


RESEARCH ARTICLE

Allelopathic effects of *Egeria densa* on the growth and morphology of *Chlorella vulgaris*

Dujuan Dai^{1,a}, Yue Yang^{1,a}, Feihu Wang¹, Yang Zhang¹, Man Zhang¹, Yunni Gao¹ , Xiaofei Gao¹, Jing Dong^{1,*}, Xuejun Li^{1,*} and Mengyang Chang^{2,*}

¹ College of Fisheries, Henan Normal University, Xinxiang 453007, China

² School of Software Engineering, Anyang Normal University, Anyang 455000, China

Received: 5 August 2022; Accepted: 13 March 2023

Abstract – Interspecific interaction between submerged macrophytes and phytoplankton is of great significance in sustaining ecological balance and restoring eutrophic water regions. In consideration of the diversified algal density and macrophytes biomass, the present study selected two biomass levels of *Egeria densa* and gradient densities of *Chlorella vulgaris* for simulated cultivation experimentation. *E. densa* could significantly suppress the growth and affect the morphology of *C. vulgaris*. The allelopathically inhibitory growth of *C. vulgaris* was suggested to increase with increasing biomass of *E. densa* and decreased with the elevating density of *C. vulgaris*. Higher amount of colonies and bigger colony size of *C. vulgaris* were detected after the treatment with 5 g/L of *E. densa* together with *C. vulgaris* ($OD_{680} = 0.2$) and treatment with 10 g/L *E. densa* together with *C. vulgaris* ($OD_{680} = 0.05$). Elevated extracellular and intracellular polysaccharides were detected, which possibly contributed to the morphological changes of *C. vulgaris* induced by *E. densa*.

Keywords: Allelopathy / *Egeria densa* / morphology / *Chlorella vulgaris* / growth

1 Introduction

As an important primary producer and a key biological factor in sustaining the healthy function of aquatic ecosystems, the submerged macrophytes can assist in purification and improvement of water transparency, can provide a good habitat for invertebrates and can shape phytoplankton community structure (Amorim and Moura, 2020). The occurrence of eutrophication and cyanobacterial blooms is closely related to the decline of submerged plants in recent decades (Qin, 2020). Indoor and field experiments have shown that the chemically active substances secreted by submerged plants, including polyphenols, fatty acids, terpenes and alkaloids, could significantly inhibit the growth of cyanobacteria, especially *Microcystis* (Techer *et al.*, 2016; Santonja *et al.*, 2018; Li *et al.*, 2021). However, considerable controversial results exist in the effects of submerged macrophytes on green algae (Pakdel *et al.*, 2013; Santonja *et al.*, 2018). Some studies did not detect significant inhibition on green algae by submerged macrophytes (Hong and Hu, 2007; Hilt and Gross, 2008; Zhu *et al.*,

2010; Pakdel *et al.*, 2013; Jeong *et al.*, 2021; Zhu *et al.*, 2021). Instead, chlorophytes were stimulated by allelochemicals (Jasser, 1995; Mulderij *et al.*, 2007). Toporowska *et al.* (2008) found that green algae dominated in the water regions with abundant *Ceratophyllum demersum* or *Potamogeton lucens*. Similarly, our previous analysis of historical phytoplankton changes in Lake Dianchi (Kunming, China) also indicated that during the period in which the submerged macrophytes were many, it was the green algae, such as *Scenedesmus*, *Pediastrum* and *Coleastrum*, that dominated (Dong *et al.*, 2014). However, *Najas minor* and *Potamogeton malaiianus* could significantly suppress the growth of *Scenedesmus obliquus* (He *et al.*, 2008). Combined with the previous studies, it was assumed that the contradicting results depend on species-specific interaction, algal density and macrophyte biomass (Zhao *et al.*, 2012; Jiang *et al.*, 2015; Zuo *et al.*, 2015; Dong *et al.*, 2018). Therefore, in consideration of diversified density distribution of phytoplankton in natural shallow freshwater ecosystems, the interactions between algae with varying densities and gradient biomass of aquatic macrophytes should be discussed to comprehensively evaluate the allelopathic effects on aquatic ecosystems (Zheng *et al.*, 2013; Jiang *et al.*, 2015; Zuo *et al.*, 2015; Donadi *et al.*, 2019; Nezbrytska *et al.*, 2022).

Similar to cyanobacteria, fierce competition also exists between green algae and submerged macrophytes. So, what is

*Corresponding author: happydj111@163.com; xjli@htu.cn; HSSTCMY@163.com

^a Co-first authors.

the ecological strategy for coexistence of green algae and submerged macrophytes in some water regions? The traditional environmental toxicology research focuses on the environmental stress on growth, photosynthetic activities, oxidative ability and membrane damage of the target algae (Körner and Nicklisch, 2002; Leu *et al.*, 2002; Dziga *et al.*, 2007; Hong *et al.*, 2008; Zhu *et al.*, 2010). However, an increasing number of studies indicated that morphological transformation is more sensitive to environmental pressure than the growth effects. Lürling and Beekman (2002) found that growth inhibition concentration of ionic surfactants originating from the glass fibre, hybrid fibre and nitrocellulose membrane was far below than that causing the morphological changes on *S. obliquus*. Huang *et al.* (2016) suggested that low concentration of free Cu^{2+} had no significant effects on algal growth and photosynthesis but can inhibit *S. obliquus* to form colonies in response to *Daphnia* filtrate. Cheloni and Slaveykova (2021) measured the effects of four kinds of micropollutants (paraquat, perfluorooctane sulfonic acid, as well as heavy metal cadmium and copper) on *Chlamydomonas reinhardtii*. The chlorophyll fluorescence, oxidative stress and membrane injury of *C. reinhardtii* were not detected in the micropollutant concentration-induced colony formation. Zhu *et al.* (2021) revealed that the algal morphology was more sensitive than growth for indicating the phytoplankton's response to allelochemicals from macrophytes. Accordingly, in environmental studies, the growth rates effects and the morphological changes should be considered to better assess phytoplankton response.

