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Short communication

Herbicidal effects of Chinese herbal medicine *Coptis chinensis* Franch. extract on duckweed (*Spirodela polyrhiza* (L.) Schleid.)

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ABSTRACT

Dense mat formed by duckweeds is a significant threat to fisheries, landscape, ecological environment, and economies around the world. Effective biological management strategies to control duckweeds are quite limited. In the present study, we determined the effect of the extract of *Coptis chinensis*, a traditional Chinese medicinal herb, on the growth of duckweed (*Spirodela polyrhiza*). The growth of *S. polyrhiza* was strongly inhibited by the *C. chinensis* extract. The number of fronds and fresh weight of the plants were significantly decreased after a 96-h treatment. Furthermore, the chlorophyll (*a*, *b*, and *a* + *b*) content was remarkably decreased by the *C. chinensis* extract. The 24-, 48-, 72- and 96-h-IC_{50} of the *C. chinensis* extract to *S. polyrhiza* were 6.56, 1.06, 0.81, and 0.33 g DW eq. extract L^{-1}, respectively. In addition, the *C. chinensis* extract was found to be safer for the submerged macrophytes (such as *Vallisneria natans*: 96-h-EC_{50} was 10.39 g DW eq. extract L^{-1}) and aquatic animals (such as zebrafish: 96-h-LC_{50} was 20.35 g DW eq. extract L^{-1}). These results showed that the *C. chinensis* extract was inhibitory to the growth and reproduction of *S. polyrhiza*. Moreover, the field test results also confirmed that the inhibitory effect of *C. chinensis* extract on *S. polyrhiza*. Thus, we recommend the use of the *C. chinensis* extract as an effective and safe botanical herbicide for duckweed management in water ecosystems.

1. Introduction

Duckweeds (Lemnaceae) are floating aquatic plants, and their common species can be mostly represented by *Lemna minor*, *L. gibba*, *L. valdiviana*, *L. aequinoctialis*, *Landoltia punctate*, *Spirodela polyrrhiza*, which grow in various types of water, and often promote nutrient loading (Appenroth et al., 2015; Lam et al., 2014). They are considered as nuisance plants in some freshwaters (Parr et al., 2002; Pęczuła and Suchora, 2014; Wersal and Madsen, 2009). With nutrient levels increasing over time due to the continuous accumulation of pollutants, excessive fish feeding, and discharge of surface run-off, an increasing number of water-bodies (such as ditches, ponds, small lakes, and wetlands) are suffering from the overgrowth of duckweeds. The dense mats formed by duckweeds have brought a series of serious consequences, like worsening underwater light climate (Parr et al., 2002), increasing diel hypoxia, posing significant threat to the fisheries (Killgore and Hoover, 2001), changing the growth of phytoplankton (de Tezanos Pinto et al., 2007; Pasztaleniec and Poniewozik, 2013) and submerged macrophytes (Janes et al., 1996), reducing the aesthetics of garden ponds and even affecting the whole ecological system changes (Pęczuła and Banach, 2013). Besides, the negative changes induced by duckweeds might reduce the aesthetics of garden ponds or have a profound effect on the conservation of valuable habitats in fishponds (Pęczuła and Banach, 2013; Zhang et al., 2014a). Therefore, great attention should be paid to control duckweeds, which have been acting as nuisance species in the recent years.

When waters are suffering from the dense mats formed by duckweeds, the traditional technologies used to control the overgrowth of duckweeds include the mechanical removal (Zhang et al., 2014b), chemical herbicides (Cheshier et al., 2011; Langeland et al., 2002; Wersal and Madsen, 2009), and biological predation (Pípalová, 2006). However, the application of the above technologies was limited because of some significant weakness, for example, the mechanical removal of the free-floating duckweeds is time-consuming and often ineffective (Cooke et al., 2005; Zhang et al., 2014b). Though the chemical herbicides are fast-acting and low-cost, their negative effects on water

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quality and the safety of this method for fish and other aquatic biota might still be questioned (Pęczuła and Suchora, 2014). On the other hand, although duckweeds can be used as food for some aquatic organisms, the biological predation has always been restricted by many factors. Moreover, previous studies have shown that this method seemed to have negative environmental consequences, such as the release of nutrient-rich excrements into water and community change of aquatic plants and animals (Chilton and Muoneke, 1992; Pípalová, 2006). Hence, it is necessary to find more effective and safe ways to manage the excessive proliferation of duckweeds.

In the recent years, plant extracts have been used as botanical herbicides to control the growth of duckweeds, for example, rice hulls extract can strongly inhibit the growth of duckweed (*L. paucicostata*) (Chung et al., 2006), barley straw extract addition inhibit the growth of *L. valdiviana* (Pęczuła and Suchora, 2014), aqueous extract from shoots of dried spikerushes retard the growth of *L. paucicostata* (Sutton and Portier, 1989), aqueous extract from the Chinese traditional medicine *Euphorbia helioscopia* also inhibit the growth of *L. minor* (Zhang et al., 2014a). *Coptis chinensis* is a perennial herbaceous plant and also a common Chinese traditional medicine which belongs to *Coptis*, Ranunculaceae, Ranunculales. It has been reported to exert a number of pharmacological effects on human including antihypertensive, antibacterial, anti-oxidative, anti-inflammatory, and so on (Tjong et al., 2011). In aquatic ecosystems, *C. chinensis* is commonly used for the treatment of fishes diseases, such as red skin disease, enteritis, septicemia, and so on (Chen and Yuan, 2015; Choi et al., 2013). In addition, berberine and total alkaloids are the main constituents of *C. chinensis* (Shi et al., 2009; Wang, 2016), and the alkaloid is one of the important allelochemicals in aquatic plants (Rice, 1984). Therefore, we predict that the aqueous extracts from *C. chinensis* can inhibit the growth of duckweeds. However, to the best of our knowledge, research on the use of the *C. chinensis* extract as a botanical herbicide to control the overgrowth of duckweeds have yet to be undertaken.

