

Inhibition Effect of the *Aegiceras Corniculatum* Leaves Water Extract on the Growth of Two Red Tide Algae

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Received: 17 January 2025; Accepted: 8 March 2025; Published: 8 April 2025

Abstract:

Red tide, also known as Harmful Algal Blooms (HABs), which is the type of marine disaster. HABs can have adverse effects on offshore aquaculture, ecological environment and Marine organisms, and resulting in greater economic losses. In addition, the toxins produced by red tide algae, such as paralytic shellfish poison (PSP) and diarrhetic shellfish poison (DSP), have been harmful to human. Interestingly, red tides rarely occur in wetland or mangrove, and it is speculated that mangrove plants can produce active substances which to inhibit the HABs. Therefore, this paper uses the leaves of the mangrove plant *Aegiceras Corniculatum* as the research target, and study the inhibit effect of two common red tide algae. The results showed that, the leave water extracts from the *Aegiceras Corniculatum* had inhibitory effects on both species of red tide algae. The toxic effects of the *Aegiceras Corniculatum* water extracts on different species of algae are specific. The results showed that the sensitivity of the *Prorocentrum donghaiense* to the *Aegiceras Corniculatum* water extract of the leaves was stronger. The results can provide scientific basis for effective management of HABs.

Keywords:

Heterosigma Akashiwo, *Prorocentrum Donghaiense*, Allelopathy, *Aegiceras Corniculatum*, Harmful Algal Blooms

1. Introduction

1.1 Research Background

Against the backdrop of global climate change, red tide disasters are showing an increasingly serious trend, and the scope of their impact is gradually increasing, spreading throughout water bodies around the world [1]. Red tide disasters have become a typical marine environmental disaster that restricts the economic development of coastal areas, damages marine ecosystems, and threatens human food safety. Coastal areas are usually the most economically developed and densely

populated areas of a country. However, with global climate change, red tides are becoming increasingly frequent, and the destructive effects of red tide disasters on coastal areas of various countries are also significantly increasing. This is also a huge impact on the world economy and society [2].

For plants, photosynthesis is the foundation of all their life activities. The photosynthetic capacity of algae is mainly determined by chloroplasts, but chloroplasts are the most sensitive to environmental stress. Tang Peng et al [3] pointed out that allelochemicals released by aquatic plant can affect the physiological state of algae by destroying the photosynthetic system of algae, thus achieving the purpose of inhibiting algae. Allelochemicals have been proved to damage algal organelle by promoting the production of free radicals and reactive oxygen species. When exposed continuously or with high concentrations of allelochemicals, cells are unable to scavenge free radicals and reactive oxygen species, resulting in oxidative damage or death of algae cells [4]. In addition, the physical and chemical properties of chloroplasts in photosynthetic plants may be changed by pollutants such as polycyclic aromatic hydrocarbons and heavy metals, changing the physiological activity of chloroplasts and reducing the efficiency of photosynthesis in algae [5]. The strong inhibition of photosynthesis ability will significantly slow down the synthesis rate of organic matter in algae, which will inevitably delay the growth and development of algae, thereby achieving the effect of inhibiting algae growth and effectively controlling the occurrence of red tides.

There are numerous methods for preventing and controlling red tide, with some researchers using copper sulfate, potassium chloride, or filters and pumping techniques to remove red tide algae [6]. More common techniques include using modified clay to adsorb red algae and immersing them in the seabed to clarify water quality. However, all of these technologies have some issues, such as high cost, difficulty in obtaining materials, low efficiency, and severe secondary pollution, which limit their application. The conventional methods for controlling algae include physical control methods, chemical control methods, and biological control methods. Although physical and chemical control methods have certain effects in controlling the growth and reproduction of algae, physical methods are time-consuming and labor-intensive, while chemical methods pose high ecological security risks and may cause secondary pollution. Currently, biological methods that utilize plant chemical induction should be a valuable algal control technique. Tang Peng et al mentioned that allelochemicals are the key to allelopathy and algal suppression of aquatic plant [3]. At present, the research on the mechanism of allelopathy and algal inhibition of aquatic plant mostly focuses on the cell level, mainly on the aspects of influencing the photosynthetic system, cell membrane permeability, enzyme activity, etc. of algae. Numerous studies have shown that the effects of allelochemicals on the photosynthetic system of algae cells can be mainly divided into two aspects: one is to reduce the chlorophyll content in algae cells, and the other is to block electron transfer. This study aims to provide important support for further discussion on the mechanism of harmful algal blooms caused by red tide heterobenthic algae and the prevention and prediction of red tides.

