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Table: -, Figs : 4

Allelopathic effects of mangrove plant *Bruguiera gymnorrhiza* on microalgae

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ABSTRACT

We assessed the algaicidal potential of mangrove plant *Bruguiera gymnorrhiza* on microalgae *Phaeocystis globosa* and *Heterosigma akashiwo* as target species. The aqueous leaf extracts of *B. gymnorrhiza* significantly inhibited the microalgae growth. The inhibitory effects of *B. gymnorrhiza* on microalgae were algae species dependent. Peak inhibitory rates (IR) in microalgae *P. globosa* and *H. akashiwo* were 97.4% and 90.1%, respectively. The sensitivity of both microalgal species to *B. gymnorrhiza* allelopathy were variable, *P. globosa* was more sensitive than *H. akashiwo*. Heat treatment did not change the algicidal potential of *B. gymnorrhiza* extracts ($P > 0.05$). Microscopic observation showed that the extracts severely damaged the cell membranes of microalgae and the microalgal cells were expanded and ruptured.

Key words: Allelopathic effects, *Bruguiera gymnorrhiza*, *Heterosigma akashiwo*, Inhibitory rate (IR), microalgae, *Phaeocystis globosa*

INTRODUCTION

Harmful algal blooms (HABs) are becoming an increasing problem to human health and environment (including effects on natural and cultured resources, tourism and ecosystems) all over the world (10). They caused economic huge losses in United States (13). *Phaeochystis globosa* is a harmful red tide microalgae, which is eurythermal and euryhaline (8,25). It can form HABs in the eutrophic waters in a short time, which can cause deleterious effects on the marine ecosystem structure and function, and also cause huge losses to fishery. Since 1997, many large-scale *Phaeocystis* blooms have occurred in the South China Sea (4,5). *Heterosigma akashiwo* is also important offshore red tide microalgae in China, which can produce ichthyootoxin and cause massive mortalities of fish. *H. akashiwo* is widely distributed in Dalian Bay, Jiaozhou Bay and coastal regions of Guangdong and had caused severe damage to the offshore aquaculture and marine environment in China (3, 28). It is also a threat to the health of sea birds, poultry and human through the food chain (26). The control of red tides has been a hot topic in red tide studies. Current red tide control methods were mainly designed based on the physical,

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chemical and biological aspects (29). However, only few of them are applicable due to high cost, secondary pollution and impracticability (1). Hence, other alternatives for effective control of red tides are urgently required.

The discovery of allelopathy hinted that the antialgal allelochemicals produced by macrophytes might be used as algal growth inhibitors (6,27). Allelopathy has been used to control harmful microalgae with allelopathic substances (7,22). The aquatic plants [water milfoil (*Myriophyllum spicatum*), fanwort (*Cabomba caroliniana*), bulrush (*Typha augustifolia*), hornwort (*Ceratophyllum demersum*), pondweed (*Potamogeton pectinatus*), Chinese water chestnut (*Eleocharis tuberose*), water hyacinth (*Eichhornia crassipes*) and duckweed (*Lemna minor*)] are inhibitory to the growth of some algae spp (*Chlorella pyrenoidosa, Scenedesmus obliquus, Chlamydomonas reinhardtii*) (29). Although red tide occurrences have become frequent and resulted in much more detrimental effects to human beings, but studies on its control with allelopathic substances have been done largely in the area of fresh water and only few has involved the marine red tides. Nan *et al*. (23) reported that an algaecidal substance was obtained from the marine microalgae *Ulva pertusa* and a water soluble gross extract showed strong inhibitory and killing effects on the growth of red tide algae *Heterosigma akashiwo*, *Skeletonema costatum* and *Alexandrium tamarense.*

Mangroves are woody plant communities in intertidal zones along tropical and subtropical coastlines (16,17). Their important functions are water purification, coastal protection from wind and waves, and biodiversity enhancement (18). Many mangrove plants can produce allelochemicals that affect the growth of other plants. Interspecific allelopathic effects between mangrove plants *Kandelia candel* and *Bruguiera gymnorrhiza* have been reported (20). *Sonneratia apetala* can produce allelopathic effects on the native mangroves (14). Li *et al.* (12) found that the root exudates of mangrove plants can produce allelopathic inhibition on the *Skeletonema costatum.* Till now there are few reports on the use of extracts from fresh tissues of mangrove plants to control red tide algae.

This study aimed to assess the allelopathic effects of mangrove plant *B. gymnorrhiza* on microalgae, which may be used as an alternative for red tide control and provide new ideas for further use of mangrove resources to improve ecological environment of coastal waters. Two typical red tide algae *P. globosa* and *H. akashiwo* were chosen as target species.

MATERIALS AND METHODES

Algal materials

Experimental algae *P. globosa* and *H. akashiwo* were obtained from Research Centre of Hydrobiology, Jinan University, Guangzhou, China. Algal cultures were incubated in F/2 media (9) prepared with sterile-filtered artificial seawater under the growth condition of (23±1) ^{o}C and 4,000 lux, with 12h dark/light in a light incubator.

Allelopathic effects of mangrove plant on red tide algae

Laboratory bioassay experiment was done assess the allelopathic effects. Fresh leaves of mangrove plant *B. gymnorrhiza* were collected from Qi'ao Island Mangrove Natural Reserve of Zhuhai, Guangdong Province. The fresh leaves were cleaned with distilled water and dried naturally. Then, the leaves were cut into small pieces, mixed with

seawater in a ratio of 1g leaves per 10 mL seawater, placed in a constant temperature water bath pot of 35 ^{o}C for 48 h, and then gently shaken 3 times daily. The water extract was then filtered through filter papers and centrifuged for 15 min at 5000 rpm. The supernatant was filtered with syringe filters (0.22 μm) to remove microorganisms and was kept at 4 ^{o}C as stock extract until further use in experiments.

Bioassay experiments were conducted in 100 mL clean and sterilized conical flasks. The stock extract was diluted into a series of extracts with different concentrations of 100, 50, and 25 g/L. One mL diluted extracts of different concentrations was added into conical flasks with 49 mL algal liquid. Final experimental volume was 50 mL and final concentrations of plant extracts were 2.0, 1.0 and 0.5 g/L. One mL sterilized seawater was added to 49 ml algal liquid as the control. Each treatment was replicated three times. The cultural condition was the same as in section algal materials. Samples (0.1 mL) were taken every 24 h, and the number of algal cells was counted under a microscope. Samples were taken 1 cm below the water surface, with movement, to reduce erroneous results. The experiment lasted 5-days. Inhibitory rate (IR) was calculated as under:

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

Where, IR: Inhibitory rate; N: Algal density of treatment group (cells·mL^{-1}); N_0: Algal density of control group (cells/mL).

Effect of heat treatment on *B. gymnorrhiza* allelopathic potential

25 mL stock extract was added into 100 mL clean and sterilized conical flasks. And the flasks were covered with aluminum foil and aseptic sealing film, cooled down after boiling for 30 min, added sterilized purified water to the final volume of 25 mL and then gently shaken by hand. One mL extract was added into 49 mL algal liquid and the concentration of final extract was 2.0 g/L. In control, one mL stock extract without being boiled was added into 49 mL algal liquid. The cultural condition was the same as in section algal materials. Samples (0.1 mL) were taken every 24 h, and the number of algal cells was countered with a hemocytometer under a microscope.

Statistical analysis

Statistical analysis of data was done using software of SPSS 13.0. The difference between treatment group and control group was assessed by One-Way ANOVA and P<0.05 was considered significant. Post hoc tests were used for multiple comparisons.

RESULTS AND DISCUSSION

Leaf extracts allelopathic potential

The algal cells of control group and treatment groups grew slowly on first day and thereafter grew faster (Fig.1). The 1.0 g/L and 2.0 g/L extracts significantly inhibited the growth of algal cells compared with control (P <0.05). After 3-days, the algal cells density in treatment groups of 0.5 g/L was higher than in control showing a stimulatory effect (P <0.05). During the whole experimental period, the cell density of the treatment group of 2.0 g/L was lowest than other groups.

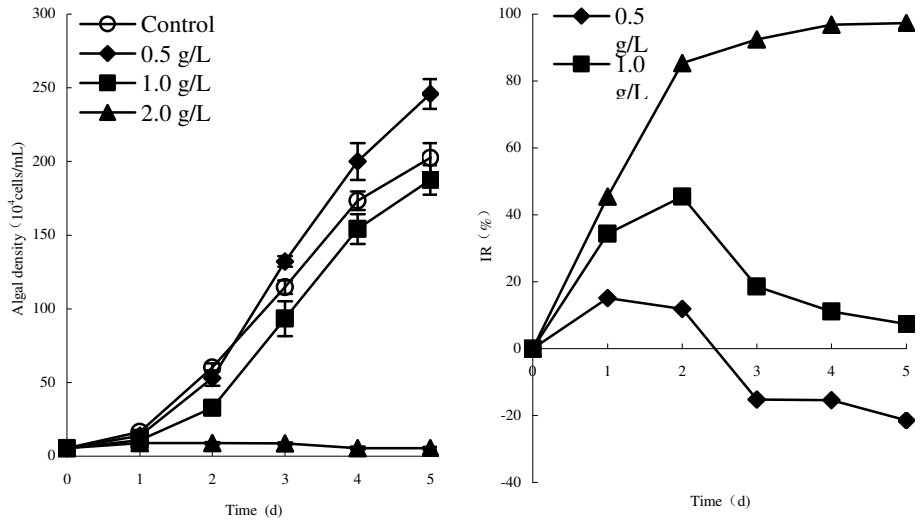

Figure 1. Growth curves of *P. globosa* and the IR after being treated with leaf extracts of *B. gymnorrhiza*

All treatment groups showed inhibitory effects on algal cells in the first two days (Fig.1). From the third day, the treatment group of 0.5 g/L extracts showed stimulatory effects on the growth of *P. globosa*. The treatment group of 1.0 g/L reached the maximum IR of 45.4% on the third day and then the IR decreased gradually. The treatment group of 2.0 g/L reached the maximum IR of 97.4% on the fifth day. Overall in 5-days, the IR of treatment group of 2.0 g/L ranged from 45.5% to 97.4%.

The algal cell density of *H. akashiwo* decreased gradually with the increasing concentrations of extracts (Fig.2). On first day, the algal cells in control group and the treatment groups were growing slowly and the treatment group of 2.0 g/L showed negative growth i.e., strong inhibition of algal cell growth. After wards, the growth rate of control group increased and the cell density of treatment groups was significantly lower than control (P <0.05). In first 3-days, the algal cell density of treatment group of 2.0 g/L was always lower than initial inoculation density of 5.0×10^4 cells/mL, showing a negative growth.

During the experiment, the IR of treatment groups was positive, showing inhibitory effects (Fig. 2). The treatment group of 0.5 g/L reached maximum IR (55.3%) on third day and thereafter the IR began to decrease. The treatment groups of 1.0, 2.0 g/L reached maximum IR (77.9% and 90.1%, respectively) on fourth day and showed declining trend on fifth day. Overall in treatment group of 2.0 g/L, the IR was 44.4% to 90.1% during the experiment.

In this study, extracts of *B. gymnorrhiza* leaves had significant inhibitory effects on *P. globosa* and *H. akashiwo*, which is consistent with literatures. Li *et al.* (12) found that water extracts of mangrove powder had significant inhibitory effects on the growth of *Skeletonema costatum*. Chai *et al.* (2) found that extract of four kinds of

