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PRIMARY RESEARCH PAPER

# Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus*

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**Abstract** Allelopathic effects of the submerged macrophyte *Potamogeton malaianus* on *Scenedesmus obliquus* were assessed using a two-phase approach under controlled laboratory conditions. In the co-culture experiment (phase I), the growth of *S. obliquus* at two different initial cell densities was significantly inhibited by *P. malaianus*. Moreover, the growth inhibition was dependent on the biomass density of *P. malaianus*. Antioxidant enzymes (SOD, CAT and POD), MDA, APA, total soluble protein, protein electrophoretic pattern and morphology of *S. obliquus* were determined after the co-culture experiment was terminated. The activities of SOD, CAT, POD and APA at the low initial cell density were stimulated, the contents of MDA and total soluble protein were increased, and some special protein bands disappeared in *P. malaianus* treatments. The macrophyte had no effect on the activities of SOD and APA at the high initial cell density, but significantly influenced other physiological parameters of *S. obliquus* with the increase of biomass density. The morphology of *S. obliquus* showed no difference in the macrophyte treatments and the controls, and the cultures were dominated by 4-celled coenobia. The results indicated *P. malaianus* had significant allelopathic effects on the growth and physiological processes of *S. obliquus*. Moreover, the allelopathic effects depended on initial algal cell density, biomass density of the macrophyte, and their interaction. In the experiment of *P. malaianus* culture filtrates (phase II), filtrates from combined culture of plant and *S. obliquus* at the low initial cell density exhibited no apparent growth inhibitory effect on *S. obliquus*. The result showed that initial addition of growth-inhibiting plant filtrates had no allelopathic effect on *S. obliquus*. We concluded that the allelopathic effects on *S. obliquus* were found in the presence of *P. malaianus*, but not in *P. malaianus* filtrates. However, the absence of allelopathic effect on *S. obliquus* might be due to the very low concentrations of allelochemicals in the filtrates.

**Keywords** Allelopathy · *Potamogeton malaianus* · *Scenedesmus obliquus* · Growth inhibition · Physiological changes

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## Introduction

Submerged macrophytes play important roles in material cycling, and in both abiotic and biotic processes in shallow lakes, many of which are used as the pioneering species for lake restoration (Qiu et al., 2001; Lauridsen et al., 2003). Macrophytes are important primary producers competing for nutrients and other resources with phytoplankton and periphyton (Ozimek et al., 1990; Van Donk et al., 1993). Macrophytes may inhibit sediment resuspension (Scheffer et al., 1993), and provide structure and shelter for other organisms (Lauridsen & Buenk, 1996). Furthermore, macrophytes can reduce biomass of epiphyte and phytoplankton by releasing allelopathic compounds (Erhard & Gross, 2006; Mulderij et al., 2006).

It has been confirmed that many submerged macrophytes, such as *Myriophyllum spicatum* (e.g., Gross et al., 1996), *Ceratophyllum demersum* (e.g., Körner & Nicklisch, 2002), *Chara* (e.g., Mulderij et al., 2003), *Najas marina* (Gross et al., 2003), *Stratiotes aloides* (e.g., Mulderij et al., 2005a, b) and *Elodea* (e.g., Erhard & Gross, 2006) may secrete allelochemicals to inhibit algal growth. Exudates of *S. aloides* affected cell morphology of *Scenedesmus obliquus* (Mulderij et al., 2005b). In addition to effects on algal morphology and biomass, allelochemicals probably also interfere with many physiological functions of algal cells (Gross, 2003). For example, the activity of alkaline phosphatase (APA) in *Trichormus* was affected by allelopathic compounds (Gross et al., 1996). The excretion from root system of *Eichhornia crassipes* decreased total soluble protein content of *Scenedesmus* (Tang et al., 2000). Oxidative stress in *Chlorella pyrenoidosa* and *Microcystis aeruginosa* was promoted by allelochemicals isolated from *Phragmites communis* (Li & Hu, 2005). Reactive oxygen species (ROS) may bring about lipid peroxidation. To mitigate the oxidative stress caused by ROS, algae have an elaborate system involving antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) (Baranenko, 2001; Roginsky & Barsukova, 2001).

