

缘管浒苔对球等鞭金藻生长的克生作用

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摘要: 研究了缘管浒苔新鲜组织、干粉末、培养水过滤液和水溶液抽提液对球等鞭金藻生长的克生作用。结果表明,缘管浒苔新鲜组织、干粉末和水溶性抽提液对球等鞭金藻的生长具有强烈的克生作用。当培养液中缘管浒苔新鲜组织、干粉末和水溶性抽提液的浓度为 5.0 g-wet/L、1.2 g-dry/L 和 1.0 g/L 时,球等鞭金藻不能够维持正常的生长或全部死亡。在缘管浒苔培养水过滤液培养实验中,一次性添加缘管浒苔培养滤液,不能持久地抑制球等鞭金藻增殖,而半连续添加缘管浒苔培养滤液,其克生作用较强,说明克生物质的连续分泌是有效抑制球等鞭金藻生长的关键。经高温高压处理后的缘管浒苔培养滤液对球等鞭金藻无显著的抑制作用,说明缘管浒苔所分泌的克生物质在高温高压下不稳定和易分解。

关键词: 缘管浒苔; 球等鞭金藻; 克生作用

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由于沿海区域的富营养化而导致的大型绿藻过量生长已经成为世界沿海范围内越来越普遍的现象,这种现象被称为“绿潮”^[1]。很多研究证明,大型绿藻的藻华从不同方面对海洋潮间带生态系统造成了有害影响,包括生态系统结构的改变^[2]和生物多样性的丧失^[3]。另外,绿潮也能影响当地的渔业、水产养殖业和滨海旅游业^[4-5]。在近海生态系统中,大型绿藻生物量与其富营养化程度之间有着显著的正相关关系,可以作为近海海域水体富营养化的生物指示物^[6],并且大规模生长的大型绿藻可以吸收过量的营养盐和一些毒素,扮演着重要的“反硝化反应器”的作用^[7]。研究证明,水生生物间的克生作用确实能够改变水域生态系统的结构和交替顺序^[8-11]。因此,大型绿藻生长过程中能起到生态修复的作用。

2008年以来,在我国黄海海域爆发了主要由浒苔绿藻爆发性增殖引发的绿潮灾害,其规模之大,历史罕见,已对我国海洋生态环境和海洋经济

造成了一定的影响。但目前对我国绿潮的溯源、成因、过程和对海洋生态系统的影响等问题缺乏足够的科学认识。缘管浒苔(*Ulva linza*)是普遍的能形成绿潮的大型绿藻^[4,12-13],也是形成我国黄海绿潮的大型绿藻之一^[14]。本文比较系统地研究了缘管浒苔对球等鞭金藻(*Isochrysis galbana*)生长的克生作用,为深入探讨沿海区域大型绿藻与微藻之间的克生作用,解释绿潮对海洋生态环境的影响提供科学依据。

1 材料与方法

1.1 藻种和培养条件

缘管浒苔于2009年5月采自江苏如东海区紫菜栽培架上,采集后用经高温高压消毒过的海水仔细地清洗,以去除泥污和附生生物,无菌培养于VSE培养液中。球等鞭金藻购自中国科学院海洋研究所藻种库,培养于f/2培养液中。以上藻种均培养于温度为20℃,盐度30,光强为60 μmol photons/

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($\text{m}^2 \cdot \text{s}$),光周期为 12L:12D 的培养箱中。每天定时晃动微藻培养瓶两次,以防止其附壁生长。

1.2 球等鞭金藻生长曲线绘制

将球等鞭金藻接种于 200 mL 经 f/2 营养盐加富的 100 mL 培养基中,初始密度为 1×10^4 ind/mL,实验设置 4 个重复。每天定时摇动培养瓶两次,以防止其附壁生长。每天定时在每个培养瓶中采样 1 mL,用 Lugol 氏液固定,并用血球计数板在 Olympus 光学显微镜下计数。然后每个培养瓶中加入 1 mL 含有 100 mL 培养液所有 f/2 成分营养液以避免营养耗尽。实验进行 23 d,根据藻类细胞计数结果绘制球等鞭金藻的生长曲线。

1.3 实验设计

缘管浒苔新鲜组织对球等鞭金藻生长的影响

实验采用共培养系统。实验在盛有 200 mL 培养液的 250 mL 三角瓶中进行。将处于对数生长期的球等鞭金藻接种到新鲜培养液中,初始密度为 5×10^4 ind/mL。缘管浒苔新鲜组织的接种量设定为(湿重)0、0.5、1.0、2.0 和 5.0 g/L。每组均设 4 个重复,实验进行 7 d。营养盐补充和微藻细胞计数方法同 1.2。