2. Materials and Methods

2.1. Cultivation of algae

This study selected the red tide algae species, *Heterosigma akashiwo* and *Prorocentrum donghaiense*, provided by the Algae Species Laboratory of the School of Chemistry and Environment, Guangdong Ocean University. Before the experiment begins, the red tide algae were cultured three times in logarithmic phase (the logarithmic phase can be determined based on the plotted growth curve). After the cultivation is completed, inoculate the red tide algae into a 250 mL medium with a cultivation volume of 200mL with f/2 medium [7]. Place the inoculated conical flask into a light incubator for cultivation at a temperature of 23 ± 1 °C, light intensity of 3000 lx, and a light to dark ratio of 12h: 12h.

2.2. Experimental Plan

The leaves of the mangrove plant *Aegiceras corniculatum* were collected from the Zhanjiang Mangrove National Nature Reserve in Zhanjiang City, Guangdong Province. The fresh leaves of the picked *aegiceras corniculatum* flower leaves are first rinsed with running tap water, and all impurities such as soil and sand on the surface of the *aegiceras corniculatum* leaves are rinsed thoroughly. Then, distilled water is rinsed three times to ensure that there are no visible impurities on the surface of *aegiceras corniculatum* leaves. The cleaned leaves are then transferred to a dry and ventilated environment, and the surface moisture is dried at room temperature. Finally, freeze dry the dried leaves of the *aegiceras corniculatum* tree at -20 °C, and use a flour mill to grind them into dry powder.

Prepare 1L f/2 culture medium, sterilize it in an autoclave at high temperature, and then Free cooling it. Use the Analytical balance of the laboratory to accurately weigh 100g of dried powder of *aegiceras corniculatum* leaves, add it to the cooled f/2 medium, seal it with plastic sealing film, seal the bottle mouth with newspaper, and then heat it for 6h in a 70 °C water bath. The water extract after heating in the water bath is cooled at room temperature, and then subjected to ultrasonic treatment for 2 hours using an ultrasonic instrument. The treated water extract is first filtered with a rapid quantitative filter membrane to remove large particle residues. Then, a 50mL syringe sterilized at high temperature is connected to a pore size of 0.22 μL. The filter head of 0.22 μm is used to filter the water extract to remove impurities such as microorganisms and fine particles, and ultimately obtain a concentration of 100 g/L of the mother solution of the water extract from *aegiceras corniculatum* tree leaves. It is then placed in a brown reagent bottle and stored in a dark place at 4 °C.

Set gradients of Control (blank), 0.3125g/L, 0.625g/L, 1.25g/L, 2.5g/L, 5g/L, and 10 g/L respectively for the cultivation of red tide algae. Three duplicate samples were set for each group in the experiment, with a vaccination density of 5.5×10^4 pieces/L, regularly shake the conical flask 3 times a day and randomly switch positions. Take 100 every 24 hours μ Count the algal solution of L in the plankton counting box, draw its growth curve based on the recorded data, and calculate the inhibitory rate of the water extract of paulownia tree leaves on algal cells according to the following formula:

$$IR = \frac{X_0 - X_t}{X_0} \times 100\%$$

In the formula, IR is the inhibitory rate of the water extract of *aegiceras corniculatum* leaves on the red tide heterobenthic algae; X_0 represents the algae density of the blank control group; X_t is the algae density of the treatment group.