The submerged macrophyte *Potamogeton malaianus*, belonging to Potamogetonaceae, is a very common summer species in Chinese lakes (Sun, 1992). *P. malaianus* starts growing in spring and reaches a biomass maximum in July–August in temperate regions. Many species of Potamogetonaceae showed inhibitory effects on the growth of algae in laboratorial assays (Chen et al., 1994; Nakai et al., 1999; Chen et al., 2004; Xian et al., 2005), and several anti-algal compounds were isolated from Potamogetonaceae (DellaGreca et al., 2000, 2001; Cangiano et al., 2001, 2002; Waridel et al., 2003, 2004). However, the inhibition of algae by *P. malaianus* is seldom investigated. Intact *P. malaianus* showed inhibitory effect on the growth of *Selenastrum capricornutum* (Chen et al., 1994). Only two antiviral furanoid labdane deterpenes were extracted from *P. malaianus* (Kittakoop et al., 2001). *Scenedesmus*, as common genera of freshwater algae (Trainor, 1998), is expected to occur in the growth period of *P. malaianus*.

In the present study, the allelopathic effects of *P. malaianus* on *S. obliquus* were tested using a two-phase approach. In the co-culture experiment (phase I), in addition to effect of *P. malaianus* on the growth of *S. obliquus* at two different initial cell densities, effects on some physiological processes and morphology were also examined after the co-culture experiment was terminated. In order to elucidate the allelopathic effect of plant culture filtrates (phase II), the response of *S. obliquus* to co-culture filtrates was investigated.

## Materials and methods

### Macrophyte culture

*P. malaianus* originated from Wuhan botanical garden, Chinese Academy of Sciences in 2004. The plants used in the study were derived from the cement ponds outdoor. The water and the sediment of the ponds used originated from Lake Donghu (Wuhan, China).

Three days before the co-culture experiment, plants were transferred into the laboratory, carefully rinsed with tap water to remove adhering epiphyte and zooplankton, acclimated in 4 l glass aquaria filled with modified 10% Hoagland (Table 1) medium (Reddy & Tucker, 1983) near windows by natural light at room temperature.

**Table 1** Composition of 10% Hoagland medium (modified)

| Major nutrients (mg $l^{-1}$) | | Trace elements (mg $l^{-1}$) | |
|---|---|---|---|
| $Ca(NO_3)_2{\cdot}4H_2O$ | 94.5 | $H_3BO_3$ | 0.286 |
| $KNO_3$ | 60.7 | $ZnSO_4{\cdot}7H_2O$ | 0.008 |
| $KH_2PO_4$ | 13.6 | $MnCl_2{\cdot}4H_2O$ | 0.022 |
| $MgSO_4$ | 49.3 | $CuSO_4{\cdot}5H_2O$ | 0.181 |
| $FeSO_4{\cdot}7H_2O$ | 1.39 | $Na_2MoO_4{\cdot}2H_2O$ | 0.00242 |
| $Na_2EDTA$ | 1.865 | | |

## Alga culture

The axenic strain of *S. obliquus* was obtained from the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Sciences. Prior to the initiation of the experiments, short-term batch cultures of *S. obliquus* were grown in 2000 ml Erlenmeyer flasks filled with 1600 ml 10% Hoagland medium and aerated permanently to provide an optimal concentration of $CO_2$. Light source was supplied by fluorescent tubes of one side with a daylight similar emission spectrum (70 μmol $m^{-2}$ $s^{-1}$) at 12:12 L/D cycle. The temperature was maintained at 25°C in an air-conditioned growth chamber. Cells in the exponential growth phase were collected from batch cultures and used as the inocula for the following experiments.

The culture conditions in the following experiments were the same as for the algal culture unless it is mentioned.

## Co-culture experiment

When the experiment started (day 0), the fresh weight (FW) of *P. malaianus* was determined after blotting. Then the plants were placed into glass aquaria (20 × 20 × 30 cm) containing 8 l of 10% Hoagland nutrient solution. Different biomass densities of macrophyte were used (2.5 and 3.75 g FW $l^{-1}$ at the low initial algal cell density; 3.75 and 5 g FW $l^{-1}$ at the high initial algal cell density). Biomass densities of *P. malaianus* were set according to Nakai et al. (1999). Aquaria containing plastic plants were used as controls to simulate the shading effect. Aquarium aerators were used for circulation of the water to prevent sedimentation and provide an optimal concentration of $CO_2$. *S. obliquus* was inoculated with two different cell densities ($7 \times 10^7$ / $7 \times 10^8$ cells $l^{-1}$) in aquaria.

Each treatment had three replicates. The high initial cell density ($7 \times 10^8$ cells $l^{-1}$) was chosen in accordance to $OD_{650}$ (0.1) of *Scenedesmus* in the coexistence assay, in which the growth of *Scenedesmus* was inhibited by *E. crassipes* (Tang et al., 2000). The high initial cell density reached the onset of the stationary phase in 7 days incubation, while the low initial cell density nearly reached the onset of the stationary phase in 10 days incubation in our experiments. Thus, we respectively chose incubation of 7 ($7 \times 10^8$ cells $l^{-1}$) and 10 ($7 \times 10^7$ cells $l^{-1}$) days in the coexistence experiment.

## Experiment of macrophyte culture filtrates

According to the result of growth inhibition in the co-culture experiment, more strongly inhibitory co-culture filtrates were chosen. After 10 days of co-culture, the control and *P. malaianus* treatments (at 2.5 and 3.75 g FW $l^{-1}$) were filtered through a glass-fiber filter (pore size 0.22 μm, Jinteng, China) to remove bacteria from the culture media. The concentrations of nitrate and phosphate in the filtrates were added in excess to avoid nutrient limitation during the experiment. Then homogeneously mixed medium was divided into three 250 ml Erlenmeyer flasks containing 100 ml filtrate enriched with 6 mg $NaHCO_3$. In the experiment, *S. obliquus* was inoculated in medium with (filtrates from *P. malaianus* treatments) and without *P. malaianus* water (filtered control). All treatments were performed with the low initial algal cell density ($7 \times 10^7$ cells $l^{-1}$). The incubation time was 10 days.

## Sampling and analysis

Growth was monitored daily by measuring cell numbers at a set time in the co-culture experiment and the filtrates experiment. Cell numbers of the algae were counted on a hemocytometer under a Laboval-4 microscope (Carl Zeiss, Jena, Germany) after staining sample of 1 ml algae with Lugol's Iodine solution.

Growth rates of the low initial algal cell density in the co-culture experiment and the filtrates experiment were calculated from the increase of cell numbers in 10 days incubation applying a logistic growth model (Mulderij et al., 2003).

After the co-culture experiment was terminated, the 250–350 ml algae samples were filtered with glass-fiber filters (pore size 0.45 $\mu$m, Jinteng, China). All filters were immediately placed on ice and quickly transferred to –20°C until analysis (<4 weeks).

For assays of antioxidant enzymes, the filters were ground in an ice bath with 1.5 ml of 50 mM potassium phosphate buffer (pH 7.8) and quartz sand. The homogenate was centrifuged for 15 min at 15,000 rpm at 4°C, and the supernatant was collected for enzyme assays. SOD activity was determined by NBT test (Giannoplities & Ries, 1977), which was expressed as U $mg^{-1}$ protein. CAT activity was assayed with a UV 754N spectrophotometer (Shanghai, China) by measuring the decrease of absorbance at 240 nm due to $H_2O_2$ decomposition (Rao et al., 1996). POD activity was measured according to the method of Dias & Costa (1983).

Lipid peroxidation was determined by measuring the content of total malondialdehyde (MDA) according to the methods reported by Heath & Parker (1968).