缘管浒苔培养滤液对球等鞭金藻生长的影响

(1) 一次性培养。将生物量为 10 g-wet/L 的缘管浒苔在 f/2 培养液中培养 3 d 后,将培养液用高压灭菌过的滤膜(Whatman GF/C, 0.22 μm 孔径)过滤,并且用 40 倍 f/2 营养液重新加富,立即接种处于对数生长期的球等鞭金藻,初始密度为 1×10^5 ind/mL。以相同条件下培养于 f/2 加富海水中的球等鞭金藻为对照组。实验于盛有 40 mL 培养液的 100 mL 三角烧瓶中进行,每组设置 4 个重复,实验进行 7 d。

(2) 半连续培养。按照(1)中的方法将球等鞭金藻接种于缘管浒苔培养水过滤液中。每天从培养瓶中移出 10 mL 培养液,然后加入 10 mL 营养重新加富的大藻培养水过滤液以保持培养液体积的恒定。作为对照,以 f/2 培养液代替重新加富的大藻培养水过滤液。实验设置同(1)。

(3) 经高温高压处理的缘管浒苔培养水过滤液。同(1)的方法制备缘管浒苔培养滤液,于 121 $^{\circ}\text{C}$ 高压灭菌 20 min 后冷却并加富,接种球等鞭金藻。以相同条件下培养于 f/2 加富消毒海水中的球等鞭金藻为对照组。实验设置同(1)。

缘管浒苔干粉对球等鞭金藻生长的影响

新鲜的缘管浒苔组织于 60 $^{\circ}\text{C}$ 恒温干燥 3 d,然后用研钵研磨成粉末。实验在盛有 200 mL 培养液的 250 mL 三角瓶中进行。将处于对数生长期的球等鞭金藻接种到新鲜培养液中,初始密度为 1×10^5 ind/mL。缘管浒苔干粉末的初始接种浓度从 0、0.2、0.4、0.6 和 1.2 g-dry/L。每组设置 4 个重复,实验进行 6 d。营养盐补充和微藻细胞计数方法同 1.2。

缘管浒苔水溶性抽提液对球等鞭金藻生长的影响 将 10 g-wet/L 的缘管浒苔加少许蒸馏水研磨成浆,用消毒海水 4 $^{\circ}\text{C}$ 下以 6 000 r/min 离心 10 min,重复 3 次,收集上清液共 100 mL 作为母液,再用消毒海水作不同稀释倍率,浓度分别为 0.1、0.2、0.5 和 1.0 g/L,后用 40 倍 f/2 营养液重新加富,立即接种处于对数生长期的球等鞭金藻,初始密度为 2×10^5 ind/mL。以相同条件下培养于 f/2 加富消毒海水中的球等鞭金藻为对照组。每组均设 4 个重复,实验进行 6 d。

1.4 数据处理

数据用 SPSS 13.0 软件做 ANOVA 分析,并进行 Duncan 氏多重比较,以 $P < 0.05$ 作为差异显著水平,所得数据结果均以平均值 \pm 标准差表示。

2 结果与分析

2.1 球等鞭金藻的生长曲线

由图 1 可知,球等鞭金藻在接种后的 12 ~ 13 d 后达到对数生长期,对数生长期的持续时间为 5 ~ 6 d。

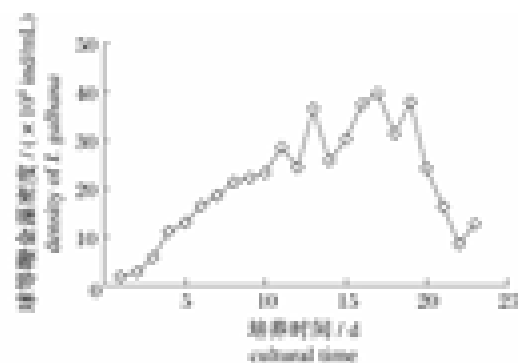


图 1 球等鞭金藻的生长曲线

Fig. 1 Growth curve of *I. galbana*

2.2 缘管浒苔鲜组织对球等鞭金藻生长的作用

与对照组相比,各实验组缘管浒苔对共培养的

球等鞭金藻生长均有显著抑制作用($P < 0.01$),尤其是5 g-wet/L组,球等鞭金藻不能维持正常生长。实验结束后,0.5、1.0、2.0和5.0 g-wet/L组