Therefore, the main objectives of the present study were to (1) determine the effects of *C. chinensis* extract on the growth of *S. polyrhiza* (also named as giant duckweed, one of the common duckweeds in the freshwater system); (2) evaluate the feasibility of applying the technology based on the assessment of ecological effects of the *C. chinensis* extract on aquatic organisms.

2. Materials and methods

2.1. Spirodela polyrhiza culture

Fresh samples of *S. polyrhiza* were collected from a eutrophic pond near Shanghai Ocean University and cultured in the laboratory for several weeks before the assays. Prior to experiment, sufficient plants were removed and grown in glass beakers (1000 mL) for 7 days (pre-cultivation) in illuminating incubators at 25 °C under 60 μmol photons $m^{-2} s^{-1}$ cool fluorescent light (8:16 h LD cycle) in 1/4 Hoagland's complete nutrient solution (Li and Cheng, 2015). The nutrient solution was diluted by the water samples obtained from the same pond and filtered by using cellulose nitrate membranes (Whatman, pore size 0.45 μm).

2.2. Preparation of C. chinensis extract

Coptis chinensis was purchased from a Chinese medicine shop in Shanghai, China. The method of aqueous extracts from *C. chinensis* was according to Shao et al. (2010) and Zhang et al. (2011) with small modifications. It was crushed (approximately 100 mesh) after drying at 80 °C for 48 h. For preparing the extract, 25 g of *C. chinensis* powder was dissolved in 500 mL distilled water in a 1000-mL beaker and put in a boiling water bath for 2 h. The extract was centrifuged at 1000*g* for 30 min, and the supernatant was diluted with distilled water to 250 mL. The final dose of the *C. chinensis* extract was 100*g* dry weight equivalent extract L^{-1} (g DW eq. extract L^{-1}).

2.3. Experimental design

A total of 102 fronds of healthy *S. polyrhiza* were selected from the pre-cultivation and placed into a 1000-mL beaker as one replicate. *Coptis chinensis* extract was diluted with the water from the eutrophic pond to 600 mL to obtain the final concentrations of 0 (the control), 0.4, 0.8, 1.6, 3.2, and 4.8 g DW eq. extract L^{-1}. All experiments were carried out in the illuminating incubators at 25 °C under 60 μmol photons $m^{-2} s^{-1}$ cool fluorescent light (8:16 h LD cycle) with five biological repeats.

2.4. Determination of growth and chlorophyll concentration

The growth of *S. polyrhiza* was determined by measuring the number of fronds (FN) and frond weight (FW). FN was observed at 0, 24, 48, 72, and 96 h. FW was determined at 96 h after the frond was surface-dried by paper towels. The relative growth rate (RGR) was calculated according to the equation: $RGR = (\ln x_t - \ln x_0)/(t_t - t_0)$, where x_0 and x_t are the measured values of parameters (FN) at time t_0 (the start of the test) and t_t (24, 48, 72, and 96 h). The growth inhibitory rate (GIR) was calculated according to Coronado-Posada et al. (2013). The concentrations of chlorophyll *a* (Chl*a*), *b* (Chl*b*), and chlorophyll (*a* + *b*) [Chl(*a* + *b*)] were determined according to the method described by Huang et al. (2007). The half maximal inhibitory concentration (IC_{50}) of *S. polyrhiza* growth was calculated using the PASW Statistics 18.0 software.

2.5. Ecological safety experiment

Vallisneria natans and zebrafish (*Brachydanio rerio*) were selected as the non-target test organisms and exposed to the *C. chinensis* extract. Six *V. natans* (the average fresh weight of the plant was 5.2 ± 0.7 g) and fifteen zebrafish (the average weight of the fish was 0.15 ± 0.01 g) individuals were placed into each aquarium (capacity of 25 L) with different concentrations (0, 0.4, 0.8, 1.6, 3.2, and 4.8 g DW eq. extract L^{-1}) of the extract as one replicate. All experiments were performed in the illuminating incubators at 25 °C under 60 μmol photons $m^{-2} s^{-1}$ cool fluorescent light (8:16 h LD cycle) with three biological repeats. The aquariums were continuously aerated using an aquarium aerator. The number of dead zebrafish was recorded and timely removed at 0, 24, 48, 72 and 96 h. The fresh weight of *V. natans* was measured at the end of the experiment (96 h). The half maximal effective concentration (EC_{50}) of *V. natans* and the half lethal concentration (LC_{50}) of zebrafish were calculated using the PASW Statistics 18.0 software. The safe concentration (SC) was calculated according to Lei (2004).

2.6. Statistical analysis

Data (mean ± SD) were proved as the normal distribution by Shapiro-Wilk test, and the equal variance were evaluated by Levene's test. The data were subjected to the one-way analysis of variance (ANOVA) according to the Duncan Multiple Range Test, and $P < 0.05$ was considered statistically significant. All analyses were conducted using PASW Statistics 18.0 (IBM SPSS Software, Chicago, USA).

3. Results and discussion

3.1. Effects of the C. chinensis extract on the FN, RGR, and GIR of S. polyrhiza

Fig. 1 shows that the average FN and RGR of *S. polyrhiza* changed with time under different concentrations of the *C. chinensis* extract. We observed significantly high FN in the 0.4-g DW eq. extract L^{-1} group

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