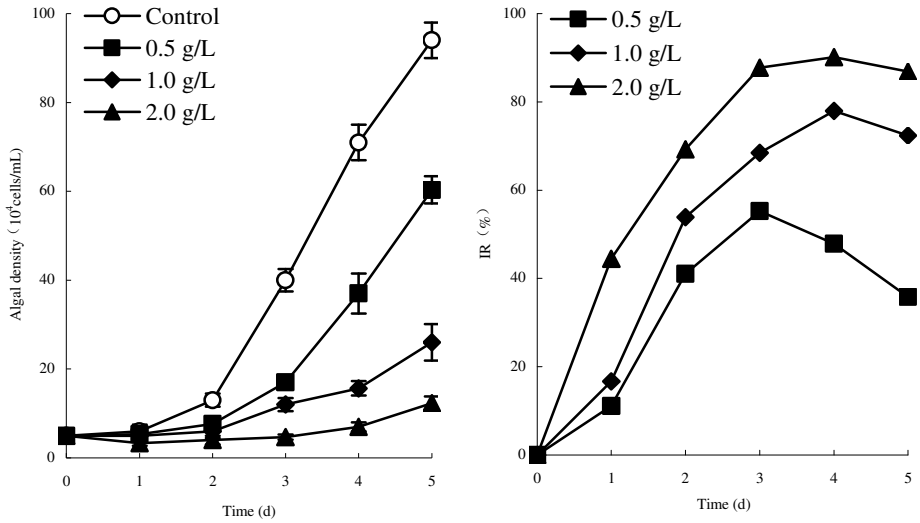

Figure 2. Growth curves of *H.akashiwo* and the IR after being treated with leaf extracts of *B. gymnorrhiza*

medicinal plants had allelopathic inhibitory effects on the growth of *Chlorella pyrenoidosa*. In our study, we used the fresh tissue of mangrove plants as experimental materials, which showed more severe inhibitory effects.

In the later stage of experiment, the IR of extracts of mangrove plants on algae declined and even the growth of algae was stimulated by mangrove extracts at low concentration, which is consistent with the findings of Men (19) and Li (15). When the extracts were added in algal cultural medium, perhaps their allelochemicals were unstable and easily degradable in aqueous solution, or the allelochemicals were degraded by resistance material produced by algae, or the allelochemicals were absorbed and transformed into nutrients that could be used by algae (15). Thus with the extension of time, the concentration of allelochemicals decreased gradually and their inhibitory effects were weakened.

The study found that *P. globosa* was less sensitive to *B. gymnorrhiza* than *H. akashiwo*, indicating that the inhibitory effects of mangrove extracts were species-specific. Jasser (11) found that cyanobacteria were more sensitive to allelochemicals from Ceratophyllum than green algae.

Heat treatment

Fig. 3 shows the growth of *P. globosa* and *H. akashiw*o with or without heat treatment of *B. gymnorrhiza* extracts. Both *P. globosa* and *H. akashiw*o grew fast in control groups, but their growth was inhibited in treatment groups. There were no significant differences in the inhibitory effects on two microalgae after heating *B. gymnorrhiza* extracts ($P > 0.05$). Our study found that heat treatments did not change the inhibitory effects of extracts from *B. gymnorrhiza*, indicating that algicidal compounds from *B. gymnorrhiza* are relatively stable.

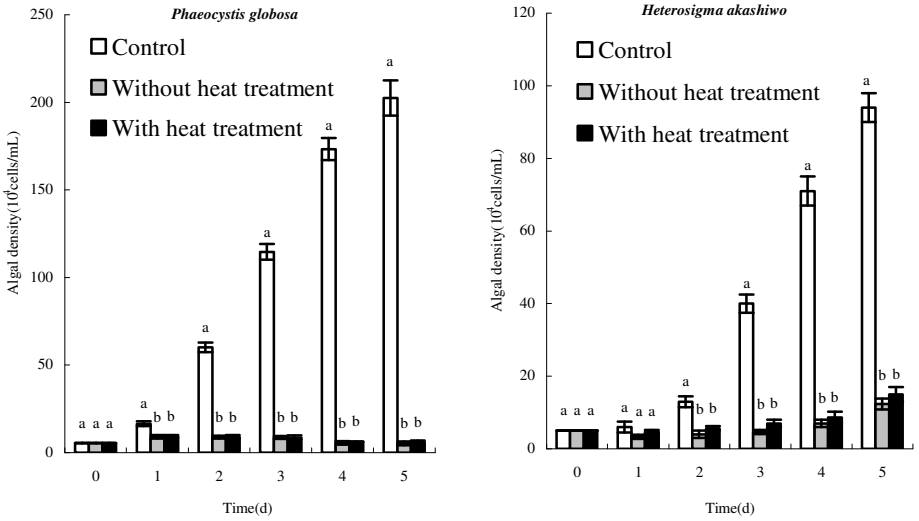

Figure 3. Effects of *B. gymnorrhiza* extracts on *P. globosa* and *H. akashiwo* before and after heat treatment.

Morphological changes

After 24 h culture, observations from microscope showed that in treatment groups the number of splitting algal cells decreased significantly and the movement of algal cells was slower. The volume of algal cells of treatment groups became larger and parts of algal cells were broken after 48 h. With the extension of time, more broken cells were found in the conical flasks of treatment groups, with a blurred microscopic vision, while the cells of control groups dispersed evenly and the vision was clear. Most of the algal cells of treatment group of 2.0 g/L were broken in 120 h (Fig. 4).

