The effect of plant extracts on growth and photosynthetic fluorescence characteristics of *Microcystis flos-aquae*

Yuxin Shi, Anglu Shen, Meng Tan, Peimin He and Liu Shao

**ABSTRACT**

The cyanobacteria *Microcystis flos-aquae* can cause harmful algal blooms in waterbodies, which threaten the normal functioning of aquatic ecosystems and human health. Some plant extracts are considered as promising algacides. In this study, the effects of ten plant extracts (*Cinnamomum camphora, Ginkgo biloba, Firmiana platanifolia, Salix babylonica, Euphorbia humifusa, Erigeron annuus, Solidago canadensis, Alternanthera philoxeroides, Thalia dealbata* and *Eichhornia crassipes*) against *M. flos-aquae* were investigated. The results showed that all ten plant extracts had a significant inhibitory effect on *M. flos-aquae* growth after 96 h (*P* < 0.01). The inhibition rates of *S. babylonica, E. humifusa, S. canadensis* and *A. philoxeroides* were over 70.00%. Furthermore, the *E. humifusa* extract had the best inhibitory effect on the photosynthesis of *M. flos-aquae*, with the effective quantum yield of photosystem II and maximal relative electron transport rate decreasing by 97.50% and 97.00%, respectively, after 96 h. Additionally, the *E. humifusa* extract was found to be non-toxic to non-target organisms such as *Brachydanio rerio* and *Vallisneria spiralis* within 96 h. This study contributes to the existing knowledge and data of freshwater cyanobacteria blooms, and provides insights for their control and the restoration of freshwater systems affected by cyanobacteria blooms.

**Key words** | algal bloom control, *Euphorbia humifusa*, growth inhibition, photosynthetic activity, plant extract

**HIGHLIGHTS**

- Algistatic mechanisms could be related to the species of allelochemicals rather than the species of plant.
- Polyphenols and flavones are likely to be the main phytotoxic substances.
- The photosynthetic system analysis is a better way to understanding the mechanisms of allelochemicals.
- *Euphorbia humifusa* had higher efficiency as an algacide and had no potential risk to the ecological safety of aquatic ecosystem.

**GRAPHICAL ABSTRACT**

[Image of flowchart showing the effects of leaf litter extract on growth inhibition, cell density, photosynthesis, and fluorescence intensity.]
INTRODUCTION

Algal blooms, especially cyanobacteria blooms, caused by eutrophication have become a widespread environmental issue that cause severe water quality problems and affect the ecological functions of water (Chen et al. 2020; Yan et al. 2020). To date, a number of approaches have been reported to eliminate or control cyanobacteria blooms. Some physical methods such as artificial mixing and sonication (Muelle et al. 2015; Visser et al. 2016), chemical alternatives, such as hydrogen peroxide (Sinha et al. 2018) and some metals (copper sulfate, iron, aluminum) (Crafton et al. 2018), and some biological methods, such as algicidal bacteria (Li et al. 2016a) have been used to prevent the growth or directly kill the cyanobacteria. However, the installation, operation and energy costs are relatively high, and most of these substances can cause secondary pollution of aquatic ecosystems.

At present, an attractive alternative to traditional approaches is the use of biologically derived substances from plants (Zerrifi et al. 2020), which is considered to be an environmentally friendly and promising approach for controlling cyanobacteria blooms in aquatic environments (Shao et al. 2015; Hua et al. 2018). A number of studies have been conducted on cyanobacteria bloom control using aquatic and terrestrial plant extracts (Zhang et al. 2016; Zhao et al. 2019). This method seems to offer a new and promising alternative for harmful algae bloom control due to its low toxicity and lower risk to the environment (Hua et al. 2018). Therefore, in this study, we selected ten familiar plants, Cinnamomum camphora, Ginkgo biloba, Firmiana platanifolia, Salix babylonica, Euphorbia humifusa, Erigeron annuus, Solidago canadensis, Alternanthera philoxeroides, Thalia dealbata and Eichhornia crassipes as algae suppressor source plants, which are common and rich in anti-bacterium substances and some phytotoxic compound (Shanab et al. 2010; Ni et al. 2011; Salem et al. 2011; Zhang et al. 2011; Yang et al. 2012; Luyen et al. 2014; Bależentienė 2015; Kleiowski et al. 2016; Le Rouzic et al. 2016; Chen et al. 2018). However, research on the control of algae blooms by plant extracts mainly focus on one plant or one type of plant. Little information is available for a comparative evaluation of the antialgal activities of different plant extracts due to different experimental conditions (Sinang et al. 2019). In addition, most studies choose cell density and chlorophyll content of algae as a monitoring index (Li et al. 2016b) and little is known about the effect of extracts on the photosystem of algae, which is probably an important target of some allelochemicals (Shao et al. 2020). Chlorophyll fluorescence analysis can provide different fluorescence parameters, such as the maximal quantum yield of photosystem II (PSII) (Fv/Fm), the effective quantum yield (YII) and the efficiency (α) and maximal relative electron transport rate capacity (rETRmax) of photosynthesis, without causing harm to the plant (Jiang et al. 2018). This method also provides a reliable assessment of photosynthetic activity on algae. It is reported that fluorescence parameters may provide an additional physiological signal, as the information can identify changes in photosynthetic status before morphological or density-based changes are evident (Wang et al. 2018).