Related studies have reported that colony responses to adverse conditions could significantly improve the resistance of target green algae to predation by zooplankton (Boraas *et al.*, 1998; Wu *et al.*, 2013; Zhu *et al.*, 2015; Fisher *et al.*, 2016; Lürling, 2021), global warming (Duan *et al.*, 2018; Zhu *et al.*, 2019), environmental pollutants (Liu *et al.*, 2010; Li *et al.*, 2013; Cheloni and Slaveykova, 2021) and competition with macrophytes (Dong *et al.*, 2018; Dong *et al.*, 2019; Zhu *et al.*, 2021). However, ecological costs also exist in the colony formation of green algae. Owing to induced colony formation, the sedimentation rates of the target algae into the bottom water increased, and the growth was limited by low temperature and little light at the bottom water region (Mulderij *et al.*, 2005; Dong *et al.*, 2013). Therefore, in inter-specific relationships of predation and competition, the morphology of phytoplankton may indirectly affect the structure of food web, and the morphology dynamics of phytoplankton would further exert profound effects on the function of aquatic ecosystems.

To increase the knowledge towards the comprehensive influences of submerged macrophytes on green algae, the macrophyte *Egeria densa* and green algae *Chlorella vulgaris* naturally occurring in fresh water ecosystems were selected in the present study. The present study aimed to compensate for the limited knowledge on algal morphological plasticity and responses of green algae with gradient density to different biomass levels of submerged macrophytes.

2 Materials and methods

2.1 Algae culture

The green algae strain *C. vulgaris* (FACHB-8) was obtained from Freshwater Algae Culture Collection of Institute

of Hydrobiology, Chinese Academy of Sciences in Wuhan, China. Prior to experimentation, the stock cultures of *C. vulgaris* were conducted under favourable conditions at room temperature (25 °C) in 500 mL Erlenmeyer flasks with 300 mL BG₁₁ medium (Rippka *et al.*, 1979) under a 12 h light:12 h dark cycle (25 $\mu\text{mol photons s}^{-1} \text{m}^{-2}$). The cultures were shaken thrice in one day to prevent algal sedimentation.

2.2 The acclimation of macrophytes

The submerged macrophytes *E. densa* were collected from a field pond for growth of experimental macrophytes in Henan Normal University, China (35°19'38.363"N, 113°54'09.482" E) and were carefully rinsed with tap water to remove all adhering epiphytes and zooplankton (Lürling *et al.*, 2006). Then, the plants were acclimated with *C. vulgaris* to simulate the co-existence of the target algae and macrophytes. The cultivation conditions were the same as those mentioned above. Finally, robust and isometric shoots (Lürling *et al.*, 2006) of the submerged *E. densa* were selected for experimentation after careful rinsing with sterile water.

2.3 Experimental design

When it reached an exponential growth phase, *C. vulgaris* was cultivated in three 2 L flasks with 1.8 L of fresh BG₁₁ medium under a 12 h:12 h light cycle (25 $\mu\text{mol photons s}^{-1} \text{m}^{-2}$). The initial algal density was set as follows: Flask one = 0.05 (OD₆₈₀); Flask two = 0.1; and Flask three = 0.2. Subsequently, each algal cultivation in 2 L flask with different initial algal densities was divided into nine 250 mL Erlenmeyer flasks with 200 mL of the algal solution per flask on average and further cultivated in the presence or absence of submerged *E. densa* (5 g FW L⁻¹ or 10 g FW L⁻¹). The control (with one or two *E. densa*-like plastic plants to exclude the potential shading effects of the macrophytes) and macrophytes treatment (5 g FW L⁻¹ or 10 g FW L⁻¹) were set in triplicate. All the cultivation flasks with three replicates were covered with parafilm and cultivated under favourable conditions as described above. The whole experiments lasted for 10 days. Each alga culture was sampled regularly to measure nutrients in the cultivation medium and the growth, photosynthetic pigments, soluble intracellular (and extracellular) polysaccharides, colony proportion and average cell numbers per colony of *C. vulgaris*.

2.4 Parameters measurements

2.4.1 Measurement of total dissolved nitrogen (TDN) and phosphorous (TDP)

The concentrations of TDN and TDP were measured according to Protocols for Standard Observation and Measurement in Aquatic Ecosystems of Chinese Ecosystem Research Network (CERN) (Huang *et al.*, 2000; Cai, 2007).

2.4.2 Optical density (OD₆₈₀)

The growth of *C. vulgaris* was determined by its OD₆₈₀ under ultraviolet/visible spectrophotometer.

