ALLELOPATHIC EFFECTS OF EICHHORNIA CRASSIPES ROOTS ON ALEXANDRIUM TAMARENSE

CHEN Zhi-Lan, YANG Wei-Dong, LIU Jie-Sheng, XIE Jin and JIANG Tian-Jiu
(*College of Life Science and Techonlogy, Jinan University, Guangzhou* 510632)

Abstract: Allelopathic effects of dry powder and extract of acetone/ethyl acetate of *Eichhornia crassipes* roots on *Alexandrium tamarense* (HK strain) were evaluated. 0.1 g, 0.5g, 1.0g, 1.5g and 2.0g/L of *E. crassipes* root powder were added, respectively. Due to low solubility of extracts, a control of slide was used as carrier to measure the actual concentration of extracts in the cultures. At first, 0.01g, 0.03g, 0.05g, 0.07g/L of extracts were put into the weighted slides. Then the slides were added to the flasks containing 100mL of *A. tamarense*. At the end of the experiment, the slides were taken out, dried and weighted. The actual concentration of extracts in the cultures was obtained from the loss in weight. The results showed that the growth of *A. tamarense* was absolutely inhibited when the algae was exposured to dry powder of *E. crassipes* roots at above 1.5g/L. When the concentration of dry powder of *E. crassipes* roots were 0.5g/L and 1.0g/L, the growth of algae increased after 3 days. The growth of *A. tamarense* was also inhibited at the actual concentration of 0.038g/L and 0.022g/L extract of acetone and ethyl acetate of *E. crassipes* roots, respectively. It suggested that *E crassipes* could be a potential algaecide to control harmful algal blooms.

Key words: *Alexandrium tamarense*; Allelopathic effects; *Eichhornia crassipes*; Harmful algal blooms
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The harmful algal blooms (HAB) were serious in recent years all over the world. They had negative impact on human activities, such as risks for human health, direct effect on natural and cultivated invertebrates, damage to aquatic ecosystem[1]. Therefore, many studies have concentrated on the control of undesired algae, and many methods have been developed, such as use of clay complex system[2], chlorine dioxide[3], bacteria[4] etc. These methods can be effective in short time, but they may have potential pollution to the environment.

Allelopathic effects are prevalent natural phenomenon in aquatic ecosystems among high plants and between high plants and microorganisms, including stimulatory or inhibitory interactions[5]. Many researchers have studied allelopathy and practised on HAB control[6—8]. However, most studies concentrated on freshwater ecosystem, little attention involved in red tide algae. *Eichhornia crassipes* is a high aquatic plant commonly distributed in the lakes and rivers. Some researchers have demonstrated that *E. crassipes* can inhibit the growth of algae in freshwater because of its allelopathic effect on the algae[9,10]. The allelopathic substances in *E. crassipes* roots have been detected. The main components of acetone extracts are N-phenyl-2-naphthylamine, linoleic acid and glycerol-1, 9-12 (ZZ)-octadecadienoic ester[10]. The main components of ethyl acetate extracts are 4α-methylation sterol[11].

A. tamarense can produce paralytic shellfish poisoning (PSP) which is toxic to human and other animals. The objective of this study was to investigate the allelopathic effects of *E. crassipes* on *A. tamarense*, to provide the information of the control of HAB.

1 Materials and methods

1.1 Culture of HAB algae and treatment of hydrophytes

A. tamarense HK strain was obtained from Institute of Hydrobiology of Jinan University in Guangzhou. The strain was

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Biography: Chen zhi-lan (1979—), female, born in Guangzhou, postgraduate, study in algae physiology and biochemistry
Corresponding author: Liu jie-Sheng, E-mail: tywd@jnu.edu.cn

cultured in k medium (salt degree 33‰, filter-sterilized through 0.22μm filter) at 22 ℃ with a 12∶12 h cycle in an illuminating incubator. All the flasks containing microalga were shaken twice everyday.

