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RESEARCH ARTICLE

Effects of *Ginkgo biloba* extract on growth, photosynthesis, and photosynthesis-related gene expression in *Microcystis flos-aquae*

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Abstract

The inhibitory effect of plants on algae offers a new and promising alternative method for controlling harmful algal blooms. Previous studies showed that anti-algal effects might be obvious from extracts of fallen leaves from terrestrial plants, which had great potential for cyanobacterial control in field tests. To investigate the anti-algal activities and main algicidal mechanisms of *Ginkgo biloba* fallen leaves extracts (GBE) on *Microcystis flos-aquae*, the cell density, photosynthetic fluorescence, and gene expression under different concentrations of GBE treatments were tested. GBE (3.00 g L^{-1}) showed a strong inhibitory effect against *M. flos-aquae* with an IC_{50} (96h) of 0.79 g L^{-1}. All the inhibition rates of maximal quantum yield (F_v/F_m), effective quantum yield (F_q'/F_m'), and maximal relative electron transfer rate ($rETR_{max}$) were more than 70% at 96 h at 3.00 g L^{-1} and more than 90% at 6.00 g L^{-1}. Further results of gene expression of the core proteins of PSII (*psbD*), limiting enzyme in carbon assimilation (*rbcL*), and phycobilisome degradation protein (*nblA*) were downregulated after exposure. These findings emphasized that photosynthetic damage is one of the main toxic mechanisms of GBE on *M. flos-aquae*. When exposed to 12.00 g L^{-1} GBE, no significant influence on the death rate of zebrafish or photosynthetic activity of the three submerged plants was found. Therefore, appropriate use of GBE could control the expansion of *M. flos-aquae* colonies without potential risks to the ecological safety of aquatic environments, which means that GBE could actually be used to regulate cyanobacterial blooms in natural waters.

Keywords *Microcystis flos-aquae* · *Ginkgo biloba* extract · Algal bloom control · Inhibition mechanisms

Introduction

Eutrophication, one of the key triggers for cyanobacterial bloom occurrences, has been identified as a major water quality management issue worldwide (Lürling et al. 2016). Cyanobacteria blooms are recognized as the greatest threat to water quality by representing the odor and malodorous metabolites associated with bloom-forming cyanobacteria (Brooks et al. 2016; Bukowska et al. 2017). Therefore, controlling cyanobacterial blooms is a crucial step for the safety of water supplies, other water users, and aquatic organisms.

Among the diverse physical (Rajasekhar et al. 2012; Visser et al. 2016), chemical (Crafton et al. 2018; Sinha et al. 2018), and biological methods (Li et al. 2016; Hua et al. 2018) explored for cyanobacterial bloom management, natural plant agents have been proven to be effective, degraded in nature, and environmentally friendly (Yakefu et al. 2018; Patino et al. 2018). Numerous plants have been reported to exhibit algal inhibition effects (Yi et al. 2012; Tazart et al. 2018; Qian et al. 2019; Xu et al. 2020). Our previous study also demonstrated that biologically derived substances that can significantly inhibit the growth of algae are common in different kinds of plants, including woody plants, herbaceous plants, and aquatic plants (Shi et al. 2020).

It is necessary to understand the inhibition mechanisms by obtaining more experimental evidence for better

Highlights

- *Ginkgo biloba* extracts was found to be an efficient botanical algaecide.
- The expression of some key photosynthesis related genes was influenced by GBE
- Photosynthetic damage is one of the main toxic mechanisms of GBE on *M. flos-aquae.*
- GBE (< 12.00 g L^{-1}) had no significant potential risk to aquatic ecological safety.

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development of this novel method. To date, many researchers have observed that biologically derived substances can interfere with *Chl*-a synthesis in algae by blocking the photosystem II (PSII) electron transport chain (Huang et al. 2015; Zhao et al. 2019; Xu et al. 2020). For example, pyrogallic acid which can be extracted from various plants, such as *Myriophyllum spicatum* (Zhang et al. 2010), can damage the oxidative and photosynthetic systems of cyanobacteria (Wu et al. 2013). Measuring gene expression is also been proved as one of the useful tools to investigate the response of algae to some stress (Shao et al. 2013; Lu et al. 2014; Wu et al. 2018). However, to the best of our knowledge, no research has provided a comprehensive evaluation of the potential mechanism in photosynthesis and gene expression of *Ginkgo biloba* extract (GBE) on *Microcystis flos-aquae*.