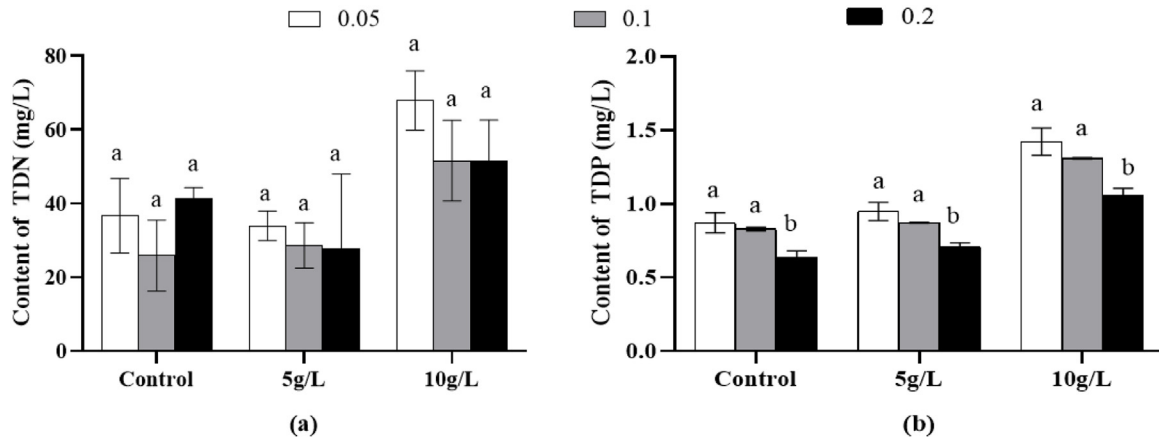


Fig. 1. The content of TDN and TDP in cultivation of *C. vulgaris* with or without *E. densa* at the end of the experimentation.

2.4.3 Photosynthetic pigments

Samples (5 mL) were centrifuged under 12 000 rpm for 10 min. The centrifuged algae cells were extracted with 95% ethanol for 24 h at 4°C in the dark. Shaking was performed after extraction for 12 h to fully extract the pigments. Then, all the extracted substances were centrifuged, and 3 mL supernatant was measured by using an ultraviolet/visible spectrophotometer. The absorbance values at 665 and 649 nm (A_{665} and A_{649} , respectively) were measured, and the concentrations of photosynthetic pigment chlorophyll a (Chla) were calculated as follows (Lichtenthaler and Buschmann, 2001):

$$\text{Chla (mg/L)} = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (1)$$

2.4.4 Algal density and the morphology composition

Samples (2 mL) were fixed with Lugol's fixative and viewed under an inverted microscope to count the cell density, the number of cells per colony and the colony proportion (minimum of three cells) (Lüring and Van Donk, 1997).

2.4.5 Inhibition on *C. vulgaris* by *E. densa*

The inhibition rate of *C. vulgaris* and *S. obliquus* in the treatment groups compared with the control at the end of the experiment were measured as follows:

$$I(\%) = (C_t - C_c) / C_c \times 100\%$$

where C_c and C_t represent the (OD_{680} , algal density, Chla) contents of *C. vulgaris* in the control and each treatment group, respectively.

2.4.6 Intracellular and extracellular polysaccharides

Algal solutions (3 mL) in each group were sampled regularly and centrifuged at a speed of 8000 rpm for 10 min. The supernatant was harvested to determine the extracellular polysaccharide. The centrifuged algal deposits were washed with sterile water and boiled for 30 min to ensure that the intracellular polysaccharide were sufficiently extracted from the cell. The procedure for determination of intracellular and extracellular polysaccharides were conducted according to anthrone colorimetry methods in accordance with Li *et al.* (2000).

2.5 Statistical analysis

Mean values and standard deviations were calculated for the different replicates ($n=3$). Two-way ANOVA was conducted to test the differences for nutrients changes and algal inhibition in each treatment. Repeated measurement of ANOVA was utilised to compare the colony proportion, average cell number per colony, soluble intracellular and extracellular polysaccharide among different algal density and *E. densa* treatments. All the statistical analyses employed SPSS 22.0 for Windows. A value of $p < 0.05$ was considered statistically significant in all analyses.

3 Results

3.1 Content of TDN and TDP

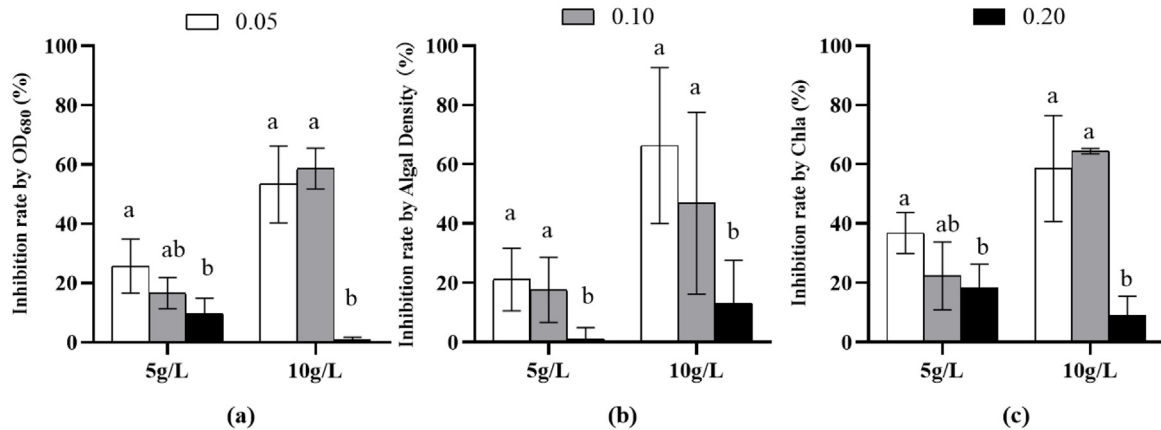
As illustrated in Figure 1, the contents of TDN and TDP in the co-cultivation of *C. vulgaris* with 10g/L *E. densa* were higher than those of the control without macrophytes (Figs. 1a and 1b). In consideration of responses owing to diversified algal density, the reduction of TDP in groups with high algal density ($OD_{680}=0.2$) was more significant than that of lower ones ($OD_{680}=0.05$ and $OD_{680}=0.1$) (Fig. 1b). Two-way ANOVA indicated that treatment with *E. densa* exerted significant influences on the content of TDN at the end of the experimentation, whereas the effects of initial algal density were not obvious in each treatment ($F=2.432$, $P=0.116$). The result also demonstrated the significant influences of treatment ($F=250.650$, $P < 0.001$) and initial algal density ($F=75.434$, $P < 0.001$) on the content of TDP at the end of the experimentation (Tab. 1).