We hypothesized that there would be one or two plant extracts that inhibit growth of M. flos-aquae, mostly by the pathway of photosystem and that would be a promising approach for controlling cyanobacteria blooms in aquatic environments. Therefore, this study compared the antialgal activity of ten types of plant extracts on M. flos-aquae under the same experimental conditions. Meanwhile, the variation in photosynthetic fluorescence characteristics of M. flos-aquae was also studied to uncover the inhibition mechanism of extracts. In addition, an assessment of the ecological safety of the extract with the best inhibition rate was carried out. The goal is to develop effective and safe algaecides for cyanobacteria bloom control.

MATERIALS AND METHODS

Algal culture

M. flos-aquae were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The algae were pre-cultured in a sterile BG11 medium and were maintained in an incubator at 25 °C under 60 μmol photons m−2 s−1 on a 12:12 h light:dark cycle (Li et al. 2016b). Stock cultures in the exponential growth phase were used in the following experiments.

Preparation of plant extracts

Ten types of plant, including four woody plants (fallen leaves of C. camphora, G. biloba, F. platanifolia and S. babylonica), three herbaceous plants (E. humifusa, E. annuus and S. canadensis), and three aquatic plants
(A. philoxeroides, T. dealbata and E. crassipes) were selected as the experimental materials, and all the materials were collected from the Shanghai Ocean University (Shanghai, China) during 2018. Extracts were prepared according to Shao et al. (2010) with small modifications. The plant materials were washed with tap water, oven-dried at 60 °C for 48 h and then made into powders (approximately 50 mesh). Then 15 g of each powder was placed in 500 mL distilled water and sonicated for 2 h. The extract was passed through 0.22 μm filter units and diluted with distilled water to 2 L. The final extract concentration was 7.5 g L\(^{-1}\) and was stored at 4 °C.

**Effect of plant extracts on growth and photosynthetic fluorescence characteristics of M. flos-aquae**

Culturing experiments were conducted in 250 mL flasks containing plant extracts and culture medium. The plant extract was diluted with culture medium to obtain a final concentration of 3 g L\(^{-1}\) and all cultures were cultivated under the aforementioned conditions. All experiments were carried out in triplicate. Samples were collected and the cell density and photosynthetic fluorescence parameters were determined at 24, 48, 72 and 96 h, respectively. Cell density was counted with a phytoplankton counter frame (CC-F; Beijing Purity Instrument Co. Ltd, Beijing, China) under an optical microscope (Nikon, Y-TV55; Nikon Tokyo, Japan) and the photosynthetic fluorescence characteristics of M. flos-aquae were measured by pulse amplitude-modulated (PAM) fluorescence monitoring system (Phyto-PAM; Walz, Effeltrich, Germany). The method used follows that described by Schreiber (1998) with minor modifications. A 3 mL sample was analyzed after 5 min dark adaption, the light measurement frequency was 2 and the maximum quantum yield \(F_{i}/F_{m}\) was calculated. The photosynthetically active radiation (PAR) of actinic light was adjusted to 80 mmol photons m\(^{-2}\) s\(^{-1}\) and after 180 s, the YII was calculated. The \(\alpha\) and rETR versus light curves were determined under 15 PAR levels (every measurement lasted for 20 s). Data acquisition and analysis were performed using PhytoWin v2.13.

