

学校编码: 10384

分类号_____密级_____

学号: B200426013

UDC_____

厦 门 大 学

博 士 学 位 论 文

基于化感作用的环境友好型抑藻剂的研究

Study on Environment-Benign HABs-Inhibitors Based on

Allelopathy

周立红

指导教师姓名: 郑天凌 教授

专 业 名 称: 微生物学

论文提交日期: 2008年6月15日

论文答辩时间: 2008年7月28日

学位授予日期:

答辩委员会主席: 李少菁 教授

评 阅 人: _____

2008年7月

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果,均在文中以适当方式明确标明,并符合法律规范和《厦门大学研究生学术活动规范(试行)》。

另外,该学位论文为()课题(组)的研究成果,获得()课题(组)经费或实验室的资助,在()实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称,未有此项声明内容的,可以不作特别声明。)

声明人(签名):

年 月 日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

1. 经厦门大学保密委员会审查核定的保密学位论文，
于 年 月 日解密，解密后适用上述授权。

2. 不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年 月 日

目 录

中文摘要	I
英文摘要	a
第一章 绪论	1
第一节 抑藻剂的类型	2
1.1 物理性抑藻剂	2
1.2 化学性抑藻剂	2
1.3 生物化学性抑藻剂	3
第二节 基于化感作用的抑藻剂的研究现状	3
2.1 研究背景	3
2.2 研究成果	4
第三节 化感物质抑藻剂的应用前景	10
第四节 本研究的技术路线、目的和意义	11
4.1 研究目的、意义和内容	11
4.2 技术路线	13
第二章 抑藻材料筛选与抑藻作用的实验研究	14
第一节 材料与方法	14
1.1 材料	14
1.2 实验方法	16
第二节 结 果	18
2.1 14 种抑藻材料干粉对赤潮藻 ATDH01 的影响	18

2.2 单种中草药对 ATDH01 细胞活力的影响	19
2.3 中草药药物浓度与抑藻率的效应关系	21
2.4 两种中草药联合对 ATDH01 活力的影响	22
2.5 相同浓度蒜液对不同赤潮藻的抑制作用	23
2.6 不同浓度蒜液对 3 种赤潮藻的抑制作用	24
2.7 大蒜素对 ATDH01 的抑制作用	26
第三节 讨 论	28
3.1 中草药对 ATDH01 细胞活力的抑制作用	28
3.2 大蒜对赤潮藻的抑制作用	31
3.3 小结	33
第三章 大蒜抑藻剂的抑制机理研究	35
第一节 材料与方法	35
1.1 材料	35
1.2 实验方法	35
第二节 结 果	42
2.1 影响大蒜抑藻能力的因素	42
2.2 大蒜抑藻剂对赤潮藻酶活性的影响	46
2.3 抑藻剂对藻细胞生化组成的影响	51
2.4 抑藻剂对藻细胞光合作用及呼吸作用的影响	55
2.5 细菌对抑藻剂抑藻效果的影响	58
第三节 讨 论	59
3.1 影响大蒜抑藻能力的因素	59

3.2 抑藻剂对赤潮藻酶活性的影响	60
3.3 抑藻剂对藻细胞生化组成的影响	62
3.4 抑藻剂对藻细胞光合作用及呼吸作用的影响	63
3.5 小结	64
第四章 抑藻剂的生态安全性研究	65
第一节 材料与方法	65
1.1 材料	65
1.2 实验方法	65
第二节 结 果	68
2.1 抑藻剂对有益单细胞藻类的影响	68
2.2 抑藻剂对浮游动物的影响	69
2.3 抑藻剂对常见水产养殖生物幼体的毒性	79
第三节 讨 论	100
3.1 抑藻材料对不同生物的毒性	100
3.2 同种动物对不同抑藻材料的抗性	101
3.3 抑藻材料综合分析	101
3.4 小结	105
第五章 抑藻剂的应用研究	107
第一节 材料与方法	107
1.1 实验材料	107
1.2 实验方法	107
第二节 结 果	110

2.1 中药抑藻剂的作用效果	110
2.2 大蒜抑藻剂的作用效果	111
2.3 不同处理方式的茶叶抑藻能力比较	113
第三节 讨 论	117
3.1 中草药抑藻剂	117
3.2 大蒜抑藻剂	118
3.3 茶叶抑藻剂	120
3.4 抑藻剂应用前景分析	122
3.5 小结	124
结语	125
参考文献	129
在学期间参加的科研项目及成果	139
致谢	141

Contents

Abstract in Chinese	I
Abstract in English	a
Chapter 1 Introduction	1
Section 1 Types of algicides	2
1.1 Physical algicides	2
1.2 Chemical algicides	2
1.3 Biochemical algicides	3
Section 2 Status of algicides research based on Allelopathy	3
2.1 Background of research	3
2.2 Fruits of study on allelopathy	4
Section 3 Outlook on application of allelopathic algicides in algal control	10
Section 4 Technical proposal, aims and significance of this study	11
4.1 Aims, significances and contents of this study	11
4.2 Technical proposal	13
Chapter 2 Selection of algicidal substances	14
Section 1 Materials and methods	14
1.1 Materials	14
1.2 Methods	16
Section 2 Results	18
2.1 Effects of 14 kinds of powder on <i>Alexandrium tamarense</i> DH01	18

2.2 Effects of Chinese herbs on mobility of <i>A. tamarense</i> DH01	19
2.3 Relationships between algal inhibitory rate and concentration of Chinese herbs	21
2.4 Effects of combined Chinese herbs on mobility of <i>A. tamarense</i> DH01	22
2.5 Inhibition of garlic solution on various algae causing redtide	23
2.6 Inhibition of garlic solution in different concentration on 3 species of algae	24
2.7 Inhibition of diallyl trisulfide on <i>A. tamarense</i> DH01	26
Section 3 Discussions	28
3.1 Inhibition of Chinese herbs on mobility of <i>A. tamarense</i> DH01 ..	28
3.2 Inhibition of garlic on algae causing redtide	31
3.3 Conclusion	33
Chapter 3 Research on the inhibitory mechanisms of garlic algicides to algae	35
Section 1 Materials and methods	35
1.1 Materials	35
1.2 Methods	35
Section 2 Results	42
2.1 Factors to influence algal inhibition of garlic	42
2.2 Effects of garlic algicides on the enzymatic activity of algae	46
2.3 Effects of algicides on the cell component of algae	51
2.4 Effects of algicides on the photosynthesis and respiration	55

2.5 Effects of bacterium on the algal inhibition with algicides	58
Section 3 Discussions	59
3.1 Factors to influence the algal inhibition of garlic	59
3.2 Effects of algicides on the enzymatic activity of algae	60
3.3 Effects of algicides on the cell component of algae	62
3.4 Effects of algicides on the photosynthesis and respiration	63
3.5 Conclusion	64
Chapter 4 Study on the ecological safety of algicides	65
Section 1 Materials and methods	65
1.1 Materials	65
1.2 Methods	65
Section 2 Results	68
2.1 Effects of algicides on useful algae	68
2.2 Effects of algicides on zooplankton	69
2.3 Toxicity of algicides on juveniles of some familiar aquicultural organisms	79
Section 3 Discussions	100
3.1 Toxicity of algicidal substances on various organisms	100
3.2 Resistance of same animals on various algicidal substances	101
3.3 Integrated analysis to algicidal substances	101
3.4 Conclusion	105
Chapter 5 Study on the algicidal applications	107
Section 1 Materials and methods	107

1.1 Materials	107
1.2 Methods	107
Section 2 Results	110
2.1 Effects of algicides made from Chinese herbs	110
2.2 Effects of algicides made from garlic	111
2.3 Comparison of algal inhibition by tea treated in different methods	113
Section 3 Discussion	117
3.1 Chinese herb algicides	117
3.2 Garlic algicides	118
3.3 Tea algicides	120
3.4 Outlook of algicidal application	122
3.5 Conclusion	124
Summary	125
References	129
Research projects involved and achievements obtained in the period of doctoral degree study	139
Acknowledgements	141

摘 要

当前,有害藻类爆发现象是一个全球性问题,能导致水环境恶化、损害水体正常利用,而抑藻剂的开发与应用被认为是效果显著和极具潜力的应对措施之一。本研究论文以化感物质抑藻的新理念为基础,广泛发掘抑藻剂资源,结合生态安全性分析等进行系列的实验研究,研制出 7 种抑藻能力强的环境友好型抑藻剂,并探讨环境条件和吸附剂等对抑藻剂抑藻作用的影响。同时,还根据藻类的生理和生化性质进一步探讨抑藻剂的抑藻作用机理,为抑藻剂的科学应用提供理论依据。通过研究获得了如下主要结果:

1、筛选出多种具有高效抑藻活性的材料

根据化感作用原理,选择 15 种常见陆生植物为原材料进行抑藻实验,通过比较抑制率、抑制时间、抑藻浓度等参数,筛选出对塔玛亚历山大藻(*Alexandrium tamarense* DH01)抑制作用较强的有乌梅(*Fructus Mume*)、黄连(*Rhizoma Coptidis*)、大黄(*Radix et Rhizoma Rhei*)、绿茶、槟榔(*Semen Arecae*)、大青叶(*Folium Isatidis*)等 6 种材料,处理浓度为 1 g L^{-1} 时 1 d IR 为 90.13-100%;其次是红茶、板兰根(*Radix Isatidis*)、苦参根(*Radix Sophorae Flavescentis*)、鱼腥草(*Herba Houttuyniae*)、鹤虱(*Fructus Carpesii*), 1 d IR 为 74.77 - 88.31%;此外,高抑藻率(90 - 100%)至少可以保持 5 d。使君子(*Fructus Quisqualis*)不具抑制该藻的能力。进一步研究了板兰根、苦参根、鱼腥草、黄连及槟榔等 5 种药物两两联用的抑藻作用效果,结果显示,板兰根+苦参根、板兰根+鱼腥草、鱼腥草+苦参根等联合使用比单种时抑藻效果明显加强。另外,还发现经反复冲泡后的绿茶渣仍然具有很好的抑藻作用,其资源再利用价值值得关注。

研究了大蒜液对常见的 6 种赤潮藻类的抑藻谱,结果显示:大蒜液可强烈抑制塔玛亚历山大藻(*A. tamarense* DH01)、锥状斯氏藻(*Scrippsiella trochoidea*)、链状亚历山大藻(*A. catenella* DH01)和 *A. satoanum* DH01 等几种赤潮藻类,其有效抑藻浓度为 0.04%。不能抑制微小亚历山大藻(*A. minutum* TW01)及牟氏角毛藻(*Chaetoceros muelleri*)。此外,还发现大蒜素也具有抑制 ATDH01 的能力,有效抑藻浓度为 1 mg L^{-1} 。藻液经大蒜液及大蒜素处理 3 h 后(有效抑藻浓度下)抑藻率一般能达到 90%以上。

2、阐释了大蒜抑藻剂的抑制机理

以 *A. tamarense* DH01 为模式藻研究了大蒜液及大蒜素对藻生理生化的影响, 结果发现实验浓度下大蒜液及大蒜素能使 ATDH01 藻的蛋白质、叶绿素、类胡萝卜素等重要组分的相对含量降低, 提高藻的 CAT 酶活力, 降低 SOD 酶及抗超氧阴离子自由基相对活性; 同时, 能明显降低藻的光合作用速率、增加呼吸速率。可见, 大蒜液及大蒜素是通过改变藻的重要的细胞物质组成, 使其生理功能异常, 进而导致细胞活动受抑制甚至发生死亡。另外, 还发现大蒜液的抑藻性能相当稳定, 100℃水浴 1 h 对其影响不大, 而保存时间及温度对其抑藻能力也没有明显影响。

3、评价了抑藻剂的生态安全性

研究了抑藻剂对常见 3 种有益藻类的生长抑制, 以及对 3 种浮游动物、2 种稚虾和 2 种幼鱼的毒害作用。根据 24-h LC_{50} 对抑藻材料生态安全性进行分析, 结果发现大蒜素浓度高达 10 mg L^{-1} 在 96 h 不会明显影响小球藻 (*Chlorella* sp.)、球等鞭金藻 (*Isochrysis galbana*) 及牟氏角毛藻的生长, 0.32% 大蒜液不会影响牟氏角毛藻生长; 大蒜液对各种生物的毒性由小到大为: 褶皱臂尾轮虫群体、真鲷幼鱼、蒙古裸腹蚤、花尾胡椒鲷、褶皱臂尾轮虫 (同龄)、5 日龄卤虫、斑节对虾稚虾、凡纳滨对虾稚虾; 大蒜素对不同动物的毒性由小到大为: 真鲷幼鱼、蒙古裸腹蚤、褶皱臂尾轮虫 (同龄)、花尾胡椒鲷幼鱼、褶皱臂尾轮虫群体、5 日龄卤虫、斑节对虾稚虾、凡纳滨对虾稚虾。

凡纳滨对虾稚虾对抑藻材料的耐受能力由强到弱为: 绿茶、槟榔、黄连、大蒜素。斑节对虾稚虾对抑藻材料的耐受能力由强到弱为: 秦皮、鱼腥草、槟榔、黄连、绿茶、鹤虱、榧子、板蓝根、乌梅、苦参根、大黄、大蒜素。

本论文所提出的效率-安全比概念综合考虑了抑藻剂的抑藻效果、生态安全性、成本等因素, 有助于完善有实际应用价值的生态安全抑藻剂的筛选, 由此发现绿茶、大蒜液、大蒜素、秦皮、鱼腥草、槟榔、黄连是抑藻效果好、环境风险低的抑藻材料。

4、提出了抑藻剂的应用策略与实施方法

通过比较抑藻材料在水浸提液、干粉、冻干粉等不同形态下的抑藻能力, 观

察不同吸附剂及载体对抑藻能力的影响,结合抑藻剂的成本分析,制备了几种抑藻剂成品,并提出了抑藻剂的使用水域及较为合理的使用方法。发现中草药等抑藻材料水浸提液与干粉的抑藻能力基本相当,前者作用速度略快,但后者作用持续较长;采用芦荟和海带干粉作为吸附剂可延长中草药抑藻剂在水层中的停留及作用时间,进而提高抑制率。

采用浮石、纱布为基质的大蒜抑藻剂抑藻能力比陶粒大蒜抑藻剂强;大蒜滤液、蒜渣及全蒜冻干粉的抑藻效果优于新鲜大蒜滤液,抑藻率约相差 10%,但是考虑到制作成本及时间等因素,使用新鲜大蒜液抑藻更切实可行。采用纱布、筛绢或无纺布拖袋方式使用大蒜抑藻剂,是高效、方便、快速的方法。

关键词: 抑藻剂; 环境友好; 生态安全; 化感作用; 中草药; 大蒜.