3.2 Inhibition on *C. vulgaris* by *E. densa*

The presence of *E. densa* could significantly inhibit the growth of *C. vulgaris*, as indicated by OD_{680} , algal density together with Chla Figure 2. Two-way ANOVA indicated that treatment with *E. densa* and initial algal density both exerted significant influences on the algal inhibition of OD_{680} ($F=33.519$, $P < 0.001$; $F=40.394$, $P < 0.001$), density ($F=11.259$, $P < 0.01$; $F=6.371$, $P < 0.05$) and Chla ($F=21.002$, $P < 0.01$; $F=29.294$, $P < 0.001$) (Tab. 1). The inhibition influences induced by *E. densa* depended on target

Table 1. Summary of two-Way ANOVA analysis on the effects of algal density, biomass of *E. densa* and their interaction on the nutrients (TDN, TDP) in algal cultivation and inhibition on *C. vulgaris* by *E. densa* at the end of the experimentation

Parameters	Treatment		Density		Treatment × Density	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
TDN	17.260	<0.001	2.432	0.116	1.051	0.409
TDP	250.650	<0.001	75.434	<0.001	1.709	0.192
Inhibition on OD ₆₈₀	33.519	<0.001	40.394	<0.001	18.601	<0.001
Inhibition on Algal density	11.259	<0.01	6.371	<0.05	1.255	0.320
Inhibition on Chl _a	21.002	<0.01	29.294	<0.001	14.269	<0.01

**Fig. 2.** Inhibition rate on OD₆₈₀, algal density and Chl_a of *C. vulgaris* induced by *E. densa* at the end of the experimentation.

algal density and macrophyte biomass, increased with increasing amount of *E. densa* and decreased with increasing algal density.

3.3 Morphological changes of *C. vulgaris*

3.3.1 Average cell number per colony

By using Repeated Measurement ANOVA, it was suggested that Treatment ($F=179.236$, $P < 0.001$), Initial algal density ($F=16.922$, $P < 0.001$), Day ($F=15.205$, $P < 0.001$), Treatment × Day ($F=9.765$, $P < 0.001$), Initial algal density × Day ($F=2.425$, $P < 0.05$) and Treatment × Density ($F=25.205$, $P < 0.01$) all exerted significant influences on the average cell number per colony of *C. vulgaris* (Tab. 2). As indicated in Figure 3, the presence of *E. densa* could significantly induce the colony formation of *C. vulgaris*, especially with 10 g/L *E. densa*. The biggest colony was detected in the group with an initial algal density of OD₆₈₀=0.05 and OD₆₈₀=0.1. The average cell number per colony was 9.38. However, in the presence of 5 g/L *E. densa*, only the group with an initial algal density of 0.2 exerted significant colony size increase compared with the control.

3.3.2 Colony proportion

By using repeated measurement ANOVA, it was suggested that Treatment ($F=204.366$, $P < 0.001$), Initial algal density ($F=30.037$, $P < 0.001$), Day ($F=68.040$, $P < 0.001$), Treatment × Day ($F=9.178$, $P < 0.001$) and Initial algal density × Day ($F=17.788$, $P < 0.001$) all exerted significant

influences on the colony proportion of *C. vulgaris* (Tab. 2). As indicated in Figure 4, the presence of *E. densa* could significantly increase the colony proportion of *C. vulgaris*. The highest number of colonies was detected in the group with initial algal density of OD₆₈₀=0.2 in the presence of 5 g/L of *E. densa*. However, with 10 g/L of *E. densa*, the highest number of colonies was detected in the group with an initial algal density OD₆₈₀=0.1.