Ginkgo biloba is one of the oldest living tree species, and extracts from *G. biloba* leaves have been widely used as herbal supplements (Zhou et al. 2004). For example, studies have shown growth-inhibitory activity on the weed species ryegrass (Kato-Noguchi and Takeshita 2013) and seeding (Song et al. 2002) through adding *G. biloba* extracts. Zheng et al. (2015) reported that the major bioactivities of *G. biloba* leaves should be flavonoids, which can interrupt electron transport in the PSII reaction center and decrease the effective quantum yield, resulting in impairment of photosynthesis (Huang et al. 2015). These observations suggest that biologically derived substances in *G. biloba* could serve as the basis for the development of relatively effective tools for algal bloom control. However, to the best of our knowledge, the effects of *G. biloba* fallen leaves extract on cyanobacteria have not yet been elucidated.

Based on the above description, we proposed several hypotheses: (1) GBE can effectively inhibit the growth of *M. flos-aquae*; (2) the potential inhibition mechanism is that GBE affects photosynthetic system, causing *M. flos-aquae* damage and death; and (3) the biologically derived substances of GBE may be eco-friendly. To test these hypotheses, growth was monitored when *M. flos-aquae* coexisted with different doses of GBE. In addition, photosynthetic fluorescence parameters and gene expression profiles (photosynthesis-related genes of *psbD*, *rbcL*, and *nblA*) were derived to analyze the damage mechanisms of the photosystem. The toxicity of GBE to other species in the aquatic ecosystem was identified by a toxicity test involving zebrafish and several submerged plants as the target organisms. This work not only provided some insights into employing GBE to suppress the growth and photosynthetic system of *M. flos-aquae*, but also supported that using GBE to control toxic cyanobacterial blooms may be a promising approach in the field. Besides, in our opinion, using *G. biloba* fallen leaves extract as an algaecide also provide an alternative and promising way to reuse plant litter.

Materials and methods

Algal culture and preparation of GBE

The cyanobacteria species *M. flos-aquae* were obtained from the Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). The algae were cultured at (25 ± 1) °C and a photoperiod of 12 L:12 D with 60 μmol photons m^{-2} s^{-1} in the laboratory. Culture flasks were gently swirled twice daily. Stock cultures in the exponential growth phase were used in the following experiments.

The fallen leaves of *G. biloba* were collected from Shanghai Ocean University in November 2019. Leaves were processed for extracts according to Shi et al. (2020) with minor modifications. Briefly, the leaves were rinsed with tap water, oven-dried at 60 °C for 48 h, and then ground into powder (approximately 50 mesh). Thirty grams dried powder and DI water were mixed at a mass/volume ration of 1/20. Then, the mixture was extracted by ultrasonication for 4 h. The solution was passed through 0.45 μm filter membrane and diluted with distilled water till 2 L to obtain a final extract concentration of 15 g L^{-1}. The extracts were stored in the dark at 4 °C before experimentation.

Assessment of the antialgal activities of GBE

Experimental cultures were conducted in 250 mL glass flasks. *M. flos-aquae* (1.63×10^6 cells mL^{-1}) cultures were treated with additions of the extract at a concentration gradient of 0.00, 0.75, 1.50, 3.00, 6.00, and 12.00 g L^{-1}. All experiments were carried out in triplicate and conducted for 96 h. The experimental conditions were the same as mentioned in the "Algal culture and preparation of GBE" section. A volume of 0.10 mL samples of *M. flos-aquae* were taken every 24 h from the flasks and counted immediately in a phytoplankton counter frame (CC-F, Beijing Purity Instrument Co., Ltd., China) with an optical microscope (Nikon, Y-TV55, Japan).

Photosynthetic fluorescence parameters of *M. flos-aquae* were measured every 24 h by a pulse amplitude-modulated (PAM) fluorescence monitoring system (Phyto-PAM, Walz, Effeltrich, Germany) under dark adaptation for 5 min (Lin et al. 2015; Zhao et al. 2015). The details of the method refer to Schreiber (1998) with minor modifications, and data analysis was performed in PhytoWin v2.13.