E. crassipes were collected on the campus of Jinan University in Guangzhou. The roots of *E. crassipes* were cleaned and dried, then skived into fine powder.

1.2 Effect of the dry powder of roots of E. crassipes on the growth of A. tamarense

The initial cell density of *A. tamarense* in the culture medium was 6000 cells/mL. 0.1g, 0.5g, 1.0g, 1.5g and 2.0g/L of *E. crassipes* root powder were added to the flasks containing 100mL cells of *A. tamarense* in the culture medium. 0.1mL cultures medium was sampled in every day and microalga cells were counted under XSZ-D2 inverted microscope. The tests were carried out in triplicates, and the culture medium without *E. crassipes* was used as the control.

1.3 Effect of acetone and ethyl acetate extract of E. crassipes roots on the growth of A. tamarense

The roots dry powder of *E. crassipes* were extracted with acetone or ethyl acetate and evaporated under 40 ℃[10]. Due to low solubility of extracts, a control of slide was used as carrier to measure the actual concentration of extracts in the cultures. At first, 0.01g, 0.03g, 0.05g, 0.07g/L of extracts were put into the weighted slides. Then the slides were added to the flasks containing 100mL of *A. tamarense*. At the end of the experiment, the slides were taken out, dried and weighted. The actual concentration of extracts in the cultures was obtained from the loss in weight. Two series control were prepared, one of which, a copy of slide was added to and the others without any treatment.

Inhibitory rate = (biomass of control-biomass of sample) ×100 %/ biomass of control

2 Results

2.1 Effect of dry powder of E. crassipes roots on A. tamarense

Effect of the dry powder of *E. crassipes* roots on the growth of *A. tamarense* is showed in Fig. 1. When the concentration of *E. crassipes* was over 1.5g/L, the biomass of *A. tamarense* was significantly less than that of control and all the cells in cultures died within 2 days ($P < 0.05$). The biomass of *A. tamarense* was significantly ($P < 0.05$) higher than that of the control after day 3 at the concentration of 0.5 and 1.0g/L, though a decrease occurred at 0.1g/L to 1.0g/L in first day.

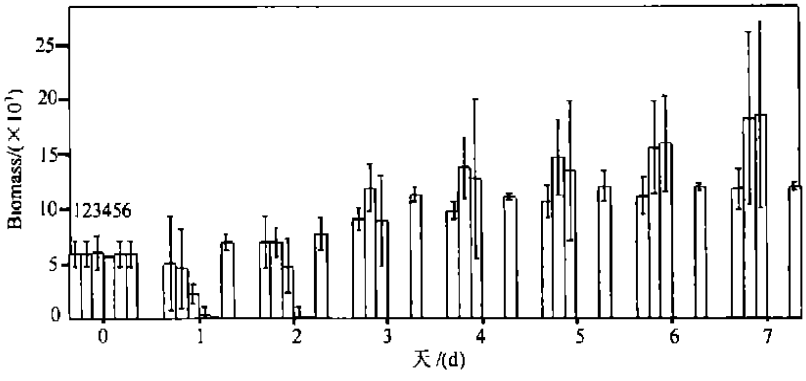


Fig. 1 Effect of different concentrations of dry powder of *E. crassipes* roots on the growth of *A. tamarense*, biomass (1×10^3 cells/mL), 1∶0.1g/L, 2∶0.5g/L, 3∶1.0g/L, 4∶1.5g/L, 5∶2.0g/L, 6∶control

2.2 Effect of the extract of acetone of E. crassipes roots on A. tamarense

Fig. 2 showed the impact of acetone extract of *E. crassipes* roots on *A. tamarense*. Significant decreases ($P < 0.05$) in growth of *A. tamarense* were observed at high concentrations in the assays. When the actual concentration of the extract was 0.038g/L, the growth of *A. tamarense* was inhibited on the day 7 ($P < 0.05$). At the concentrations of 0.018 and 0.022g/L, inhibitory rate of *A. tamarense* growth by extract was about 40 % in day 7.