**Ecological safety experiment**

*Brachydanio rerio* and *Vallisneria spiralis* were selected as non-target test organisms and exposed to *E. humifusa* extract. Fifteen *B. rerio* (the average weight of the fish was 0.18 ± 0.02 g) and three *V. spiralis* (the average fresh weight of the plant was 6.00 ± 0.50 g) were placed into each aquarium (capacity of 25 L) with different concentrations (0, 3, 6 and 12 g L\(^{-1}\)) of the extract. The experiments were conducted in triplicate. The number of dead *B. rerio* was recorded every 24 h and the photosynthetic fluorescence characteristics of *V. spiralis* was measured after 96 h using a double-modulation fluorescence monitoring system (Dual-PAM-100, Walz, Effeltrich, Germany) based on Roháček (2010) with minor modifications. *V. spiralis* were adapted to the dark for 5 min, and quantum yields of PSII were obtained by the steady state of slow kinetics mode, under the conditions of fluorescence and P700 (Zhao et al. 2015). The actinic light was adjusted to 300 mmol m\(^{-2}\) s\(^{-1}\).

**Data analysis**

The inhibitory rate (IR) was calculated using the following equation: IR (%) = \(1 - (N/N_0)\) × 100%, where \(N_0\) and \(N\) are the numbers of cell or photosynthetic parameters in the treatment and control cultures, respectively (Hua et al. 2018; Wang et al. 2018). The data (mean ± SD) had a normal distribution as determined by a Shapiro-Wilk test, and the independent t-test was applied to test the difference between control and treatment groups. Differences were considered significant at \(P < 0.05\), and highly significant at \(P < 0.01\). All analyses were conducted using Origin 8.0 (OriginLab, USA) and SPSS 24 (IBM SPSS Software, Chicago, IL, USA).

**RESULTS**

**Effect on the growth of M. flos-aquae**

The growth inhibition rate of each group at 24 h, 48, 72 and 96 h are shown in Table 1. Compared with the control group, all experimental groups exhibited highly significant antialgal activity at 48 h (\(P < 0.01\)). In terms of four woody plants, *S. babylonica* had the highest inhibition rate with an IR value of 73.18% at 96 h. Of the three aquatic plants, the inhibition rate of *E. annuus* at 24 h is insignificant. *E. humifusa* and *S. canadensis* exhibited better inhibition rates than *E. annuus* at 96 h. The three aquatic plants (A. philoxeroides, T. dealbata and E. crassipes) showed similar inhibition rates after 48 h. The growth inhibition rate of *A. philoxeroides* and *T. dealbata* increased to 77.30% and 58.88% at 96 h, respectively. However, the growth inhibition rate of *E. crassipes* decreased to 28.75% and 47.64% at 72 and 96 h. Of the plant extracts tested,
The YII values of tests decreased after exposure to four plant extracts (Figure 2). The extracts, both the efficiency (α) and capacity (rETR$_{max}$) of photosynthesis were significantly decreased compared to the control (P < 0.05). The highest reduction was observed under E. humifusa extract and the α and rETR$_{max}$ inhibition rates of M. flos-aquae were 97.00% and 95.81%, respectively, at 96 h (Figures 3 and 4).

Effect on the photosynthetic parameters of M. flos-aquae

The inhibitory effects of S. babylonica, E. humifusa, S. canadensis and A. philoxeroides extracts were found to have higher antialgal activity at 5 g L$^{-1}$ at 96 h, with the IR values over 70.00%.

Ecological safety of E. humifusa

The effects of E. humifusa extracts on non-target aquatic organisms (B. rerio and V. spiralis) at 96 h are presented in Table 2. During the 96-h experiment, insignificant differences were observed in the photosynthetic efficiency of V. spiralis compared to the control at all E. humifusa extract concentrations (P > 0.05). F$_{v}$/F$_{m}$ in all groups was above 0.7 and YII was above 0.2. Additionally, the mortality of B. rerio was zero in all groups after 96 h exposure.