RNA extraction, reverse transcription, and real-time PCR analysis

The culture of *M. flos-aquae* in an amount of 150 mL was centrifuged at 8000 rpm for 5 min at 4 °C to collect algal

cells and stored at −80 °C for total RNA isolation. Total RNA was extracted using a Cell/Bacteria RNA Extraction Kit (Tiange, China), treated with RNase-free DNase (Tiange, China), and then reverse transcribed into first-strand cDNA using a FastKing RT Kit (with gDNase) (Tiange, China) according to the manufacturer's directions. Quantitative real-time PCR (RT-qPCR) was applied to determine the transcriptional level of three genes (*psbD*, *rbcL*, *nblA*) in *M. flos-aquae* treated by the extraction of *G. biloba* for 24 h, 48 h, and 96 h. The samples were performed in an FTC-3000 real-time PCR system (Funglyn Biotech, Canada) with a SYBR Green RT-PCR Kit (Tiangen, China) in a final 20 μL volume. The 16S rRNA gene of *M. flos-aquae* served as the reference gene, and primers used for RT-qPCR were designed (Table 1). The amplification reaction was performed under the following conditions: after heating at 95 °C for 2 min, amplification was programmed for 40 cycles of 15 s at 95 °C, 30 s at 55 °C, and 15 s at 72 °C. Gene expression data were evaluated using the Ct value, and the reference gene was used to normalize the expression levels of target genes (Shao et al. 2009). The experiment was carried out in triplicate, and the average was reported. The relative gene transcription was calculated using the $2^{-\Delta\Delta Ct}$ method (Wu et al. 2018), where ΔΔCt was calculated using the following equation.

$$\Delta\Delta Ct = \left(Ct_{\text{target gene}} - Ct_{16Srm}\right)_{\text{stress}} - \left(Ct_{\text{target gene}} - Ct_{16Srm}\right)_{\text{control}}$$

Ecological safety experiment

Zebrafish (*Danio rerio*) and submerged plants (*Vallisneria natans*, *Elodea nuttallii*, and *Myriophyllum verticillatum*) were selected as the nontarget test organisms and exposed to different concentrations of GBE (0.00, 3.00, 6.00, and 12.00 g L^{-1}). Fifteen zebrafish (the average weight of the fish was 0.18 ± 0.02 g) and three selected submerged plants (the average fresh weight of the plant was 6.00 ± 0.50 g) were placed into each aquarium (capacity of 25 L). Triplicate treatments were cultivated under the same conditions. The total number of dead zebrafish was recorded every 24 h for the mortality rate, and the effect of GBE on submerged plants was measured by analyzing photosynthetic fluorescence parameters at 96 h. The method refers to Roháček (2010) with minor modifications. Three selected plants were adapted to the dark for 5 min, and the quantum yields of PSII were obtained by the steady state of slow kinetics mode under the condition of fluorescence and P700 mode (Zhao et al. 2015) in which the actinic light was 300 μmol m^{-2} s^{-1}.

Data statistic and analysis

When algal growth was inhibited, the IC_{50} at the 95% confidence interval with an upper confidence limit and a lower confidence limit was calculated using probit analysis. The data are presented as the mean ± SD of triplicates, which were normally distributed according to the Shapiro-Wilk test, and homogeneity of variances was tested by Levene's test. One-way ANOVA followed by least significant difference (LSD) was applied to test the difference between the control and treatment groups at a significance level of $P < 0.05$. Statistical analysis was performed using SPSS 24 (IBM SPSS Software, Chicago, USA), and figures were generated using Origin 8.0 (Origin Lab, USA). The inhibitory effect was estimated by the inhibition rate, which is defined by the following equation: IR (%) =[1-(N_0/N)] ×100%, where N_0 and N are the cell numbers in the treatment and control cultures, respectively.

Results

Effect of GBE on growth of M. flos-aquae

When no extracts were added into the system, *M. flos-aquae* density increased from around 1.63 × 10^6 cells mL^{-1} to 4.08 × 10^6 cells mL^{-1}, indicating a rapid growth and reproduction (Fig. 1). However, as can be seen from Fig. 1, the growth and reproduction of *M. flos-aquae* steadily decreased in the systems when GBE were added. At GBE concentration level greater than 0.75 g L^{-1}, a rapid decline in cell density was found. And the inhibitory increased along with increasing GBE dosages. For example, the inhibition rates of 3.00 g L^{-1} and 6.00 g L^{-1}

Table 1 Primers designed for real-time PCR

Gene name	Forward primer (5′-3′)	Reverse primer (5′-3′)
16S RNA[a]	GGACGGGTGAGTAACGCGTA	CCCATTGCGGAAAATTCCCC
psbD[b]	TCTTCGGCATCGCTTTCTC	CACCCACAGCACTCATCCA
rbcL[b]	CGTTTCCCCGTCGCTTT	CCGAGTTTGGGTTTGATGGT
nblA[c]	AAATGAGTCAGGAGCAAGCG	CTAGACCCCACTGGTGTTTGAG