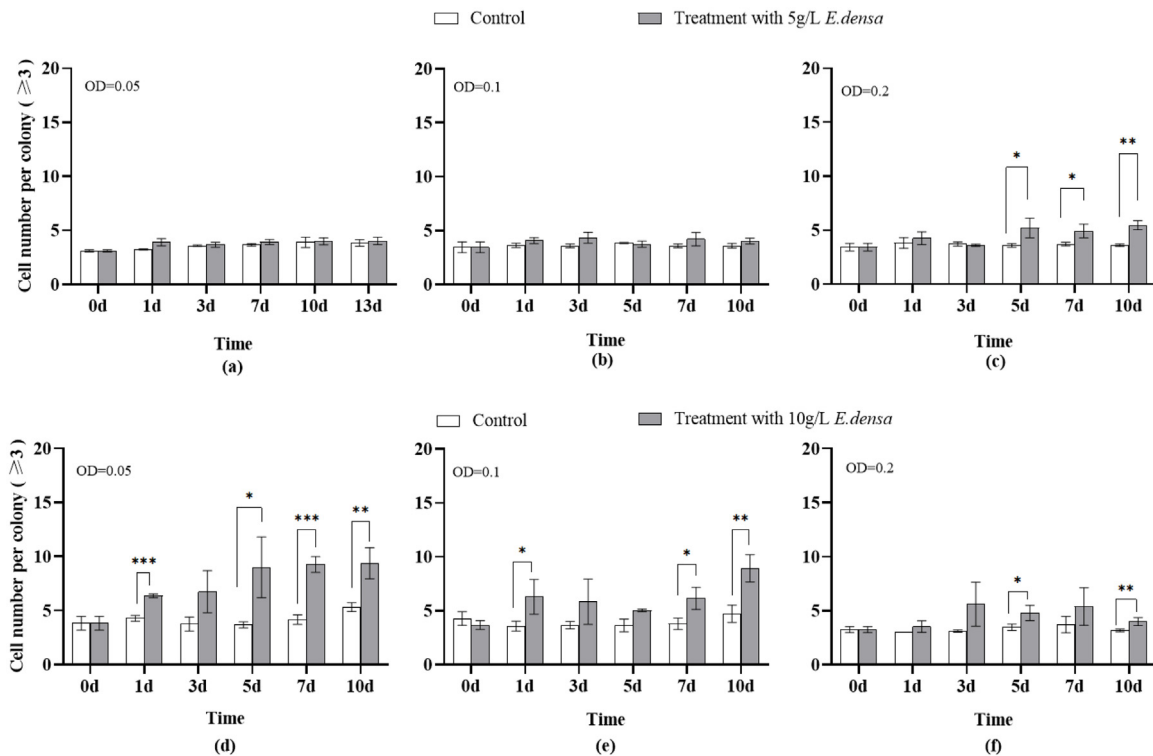
3.3.3 Soluble extracellular and intracellular polysaccharide

Resorting to Repeated Measurement ANOVA, it was suggested that Treatment ($F=57.955$, $P < 0.001$; $F=80.733$, $P < 0.001$), Initial algal density ($F=21.772$, $P < 0.001$; $F=16.331$, $P < 0.001$), Day ($F=33.756$, $P < 0.001$; $F=43.445$, $P < 0.001$), Treatment × Day ($F=5.250$, $P < 0.001$; $F=4.054$, $P < 0.001$) and Initial algal density × Day ($F=7.841$, $P < 0.001$; $F=2.273$, $P < 0.05$) all exerted significant influences on the extracellular and intracellular polysaccharide in *C. vulgaris* (Tab. 2).

As illustrated in Figures 5 and 6, *E. densa* could elevate the contents of intracellular and extracellular polysaccharides in *C. vulgaris* in each group, and the effect increased with the biomass of *E. densa*. With 5 g/L of *E. densa*, algal density effects on soluble extracellular polysaccharide was not significant (Figs. 5a–5c), whereas soluble intracellular polysaccharide in the group of OD₆₈₀=0.05 was higher than that of OD₆₈₀=0.1 and OD₆₈₀=0.2. In the presence of 10 g/L of *E. densa*, the increased extracellular polysaccharide in

Table 2. Repeated measurement analysis of variance comparing algal morphology (Colony proportion and cell number per colony), soluble intracellular polysaccharide and extracellular polysaccharide under gradient initial algal cultivation density and different biomass of *E. densa* during the experimental period.

Source of variation	Colony proportion		Cell number per colony		Soluble intracellular polysaccharide		Soluble extracellular polysaccharide	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Treatment	204.366	<0.001	179.236	<0.001	80.733	<0.001	57.955	<0.001
Density	30.037	<0.001	16.922	<0.001	16.331	<0.001	21.772	<0.001
Day	68.040	<0.001	15.205	<0.001	43.445	<0.001	33.756	<0.001
Treatment × Day	9.178	<0.001	9.765	<0.001	4.054	<0.01	5.250	<0.001
Density × Day	17.788	<0.001	2.425	<0.05	2.273	<0.05	7.841	<0.001
Treatment × Density	0.235	0.791	5.205	<0.01	12.448	<0.001	0.789	0.458
Treatment × Day × Density	4.374	<0.001	1.611	0.121	1.153	0.337	1.395	0.200

**Fig. 3.** The average cell number per colony of *C. vulgaris* with gradient concentration of initial algal cultivation density in the absence or presence of *E. densa* during the cultivation period.

C. vulgaris was most significant in the group of initial algal density $OD_{680}=0.2$, and the highest amount of intracellular polysaccharide was detected in the group of $OD_{680}=0.1$.

4 Discussion

4.1 Growth inhibition of *E. densa* on green algae *C. vulgaris*

When considering the allelopathic interaction between submerged macrophytes and phytoplankton in freshwater

ecosystems, discussing the influences of shading and nutrient competition of macrophytes with target algae was inevitable. Many previous studies have discussed nutrient competition and the shading effects in co-cultivation experiments of submerged macrophytes-phytoplankton (Gross *et al.*, 2003; Mulderij *et al.*, 2007; Hilt and Gross, 2008; Lombardo *et al.*, 2013; Jeong *et al.*, 2021). We excluded shading effects by adding plastic-simulated plants as the control and also got rid of the nutrient depletion by using medium with sufficient nutrients. According to the nutrient analysis at the end of the experiment, TDN in each groups exhibited no significant