中的球等鞭金藻的种群密度分别比对照组降低了16.32%、27.38%、43.37%和85.12%(表1)。

表1 与不同接种密度的缘管浒苔接触7 d的球等鞭金藻的种群密度
Tab.1 The densities of *I. galbana* coexisting with different inoculation density of *U. linza* fresh tissue during seven days

缘管浒苔新鲜组织的 接种密度(湿重)(g/L) inoculation of <i>U. linza</i> fresh tissue	球等鞭金藻种群密度 the densities of <i>I. galbana</i> (10^4 ind/mL)						
	1 d	2 d	3 d	4 d	5 d	6 d	7 d
0.0	7.0 ± 3.5 ^a	9.0 ± 1.9 ^a	14.6 ± 2.3 ^a	14.7 ± 3.4 ^a	26.6 ± 1.9 ^a	23.7 ± 2.3 ^a	28.0 ± 1.0 ^a
0.5	7.0 ± 3.5 ^a	8.3 ± 3.3 ^b	11.9 ± 3.9 ^b	10.5 ± 1.9 ^b	12.7 ± 2.0 ^b	17.7 ± 2.7 ^b	23.4 ± 3.5 ^b
1.0	7.0 ± 3.5 ^a	7.0 ± 2.8 ^c	7.8 ± 1.7 ^c	8.8 ± 2.5 ^c	12.0 ± 4.0 ^b	15.0 ± 4.2 ^c	20.3 ± 3.7 ^c
2.0	7.0 ± 3.5 ^a	7.1 ± 2.0 ^c	7.3 ± 1.4 ^c	9.6 ± 3.4 ^d	8.5 ± 3.2 ^c	12.2 ± 3.7 ^d	15.9 ± 2.8 ^d
5.0	7.0 ± 3.5 ^a	7.2 ± 1.9 ^c	7.3 ± 0.9 ^c	9.6 ± 1.5 ^d	7.7 ± 3.0 ^d	5.8 ± 0.9 ^c	4.2 ± 1.0 ^c

注:表中的数值为平均值 ± 标准误,同列有不同字母表示经 ANOVA 分析后差异显著($P < 0.05$)。下同。

Notes: The data in the table indicates means ± SE ($n=4$); Means in the same column followed by different letters are significantly different by ANOVA analyse ($P < 0.05$). The same below.

2.3 缘管浒苔培养滤液对球等鞭金藻生长的作用

一次性培养 由图2可知,实验组与对照组微藻种群密度均不断递增。方差分析表明,将球等鞭金藻接种至缘管浒苔的培养滤液后的第2~3天内,实验组的球等鞭金藻种群密度均极显著低于对照组($P < 0.01$),此后培养滤液对球等鞭金藻的生长已无显著抑制作用($P > 0.05$)。

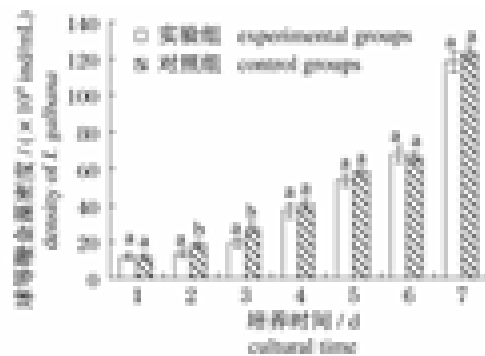


图2 缘管浒苔培养过滤液一次性添加对球等鞭金藻生长的影响

Fig.2 Allelopathic effects of *U. linza* culture medium filtrate under initial filtrate addition on the growth of *I. galbana*

半连续培养 在半连续培养方式下,实验组球等鞭金藻种群密度增长缓慢,在第5天时达到 3.8×10^5 ind/mL,然后逐渐降低,7 d后,种群密度比对照组降低了82.64%(图3)。方差分析

表明,从实验开始的第3天开始,实验组球等鞭金藻的种群密度显著低于对照组($P < 0.01$)。

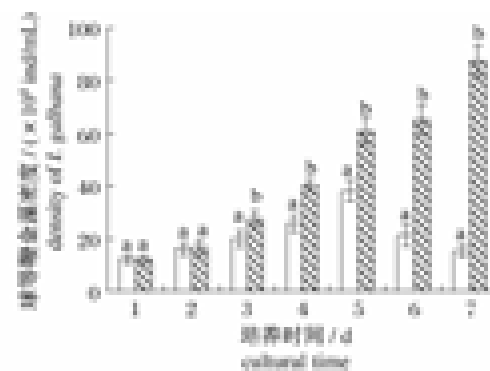


图3 缘管浒苔培养过滤液连续性添加对球等鞭金藻生长的影响

Fig.3 Allelopathic effects of *U. linza* culture medium filtrate under semicontinuous filtrate addition on the growth of *I. galbana*