[a]Primer sequence obtained from Shao et al. (2010)

[b]Primers for psbD and rbcL were obtained from Zhang et al. (2014)

[c]Primer sequence obtained from Lu et al. (2014)

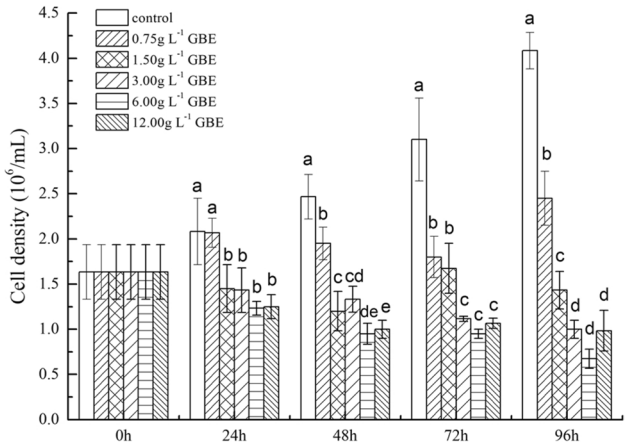

Fig. 1 Inhibitory effect of GBE on the growth of *M. flos-aquae*. The means ± SD are based on triplicates, and columns associated with difference letters indicate the difference was significant at the *P*=0.05 level

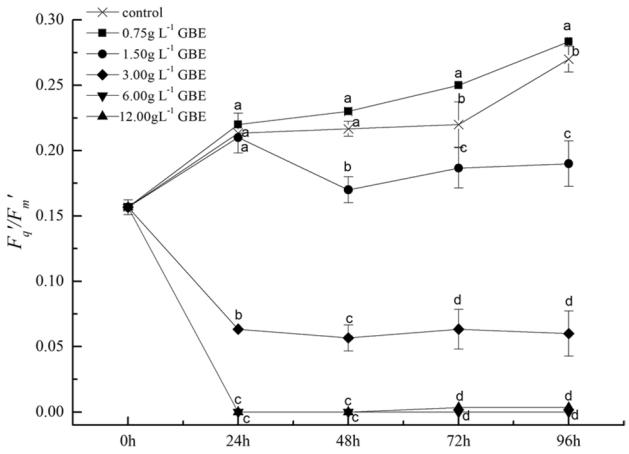

Fig. 3 Inhibitory effect of *G. biloba* extract on the F_q'/F_m' of *M. flos-aquae*. The means ± SD are based on triplicates, and columns associated with difference letters indicate the difference was significant at the *P*=0.05 level

reached 75.51% and 83.47% at 96 h, respectively. Based on the collected experiment data, the $IC_{50,96h}$ value of GBE was found to be approximately 0.79 g L^{-1}. These data suggested that GBE have a strong inhibitory effect on *M. flos-aquae*.

Effect of GBE on photosynthetic activity of M. flos-aquae

It has been reported that PAM fluorometry can effectively measure PSII activity in algae for better identification of physiological mechanisms (Wang et al., 2016b). Phyto-PAM was used to determine the F_v/F_m, F_q'/F_m', and $rETR_{max}$ of photosynthesis. After 24 h of exposure, no marked difference

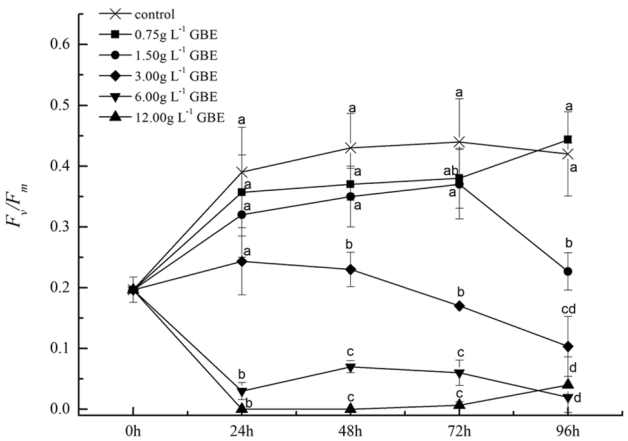

Fig. 2 Inhibitory effect of *G. biloba* extract on F_v/F_m of *M. flos-aquae*. The means ± SD are based on triplicates, and columns associated with difference letters indicate the difference was significant at the *P*=0.05 level