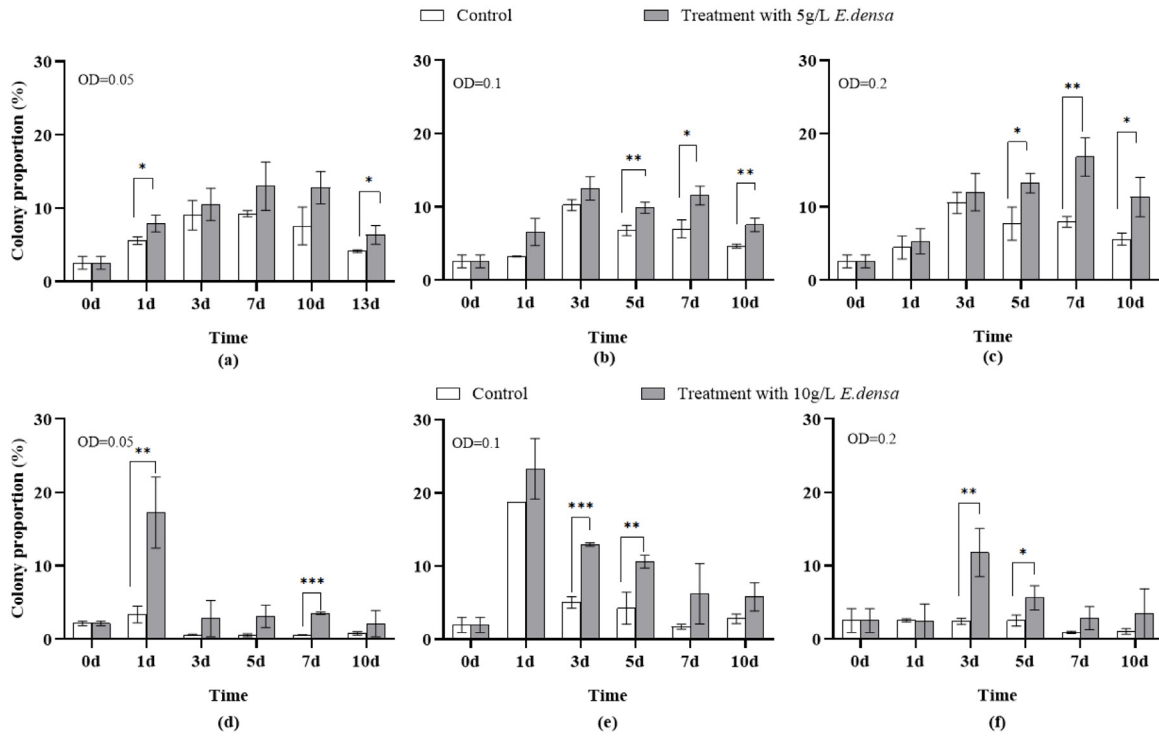


Fig. 4. The colony proportion of *C. vulgaris* with gradient concentration of initial algal cultivation density in the absence or presence of *E. densa* during the cultivation period.

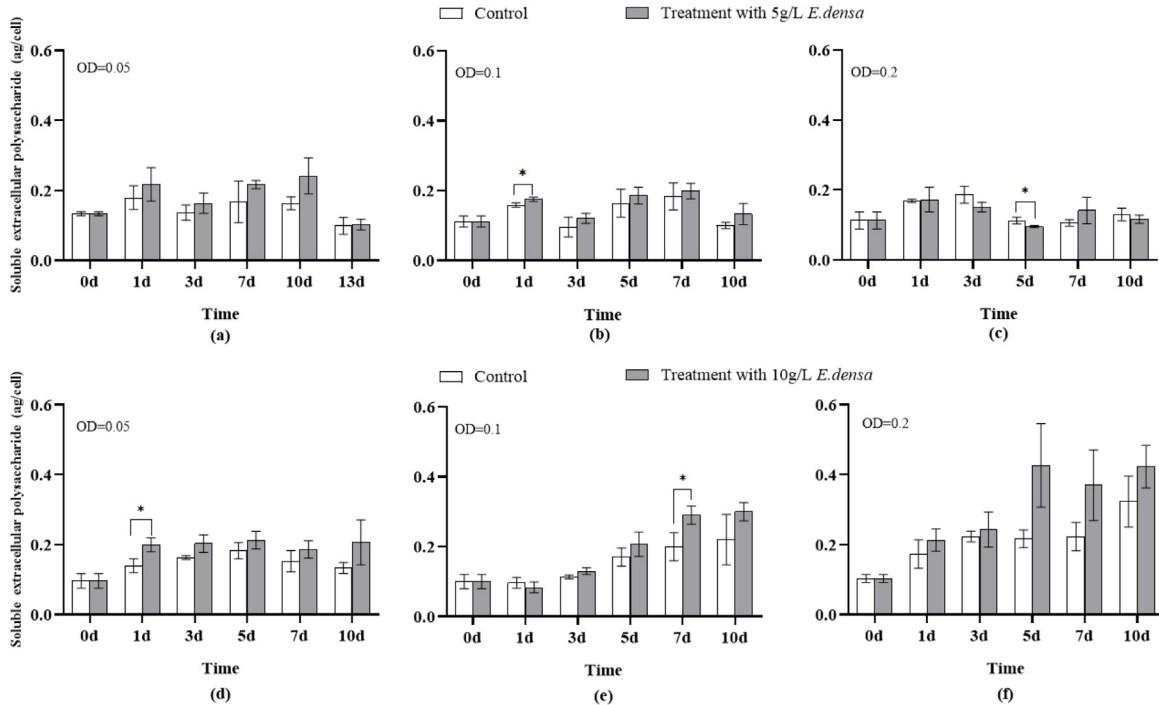


Fig. 5. The soluble extracellular polysaccharide in *C. vulgaris* with gradient concentration of initial algal cultivation density in the absence or presence of *E. densa* during the cultivation period.