经高温高压处理的缘管浒苔培养滤液 实验组与对照组的球等鞭金藻种群密度日渐递增(图4),方差分析表明经高温高压处理后的缘管浒苔滤液对球等鞭金藻生长无显著影响($P > 0.05$)。

2.4 缘管浒苔干粉末对球等鞭金藻生长的作用

在添加缘管浒苔干粉末最高浓度5.0 g-dry/L的实验组,球等鞭金藻经培养6 d后完全死亡(表2);添加浓度为0.6 g-dry/L时,球等鞭金藻的生长受到显著抑制($P < 0.01$),实验结束后,种群密度比对照组降低了59.68%;添加相对低浓度

(0.4和0.2 g-dry/L)干粉末后,与对照组相比,球等鞭金藻生长受到显著抑制($P < 0.01$),但4 d后细胞增长速率明显加快,实验结束后,种群密度仅比对照组分别降低了9.18%和3.22%。

2.5 缘管浒苔水溶性抽提液对球等鞭金藻生长的作用

不同浓度的缘管浒苔水溶性抽提液对球等鞭金藻生长均有显著抑制作用,抑制作用随着抽提液浓度增加而加强(表3)。在1.0 g/L抽提液处理下,第6天可使球等鞭金藻完全致死。抽提液浓度为0.5 g/L可使球等鞭金藻种群密度始终保持较低水平,第6天时抑制率为89.42%。相对低浓度(0.2和0.1 g/L)抽提液组,球等鞭金藻种群密度仍在逐渐增加,培养至第6天时抑制率分别为30.77%和12.50%。

表2 与不同密度的缘管浒苔干粉末接触6 d的球等鞭金藻的种群密度

Tab.2 The densities of *I. galbana* coexisting with different concentration of *U. linza* dry powder during six days

缘管浒苔干粉末的浓度(干重)(g/L) concentration of <i>U. linza</i> dry powder	球等鞭金藻种群密度 the densities of <i>I. galbana</i> (10^4 ind/mL)					
	1 d	2 d	3 d	4 d	5 d	6 d
0.0	10.0 ± 2.7 ^a	16.3 ± 1.3 ^a	26.2 ± 3.3 ^a	31.3 ± 2.3 ^a	39.7 ± 4.7 ^a	43.6 ± 2.4 ^a
0.2	10.0 ± 2.7 ^a	14.1 ± 2.3 ^b	20.0 ± 4.9 ^b	25.5 ± 1.6 ^b	34.3 ± 2.8 ^b	42.2 ± 2.0 ^{ab}
0.4	10.0 ± 2.7 ^a	13.3 ± 3.2 ^b	17.2 ± 2.2 ^c	21.6 ± 1.5 ^c	30.7 ± 1.5 ^c	39.6 ± 1.8 ^b
0.6	10.0 ± 2.7 ^a	11.7 ± 1.9 ^c	11.1 ± 3.6 ^d	12.3 ± 2.1 ^d	14.8 ± 1.1 ^d	17.5 ± 1.0 ^c
1.2	10.0 ± 2.7 ^a	8.7 ± 1.1 ^d	5.0 ± 1.2 ^c	1.2 ± 1.1 ^c	0.3 ± 0.1 ^c	0.0 ^d

表3 与不同浓度的缘管浒苔水溶性抽提液接触6 d的球等鞭金藻的种群密度

Tab.3 The densities of *I. galbana* coexisting with different concentration of *U. linza* aqueous extracts during six days