in F_v/F_m was observed between control and treatments with GBE concentration lower than or equal to 3.00 g L^{-1} (Fig. 2) ($P > 0.05$). However, for 96 h data, a significant decline in F_v/F_m was observed for the treatments of 1.50 g L^{-1} and 3.00 g L^{-1}.This result can be attributed to an initial delay in the GBE effects on the photosynthetic system of *M. flos-aquae*. Effects of GBE on the parameter of F_q'/F_m' in *M. flos-aquae* were investigated and depicted in Fig. 3. After 48 h of exposure, a significant reduction in F_q'/F_m' was denoted at systems with GBE concentration greater than 0.75 g L^{-1} ($P < 0.05$). The inhibition rate of 1.50 g L^{-1} and 3.00 g L^{-1}reached 21.54% and 75.36%, at 48 h, respectively. The value of F_q'/F_m' also reached almost zero at 6.00 g L^{-1} and 12.00 g L^{-1} and maintained till the end of experiment. Similar to the results of F_q'/F_m', $rETR_{max}$ values significantly reduced at systems with GBE concentration greater than 0.75 g L^{-1} after 48 h exposing ($P < 0.05$). Furthermore, only after 24-h exposure, the value of F_q'/F_m' decreased to nearly zero in systems with GBE at 6.00 g L^{-1} and 12.00 g L^{-1}. However, compared with the control, GBE concentration at 0.75 g L^{-1} had no significant effect on $rETR_{max}$ during the investigation (Fig. 4). The trend in change of F_v/F_m, F_q'/F_m', and $rETR_{max}$ appears to be consistent with the results of cell density. These results suggested that GBE caused substantial damage to photosynthesis in *M. flos-aquae*, thereby inhibiting cellular growth.

Effect of GBE on gene expression

This study investigated the effects of GBE on several photosynthesis genes that may be responsive to stress. From Fig. 5 a and b, expression of *psbD* and *rbcL* was found to be downregulated significantly with the treatment of GBE ($P < 0.05$). The relative expression levels of *psbD* were 15.47%,

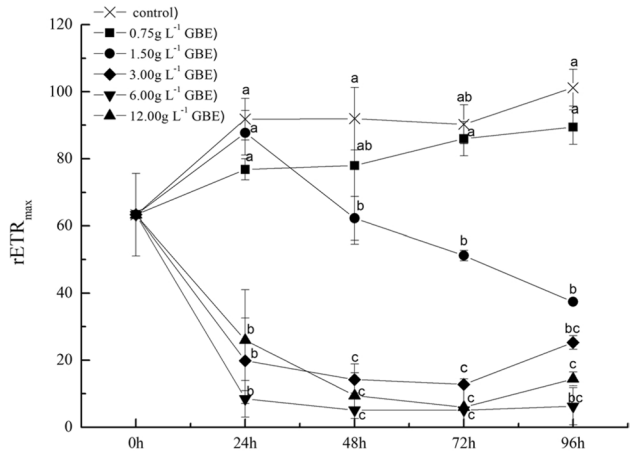

Fig. 4 Inhibitory effect of *G. biloba* extract on $rETR_{max}$ of *M. flos-aquae*. The means ± SD are based on triplicates, and columns associated with difference letters indicate the difference was significant at the P=0.05 level

25.03%, and 17.27% of the control at 24 h, 48 h, and 96 h, respectively. And this result was similar to the change of *rbcL* expression, which decreased by 87.64%, 90.07%, and 76.26% at 24 h, 48 h, and 96 h, respectively. For *nblA* expression, no significant change was observed at shorter exposure (24 h) but decreased by 21.12% and 38.18% after 48 h and 96 h exposure (Fig. 5c). The downregulation of photosynthesis genes is a marker of the photoinhibition. The result further proved that GBE reduced the efficiency of photosynthesis in *M. flos-aquae*.

Effect of GBE on zebrafish and submerged plants

Table 2 showed the effect of GBE on zebrafish and three submerged plants after 96-h exposure. The F_v/F_m values of *V. natans*, *E. nuttallii*, and *M. verticillatum* were between 0.60 and 0.75 in control and treated systems. No insignificant differences were found between the treated groups and the control ($P > 0.05$). In addition, there were no deaths of zebrafish in any group after 96 h of exposure. These results indicated that the effective algal inhibitory concentration of GBE (3.00–12.00 g L^{-1}) exerted no biotoxicity to zebrafish and submerged plants. Thus, employing GBE as a strategy in controlling algae bloom in aquatic environments would virtually have no adverse effect on other aquatic organisms in the ecosystem.