In later stage, most of the algal cells of treatment groups formed sedimentation at the bottom of conical flasks and the phenomenon of sticking to wall of the flasks was found, while in the control group most of the algal cells were suspended in the culture medium. Microscopic observation showed that the algal cells treated with extracts of mangrove plants were expanded and broken down. The active components of allelochemicals can oxidise the major fatty acids present in the cell membrane, increase the degree of unsaturation, increase the fluidity of membrane and reduce the selection of materials through the membrane (13). The allelochemicals may disintegrate the organelles with membrane or nuclear, resulting in leakage of intracellular contents (15). The entry of extracellular substances and the discharge of inner contents of organelles increased the amount of materials in the algal cells, resulting in expansion and rupture of algal cells.

Many mangrove plants are also medicinal plants, rich in secondary metabolites. These secondary metabolites are distributed in various organs [roots, stems, leaves, flowers and seeds] of these plants. The leaves of *B. gymnorrhiza* have important medicinal value, because they contains glycoside, long-chain fatty acids, hydrocarbons, lignin, tannins, terpenes, sterols and flavonoids (24). Some active chemicals in *B. gymnorrhiza* show anti-inflammatory, antifungal, termite resistance, insecticide and anti-tumor effects.

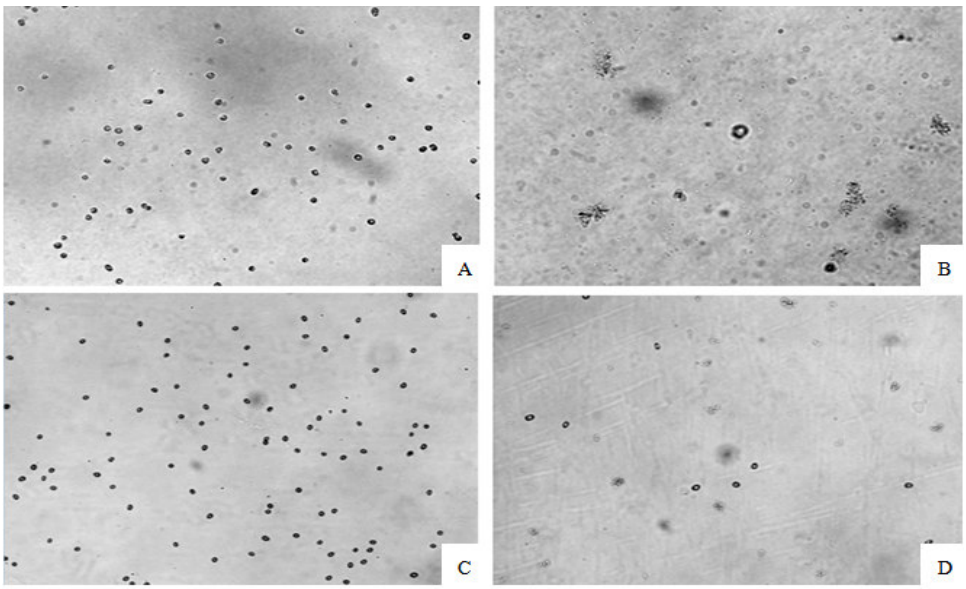

Figure 4. Micrograph of *P. globosa* (10×40) and *H. akashiwo* (10×10) with different treatments. A. *P. globosa* without extracts; B. *P. globosa* with 2.0 g/L *B. gymnorrhiza* extract; C. *H.akashiwo* without extracts; D. *H. akashiwo* with 2.0 g/L *B. gymnorrhiza* extract.

Our experiment may have lead to an underestimation of the allelopathic effect because there was no continuous addition of extract and some allelochemicals are unstable and easily decompose at room temperature (21). Considering that the content of allelochemicals extracted from mangrove plants may be low, thus a large quantity of fresh leaves of mangrove plants were used in our experiment. The crude extracts would be purified for practical application and the allelopathic effects would be greatly improved, making it possible to practise in nature. Further research is needed to isolate and identify the active algicidal compounds from the mangrove plants.

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