APA was extracted according to Berman (1970). The filters were ground in ice bath and extracted with 0.1 M Tris–HCl (pH 7.4). The activity was estimated using the reaction mixture: 0.3 M *p*-Nitrophenyl phosphate and crude enzyme.

Total soluble protein content was determined according to the method of Bradford (1976), using bovine serum albumin as the standard, in a VIS-722 spectrophotometer (Shanghai, China).

The method of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used as described by Laemmli (1970), for the determination of protein electrophoretic pattern. The gels were subjected to a staining solution for 1.5 h, followed by destaining solution overnight. Finally the destained gels were photographed while wet.

To check for colony formation, samples from *S. obliquus* were subjected to microscopic analysis at the end of the coexistence experiment. The cell numbers were counted at least 100 coenobia under a Laboval-4 microscope (Carl Zeiss, Jena, Germany) at a magnification of 640X.

Nitrogen (N) or phosphorus (P) depletion of the media was prevented by addition of Ca $(NO_3)_2{\cdot}4H_2O$ (756 mg), $KNO_3$ (485.6 mg) and $KH_2PO_4$ (108.8 mg) over the first 3 days of co-culture. $NO_3$-N and $PO_4$-P concentrations were measured in the aquaria water at the end of incubation period according to the Chinese Norms (SEPA, 1989). The pH was determined by using the portable pH meter (Shanghai, delta320, China) at the end of the coexistence assay.

### Data analysis and statistics

All experiments were carried out three times. Mean values and standard deviations were calculated from the different replicates ($n = 3$). Statistical analyses of the data were performed using SPSS 13.0. The growth curves between control and *P. malaianus* treatments were compared using repeated measure ANOVA (RM ANOVA). Physiological parameters between control and *P. malaianus* treatments were compared by two-way ANOVA. Growth rates of the low initial algal cell density in phase I and II were analyzed by two-way ANOVA, taking $p < 0.05$ as significant. Tukey's tests ($a = 0.05$) were used to group homogeneous means.

## Results

### Effect on growth of *S. obliquus* in coexistence culture

In the co-culture experiment, the investigated biomass densities of *P. malaianus* exhibited obviously inhibitory effects on the growth of *S. obliquus* at two different initial cell densities (Fig. 1). A RM ANOVA on growth of the low initial algal cell density indicated a significant time effect ($p < 0.01$), a significant treatment and time interaction ($p < 0.01$), and a significant treatment effect ($p < 0.01$). Cell numbers were decreased respectively by 92.45% and 97.23% of the control in 2.5 and 3.75 g FW $l^{-1}$ *P. malaianus* treatments at the end of the experiment. The RM

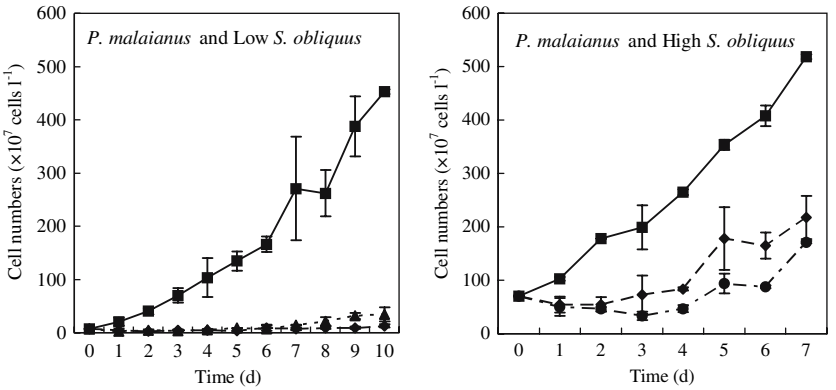


**Fig. 1** Growth curves of *S. obliquus* coexisting with *P. malaianus* in control (solid line), 2.5 g FW $l^{-1}$ (dotted line), 3.75 g FW $l^{-1}$ (dashed line), 5 g FW $l^{-1}$ (dot-dash line). 'Low' indicates an inoculated cell density of $7 \times 10^7$ cells $l^{-1}$, whereas 'High' stands for $7 \times 10^8$ cells $l^{-1}$. Data represent means ($n = 3$) with associated error bars (S.D.)