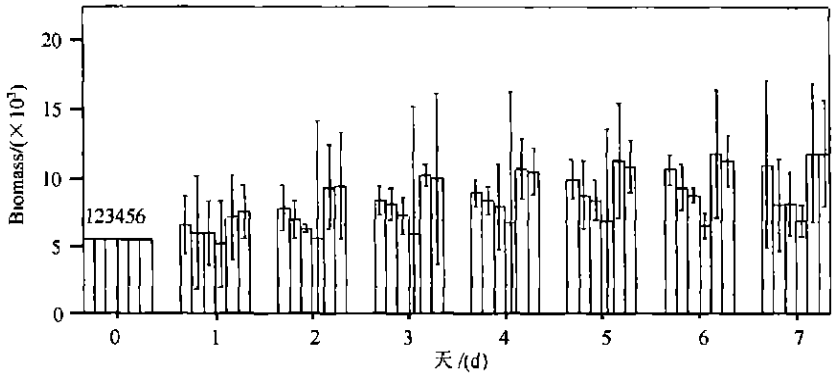


Fig. 2　Effect of different concentrations of the extract of acetone of *E. crassipes* roots on the growth of *A. tamarense*, biomass (1 ×10³ cells/mL) 1: 0.012g/L, 2: 0.018g/L, 3: 0.022g/L, 4: 0.038g/L, 5: control 1, 6: control 2

2.3　Effect of the extract of ethyl acetate of E. crassipes roots on A. tamarense

The extract of ethyl acetate of *E. crassipe* roots had a significantly negative impact on *A. tamarense* growth (Fig. 3). The biomass were less ($P < 0.05$) than that of control in cultures at high concentrations of ethyl acetate extracts. The inhibitory effects on *A. tamarense* were increased with the increased concentrations of the ethyl acetate extract of *E. crassipes* roots. When the concentration of the extracts was 0.022 and 0.035g/L, respectively, the growth of *A. tamarense* was inhibited above 50% on the day 7. At the concentration of 0.005g/L, the growth of algae was above 20% inhibited in day 7.

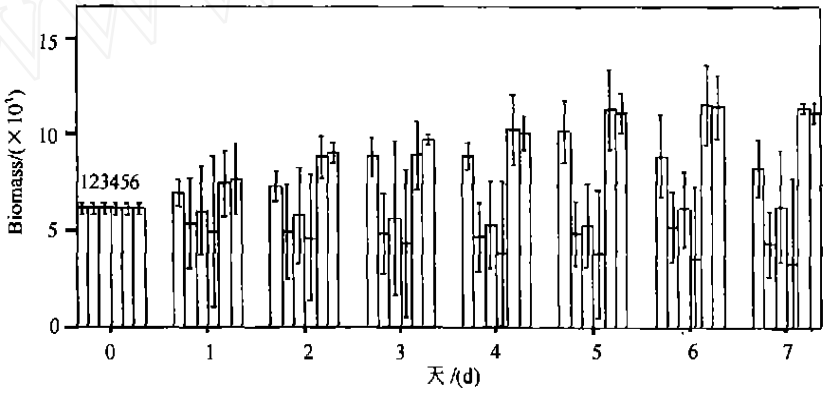


Fig. 3　Effect of different concentrations of the extract of ethyl acetate of *E. crassipes* roots on the growth of *A. tamarense*, biomass (1 ×10³ cells/mL) 1: 0.005g/L, 2: 0.022g/L, 3: 0.020g/L, 4: 0.035g/L, 5: control 1, 6: control 2

3　Discussion

This study illuminated allelopathic effects of *E. crassipes* roots on HAB alga, *A. tamarense*. The results showed that *A. tamarense* growth was strongly inhibited by the dry powder of *E. crassipes* roots at high concentrations. On the contrary, the growth of algae was stimulated after several days at certain concentrations. The results provided strong support for the ideas[13] that the allelopathic substances had stimulatory effects at low concentrations, yet had inhibitory effects at high concentrations.