缘管浒苔水溶性抽提液的浓度(g/L) concentration of <i>U. linza</i> aqueous extracts	球等鞭金藻种群密度 the densities of <i>I. galbana</i> (10^4 ind/mL)					
	1 d	2 d	3 d	4 d	5 d	6 d
0.0	21.0 ± 1.5 ^a	43.3 ± 3.5 ^a	53.2 ± 4.2 ^a	65.7 ± 5.3 ^a	93.4 ± 4.8 ^a	104.0 ± 7.1 ^a
0.1	21.0 ± 1.8 ^a	37.3 ± 12.3 ^a	46.1 ± 3.7 ^b	54.4 ± 11.9 ^a	71.7 ± 3.3 ^b	91.2 ± 3.4 ^b
0.2	21.0 ± 4.9 ^a	31.2 ± 3.3 ^b	40.3 ± 2.9 ^c	46.2 ± 5.5 ^b	60.2 ± 5.1 ^c	72.5 ± 3.2 ^c
0.5	19.0 ± 1.6 ^a	25.1 ± 2.0 ^c	21.3 ± 3.5 ^d	19.2 ± 2.8 ^c	15.3 ± 3.4 ^d	11.2 ± 2.3 ^d
1.0	19.0 ± 2.6 ^a	16.4 ± 1.1 ^d	10.3 ± 1.7 ^c	5.1 ± 2.4 ^d	0.5 ± 0.1 ^c	0.0 ^c

3 讨论

克生作用,是一种植物(包括微生物)通过向环境释放化学物质而对其附近的另一些植物产生直接或间接的、有利或有害的现象。海洋生态系统中,利用大型海藻对海洋微藻的克生作用,对赤潮进行生物防治,近年来受到较大关注。大型绿藻对赤潮微藻抑制作用的研究多见于石莼属(*Ulva*)的石莼和浒苔等大型海藻^[15-17]。许妍等^[18-19]和Jin等^[11]研究发现缘管浒苔组织内存

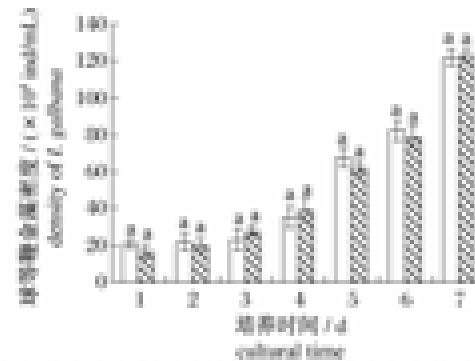


图4 经高温高压处理后的缘管浒苔培养过滤液对球等鞭金藻生长的影响

Fig.4 Effects of *U. linza* culture medium filtrate under high temperature and high pressure on the growth of *I. galbana*

在克生物质,可有效抑制赤潮异弯藻(*Heterosigma akashiwo*)生长。但关于缘管浒苔对其他海洋微藻的克生作用,还未见报道。在本研究中,我们排除了营养盐及光照竞争和微生物的作用,在实验室可控环境条件下进行缘管浒苔对球等鞭金藻克生作用的实验研究。结果表明,缘管浒苔的鲜组织、干粉末、水溶性抽提物对球等鞭金藻表现出了较强的克生作用。

在共培养实验中,高生物量的缘管浒苔可有效抑制球等鞭金藻细胞的生长。在共培养时大藻

可能会改变其生长培养液的 pH 值,从而使其不适合于微藻的生长^[20]。在共培养实验的开始和末尾,我们测定了培养液的 pH 值,并没有发现培养液 pH 值有很大的变化。因此,缘管浒苔能分泌对球等鞭金藻的生长起抑制作用的克生物质是所观察到的球等鞭金藻生长抑制现象最有可能的解释。

在培养介质中添加缘管浒苔干粉末和水溶性抽提液均可对球等鞭金藻细胞的生长产生显著抑制作用,并在高浓度下产生致死作用,表明该克生作用存在着浓度效应,当抑制物浓度达到一定阈值才出现明显的克生作用,在阈值之上浓度越高克生作用越显著^[19]。在本实验条件下,当缘管浒苔干粉末和水溶性抽提液的浓度达到 1.2 g/L 和 1.0 g/L 时,球等鞭金藻在实验进行到 6 d 后已全部死亡。

缘管浒苔培养水过滤液培养实验中,一次性添加缘管浒苔培养滤液,不能持久地抑制球等鞭金藻增殖。而半连续添加缘管浒苔培养滤液,其克生效应较强,表明缘管浒苔培养滤液中所含的克生物质含量较低,即存在于组织中克生物质浓度高于生长过程所释放的总量,因此保持环境中克生物质的浓度是抑制球等鞭金藻增殖的关键。本实验结果与 Nakai 等^[21]报道的大型水生植物穗状狐尾藻 (*Myriophyllum spicatum*) 对蓝藻生长的克生效应和 Jin 等^[11]报道的缘管浒苔对海洋原甲藻 (*Prorocentrum micans*) 生长克生作用的结果是一致的,这表明从水生生物活体中连续分泌少量易降解的克生物质可能是水域生态系统中普遍存在的一种现象。

本实验中,缘管浒苔培养滤液经高温高压处理后,对球等鞭金藻生长无显著抑制作用,表明该克藻物质不稳定、易分解。Jin 等^[11]研究表明孔石莼 (*Ulva pertusa*) 组织中含有多种不饱和脂肪酸,其中一些对赤潮异弯藻具有强烈的杀藻作用,并认为不饱和脂肪酸可能是孔石莼组织内克生物质的重要组成成分之一。不饱和脂肪酸易氧化,且被氧化后杀藻活性降低^[22-23]。缘管浒苔培养滤液中是否也存在以不饱和脂肪酸为主要成分的克藻物质,还有待通过实验进一步证实。

缘管浒苔广泛分布于沿岸低潮区,易在富营养化的河口、海湾等浅水区域中大量繁殖形成绿潮。本研究的实验结果证明,能形成绿潮的大型

绿藻——缘管浒苔对球等鞭金藻的生长具有较强的克生作用。因此,大型绿藻与其他海洋生物间化学性的相互作用在绿潮爆发期间浮游生物的组成及动态变化中可能起重要的作用,并且这也能够部分地解释这些大型绿藻之所以能在全世界沿海众多的区域内形成绿潮的原因与机制。但是,这种对海洋微藻,尤其是对海洋赤潮微藻的克生作用也使得缘管浒苔在形成绿潮的过程中改变着海洋生态系统的结构,这种改变在由富营养化引起的赤潮多发海域中无疑起着生态修复的作用。

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The allelopathic effects of *Ulva linza* on *Isochrysis galbana*

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Abstract: The excessive growth of some species from green algae, such as, *Ulva*, *Chaetomorpha* and *Cladophora*, has been reported in the formation of macroalgal blooms or green-tide events in many parts of the world including Europe, North America, South America, Japan and Australia. In June 2008 the world's largest green-tide ever covering about 600 km² occurred along the coast of the Yellow Sea. Over one million tonnes of green algae were removed from the beach and coast, which caused huge economic loss to the local government. The cause, origin tracked and threatening to the marine environment of the green-tide and the possibility of recurrence have greatly concerned government officials and scientists. During the period of green algal bloom, macroalgae could inhibit the growth of phytoplankton, which could modify the trophic mode of marine ecosystem. *Ulva linza* was a popular species of green tides that occurred along the coast of the Yellow Sea. This paper studies the allelopathic effects of the macroalgae *U. linza* on the microalgae *I. galbana*, in order to provide some theoretical proof for evaluating the effects of green algal bloom on the marine environment. Allelopathic effects of fresh tissue, dry powder, macroalgae culture medium filtrate and aqueous extracts of *U. linza* on *I. galbana* were studied in the laboratory using coexistence culture system. Different initial inoculation concentrations (0, 0.5, 1.0, 2.0 and 5.0 g-wet/L) of *U. linza* fresh tissue, (0, 0.2, 0.4, 0.6 and 1.2 g-dry/L) of *U. linza* dry powder and (0, 0.1, 0.2, 0.5 and 1.0 g/L) of *U. linza* aqueous extracts were used in coexistence culture systems. The results of the coexistence assays showed that the growth of *I. galbana* was strongly inhibited by fresh tissue, by dry powder and by aqueous extracts of *U. linza*. When the inoculation concentration of fresh tissue, dry powder and aqueous extracts was 5.0 g-wet/L, 1.2 g-dry/L and 1.0 g/L, respectively, the cells of *I. galbana* could not grow normally or died completely within six to seven days. The effects of the *U. linza* culture medium filtrate on the *I. galbana* were also investigated to confirm the existence of allelochemicals. The growth of *I. galbana* was not significantly ($P > 0.05$) lowered by macroalgae culture medium filtrate under initial filtrate addition for long time. On the contrary, the growth of *I. galbana* was strongly inhibited by *U. linza* culture medium filtrate under semicontinuous filtrate addition. It was speculated that continuous addition of allelochemicals was important to effectively control the growth of *I. galbana*. *I. galbana* was not inhibited when inoculated in the *U. linza* culture filtrate which was boiled at the high temperature and high pressure, which suggested that allelochemicals from the fresh tissue of *U. linza* were unstable and degradable at the high temperature. These tests prove that *U. linza* has allelopathic effects on *I. galbana*. These results not only provide insight into the cause and mechanism of green algal bloom, but also lead us to speculate and evaluate the effects of free-floating green macroalgae on the marine environment.

Key words: *Ulva linza*; *Isochrysis galbana*; allelopathic effects

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