ABSTRACT

Nowadays, harmful algae blooms (HABs) is a worldwide phenomenon that has led to the deterioration of aquatic environments, prejudicing the many uses to which the water is put, and the development and application of algicides has been considered as one of the potential methods to control HABs available. Based on the allelopathy and using algicidal test and ecological safety analysis of some natural plant materials, seven HABs-inhibitors showing strong algal inhibition and low risk for aquatic environment were developed, and the effects of environmental conditions and sorbents on the algicides were studied. Meanwhile, the mechanisms of algal inhibition have been studied by means of the research to the algal physiology and biochemistry. The dissertation provides the basic knowledge for further application of these algicides. The main results of this study were as follow:

1. According to the allelopathy relationship, several materials with strong algal inhibition have been screened out from fifteen species of familiar vegetation according to the inhibitory rates (*IR*), the inhibitory time, the effective inhibitory concentrations and so on. As for a red-tide causing alga-*Alexandrium tamarense* strain DH01, the most effectual inhibitory materials were found to be Fructus Mume, Rhizoma Coptidis, Radix et Rhizoma Rhei, green tea, Semen Arecae, Folium Isatidis, with the 1 d *IR* at concentration 1 g L⁻¹ from 90.13% to 100%; followed by red tea, Radix Isatidis, Radix Sophorae Flavescentis, Herba Houttuyniae and Fructus Carpesiim, which 1 d *IR* was more than 70% (74.77 – 88.31); moreover, the high *IR* (90 - 100%) could last at least 5 days. However, Fructus Quisqualis couldn't inhibit ATDH01.

The algal inhibition test showed that herb combinations such as Radix Isatidis + Radix Sophorae Flavescentis, Radix Isatidis + Herba Houttuyniae, Herba Houttuyniae + Radix Sophorae Flavescentis and so on inhibited algae more effectively than that these herbs individual. In addition, the residua of green tea dunked many times, having still good algicidal effect, should be paid attention to because it may be a cheap resource of algicides.

The inhibition of six familiar red-tide causing algae by garlic solution were researched, and the results showed that garlic solution can strongly inhibit *A.tamarensis* strain DH01, *Scrippsiella trochoidea* strain XM01, *A.catenella* strain DH01 and *A.satoanum* strain DH01 with an effective concentration 0.04%, but it endows no inhibition to *A. minutum* strain TW01 and *Chaetoceros muelleri*. Moreover, diallyl trisulfide was also found the inhibition to ATDH01 and the effective concentration was 1 mg L⁻¹. Both garlic solution and diallyl trisulfide can inhibit algae fast, generally, the algal inhibitory rate were more than 90% after 3 hours experiment at the effective concentrations.

2.The mechanisms of garlic algicide were also clarified. *A.tamarensis* strain DH01 was used as the model alga to study the effect of garlic solution and diallyl trisulfide on the algal physiology and biochemistry. The results showed that both garlic solution and diallyl trisulfide at the concentrations tested could decrease the relative contents of key components in algae such as protein, chlorophyll and carotenoids. With the increase of the concentrations of garlic solution or diallyl trisulfide, the activity of algal catalase increased also. By contrary, the activities of superoxide dismutase and anti-superoxide anion free radical in the algae of test groups were mostly lower than the control with a fluctuation. In general, garlic solution and diallyl trisulfide could significantly decrease the photosynthetic rate and increase the respiratory rate. It can be inferred that garlic solution and diallyl trisulfide can inhibit algal mobility and even bring to death by changing the components of cells and making the algal metabolism abnormal. In addition, the algicidal effect of garlic solution was steady; it reduced a little even if garlic solution was boiled in 100°C for one hour. Indeed no significant difference of IR values was found among times or temperatures for garlic solution store.

3. The ecological safety of these algicidal materials has been assessed using 24-h LC_{50} values obtained in the growth tests of three familiar species of algae, the toxicity tests for three species of zooplanktons, two species of juvenile prawns and two species of juvenile fish. The results showed that the growth of *Chlorella* sp., *Isochrysis galbana*

Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.

厦门大学博硕士论文摘要库