The decrease in growth of *A. tamarense* by the extract of acetone/ethyl acetate of *E. crassipes* roots was significant with the increase in concentrations of the extract, but the inhibition was weaker than that by the dry powder of *E. crassipes*. These could be mainly put down to the synergism of various allelopathic substances[14]. The mixture of allelopathic substances might have stronger allelopathic effects than that of the single. The dry power of *E. crassipes* roots had more kinds of allelopathic substances than that of the extracts[9]. In addition, contact effects such as adsorption might involve in the inhibitory effect.

During the short-term algicidal effect assays, the

change in shape of *A. tamarense* was observed under an inverted microscope. The algal cells were turgid and ruptured finally at high concentrations of powder and extracts, whereas few cells shape or mobility changes occurred at low concentrations. These results were consistent with the speculation by Li and Shen[15,16] that *E. crassipes* might inhibit the growth of algae through the destruction of chlorophyll a, plasma membrane and negative impact on some enzymes by allelochemicals such as naphthalene. Compared to the strong inhibition of *E. crassipes* on algae in freshwater such as *Scenedesmus arcuatus*, the negative impact on HAB alga was relatively weak. Tang[12] described that *E. crassipes* could destroy the *S. arcuatus* cells membranes and solubilize protein, at last, kill the cells. The difference in effect of *E. crassipes* on various algae may be a result of outermost layer structures between *S. arcuatus* and *A. tamarense* cells. The *A. tamarense* cells are covered by hard shells, which protected the cells from the allelochemicals. However, the inhibitory effects of powder and extracts of *E. crassipes* on *A. tamarense* demonstrated that allelochemicals from *A. tamarense* were active even against phytoplankton with rigid cell walls, not as the allelochemicals in *U. pertusa*, which were active only against phytoplankton without cell coverings[8].

E. crassipes are widely distributed all over the world and is easy to be collected and cultivated. In this study it had been showed that both the dry powder and the extract of acetone/ethyl acetate of *E. crassipes* roots can effectively inhibit the growth of *A. tamarense*. However, it was impossible for *E. crassipes* to be directly used to control HAB due to its disturbance to environment. These suggest that allelochemicals from *E. crassipes* roots may be a potential candidate in HAB control.

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凤眼莲根系分泌物对塔玛亚历山大藻的化感作用

陈芝兰　杨维东　刘洁生　谢　瑾　江天久
(暨南大学生命科学技术学院,广州　510632)

摘要:以塔玛亚历山大藻香港株(*Alexandrium tamarense*,HK strain)为材料,研究了凤眼莲(*Eichhornia crassipes*)根系分泌物及其丙酮、乙酸乙酯提取物对塔玛亚历山大藻的化感作用(Allelopathic effect)。凤眼莲根粉末的用量为 0.1g/L、0.5g/L、1.0g/L、1.5g/L 和 2.0g/L;丙酮、乙酸乙酯提取物的用量为 0.01g/L、0.03g/L、0.05g/L 和 0.07g/L。由于凤眼莲根系丙酮、乙酸乙酯提取物水溶性比较低,实验中,溶剂提取物置于盖玻片上。培养体系中溶剂提取物的实际浓度通过实验前后盖玻片的差值获得。实验结果表明,凤眼莲根粉末用量大于 1.5g/L,对塔玛亚历山大藻抑制率 100%,0.5g/L 和 1.0g/L 4d 后开始有促进生长的作用。凤眼莲根系丙酮、乙酸乙酯提取物的实际浓度达到 0.038g/L、0.022g/L时,对塔玛亚历山大藻抑制超过 50%。凤眼莲作为杀藻剂的材料有一定的应用前景。

关键词:凤眼莲;塔玛亚历山大藻;化感作用;有害藻华

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