ANOVA on growth of the high initial algal cell density indicated a significant time effect ($p < 0.01$), a significant treatment and time interaction ($p < 0.01$), and a significant treatment effect ($p < 0.01$). Cell numbers were reduced by 58.09 and 66.98% in 3.75 and 5 g FW $l^{-1}$ *P. malaianus* treatments at the end of incubation period, respectively.

## Physiological and morphological changes of *S. obliquus*

Physiological parameters (SOD, CAT, POD, MDA, APA and total soluble protein) of *S. obliquus* at the end of the coexistence experiment are shown in Fig. 2.

*P. malaianus* only stimulated SOD activity at the low initial cell density (two-way ANOVA, $p < 0.01$), but had no effect on SOD activity at the high initial cell density. SOD activity was not affected by initial cell density.

CAT activity at the low initial cell density evidently rose in the macrophyte treatments (two-way ANOVA, $p < 0.01$), while CAT activity at the high initial cell density only showed a slight increase in the macrophyte treatments (two-way ANOVA, $p < 0.05$). Initial cell density significantly influenced CAT activity as well as the interaction treatment-cell density (two-way ANOVA, $p < 0.001$).

POD activity at the low initial cell density was much higher in the treatments than that in the control (two-way ANOVA, $p < 0.05$), while POD activity at the high initial cell density was only enhanced by 5 g FW $l^{-1}$ *P. malaianus* (two-way ANOVA, $p < 0.001$). POD activity was not influenced by initial cell density.

MDA content at the low initial cell density was significantly increased in the macrophyte treatments (two-way ANOVA, $p < 0.01$). Only 5 g FW $l^{-1}$ *P. malaianus* increased MDA content at the high initial cell density (two-way ANOVA, $p < 0.05$). MDA content was not affected by initial cell density.

The macrophyte significantly stimulated APA activity at the low initial cell density (two-way ANOVA, $p < 0.05$). No significant difference of APA activity at the high initial cell density was found in the macrophyte treatments and the control. APA activity was significantly affected by initial cell density (two-way ANOVA, $p < 0.05$).

Total soluble protein content at the low initial cell density was significantly increased in *P. malaianus* treatments (two-way ANOVA, $p < 0.001$). 3.75 g FW $l^{-1}$ *P. malaianus* significantly induced an increase in total soluble protein content at the high initial cell density (two-way ANOVA, $p < 0.05$), while 5 g FW $l^{-1}$ *P. malaianus* had no effect. Initial cell density did not affect total soluble protein content.

The protein electrophoretic pattern at the low initial cell density showed the disappearance of 39.8 kDa band in 2.5 g FW $l^{-1}$ *P. malaianus* treatment, while the 39.8 kDa and 77.1 kDa bands disappeared in 3.75 g FW $l^{-1}$ *P. malaianus* treatment (Fig. 3). The protein electrophoretic