DISCUSSION

The inhibitory activities of ten plant extracts on M. flos-aquae

A number of studies have suggested that allelochemicals may be implicated in the inhibitory effects of plant extracts on algae (Hou et al. 2019). The results of the present research confirmed that all ten plant extracts had a significant inhibitory effect on M. flos-aquae at 96 h (P < 0.01), indicating that the allelochemicals that can effectively

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**Table 1** The growth inhibition rate of different plant extracts on M. flos-aquae

<table>
<thead>
<tr>
<th>Plants</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. camphora</td>
<td>27.42 ± 0.05**</td>
<td>50.00 ± 0.13**</td>
<td>59.95 ± 0.38**</td>
<td>67.73 ± 0.23**</td>
</tr>
<tr>
<td>G. biloba</td>
<td>24.19 ± 0.08**</td>
<td>48.18 ± 0.23**</td>
<td>60.92 ± 0.10**</td>
<td>69.09 ± 0.19**</td>
</tr>
<tr>
<td>F. platanifolia</td>
<td>29.03 ± 0.16**</td>
<td>49.39 ± 0.21**</td>
<td>62.86 ± 0.06**</td>
<td>67.73 ± 0.03**</td>
</tr>
<tr>
<td>S. babylonica</td>
<td>46.77 ± 0.16**</td>
<td>61.21 ± 0.10**</td>
<td>70.15 ± 0.03**</td>
<td>73.18 ± 0.12**</td>
</tr>
<tr>
<td>E. humifusa</td>
<td>21.86 ± 0.13**</td>
<td>45.96 ± 0.24**</td>
<td>61.66 ± 0.18**</td>
<td>76.85 ± 0.15**</td>
</tr>
<tr>
<td>E. annuus</td>
<td>8.74 ± 0.19</td>
<td>56.17 ± 0.23**</td>
<td>57.51 ± 0.31**</td>
<td>68.09 ± 0.26**</td>
</tr>
<tr>
<td>S. canadensis</td>
<td>14.75 ± 0.20**</td>
<td>57.45 ± 0.09**</td>
<td>71.25 ± 0.13**</td>
<td>72.58 ± 0.05**</td>
</tr>
<tr>
<td>A. philoxeroides</td>
<td>18.03 ± 0.09**</td>
<td>51.91 ± 0.19**</td>
<td>67.73 ± 0.06**</td>
<td>77.30 ± 0.15**</td>
</tr>
<tr>
<td>T. dealbata</td>
<td>27.32 ± 0.10**</td>
<td>51.91 ± 0.08**</td>
<td>58.47 ± 0.18**</td>
<td>58.88 ± 0.30**</td>
</tr>
<tr>
<td>E. crassipes</td>
<td>44.26 ± 0.13**</td>
<td>51.91 ± 0.08**</td>
<td>28.75 ± 0.1**</td>
<td>47.64 ± 0.16**</td>
</tr>
</tbody>
</table>

Data are means of three independent experiments ± standard deviation (SD). At 24 h, no significant difference was found between E. annuus and the control.

*Indicates a significant difference (P < 0.05) when compared with the control group.

**Indicates a highly significant difference (P < 0.01) when compared with the control group.
inhibit the growth of algae are common in woody plants, herbaceous plants and aquatic plants. Therefore, when an emergency cyanobacteria bloom occurs, local plants rather than designated plants should be selected as algacides. Comparing the inhibitory activities of the ten plant extracts, it was observed that extracts from *Salix babylonica*, *Euphorbia Humifusa*, *Solidago canadensis* and *Alternanthera philoxeroides* showed the best algicidal activities. This could be due to the presence of different allelochemical compounds, which vary in water solubility. Of the four plant species that produced inhibition rates over 70.00%, polyphenols and flavones are likely to be the main phytotoxic substances (Luyen et al. 2014; Baležentienė 2015; Salem et al. 2011). Several researchers have suggested that phenols play an important role in
algal inhibition (Chen et al. 2012; Huang et al. 2015). For example, Wang et al. (2016) reported that polyphenols inhibit photosynthesis by blocking electron flow in the photosystem. The flavonoid–cyanobacteria interaction likely involves the interruption of electron transport in the PSII reaction center by disrupting the function of the secondary electron acceptor complex and reducing the effective quantum yield (Huang et al. 2015). Guo et al. (2015) also confirmed that M. flos-aquae are more sensitive to polyphenols. Zhu et al. (2010) demonstrated that PSII is an important target site for the allelopathic inhibition of polyphenols in Microcystis aeruginosa. Further isolation
and identification are needed to verify the different active antialgal components among different plants.

