranged from 250 to 550 nm were evaluated in 1 nm step. The slits for both Ex and Em were 5 nm with scan rate fixed at 2400 nm min^{-1}. All data were analyzed using Matlab 2013b (MathWorks Inc., Natick, MA). Extracellular microcystins content was measured using a Beacon Microcystin ELISA kit (Beacon Analytical Systems Inc, Maine, USA).

2.6. Statistical analysis

Statistical analysis was performed using the SPSS program (SPSS Inc., Chicago, IL, USA; Version 13.0). Significant differences between data sets were detected by one-way analysis of variance. Duncan's test was performed to detect the statistical significance of differences ($p > 0.05$) between means of treatments.