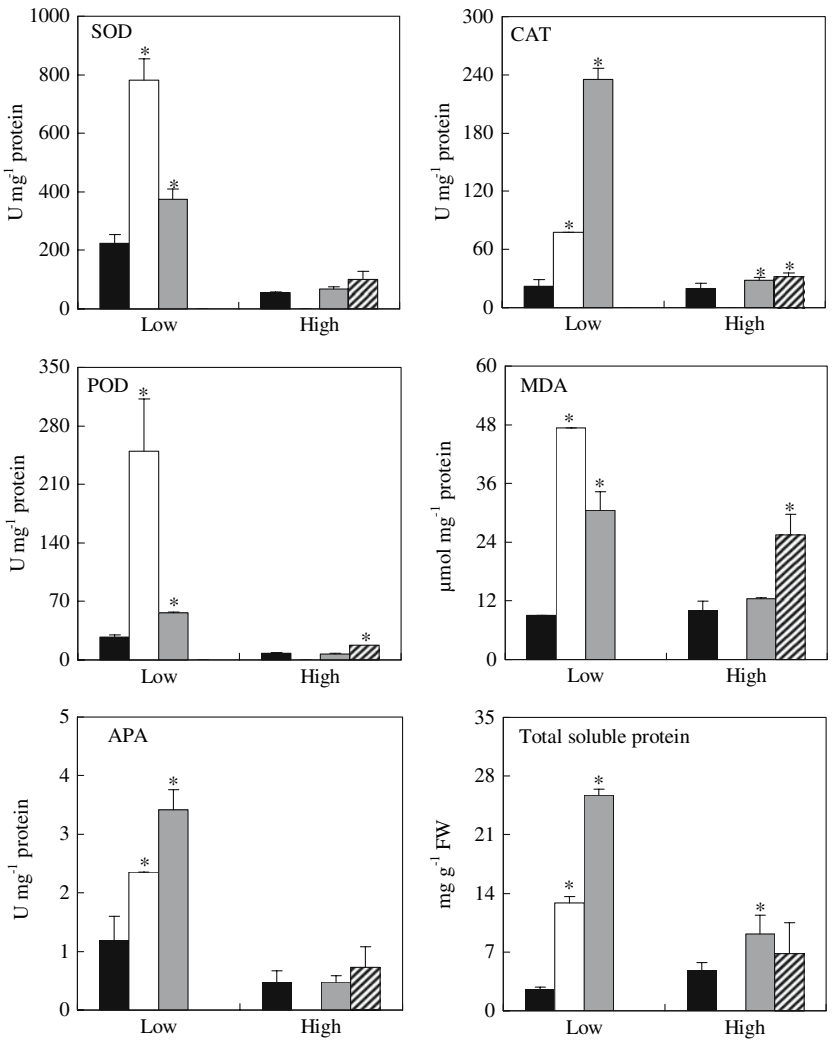


**Fig. 2** Effects of coexistence culture with *P. malaianus* on SOD, CAT, POD, MDA, APA and total soluble protein of *S. obliquus* in control (black bars), 2.5 g FW $l^{-1}$ (white bars), 3.75 g FW $l^{-1}$ (grey bars), 5 g FW $l^{-1}$ (shaded bars). 'Low' indicates an initial cell density of $7 \times 10^7$ cells $l^{-1}$, whereas 'High' stands for $7 \times 10^8$ cells $l^{-1}$. *significantly different ($p < 0.05$) from control

pattern at the high initial cell density showed that a band of 39.8 kDa disappeared in 5 g FW $l^{-1}$ *P. malaianus* treatment.

Algal cell morphology showed no difference in the macrophyte treatments and the controls when observed under the optical microscope. Many 4-celled coenobia (ca. 70%) were observed in the cultures.

Nutrient concentrations ranged from 0.8 to 3.8 (2.11 ± 1.18) mg $l^{-1}$ $PO_4$-P and 12.3 to 32.7 (23.09 ± 8.70 [mean values ± S.D.]) mg $l^{-1}$ $NO_3$-N in the controls, and 3.1 to 4.1 (3.19 ± 0.91) mg $l^{-1}$ $PO_4$-P and 14.2 to 28.1 (21.14 ± 6.09) mg $l^{-1}$ $NO_3$-N in *P. malaianus* treatments. The pH value in the co-culture was 8.20 ± 0.07.

## Effect of *P. malaianus* culture filtrates

The growth rate of *S. obliquus* at the low initial cell density was not inhibited by *P. malaianus* culture filtrates (Fig. 4). The average growth rate of the low initial algal cell density was 0.65 $day^{-1}$.