A rapid and useful indicator-chlorophyll fluorescence

Photosynthesis is an important process involving energy flow and mass transfer in algae (Xu et al. 2017; Wu et al. 2018). An efficient way to control increases in algal abundance is to influence the efficiency of photosynthesis (Xu et al. 2017). Chlorophyll fluorescence measurement has therefore become a useful method to study the effect of plant extracts on algal photosynthesis (White & Critchley 1999). PSII plays a key role in energy capture and transformation in cyanobacteria, and is one of the important targets of allelochemicals (Körner & Nicklisch 2002). In the present study, PAM was used to assess the effect of plant extracts on algal photosynthesis. A rapid reduction in $F_v/F_m$ and YII was found after the addition of four plant extracts, which indicates the immediate inhibition of the trapping capacity in the photosynthetic system. According to the results, fluorescence parameters are more sensitive than morphological or density-based parameters. For example, the IR of $F_v/F_m$ and YII values were higher than that of growth after 48 h exposure to $E. \text{humifusa}$ extract, with values of 90.00% (Figure 1) and 93.94% (Figure 2) in fluorescence parameters and 45.96% in growth (Table 1). In addition, when compared with traditional indices (such as cell density and chlorophyll concentration), chlorophyll fluorescence can evaluate the physiological condition of PSII rapidly in a non-invasive way, while measurements of cell density and chlorophyll are extremely time consuming (Kalaji et al. 2016).

### Efficiency and safety of $E. \text{humifusa}$

A good algaecide for control of cyanobacteria blooms should be effective and rapid. This research has shown that $E. \text{humifusa}$ extract can inhibit algae growth quickly. After exposure to $E. \text{humifusa}$ extract, the $F_v/F_m$ and YII inhibition rate of $M. \text{flos-aquae}$ were 90.00% and 93.94%, respectively, after 48 h and rose to 96.67% and 97.50%, respectively, after 96 h (Figures 1 and 2). These results suggested that $E. \text{humifusa}$ extract had an irreversible effect on photosystem function. Thus, it may be suitable for controlling cyanobacteria blooms in emergency situations.

It is important to consider the environmental risks posed by introducing plant extracts into waterbodies. Several studies have shown that allelochemicals from plants do not pose a threat to ecosystems (Shao et al. 2018; Wu et al. 2018). The results of the present study also demonstrated that $E. \text{humifusa}$ extract had no notable toxic effects on non-target organisms. Furthermore, considering that the extract is biodegradable and does not persist at high levels in water bodies, it is likely that $E. \text{humifusa}$ extract has limited adverse effects on the aquatic ecosystem. The extract of $E. \text{humifusa}$ could therefore be used to control $M. \text{flos-aquae}$ blooms in natural waterbodies.

### CONCLUSION

This study showed that ten plant extracts all showed significant inhibitory effects on $M. \text{flos-aquae}$ after 96 h ($P < 0.01$). The results of this study indicated that the aqueous extract of $E. \text{humifusa}$ may be a preferred algaecide due to its high efficiency and ecological safety. In addition, the results proved that the photosynthetic system analysis was a better understanding of the mechanisms of allelochemicals. Compared with traditional indices (such as algal density and chlorophyll-a concentration), using chlorophyll fluorescence to evaluate the physiological condition of PSII was convenient and time efficient. Although many allelochemicals have inhibitory effects on cyanobacteria, few of their inhibitory mechanisms have been elucidated systematically. Other factors, such as gene expression and cell ultrastructural examination should be investigated to further clarify the inhibitory mechanisms of plant extracts on cyanobacteria.

### ACKNOWLEDGEMENTS

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**Table 2** | Effect of $E. \text{humifusa}$ aqueous extract on mortality rate of $B. \text{rerio}$ and the photosynthetic II of $V. \text{spiralis}$ at 96 h of exposure

<table>
<thead>
<tr>
<th>Different concentration (g L$^{-1}$)</th>
<th>Mortality rate of $B. \text{rerio}$</th>
<th>$F_v/F_m$ of $V. \text{spiralis}$</th>
<th>YII of $V. \text{spiralis}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.714</td>
<td>0.226</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.752</td>
<td>0.226</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0.698</td>
<td>0.261</td>
</tr>
<tr>
<td>12</td>
<td>0</td>
<td>0.644</td>
<td>0.331</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.749</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.728</td>
<td>0.251</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.676</td>
<td>0.334</td>
</tr>
</tbody>
</table>
and the Major Projects of Water Pollution Control and Management of China (2017ZX07205003).

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