2.3. Data processing and analysis

All experimental results were obtained from three parallel experimental analyses and expressed as mean $\pm$ SD. Use Microsoft Excel and Origin 9.0 for data processing and drawing production. The significance analysis of intra group differences was conducted using one-way ANOVA combined with LSD for statistical analysis, with $P < 0.05$ indicating significant differences.

3. Results and Analysis

3.1. The *aegiceras corniculatum* Leaves Water Extract on the growth of *heterosigma akashiwo*

The aqueous extract of *aegiceras corniculatum* leaves can effectively inhibit the growth of *heterosigma akashiwo* (Figure 1). From Figure 1, it can be seen that as the concentration of the water extract increases, the density of *heterosigma akashiwo* decreases. At 24 hours, there was no significant difference in algae density, but as the experimental time increased, the differences between the experimental groups and the control group gradually began to become apparent. After 48 hours, the *heterosigma akashiwo* showed a downward trend at the concentrations of 2.5g/L, 5g/L, and 10 g/L of the water extract of *aegiceras corniculatum* leaves, especially at the concentrations of 5 and 10 g/L of *aegiceras corniculatum* leaves, which were basically in a completely inhibitory effect. At 96 hours, it can be seen from Figure 2 that the inhibition rate of the water extract of *aegiceras corniculatum* leaves on *heterosigma akashiwo* was 100% at 10 g/L, and the inhibition rate of the water extract of *aegiceras corniculatum* leaves at 5 g/L was as high as $98.9 \pm 0.14\%$, indicating that the water extract of *aegiceras corniculatum* leaves has a very good inhibitory effect on the growth of *heterosigma akashiwo*. According to the data obtained in Figure 2, the EC30 and EC50 for 96 hours were calculated to be 0.70g/L and 2.03g/L, respectively. Therefore, subsequent experiments were conducted using EC30 and EC50.

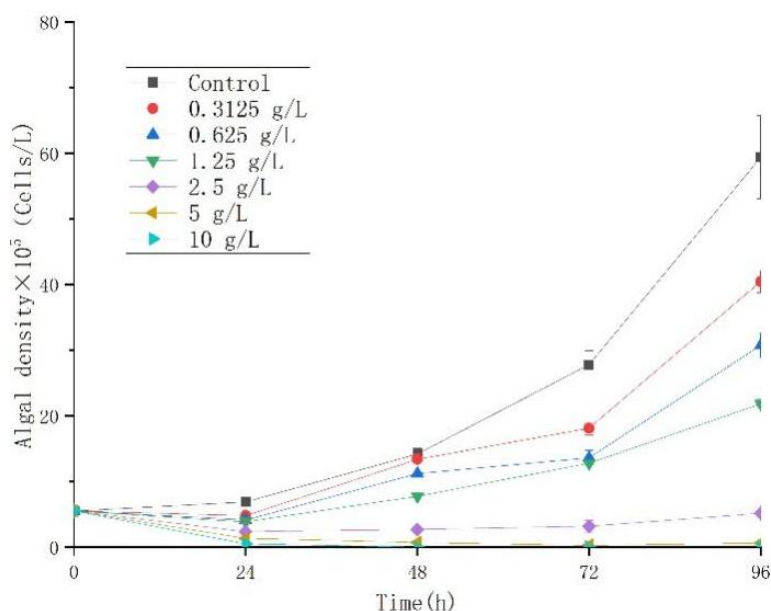


Figure 1. Effect of Water Extract from *Aegiceras corniculatum* Leaves on the Growth of *Heterosigma akashiwo*.