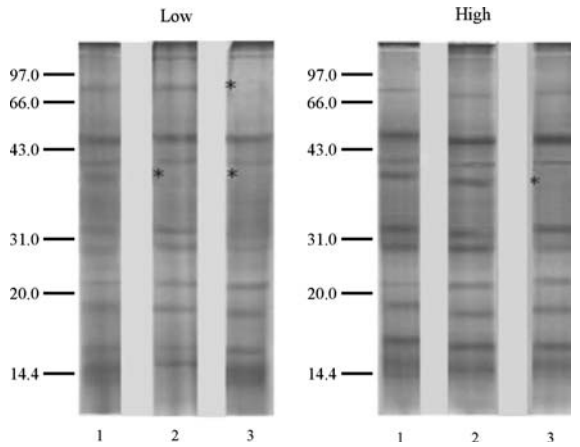


**Fig. 3** The SDS-PAGE profile of soluble protein of *S. obliquus*. 'Low' indicates an inoculated cell density of $7 \times 10^7$ cells $l^{-1}$, whereas 'High' stands for $7 \times 10^8$ cells $l^{-1}$. Lane 1: control; lane 2: 2.5 (or 3.75 in High) g FW $l^{-1}$ *P. malaianus* -treated; and lane 3: 3.75 (or 5 in High) g FW $l^{-1}$ *P. malaianus* -treated cells. *the disappearance of protein band

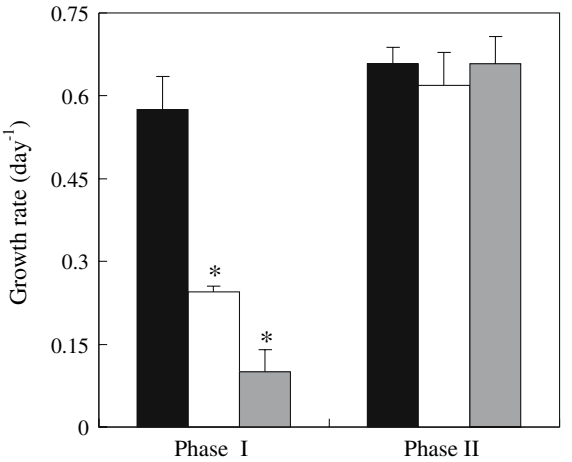


**Fig. 4** Growth rates of *S. obliquus* at the low initial cell density in control (black bars), 2.5 g FW $l^{-1}$ (white bars), or 3.75 g FW $l^{-1}$ (grey bars). 'Phase I' indicates the co-culture experiment, whereas 'Phase II' stands for the culture filtrates experiment. *significantly different ($p < 0.05$) from control

### Comparison between phase I and II: the low initial cell density

Two-way ANOVA of the growth rate of the low initial algal cell density indicated a significant difference in phase I and II ($p < 0.001$), a significant phase and treatment interaction ($p < 0.001$), and a significant treatment effect ($p < 0.001$). Significant difference between the two phases was found for two biomass densities of *P. malaianus*, but not for the controls (Fig. 4). The significant interaction was caused by the obviously reduced growth in the presence of *P. malaianus*, but disappearance of this effect in *P. malaianus* culture filtrates. The difference between control and *P. malaianus* treatments was only significant in phase I.

## Discussion

The growth of *S. obliquus* at two different initial cell densities was significantly reduced in the presence of *P. malaianus*. Although most coexistence studies have demonstrated allelopathic effects of many macrophytes on algae (Nakai et al., 1999; Tang et al., 2000; Körner & Nicklisch, 2002; Van Donk & Van de Bund, 2002; Lürling et al., 2006; Mulderij, 2006), in such a system it is very important to rule out the competition for nutrients or light between macrophyte and alga. Sufficient nitrogen and phosphorus remained for the growth of *S. obliquus*. 10% Hoagland medium lacked an inorganic carbon source, but cell numbers of *S. obliquus* in the controls showed a relatively high increase by permanent aeration. Thus, the carbon was not limiting. During the experiment *P. malaianus* and plastic plants were always at the other side far from the light source, which excluded shading effects from the macrophyte on *S. obliquus*. Hence, allelochemicals secreted by *P. malaianus* might impair normal growth of co-occurring *S. obliquus*. The result also showed that the growth inhibition was dependent on the biomass density of *P. malaianus* (2.5 g FW $l^{-1}$ *P. malaianus* was not listed because of an inhibitory rate less than 50% at the high initial cell density).

Algae may respond by a burst of reactive oxygen species (ROS) under stress conditions (Küpper et al., 2001; Choo et al., 2004). Recent studies suggest that some allelochemicals, which act as environmental stress, can increase the production of ROS in algal cells (Vardi et al., 2002; Li & Hu, 2005). In the present study, *P. malaianus* stimulated SOD, CAT and POD activities and increased

MDA content of *S. obliquus* at the low initial cell density. Higher biomass density of *P. malaianus* stimulated CAT and POD activities and increased MDA content of *S. obliquus* at the high initial cell density, while lower biomass density of *P. malaianus* only stimulated CAT activity. The enhancement of antioxidant enzymes activities suggested that the oxidative stress condition led to an increased antioxidant capability of algal cells. However, this increase might not match the production of ROS, thus resulting in an increased lipid peroxidation in algal cells.

APA activity of *S. obliquus* at the low initial cell density was increased by *P. malaianus*. The result was different from the study of Gross et al. (1996). Rice (1984) mentioned that certain allelochemicals could stimulate target organisms at lower concentrations but inhibit at higher concentrations. We assume that concentrations of *P. malaianus* exudates were below the inhibition threshold of APA activity.

In the coexistence experiment, total soluble protein content of *S. obliquus* at the low initial cell density was significantly increased by *P. malaianus*, but some protein bands disappeared. Lower biomass density of *P. malaianus* increased total soluble protein content at the high initial cell density, while higher biomass density of *P. malaianus* caused the disappearance of certain protein band. Accumulation of protein might be one of the ways through which *S. obliquus* abolish toxic effects of allelochemicals as reported for algae under heavy metal stress (Sultan & Fatma, 1999; Mohamed et al., 2004). However, the absence of protein bands showed that *P. malaianus* exudates inhibited the biosynthesis of distinct proteins.

*Scenedesmus* is characterized by coenobia formation of four and eight cells. Phenotypic plasticity in *Scenedesmus* was found in response to a wide variety of ecological conditions (Trainor, 1998). However, studies on the allelopathic effect on morphology of *Scenedesmus* are very limited. Mulderij et al. (2005b) observed that exudates of *S. aloides* affected cell morphology of *S. obliquus*, whereas Lürling et al. (2006) found exudates of *Chara*, *Elodea* and *Myriophyllum* had no morphological effect on *S. obliquus*. At the end of the co-culture experiment, 4-celled coenobia were found in the controls and the treatment groups. Therefore, the morphology of *S. obliquus* was not significantly affected by *P. malaianus* exudates.

The coexistence culture was not completely free of epiphytes or bacteria. Careful rinsing followed by microscopical checks proved that the amount of epiphytes on *P. malaianus* was considered negligible. However, it was observed in previous studies (Keating, 1978; Schagerl et al., 2001), that allelopathic effects were lower when bacteria were present. This could be partly explained by possible degradation process of the allelopathic compounds by bacteria in the co-culture experiment.

*P. malaianus* obviously inhibited the growth of *S. obliquus* at the low initial cell density in phase I, but the macrophyte culture filtrates had no growth inhibitory effect. Van Donk & Van de Bund (2002) and Lürling et al. (2006) made similar observations for *Chara*. Lürling et al. (2006) listed several possible causes for the absence of allelopathic effect of filtrates. However, Nakai et al. (1999) found that $EC_{50}$ of *M. spicatum* on *M. aeruginosa* was much higher in the filtrate assay than that in the coexistence assay. Thus, the disappearance of negative effect on *S. obliquus* might be due to the very low concentrations of allelochemicals in the filtrates.

In conclusion, coexistence assay supported the existence of allelopathic effects of *P. malaianus* on the growth and physiological processes of *S. obliquus*, whereas initial addition of plant culture filtrates showed no allelopathic effect on growth of *S. obliquus*. Further laboratory studies are needed to gain more information on allelopathic effects of *P. malaianus* on growth and physiological processes of algae, identification of allelochemicals and allelopathic mechanisms.

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