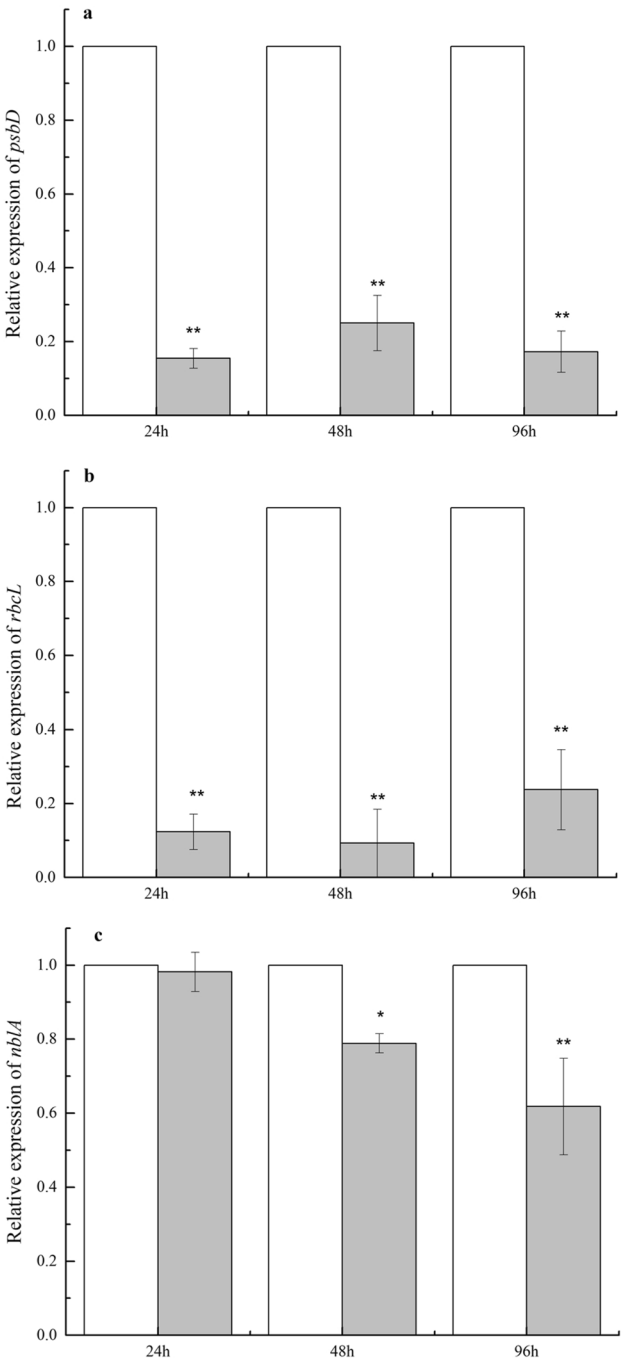

Fig. 5 The normalized expression of *psbD* (**a**), *rbcL* (**b**), and *nblA* (**c**) in *M. flos-aquae* under *G. biloba* extract at 3 g L^{-1}. Open bars represent control treatments, and gray bars represent treatments. Asterisks (*) and double asterisks (**) indicate significant ($P < 0.05$) and extremely significant ($P < 0.01$) differences between groups, respectively

Discussion

For the past few years, many researches have focused on the algicidal activity of terrestrial plants, including eucalyptus (Zhao et al. 2019), *Spartina alterniflora* (Xu et al. 2020) and *Ailanthus altissima* (Meng et al. 2015). For example, the cell multiplication of *Microcystis* was reported to be markedly inhibited by the extract of *Cinnamomum camphora* fallen

Table 2 Effect of *G. biloba* extract on the mortality rate of zebrafish and the photosynthetic fluorescence characteristics of submerged plants at 96 h