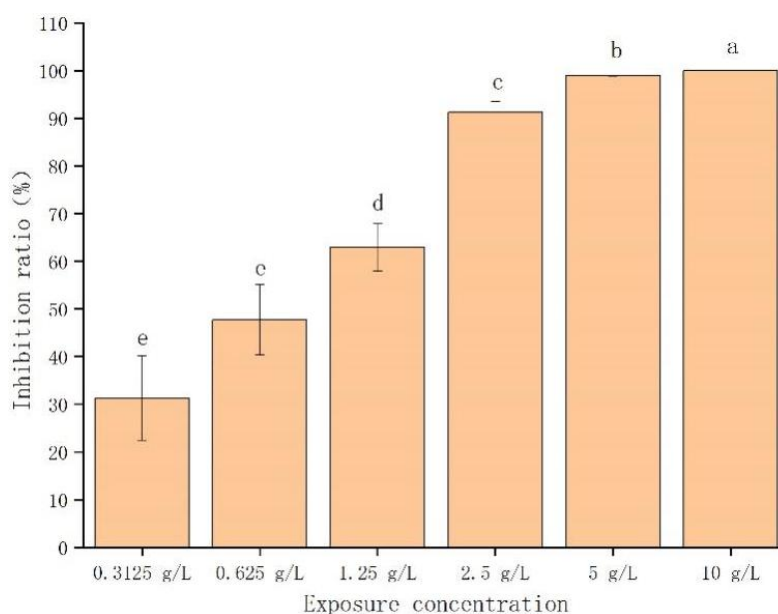


Figure 2. Effect of Water Extract from *aegiceras corniculatum* Leaves on the Inhibition Efficiency of *Heterosigma akashiwo* (96h).

3.2. The *aegiceras corniculatum* Leaves Water Extract on the growth of *prorocentrum donghaiense*

The aqueous extract of *aegiceras corniculatum* leaves can effectively inhibit the growth of *prorocentrum donghaiense* (Figure 3). As Figure 3, the density of *prorocentrum donghaiense* decreased with the increase of the water extract concentration of the leaves of *aegiceras corniculatum*.

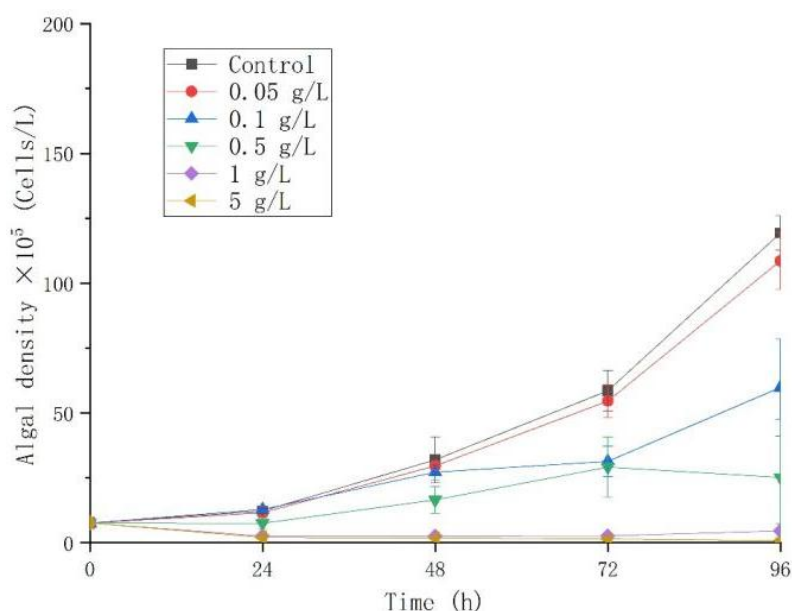


Figure 3. Effect of Water Extract from *Aegiceras corniculatum* Leaves on the Growth of *Prorocentrum donghaiense*.

At the concentrations of 0.5, 1 and 5 g/L at 24h, the density of *prorocentrum donghaiense* showed a significant decline. At 48h, the concentration of *prorocentrum donghaiense* increased at 0.5 g/L. At 96h (Figure 4), the inhibition rate was 100% at the concentration of 1 and 5 g/L of the water extract from the leaves of the *aegiceras corniculatum*. The inhibition rate was $82.36 \pm 0.12\%$ at 0.5 g/L concentration of

aegiceras corniculatum leaves water extract. The results showed that EC30 and EC50 of water extracts from the leaves of *aegiceras corniculatum* were 0.068 and 0.22g/L, respectively.