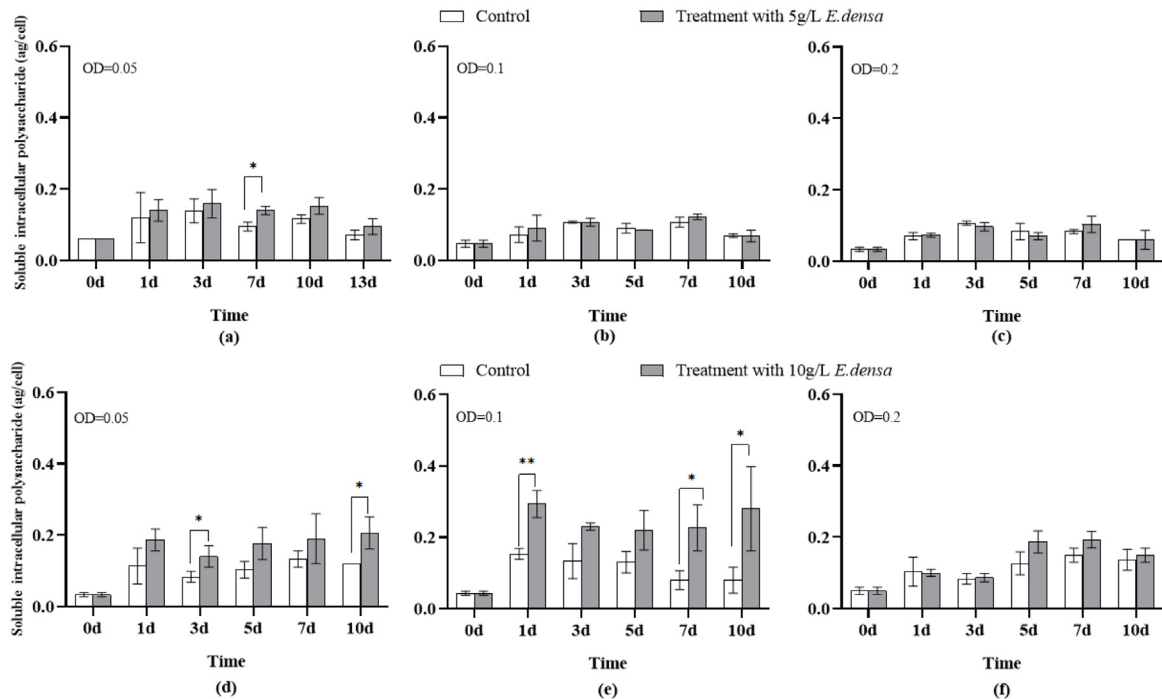


Fig. 6. The soluble intracellular polysaccharide in *C. vulgaris* with gradient concentration of initial algal cultivation density in the absence or presence of *E. densa* during the cultivation period.

differences, and TDP content was negatively correlated with initial algal density and positively related with macrophytes biomass. Therefore, considering the hypothesis that nutrient limitation would affect the allelopathic effects of submerged macrophytes on target organisms (Mulderij *et al.*, 2007; Hilt and Gross, 2008; Jeong *et al.*, 2021), it was necessary to pay attention to the nutrient changes in the co-existence cultivation systems.

Existing studies considerably discussed the inhibition effects on cyanobacteria induced by submerged macrophytes, especially for *M. aeruginosa* (Techer *et al.*, 2016; Santonja *et al.*, 2018; Li *et al.*, 2021), whereas controversial results were reported on the effects of submerged macrophyte on green algae. One of the consensus was that cyanobacteria were the most sensitive to the allelochemicals from macrophytes that have been tested, followed by diatoms. Chlorophytes (green algae) are known to be less sensitive (Körner *et al.*, 2002; Berger and Schagerl, 2004; Hilt and Gross, 2008; Pakdel *et al.*, 2013; Jeong *et al.*, 2021). Actually, whether the growth of green algae were stimulated or inhibited by submerged macrophytes depended on algal density and macrophytes biomass (Jiang *et al.*, 2015). Our study comprehensively discussed the macrophytes biomass and algal density effects between the allelochemical interaction. *E. densa* could significantly inhibit the growth of *C. vulgaris*. Vanderstukken *et al.* (2011), Cristian *et al.* (2016) and Rodríguez *et al.* (2016) have also observed that the presence of *E. densa* had an adverse impact on the growth of *Scenedesmus*. Based on the fact that the biomass density of macrophytes was only about 10 g/L in the natural environment (Zhu *et al.*, 2010), our present study demonstrated that *E. densa* could significantly suppress the growth of *C. vulgaris*, and the negative effects increased with

increasing amount of macrophytes. Consistent with our expectation, the present study also indicated that the allelopathic inhibition of *E. densa* on *C. vulgaris* depended on algal density. The inhibition effects were reduced with high initial algal concentration. A related study by Jiang *et al.* (2015) also indicated that when the initial phytoplankton density was high (1.9×10^8 cells/L), *Hydrilla verticillata* did not effectively control phytoplankton cell density. TDP content was negatively correlated with initial algal density. Thus, the lower allelopathic effects of the submerged macrophyte on phytoplankton with high density might be due to the combined effects of allelochemicals and phosphorus reduction. Previous studies have also demonstrated the lower allelopathic effects of *M. verticillatum* on cyanobacteria under phosphorus deficiency (Hilt, 2006; Hilt *et al.*, 2006).