Different concentration (g L^{-1})	Mortality rate of zebrafish (%)	F_v/F_m		
		V. natans	*E. nuttallii*	*M. verticillatum*
0	0	0.74 ± 0.06	0.66 ± 0.10	0.77 ± 0.03
3	0	0.67 ± 0.01	0.71 ± 0.01	0.78 ± 0.02
6	0	0.61 ± 0.08	0.71 ± 0.21	0.71 ± 0.07
12	0	0.69 ± 0.26	0.66 ± 0.04	0.69 ± 0.28

leaves and fresh leaves, with inhibition rates of 43% and 67% after 24 h with 15 g L^{-1}, respectively (Chen et al. 2018; Yakefu et al. 2018). In our studies, the results confirmed that the number of cells of *M. flos-aquae* was efficiently inhibited by GBE, with a maximum inhibition rate higher than 83% (6.00 g L^{-1}, 96 h). The present study also indicated that the inhibitory effect on *M. flos-aquae* occurred when exposed to GBE at lower concentrations (< 1 g L^{-1}). However, it is unreasonable to conclude that GBE had more inhibition efficiency to *M. flos-aquae* than others because of the different extract process and bioassay protocols. In addition, Zhang et al. (2014) demonstrated that ginkgolic acids, which are extracted from *G. biloba* exocarp, inhibited *M. aeruginosa* growth effectively with $IC_{50,3d}$ and $IC_{50,7d}$ of 3.26 and 2.03 mg L^{-1}, respectively, which is pretty lower than our result, which $IC_{50,96h}$ value was 0.79 g L^{-1}. The huge gap means that perhaps many biologically derived substances in crude plant extract are ineffective to *Microcystis*. Therefore, it is meaningful to further research finding effective components for the sake of improving inhibition efficiency.

Photosynthetic parameters, such as F_v/F_m, F_q'/F_m', and $rETR_{max}$, are often used as significant indexes to indicate the photoacclimation state of phytoplankton photosynthesis, which is associated with biomass and decreased earlier, meaning that phytoplankton photosynthesis changes more rapidly than cell density (Zhu et al. 2010). F_v/F_m usually is used to estimate the maximum quantum yield of PSII photochemistry (Baker 2008). When electron transfer between electron acceptors and donors in PSII was inhibited, F_v/F_m and the accumulation of carbohydrates decreased, which may slow algal cell growth (Leu et al., 2002 Chen et al. 2018). F_q'/F_m' is a measure of PSII photosynthetic efficiency when the cells are exposed to ambient light, and the value represents a balance between the amount of light energy being funneled into the PSII reaction centers and the flow of electrons away from PSII (Mackey et al. 2013). As one of the parameters of rapid light curves, $rETR_{max}$ is a measure of the capacity of the photosystems to utilize the absorbed light energy (Belshe et al. 2007). The depressions of F_q'/F_m' and $rETR_{max}$ demonstrated that electron transport was limited and that the efficiency of light capture and utilization declined (Kalaji et al. 2016, Terada et al. 2016). For example, some biologically derived substances such as pyrogallic acid and naphthoquinones interrupt the electron transport, resulting in the reduction in the effective quantum yield and photosynthesis damage (Wu et al. 2013; Hou et al. 2019). Some plant extracts, such as *Dracontomelon duperreanum* leaf litter extract (Wang et al. 2018) and eucalyptus extract (Zhao et al. 2019), were observed to affect the photosynthetic system and impair the PSII reaction center. In this study, the value of F_v/F_m, F_q'/F_m', and $rETR_{max}$ showed similar responses. With the increasing exposure time and doses of GBE, the photosynthetic parameters decreased roughly. Compared with temporary impairment at low concentrations, irreversible damage to photosynthesis was observed only if the dose was above a critical value (Wang et al., 2016a). Our results are generally consistent with these findings. As shown in Figs. 2–4, although the photosynthesis parameters of *M. flos-aquae* were significantly inhibited at low concentrations (1.50 g $L^{-1}$and 3.00 g L^{-1}), a full damage was observed at high concentrations (6.00 g L^{-1} and 12.00 g L^{-1}) with photosynthetic parameters down almost to zero, which indicated the irreversible dysfunction of the PSII reaction center of *M. flos-aquae* at high extract concentrations.

Analysis of targeting gene sites at the molecular level is also a useful tool for elucidating toxic mechanisms (Qian et al. 2010). Interfering with the intracellular electron transfer rate and reducing the expression of the core proteins were the pathways of plant polyphenols affecting photosynthetic activity (Shao et al. 2009). The results of the present research showed that under GBE exposure, the expression of *psbD*, *rbcL*, and *nblA* was all downregulated, which is in agreement with photosynthetic parameters results. *psbD* is a gene encoding the D2 core protein subunits of PSII, which can form the reaction center of PSII (Lu et al. 2014; Zhang et al. 2014). The downregulated *psbD* under GBE may indicate that there is not enough D2 core protein to transfer electrons and replace damaged proteins. This result is in accordance with the phenomenon of *M. aeruginosa* exposure to ampicillin, atrazine, and cadmium chloride by Qian et al. (2012), who assumed that electron transport-related proteins were not sufficient to transport electrons and led to a decrease in F_v/F_m, F_q'/F_m', and $rETR_{max}$. *rbcL* encodes the large subunit of Rubisco, a key protein involved in the carbon assimilation process whose downregulation would cause the accumulation of excess electrons and induce oxidative stress, meaning reactive oxygen species (ROS) production (Zhang et al. 2013). Similar phenomena were shown

when *M. aeruginosa* was exposed to neo-przewaquinone A, extracted from *Salvia miltiorrhiza* Bung (Zhang et al. 2013) and sodium chloride (Chen et al. 2015). Accordingly, the downregulation of *psbD* and *rbcL* expression demonstrated that GBE can block electron transport and affect PSII function and carbon assimilation, which are consistent with the results of photosynthetic parameters. The *nblA* gene encodes the phycobilisome degradation protein, whose upregulation means that phycobiliproteins degrade rapidly, leading to photoinhibition. The degradation of numerous phycobiliproteins results in a decline in the ability to capture and absorb light, thereby inhibiting photosynthetic activity (Lu et al. 2014). The relative copies of *nblA* were significantly different from the copies of the control group at 96 h, which attest to the decrease in the number of phycobilisome degradation protein, indicating that the ability of algae cells to capture and absorb light increased and the photosynthetic activity was restored to some degree. In addition, many studies have shown that some algaecides exhibit temporary and reversible effects on photosynthesis at low concentrations (Guo et al. 2015; Wang et al. 2018). In this study, the relative copies of *nblA* decreased gradually; however, photosynthetic parameters still showed a downward trend. One possible reason for this result is that the light repair rate is lower than the damage rate of D2 protein. At the same time, the translation elongation of the D2 protein could be inhibited by singlet oxygen molecules (Nishiyama et al. 2006), which may lead to oxidative damage. To the best of our knowledge, the equilibrium state of intracellular oxidation plays a necessary role in algae growth. The formation of ROS is enhanced by the leakage of electrons from the photosynthetic electron transport chain to oxygen (Zhou et al. 2014). Zhang et al. (2014) demonstrated that the inhibition of photosynthesis would cause an imbalance or even destruction in the antioxidant system by disrupting the electron transport. However, in this study, further research is needed to verify how the GBE caused oxidative damage in *M. flos-aquae* and the impacts between photosynthesis and ROS levels.

It is important to consider the environmental risks posed by introducing plant extracts into waterbodies. In this study, acute zebrafish and three submerged plants toxicity tests were carried out to ascertain the GBE toxicity, which gained better empirical insights on the validity of using GBE in aquatic ecosystem. As the results showed in Table 2, we did not note a remarkable toxicity of GBE toward nontarget organisms at concentration less than 12.00 g L^{-1}, indicating the safety of GBE in aquatic ecosystems. Several studies also have shown that extracts from plants do not pose a threat to ecosystems (Shao et al. 2018; Wu et al. 2018). Thus, employing GBE as a strategy in controlling algae would virtually have no adverse effect on other aquatic organisms in aquatic ecosystem. Taken together, the GBE could replace other chemical approaches providing an environmentally safe way to control algal blooms.

Conclusions

This work showed that GBE is a promising algaecide that can be used to control harmful algal blooms. The growth of *M. flos-aquae* was effectively inhibited after the addition of GBE. The results from photosynthetic fluorescence and the expression of photosynthesis genes support the opinion that photosynthesis is one of the potential mechanisms for the inhibitory effect of GBE. The reaction center of PSII and the electron transfer process are important targets for GBE damage. In addition, GBE has been confirmed to be safe and environmentally friendly in aquatic ecosystems. In further researches, more experiments are needed to analyze the biologically derived substances in GBE to improve the inhibition efficiency.

Author contribution All authors contributed to the study conception and design. Yuxin Shi, Liu Shao, and Peimin He planned and constructed the experimental setup. Material preparation, data collection, and analysis were performed by Yuxin Shi. The first draft of the manuscript was written by Yuxin Shi and Liu Shao. Anglu Shen and Peimin He reviewed and edited the manuscript together. All authors read and approved the final manuscript.

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Data availability The related data generated during this study are included in this published article and are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate Not applicable.

Consent for publication Not applicable.

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