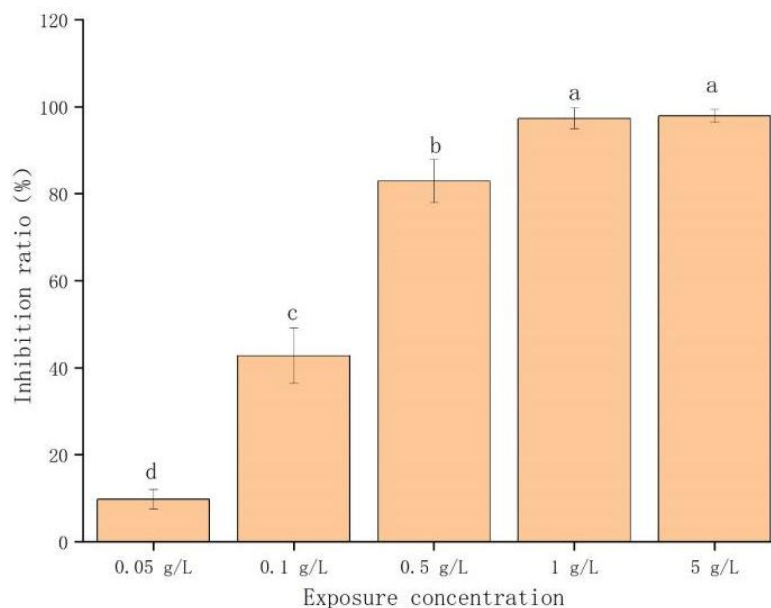


Figure 4. Effect of Water Extract from *aegiceras corniculatum* Leaves on the Inhibition Efficiency of *Prorocentrum donghaiense* (96h).

4. Discussion

In recent years, with the acceleration of urbanization and rapid economic development in China, a large amount of high nitrogen and phosphorus water bodies such as urban sewage, industrial and agricultural wastewater [8], and aquaculture wastewater have been discharged into the coastal waters of China, causing serious damage to the ecological environment of the coastal waters. Among them, eutrophication is becoming increasingly serious, leading to a large outbreak of algal blooms [9]. Research has shown that plants have significant allelopathic effects [10]. The use of biotechnology (allelopathy) to inhibit the growth of red tide algae is of great research space and value, and also provides a new approach for the prevention and control of red tide. This study found that the water extract of *aegiceras corniculatum* leaves can significantly inhibit the growth of red tide algae, showing a typical dose-effect relationship. This is consistent with the previous research results of the research group [3,10,11], which both describe that mangrove plants and their water extracts can significantly inhibit the growth of red tide algae species.

5. Conclusions

The toxic effects of the water extracts from the leaves of *aegiceras corniculatum* on different species of red tide algae are specific. Figure 2 and Figure 4 showed that the sensitivity of *Prorocentrum donghaiense* to the water extract of *aegiceras corniculatum* leaves was stronger than *Heterosigma akashiwo*. It can achieve a strong inhibition rate at a lower concentration of *aegiceras corniculatum* leaves water extract. This results consistent with previous researches [11]. Therefore, the time of action of water extracts from the leaves of *aegiceras corniculatum* on the two species of red tide algae was also different. In each treatment group, the inhibitory effect of *Prorocentrum donghaiense* appeared in 24 hours, while the inhibitory effect of

Heterosigma akashiwo appeared in 48 hours. Some studies have found that this may be related to the structure, adaptation and physiological structure of microalgae, and the production of ROS is also related [12,13].

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Funding

This work was supported by College Students' Innovative Entrepreneurial Training Plan Program of Lingnan Normal University (S202310579019); Guangdong Provincial College Students' Innovation and Entrepreneurship Training Program Project (2023).

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