4.2 Morphological changes induced by *E. densa*

Submerged macrophytes could allelopathically affect phytoplankton, including their physiological processes, such as photosynthesis, respiration (Körner and Nicklisch, 2002; Zhu *et al.*, 2010) and their antioxidant system (Wu *et al.*, 2007; Hong *et al.*, 2008). Distinct from the growth effects, Mulderij *et al.* (2005) firstly reported the induced colony formation of *S. obliquus* by aquatic macrophytes *Stratiotes aloides*. One recent study by Zhu *et al.* (2010) stated that although the exudates from *Cabomba furcata*, *Nymphoides hydrophylla* and *Chara* sp. had no significant effects on the algal growth of *S. obliquus*, the colony formation of the target algae was induced. The study firstly demonstrated that the morphological changes were better indicators of sensitivity to allelochemicals from

macrophytes than the growth parameters. In the present study, we suggested that *E. densa* could also affect the morphology of *C. vulgaris*. The study results contradicted with those of Wu *et al.* (2007), who suggested that *Potamogeton malianus* could inhibit the growth but did not induce morphological changes in *S. obliquus*. However, our previous study demonstrated the significantly induced colony formation of *C. vulgaris* (Dong *et al.*, 2018; Dong *et al.*, 2019) and *S. obliquus* (Dong *et al.*, 2013) by *Ceratophyllum demersum*. The colony formation induced by allelochemicals might be specific to macrophytes and target algae species.

An obvious ecological cost of algal colony formation was the increased sedimentation rate (Lürling and van Donk, 2000; Lürling, 2003a: b). Compared with colonial algae, single cells could be maintained at the upper layer owing to their better buoyancy (Conway and Trainor, 1972). However, the growth of colonial algae was inhibited due to light and temperature limitations at the bottom water (Zhu *et al.*, 2016; Albini *et al.*, 2019). In addition, compared with single cells, the reduction ratio of surface area to volume in colonial cells also affected the nutrients absorption, called encapsulation effect (Kirk, 1994). One question emerges. Is it a mistake for green algae to form colonies when encountering allelochemicals from macrophytes? Colony formation of green algae can be an inducible defence mechanism against predation (Hessen and Van Donk 1993; Herron *et al.*, 2019; Cheloni and Slaveykova, 2021; Lürling, 2021) in freshwater ecosystems. The ability to resist predation affects the algal survival directly and influences the trophic level via food chains (Van Donk, 2006; Van der Stap, 2007: 2008: 2009). In spite of increased sedimentation rate to bottom region, the competition between macrophytes and green algae could be alleviated. In addition, a previous study by Jeong *et al.* (2021) interpreted that allelochemicals produced by *Myriophyllum spicatum* could not penetrate the mucilage of colonial *M. aeruginosa* and *Anabaena circinalis*. Numerous studies have demonstrated that colony formation of green algae could increase their resistance to global warming (Zhu *et al.*, 2019) and micro-pollutants (Cheloni and Slaveykova, 2021). The present study suggested that the morphology of *C. vulgaris* was sensitive to allelochemicals from *E. densa*, indicating the presence of ecological strategies utilised *C. vulgaris* when encountering allelochemicals.

4.3 How to form colonies?

In consideration of the ecological consequences induced by interspecific interaction, one issue emerges: how do the colonies form? In the present study, the colony proportion and cell number per colony of *C. vulgaris* ($OD_{680}=0.1$) in the treatment with 10 g/L of *E. densa* were significantly higher than those of the control. Although many abiotic and biotic factors reportedly play roles in the colony formation of green algae, the inherent mechanisms are still unclear. One consensus was that the increased secretion of polysaccharide helps with cell adhesion and might be of great significance in the colony formation of green algae. Although no significant linear regression between the algal morphology and the content of polysaccharide was observed, it was indicated that

the content of intracellular and extracellular polysaccharide per cell in *C. vulgaris* were elevated in the presence of *E. densa* during the whole cultivation period, especially in the treatment of *C. vulgaris* ($OD_{680}=0.1$) by 10 g/L of *E. densa* with obvious algal morphological changes. The induced colony formation of green algae by abiotic and biotic factors were related to polysaccharide (Yang *et al.*, 2010; Li *et al.*, 2013; Bisova and Zachleder, 2014; Khona *et al.*, 2016; Dong *et al.*, 2018). The result was also verified by Liu *et al.* (2010), who demonstrated that the addition of glyoxylic acid could stimulate the polysaccharide production in *S. obliquus*, and the increased polysaccharide level was significantly positively correlated with the colony size of *S. obliquus*. Sun *et al.* (2020) indicated that the inhibitory expression of genes regulating the precursor synthesis during polysaccharide production could interrupt colony formation of *S. obliquus*.

Ethics approval and consent to participate

All authors agree with this submission.

Consent for publication

All authors agree with this publication.

Conflict of interest

The authors declare no conflicts of interest.

Funding information

This work was financially supported by the Young Backbone Teachers Project of Henan Province (No. 2020GGJS064), the National Natural Science Foundation of China (No. 31500380), the Scientific Fund of Henan Normal University (No. 2020QK02), the Major public welfare projects in Henan Province (No. 201300311300) and China Agriculture Research System (CARS-50).

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Cite this article as: Dai D, Yang Y, Wang F, Zhang Y, Zhang M, Gao Y, Gao X, Dong J, Li X, Chang M. 2023. Allelopathic effects of *Egeria densa* on the growth and morphology of *Chlorella vulgaris*. *Int. J. Lim.* 59: 4: