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Table: 2, Figs : 6

Inhibitory effects of aqueous extracts of *Eucalyptus tereticornis* on HAB causing specie, *Prorocentrum donghaiense*

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ABSTRACT

We studied the cytotoxic effects of aqueous extracts from different parts (trunk, branches, leaves and roots) of *Eucalyptus tereticornis*, against the noxious alga *P. donghaiense*. The aqueous extracts of leaves significantly inhibited ($p< 0.05$) the growth of algae, whereas other plant parts showed little inhibition effects ($p> 0.05$) even at the highest concentration (0.50 $g \cdot L^{-1}$). The leaves aqueous extracts interrupted the electron-transfer chain between plastoquinone QA and QB and made PSII reaction centre of reversible inactivation and became an energy trap, absorbing light energy rather than promoting electron transfer. It also triggered the activity of microalgae antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)], and the content of Glutathione (GSH). We found that leaves aqueous extracts of *E. tereticornis* possess algal inhibiting ability, hence, it may be potential way to control the harmful algal blooms.

Key words: Algal bloom, algal inhibitor, allelopathy, antioxidants enzymes, aqueous extracts, catalase, *Eucalyptus tereticornis*, peroxidedase, *Prorocentrum donghaiense*, superoxide dismutase.

INTRODUCTION

Severe harmful algal blooms (HABs) adversely affect the fishery, water-supply and human health through the food chain (1,2). The methods of HABs control mainly focus on physical (3,4), chemical (5,6) and biological aspects (7-9). However, application of these methods is limited due to high cost and possible ecological secondary pollution (10). As an environment friendly method, allelochemicals from plants may provide a new way to mitigate HABs and have promising future (7,11). Many laboratory and field tests have shown that adequate allelopathy exists in water (marine and freshwater) and most of the primary producers of allelopathy in water (cyanobacteria, microalgae and terrestrial plants, large algae and macrophytes) generates and release variety of allelopathic compounds (9,12-15). Different from synthetic herbicide, the allelochemicals are the secondary metabolites, which degrade naturally and are ecologically safe, therefore research on allelopathic against HABs is promising. Previous researches have been done to understand the action mechanism of these allelochemicals and their performance in environment (16).

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There are 4-mechanisms of allelochemicals against algae: destroy cell structure, hinder algal photosynthesis, hinder respiration and trigger enzymatic activities (17-19).

The *E. tereticornis* has been widely introduced worldwide from Australia due to the rapid growth and to meet rising demand for paper and plywood (20). It is widely cultivated in coastal areas of South China. The phenolic acids and eucalyptus oil released from its leaves, bark and roots of certain *Eucalyptus* spp. are harmful to other plant species (21,22). However, few researchers have investigated the effects of eucalyptus on microalgae or which parts of eucalyptus have strongest inhibitory effects on microalgae, or what is the mechanism. The *Prorocentrum donghaiense* Lu is a kind of HAB microalgae of toxic variegated single-cell species. It belongs to the original dinoflagellates branch, prorocentrum genus, and distributed in China, Japan, and South Korea Sea domain–specific (23). At high concentration, it clogs fish, shellfish's respiratory organs, and results in suffocation. Furthermore, their toxic secretions are hazardous to marine lives. It receives wide attention in recent years, because the original red tide dinoflagellate species occurs frequently in Yangtze River estuary and East China Sea (1,24,25).

This study aimed to assess: (i) Effects of *E. tereticornis* on *P. donghaiense*, (ii) which part of *E. tereticornis* is most effective against *P. donghaiense*; (iii) effects of leaves aqueous extracts of *E. tereticornis* on PSII systems and antioxidant systems of algae.

MATERIALS AND METHODS

Algal materials: Culture of *P. donghaiense* was obtained from the Hydrobiology Research Centre, Jinan University, Guangzhou, China. The algae were incubated in f/2 media prepared with sterile-filtered artificial seawater. The culture was illuminated with cool, fluorescent light at light intensity of 80 $\mu mol \cdot s^{-1} \cdot m^{-2}$ at the surface of culture medium (measured by the LI-250 Light meter, LI-COR, Inc., US), with a 12:12 h light-dark cycle in an environmental chamber at 23 ± 1°C.

The aqueous extracts of *E. tereticornis*

The samples of *E. tereticornis* were obtained from Huanjiang Observation and Research Station, Chinese Academy of Sciences, Guangxi, China. The samples were taken from different plant parts (trunks, branches, leaves and roots) of tree at fruit-bearing stage in July 2013. From each sample 300 g material was taken, dried to constant weight in oven at 70°C and then grinded into dry powder. These powders were sealed and labeled, respectively. The stock solution of the mixed aqueous extracts were made by mixing same amount (75g) of dry powder of four different plant parts in 1000 mL deionized water and soaked for 48 h, then it was filtered through filter paper to remove impurities and bacteria through an autoclaved 0.22 μm membrane filter to remove bacteria. The stock solution was diluted into 25, 50, 100 and 150 $g \cdot L^{-1}$ mixed aqueous extracts and kept at 4 °C until further use in experiments (Table 1).

The stock extracts of different plant parts (trunks, branches, leaves and roots) were made in ratio of 1:10 (w/v) dry powder and deionized water, respectively. Then the stock aqueous extracts of were diluted into 4-concentrations (5, 10, 25 and 50 $g \cdot L^{-1}$) (Table 1). The preparation methods were the same as for the mixed aqueous extracts.

Table 1. The aqueous extracts stock solution concentrations of *E. tereticornis*

Plant aqueous extracts	Concentration ($g \cdot L^{-1}$)			
Mixed aqueous extracts	25	50	100	150
Trunks	5	10	25	50
Branches	5	10	25	50
Leaves	5	10	25	50
Roots	5	10	25	50

Algal assays

In clean and sterilized conical flasks (250 ml) containing 99 ml of sterile f/2 algal culture medium, various concentrations (25, 50, 100 and 150 $g \cdot L^{-1}$) of mixed aqueous extracts (1 ml) were added to test their dose effects. The mixed aqueous extracts concentrations were 0.25, 0.50, 1.0 and 1.5 $g \cdot L^{-1}$ in the fresh cultures. The initial cell densities of *P. donghaiense* were 3.55×10^5 $cells \cdot ml^{-1}$ and the cell number was measured daily.

For algal inhibition experiment the aqueous extract concentrations of various plant parts was 0.50 $g \cdot L^{-1}$. The algal medium volume was 100 ml and the experiment lasted for 120 h. The initial cell densities of *P. donghaiense* were 2.48×10^5 $cells \cdot ml^{-1}$. Sampling and counting were done every 24 h.

In final experiments, the leaves aqueous extracts concentrations were 0.05, 0.10, 0.25 and 0.50 $g \cdot L^{-1}$. Each treatment was replicated thrice. Sterilized seawater (1 ml) was added to algal liquid (99 ml) as control. The initial algae cell densities were 1.98×10^5 $cells \cdot ml^{-1}$. Sampling and counting were done every 24 h and the experiment lasted for 120 h. Samples were taken 1 cm below the water surface without movement, to reduce error. The steps were taken to avoid the re-suspension of sunken algae cells in the experiments. Algae cell numbers were counted on a hemocytometer via a Laboval-4 microscope (Carl Zeiss, Jena, Germany) after staining sample with acidic Lugol's. Inhibitory Rate (*IR*) was computed by Eq. (i).

$$IR = (1\text{-}N_t/N_1) \cdot 100\% \quad \text{(i)}$$

Where, N_t and N_1 were the cell densities ($cells \cdot ml^{-1}$) in the treatment and control cultures, respectively.

Chlorophyll fluorescence parameters of *P. donghaiense* assays

The key parameters of OJIP chlorophyll fluorescence transient were detected by Plant Efficiency Analyzer (Handy PEA, Hansatech instruments, Norfolk, UK) in the liquid culture fluorescence measurement system (33). Before the detection, sample vials were kept in dark for 20 min to achieve complete PS relaxation at room temperature every 24h, and test solutions in tubes were shaken homogeneously for several times. Chlorophyll fluorescence was measured continuously for 1s on a logarithmic scale (26). Fast fluorescence kinetics were measured on a time scale of 10 ms-1 s. The fluorescence yield

at 50 μs was considered as the initial fluorescence (F_o), the maximal fluorescence yield as F_m and the fluorescence intensities at 300 μs as $F_{300\mu s.}$ The fluorescence rise at O, J, I and P levels were recorded respectively, which was taken as the algal growth indicator.

Based on the theory of energy fluxes in photosystem II (27, 28), absorption energy flux (ABS) correspondence to photons were absorbed by the antenna pigments and most of this excitation was transferred to the reaction centers (RC) as a trapping flux (TR). Energy was converted by the reduction of Q_A to Q_A^- which was then reoxidised to Q_A and created an electron transport flux (ET). Finally, part of the energy which was dissipated as heat, fluorescence or transferred to other systems was described by DI. JIP test parameters were used to explain the energy fluxes through PSII (29). According to Strasser (27), the following equations were used to evaluate energy fluxes in PSII (30).

Absorption flux per RC, $ABS/RC = (TR_o/RC)/(TR_o/ABS)$ (ii)
Trapped energy flux per RC, $TR_o/RC = (M_o/V_j)$ (iii)
Electron transport rate in an active RC, $ET_o/RC = (TR_o/RC)(ET_o/TR_o)$ (iv)
The effective dissipation of an active RC, $DI_o/RC = (ABS/RC)-(TR_o/RC)$ (v)
Approximated initial slope of the fluorescence transient, $M_o= 4(F_{300\mu s}-F_o)/(F_m-F_o)$ (vi)
Normalized total complentary area above the O-J-I-P transie, $S_m = (Area)/(F_m-F_o)$ (vii)
Quantum yield for electron transport, $\phi E_o= ET_o/ABS = [1-(F_o/F_m)][ET_o/TR_o]$ (viii)

Where, $V_j= (F_j-F_o)/(F_m-F_o)$, were relative variable fluorescence intensity at the J-step. Areas were the area between fluorescence curve and F_m.

Antioxidant enzymes of *P. donghaiense* assays

For assays of antioxidant enzymes, after 48 h and 96 h incubation, the algal cells in each flask were sampled and centrifuged at 4000 g for 15 min at 4°C. Then the filters were grinded in an ice bath with 1.5 ml of 50 mM potassium phosphate buffer (pH 7.8) and quartz sand. The homogenate was centrifuged for 15 min at 5000 g at 4°C and the supernatant was collected for enzyme assays.

Total soluble protein was determined by method of Bradford (31), using bovine serum albumin as control in a UV-2450 spectrophotometer (Shimadzu, Japan). SOD activity was determined by NBT test (32), which was expressed as U mg^{-1}protein. CAT activity was assayed with a UV-2450 spectrophotometer (Shimadzu, Japan) by measuring the decrease of absorbance at 240 nm due to H_2O_2 decomposition (33). The peroxidase POD activity was measured by method of Montavon and Bortlik (34).

The reduced GSH content was determined with a reagent kit (Nanjing Jian Cheng Bio Inst, Nanjing, China) using 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). The aliquot of 0.2 ml extract, 0.8 ml reaction buffer (0.1 M phosphate buffer (pH 7.0), 0.5 mM ethylene diamine tetraacetic acid (EDTA) and 50uL of 3 mM DTNB) were added to test tube and thoroughly mixed. After 5 min, the absorbance at 412 nm was measured using an UV-2450 spectrophotometer (Shimadzu, Japan). The content of GSH was calculated based on a linear standard curve ranging from 0 to 100 μM.

Statistical analysis

All experiments were done thrice. Mean values and standard deviations were calculated from the different replicates (n=3). Statistical data analysis was performed using Origin8.0 (Origin Lab, Northampton, MA). The statistical difference between control and treatment groups was analyzed by ANOVA, Tukey-kamer multiple comparisons and Wilcoxon test when nonparametric tests were necessary (when $p<0.05$ was considered significant).

RESULTS AND DISCUSSION

Algal growth

We found that leaves aqueous extracts of *E.tereticornis* were most inhibitory to *P. donghaiense* at 0.50 g·L^{-1}. As an initial study, a growth response of *P. donghaiense* to the mixed aqueous extracts was determined. The algal cells grew slowly in first 24 h and became faster thereafter (Figure 1). The mixed extracts of 0.25 g·L^{-1} showed slight inhibition on the algae but not significant. However, 1.0 g·L^{-1} and 1.5 g·L^{-1} mixed aqueous extracts of *E. tereticornis* significantly inhibited the growth of algal cells than control (p <0.05) (Figure 1). The algal growth was not inhibited at low concentration (0.50 g·L^{-1}) mixed aqueous extracts, however, it was significantly lower than control at > 1.0 g·L^{-1} mixed aqueous extracts (Figure 1). The aqueous extracts (0.50 g·L^{-1}) of trunks, branches and roots showed little effects (p >0.05) on the algal growth, while the aqueous extract of leaves significantly inhibited (p <0.05) the growth of tested algae (Figure 2).

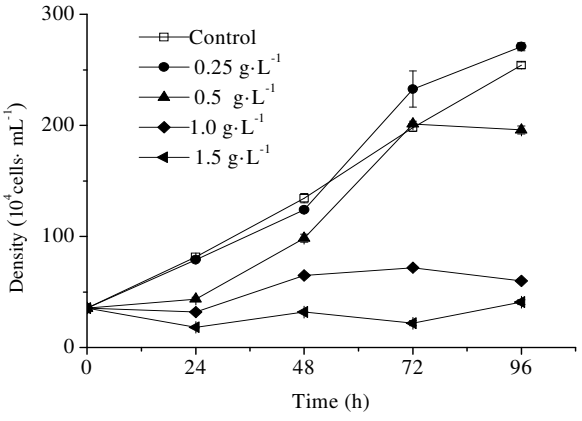

Figure 1. The effects of mixed aqueous extracts concentrations of *E.tereticornis* on the growth of *P. donghaiense.* Algae were grown hydroponically for 3 d and transferred to the fresh culture containing 0, 0.25, 0.5, 1.0 and 1.5 g·L^{-1} mixed aqueous extracts for 96 h. Data represented means(n=3) with associated error bars (S.D.).

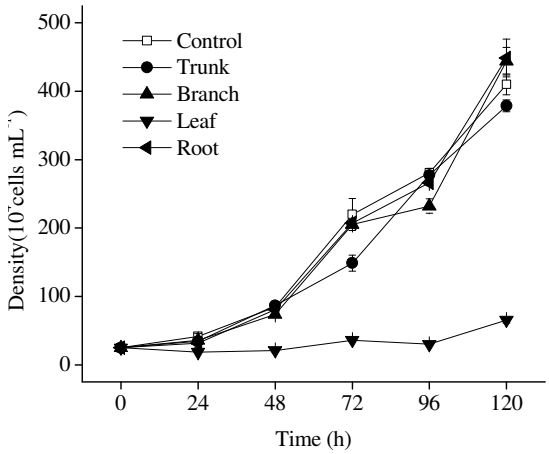

Figure 2. The effects of aqueous extracts from different parts of *E.tereticornis* on the growth of *P. donghaiense.* Algae were grown hydroponically for 3 d and transferred to the fresh culture containing 0.50 $g \cdot L^{-1}$ aqueous extracts of trunk, branch, leaf and root respectively for 120 h. Vertical bars represented standard deviation.

The leaves aqueous extracts inhibited the algal growth within 96 h (Figure 3a). After 96 h, the 0.05 $g \cdot L^{-1}$ and 0.10 $g \cdot L^{-1}$ stimulated the growth of *P. donghaiense*. The 0.25 $g \cdot L^{-1}$ reached the maximum *IR* of 40% at 72 h. However, the *IR* dropped to 0% at 120 h (Figure 3b). The *IR* of the group of 0.50 $g \cdot L^{-1}$ ranged from 40% to 75% within 120 h, which maximizes at 96 h (Figure 3b), implying leaves extracts may be rich in allelochemicals. However, the treatment groups of 0.05 $g \cdot L^{-1}$ and 0.10 $g \cdot L^{-1}$ promoted the algae growth after 96 h (Figure 3a), indicating that some allelochemicals of leaves were unstable and decomposed easily in natural conditions. Further research is needed to identify and isolate the effective and stable algicidal compounds.

Many Eucalyptus species are rich in secondary metabolites, ketones, polyphenols and phenolic acids were found in eucalyptus leaves (35-37). These substances may act as inhibitory allelochemicals. In addition, some scholars also pointed out that some fatty acids of Chinese medicinal plants were the main component of inhibition on algal growth (38-40). The fatty acids could impact the membrane system and change the membrane permeability which could damage the structure of the photosynthetic reaction centre and inhibit the fluorescence parameters of microalgae. The existence of fatty substances in Eucalyptus calls for more exploration.

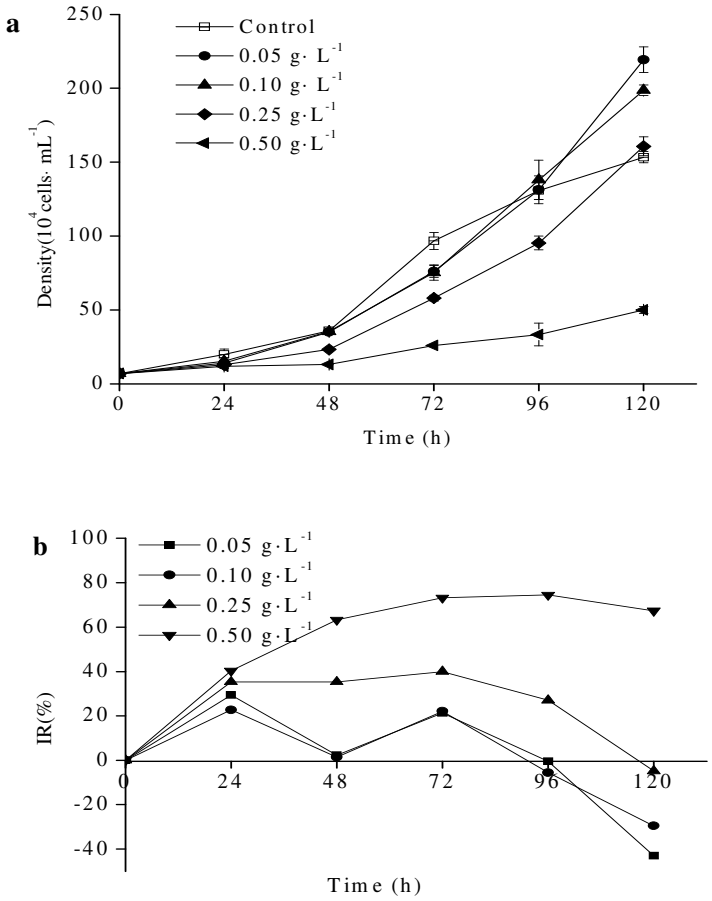

Figure 3. The effects of concentration of leaves aqueous extracts of *E.tereticornis* on the growth curves (a) and the IR (b) of *P. donghaiense.* Algae were grown hydroponically for 3 d and transferred to the fresh culture containing 0, 0.05, 0.10, 0.25 and 0.50 g·L^{-1} leaves aqueous extracts for 120 h. Vertical bars represented standard deviation.

PS Ⅱ of *P. donghaiense*

To identify the effects of leaves aqueous extracts on algal PSII, the algal rapid rise fluorescence kinetics were determined after 48 h and 96 h incubation, respectively. The microalgae exposed to 0.05g·L^{-1} leaves aqueous extracts, its fluorescence presented an increase in the characteristic transient steps (O-J-I-P) at 48 h (Figure 4a) and suppressed at 96 h (Figure 4b). The 0.50 g·L^{-1} leaves aqueous extracts at both two time points significantly decreased (p <0.05) the fluorescence (Figure 4a, b). The analysis of the

O–J–I–P fluorescence kinetic allows calculation of various parameters; it revealed how the photosystem II (PSII) energy fluxes were distributed in absorption, trapping, electron and dissipation (29). Aqueous extracts of leaves drastically suppressed the photosynthesis process of *P. donghaiense* culture and there was a clear concentration-response relationship in the O-J-I-P curve after 48 h and 96 h incubation (Figure 4a, b).

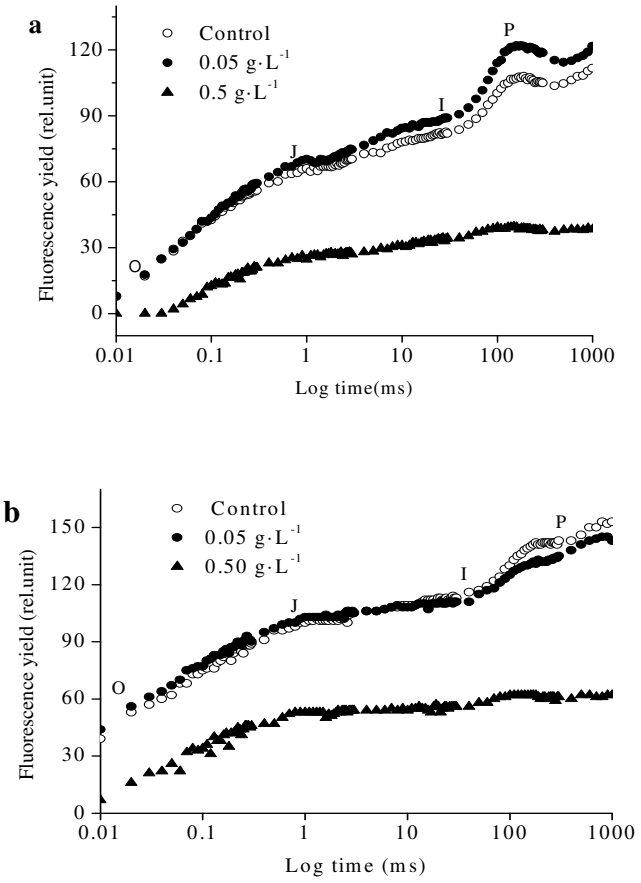

Figure 4. Rapid rise fluorescence kinetics for control (○) and algae exposed to 0.05 g·L^{-1} (●) and 0.50 g·L^{-1} (▲) leaves aqueous extracts after 48 h (a) and 96 h (b), plotted on a logarithmic time scale. 2 ml culture was taken to determine O-J-I-P chlorophyll fluorescence transient by Plant Efficiency Analyzer (Handy PEA, Hansatech instruments, Norfolk, UK) through a liquid culture fluorescence measurement system (Hansatech instruments). The fluorescence was induced by a saturating red light pulse at 1500 µmol m^{-2}s^{-1} provided from LED light source of the liquid culture attachment.

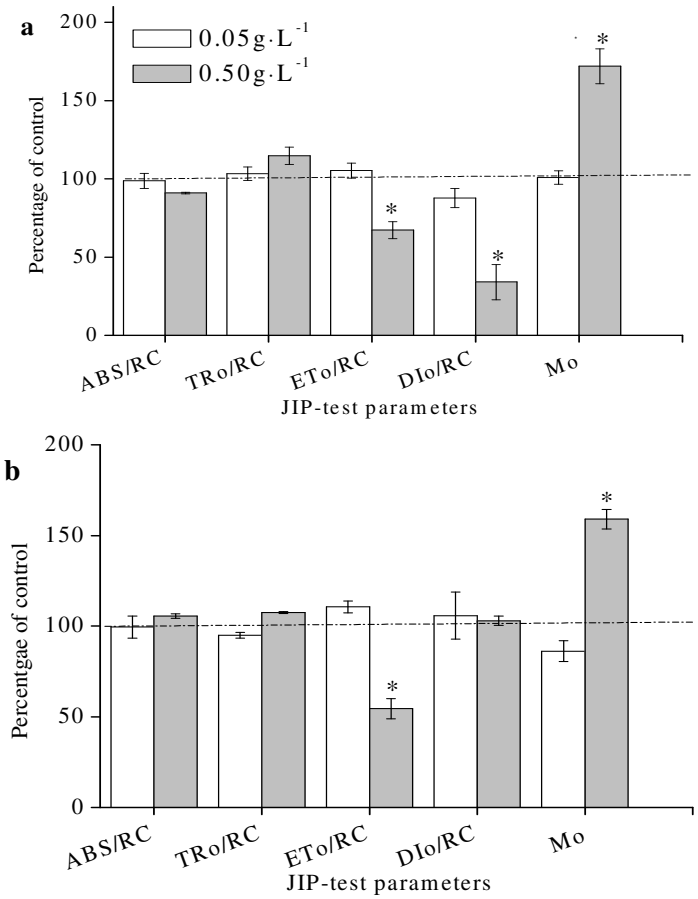

Figure 5. JIP-test parameters expressed as a percentage of the control when *P. donghaiense* were treated with 0.05g·L^{-1} and 0.50g·L^{-1} leaves aqueous extracts after 48 h (a) and 96 h (b). The dotted line indicated the value of the control treatment. Vertical bars represented standard deviation.*Significant different from the control (p <0.05).

Five of the JIP-test parameters were selected to study the algal PSII energy fluxes. The effects of leaves aqueous extracts on energy fluxes for light absorption, excitation energy trapping and electron transport were presented in the form of column diagram (Figure 5a, b). Absorption flux per RC (ABS/RC) observed were close to control both in low and high leaves aqueous extracts concentration (Figure 5a, b). At low concentration (0.05 g·L^{-1}) of leaves aqueous extracts, the maximal trapping rate of PSII (TR_o/RC) was slightly increased after 48 h. Electron transport flux per RC (ET_o/RC) was increased by 5% and 10% after 48 h and 96 h, respectively. The effective dissipation (DI_o/RC) was decreased by 12% at 48 h (Figure 5a), and recovered at 96 h (Figure 5b). M_o that implied the change in receptor side was not affected by 0.05g·L^{-1} leaves aqueous extracts. At high

concentration (0.50 $g \cdot L^{-1}$), TRo/RC was slightly increased by 14% and 7% after 48 h and 96 h, respectively. ET_o/RC was decreased by 33% and 46% after 48 h and 96 h, respectively. DI_o/RC was decreased significantly by 66% after 48 h incubation (Figure 5a), however, it recovered to the normal after 96 h (Figure 5b). M_o was increased by 72% and 59% after 48 h and 96 h incubation, respectively (Figure 5a, b). The S_m and ϕE_o reflected single-turn over Q_A reduction events and Quantum yield for electron transport from PSII to PSI (Table 2). They were not impacted by the 0.05 $g \cdot L^{-1}$ leaves aqueous extracts, except that S_m was stimulated at 96 h incubation. Both S_m and ϕE_o of 0.50 $g \cdot L^{-1}$ extracts were decreased than control (Table 2). Decrease in the values of S_m and ϕE_o on treatment groups indicated electron flow from PSII to PSI was interfered. A corresponding increase in M_o proved that the limitation of Q_A^- reoxidation was caused by poor diffusion of PQ across the thylakoid membrane.

Table 2. Changes of Sm and ϕEo in different leaves aqueous extracts concentration of *P. donghaiense* after 48h and 96h incubation

Treatment	S_m-48h	S_m-96h	ϕE_o -48h	ϕE_o -96h
Control	79.12±3.97A	68.55±16.67B	0.39±0.01a	0.28±0.03a
0.05 $g \cdot L^{-1}$	64.72±8.14A	92.62±3.59A	0.42±0.01a	0.3±0.03a
0.50 $g \cdot L^{-1}$	0.00±0.00B	13.1±3.17B	0.31±0.02b	0.13±0.01b

Note: Each value is the Mean±S.D. (n=3), different letters indicate significant difference (p<0.05) among different treatments.

In photosynthetic inhibition, leaves aqueous extracts of *E. tereticornis* blocked the electron transport chain of *P. donghaiense*, which showed an increase of M_o and a decrease of electron transport flux per RC (ET_o/RC) (Figure5a, b). It was confirmed by the approximation of the slope at the origin of fluorescence rise (M_o), represented an exciton trapped by active reaction centers (TR_o/RC), and slightly increased, indicating a further reduction of Q_A to Q_A^- (29). M_o also reflected the change in receptor sides, which was the maximum rate of reduction of Q_A, related with reaction center, caught the light colour of pigments and the status of Q_A (27,41). The Q_A^- electron transfer of *P. donghaiense* was inhibited by the 0.50 $g \cdot L^{-1}$ leaves aqueous extracts, decreasing quantum yield for electron transport. Hence light energy was used to restore the Q_A. The results showed that PSII was hindered, reducing receptor pool as suggested by S_m (42).

DI_o/RC decreased at 48 h and recovered to normal after 96 h (Figure 5a, b). This phenomenon indicated that PSⅡ reaction center of reversible inactivation became an energy trap, which absorbed light energy rather than promoted electron transfer. Studies showed that it might be a protective mechanism against the injury (43,44). For example, the over-excited energy could be consumed by Mehler reaction and photorespiration (43,45,46). Nan *et al*. (47) found that the culture filtrate of macroalga *Ulva lactuca* inhibited the growth of *S. costatum* in the early days of experiments, but the diatom grew later, suggesting that allelochemicals degraded over the time. Long term inhibition might, therefore, require continuous presence of allelopathic compounds (18).

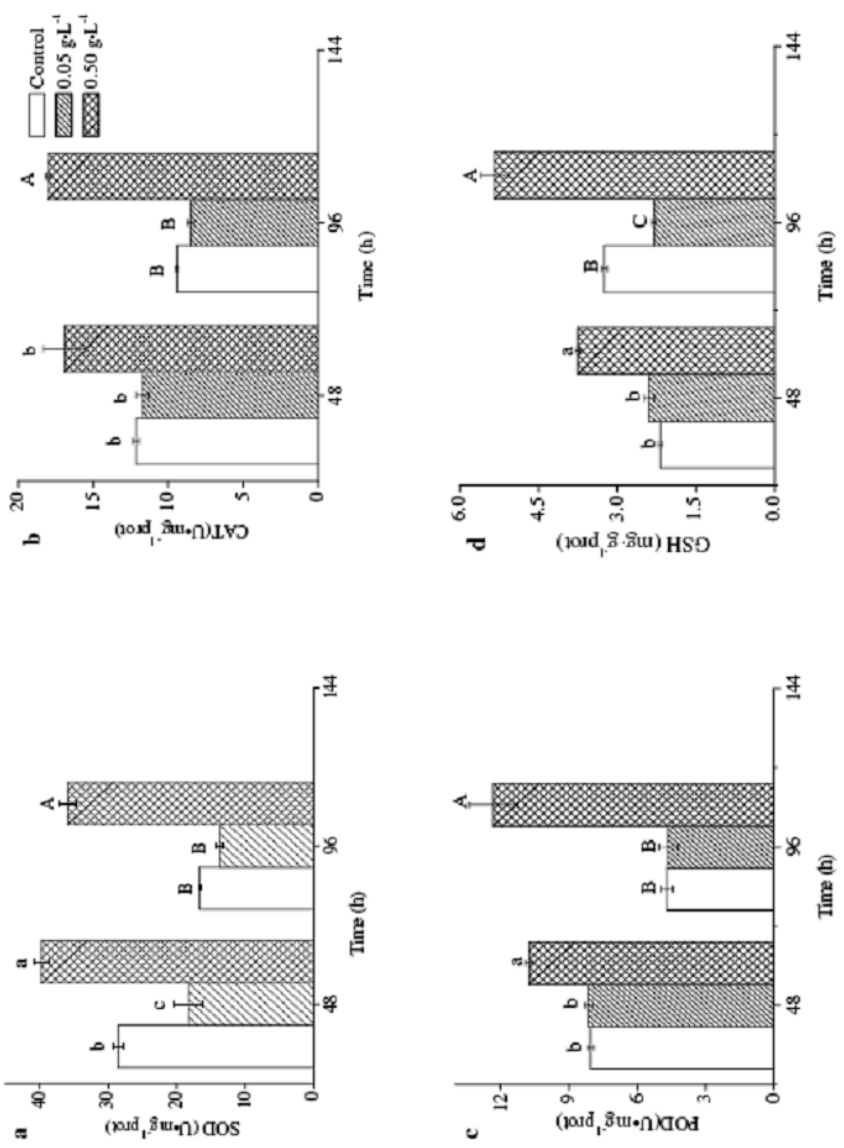

Figure 6. Effects of leaves aqueous extracts on SOD (a), CAT (b), POD (c) activity and GSH content (d) of *P. donghaiense* after 48 h and 96 h incubation. Means and standard deviation of three replicates were shown, the mean with different letters at each exposure time indicated that they were significantly different at $p < 0.05$.

Algal antioxidant systems

Physiological parameters (SOD, CAT, POD and GSH) of *P. donghaiense* after 48 h and 96 h incubation were shown in Figure 6. The leaves aqueous extracts of *E.tereticornis* at 0.50 $g \cdot L^{-1}$, stimulated the SOD activity of *P. donghaiense* ($p < 0.05$), but inhibited the SOD activity after 48 h incubation and did not affect the SOD activity. The leaves aqueous extracts at 0.50 $g \cdot L^{-1}$ were stimulatory before 96 h, while stimulated thereafter (Figure 6a). CAT activity at 0.50 $g \cdot L^{-1}$ leaves extracts rose after both 48 h and 96 h incubation ($p < 0.05$), while CAT activity at 0.05 $g \cdot L^{-1}$ showed no effect or even slight decrease (Figure 6b). The changes in POD activity of microalgae displayed a similar trend to CAT activity. The leaves aqueous extracts at 0.50 $g \cdot L^{-1}$ increased the POD activity than control ($p < 0.05$) during the incubation period, but it was not influenced by 0.05 $g \cdot L^{-1}$ leaves aqueous extracts (Figure 6c). The CAT activity with the high concentrations of leaves extracts (0.50 $g \cdot L^{-1}$) was much higher than control and low concentrations of leaves extracts. When *P. donghaiense* was incubated in high leaf extracts concentration (0.50 $g \cdot L^{-1}$) for 96 h, more GSH was produced compared with control. On the contrary, the GSH content declined significantly with low concentration (0.05 $g \cdot L^{-1}$) of leaves extracts (Figure 6d).

Several allelochemicals causes the cellular responses of antioxidant enzymes and non-enzymatic antioxidants (48-50). In this study, we found that the activities of antioxidant enzymes increased, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and the content of Glutathione (GSH) in *P. donghaiense*. The increase in antioxidant enzymes activities indicated that oxidative stress condition could increase the antioxidant capability of algal cells. However, this increase might not match the production of reactive oxygen species (ROS), leading to the increase in lipid peroxidation in algal cells (50) and inhibition of *P. donghaiense* growth. These results were consistent with those of Yu Hong *et al.* (11) who found that the activation of antioxidants in *M. aeruginosa* played an important role in resisting the stress from gramine. The inactivation of SOD was crucial to the growth inhibition of *M. aeruginosa* by gramine and *M. aeruginosa* may be damaged by the gramine due to oxidation of ROS.

CONCLUSIONS

The leaves aqueous extracts strongly inhibited the *P. donghaiense*, even at low concentration than aqueous extracts of from parts of *E.tereticornis*. The leaves aqueous extracts not only inhibited the electron flow from PSII to PSI, disrupting energy flow in PSII, but also increased the activity of antioxidant enzymes, triggering the synthesis of reactive oxygen species (ROS) and then damaging the subcellular structure of algae. Therefore the eucalyptus leaves might be valuable resource to control the HAB outbreaks. The Eucalyptus is widely cultivated in coastal area of China and the possible inhibitory effects of its leaves on oceanic algae might influence the coastal ecosystem.

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