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ARTICLE

Effects of Two Potential Allelochemicals on the Photosystem II of *Nitzschia closterium* and *Monostroma nitidum*

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ABSTRACT

In aquaculture, high-density seaweed farming brings higher economic benefits but also increases outbreaks of diatom felt. The effective control of diatom felt in high-density seaweed farming has always been a research hotspot. This study selected two potential allelochemicals 2-hydroxycinnamic acid and quinic acid to explore their effects on a diatom *Nitzschia closterium* and an economic seaweed *Monostroma nitidum*. The results showed that 2-hydroxycinnamic acid had better inhibitory effects than quinic acid on the growth, pigment content and photosynthetic efficiency of *N. closterium*. Their half-maximal inhibitory concentrations at 120 h ($IC_{50-120\ h}$) were 0.9000 and 1.278 mM, respectively. Additionally, these allelochemicals had limited inhibitory effects on the growth, pigment content and photosynthetic efficiency of *M. nitidum* before 24 h. To further explore the allelopathic effect of these chemicals, this study focused on the photosystem II energy fluxes of *N. closterium*. It was found that 3 mM 2-hydroxycinnamic acid could destroy the whole photosynthetic system by devastating the PSII reaction centre (RC) before 24 h; however, the same concentration of quinic acid could only down-regulate the electron transport efficiency by changing the effective antenna size of an active RC and downregulating the PSII reaction centre density. These experimental results are expected to provide a new strategy to control diatom felt blooms on the high-density seaweed farming areas.

KEYWORDS

Allelochemicals; 2-hydroxycinnamic acid; quinic acid; *Nitzschia closterium*; *Monostroma nitidum*; photosynthetic system

1 Introduction

Marine biofouling is the result of marine organisms setting, adhering and growing on any structural surfaces that are immersed in seawater. It has always caused severe ecological environmental issues [1]. Marine fouling organisms include microfouling organisms and macrofouling organisms, and complete microfouling always increases macrofouling [2]. Diatoms are necessary components of microfouling organisms. They secrete mucilage or produce mucilage medium to attach to a substrate, with their attachment modes classified as valve attachment, mucilage pad attachment, mucilage stalk attachment, mucilage tube attachment and mucilage film attachment [3]. According to previous studies, diatoms have no strict specificity for different attachment substrates but are classified as epilithic, epipelic, epipsammic, epiphytic and epizoic diatoms according to their attachment substrate [4–8]. Macroalgae are common

hosts of epiphytic diatoms. The attachment of large quantities of epiphytic diatoms will limit their hosts' access to light and nutrients, thereby adversely affecting the hosts' growth [9].

In pursuit of higher economic profits, aquaculturists increase the density of seaweed cultivation as much as possible, which further aggravates outbreaks of diatom felt [10,11]. According to the 2010 and 2020 Algal Diseases Review, regular outbreaks of diatom felt in aquiculture areas caused pollution, bleaching and decreased yields of economic macroalgae, which led to significant economic losses for the global algal industry [11–13]. Since the mid-1980s, diatom felt outbreaks in the aquacultures of Japan's coastal waters have commonly resulted in damages in the order of a billion yen (>10 million dollars) [14,15]. In Korea and Japan, outbreaks of diatom felt have been devastating, causing serious economic loss to *Monostroma* and *Porphyra* sea farmers by lowering auction prices [13,16]. In China, the same problem has plagued aquaculturists for years [3,17]. Regular acid-washing is still considered the most effective method to combat diatom felt [12].

With the rise of allelopathy, more and more allelochemicals have been developed as biological methods for controlling the growth of harmful algae [18–22]. Allelochemicals are economical and eco-friendly compounds for controlling harmful algae because they are easy to access, biodegrade quickly and cause minimal pollution [19]. Many reports have revealed that botanical allelochemicals can significantly suppress the growth and photosynthesis of harmful algae [23–28]. The change of chlorophyll a, chlorophyll c and carotenoids might attribute to the photosynthetic system to acclimate the stress, as they are involved in light absorption, light harvesting and photochemistry [29–31]. The suppression of algal growth, pigment content and photosynthetic efficiency are macroscopic expressions of electron transport and ATP synthesis heterogeneity [32,33]. Many allelochemicals have been confirmed as hindering electron transport in algal photosynthetic systems, thus affecting ATP synthesis [28,34,35]. The OJIP fluorescence transient and JIP test, a sensitive probe for PSII heterogeneity, can elaborate changes in photons, excitons, electrons and other metabolites [36]. However, previous studies on the application of allelochemicals have concentrated on red tide algae rather than diatoms and economic macroalgae [37,38].

Our preliminary study revealed that *Gracilaria bailiniae* extract could inhibit the growth of *N. closterium* (data not shown). Two potential allelochemicals—2-hydroxycinnamic acid, a phenolic acid, and quinic acid, an organic acid—were identified in the *G. bailiniae* extract. Therefore, the allelopathic potential of 2-hydroxycinnamic acid and quinic acid was tested on epiphytic diatom *N. closterium* and economic macroalgae *M. nitidum*, respectively. The aims of this study were (1) to determine the effect of 2-hydroxycinnamic acid and quinic acid on the growth and photosynthetic efficiency of *N. closterium*, (2) to determine the effect of 2-hydroxycinnamic acid and quinic acid on the growth and photosynthetic efficiency of *M. nitidum* and (3) to further investigate the allelopathic effect of 2-hydroxycinnamic acid and quinic acid on the photosynthetic system of *N. closterium*.

2 Materials and Methods

2.1 Preparation of Standard Solutions

Standard substances 2-hydroxycinnamic acid (98%, CAS number: 583-17-5) and quinic acid (98%, CAS number: 77-95-2) were purchased from Bide Pharmatech Co., Ltd. (Shanghai, China) and Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). The appropriate weight of each standard substance was transferred into a 10 mL centrifuge tube and immediately dissolved in 0.1% DMSO. The resulting standard solutions (5, 10 and 30 mM) were stored at 4°C.

2.2 Pre-Treatment of N. closterium and M. nitidum Samples

N. closterium was obtained from the Guangyu Biological Technology Co., Ltd. (Shanghai, China). Attached *M. nitidum* samples were collected from the Naozhou Island, on the South China Sea coast of Zhanjiang City (20°55' 0"N, 110°35' 0"E). *M. nitidum* samples were taken to the laboratory in a cooler

on ice. Before experiments, sediments and contaminants were removed using filtered seawater and a soft brush. And then, *N. closterium* and *M. nitidum* were cultured in the laboratory using filter-sterilised (0.22 μm) natural seawater enriched with a modified f/2 medium in 1 L conical flasks. All conical flasks were sterilised at 121°C for 20 min in an autoclave steriliser to reduce bacterial numbers. To promote the growth of *N. closterium*, the initial concentration of $Na_2SiO_3 \cdot 9H_2O$ in the modified f/2 medium was adjusted to 3.18×10^{-4} M. Under relatively sterile conditions, it was then cultured in a thermostatic incubator at 22 ± 1°C, with an illumination intensity of 2,500 lx, a 12:12 h light/dark regime and a salinity of 30 PSU. To reduce the effect of bacteria, a mixed antibiotic solution was added to all cultures before inoculation of diatom samples. The mixed antibiotic solution contained 200 mg kanamycin sulphate, 200 mg ampicillin, 200 mg gentamicin and 100 mg streptomycin (Beijing Solarbio Science and Technology Co., Ltd., China). According to the findings of our previous study, this antibiotic mixture had no obvious adverse effects on the survival of *N. closterium* and *M. nitidum*. All conical flasks containing *N. closterium* were lightly shaken three times daily to assist growth and prevent *N. closterium* cells from adhering to the walls of the flasks.

2.3 Growth Assessment of N. closterium and M. nitidum Exposed to Various Concentrations of Potential Allelochemicals

N. closterium in the exponential growth stage was inoculated in the groups (containing equivalent volumes of modified f/2 medium) to obtain an initial algal cell density of approximately 2×10^6 cells·mL^{-1}. The thalli of *M. nitidum* were added to the groups (containing equivalent volumes of modified f/2 medium) to obtain an initial algal cell density of approximately 0.57 g·L^{-1}. Next, the standard solutions of the potential allelochemicals (2-hydroxycinnamic acid and quinic acid) were diluted to 0.5, 1 and 3 mM to test their effect on the growth of *N. closterium* and *M. nitidum*, respectively. The control group was treated with a 0 mM standard solution. There were three replicates for all groups in the experiment and the culture conditions were the same as those for the temporary culture of *N. closterium* and *M. nitidum* for 120 h. To decrease the effects of minor differences in photon irradiance, the conical flasks contained *N. closterium* were shaken slightly three times daily and manually rearranged, randomly. The growth rate of *M. nitidum* was determined every 24 h by monitoring changes in fresh wet. The growth of *N. closterium* was determined every 24 h using a haemocytometer (Qiujing Company, Shanghai, China). The inhibition rate (IR) of *N. closterium* was determined by the following equation:

$$IR\ (\%) = \frac{N_C - N_t}{N_c} \times 100 \quad (1)$$

Here, N_c is the average number of algal cells in the control group and N_t is the average number of algal cells in the treatment group.

2.4 Pigment Content Analysis of N. closterium and M. nitidum Exposed to Various Concentrations of Potential Allelochemicals

For content analysis of pigments chlorophyll a (chl a), chlorophyll c (chl c) and carotenoid, 10 mL of *N. closterium* culture exposed to various concentrations (0, 0.5, 1 and 3 mM) of 2-hydroxycinnamic acid or quinic acid was filtered through a 0.22 μm synthetic fabric filter membrane (Xinya Company, Shanghai, China) every 24 h. Each filter membrane was immediately collected and stored in the dark for 16 h at −20°C. The pigment of *N. closterium* was extracted from the filter membranes using 5 mL 95% ethyl alcohol and analysed by ultraviolet spectrophotometry (Shimadzu UV2450, Shimane, Japan) at 480, 510, 630, 664 and 750 nm according to the method described by Chen et al. [39]. The pigment content was calculated using the following equations:

$$chl\ a\ \left(\mu g \cdot mL^{-1}\right) = 11.47 \times A_{664} - 0.40 \times A_{630} \quad (2)$$

$$chl\ c\ \left(\mu g \cdot mL^{-1}\right) = 24.36 \times A_{630} - 3.73 \times A_{664} \quad (3)$$

$$carotenoid\ \left(\mu g \cdot mL^{-1}\right) = 7.6 \times [(A_{480} - A_{750}) - 1.49 \times (A_{510} - A_{750})] \quad (4)$$

Here, A_{480}, A_{510}, A_{630}, A_{664} and A_{750} are the average optical density at 480, 510, 630, 664 and 750 nm, respectively, and the unit of pigment content is $\mu g \cdot mL^{-1}$.

Additionally, 0.1 g thalli of *M. nitidum* exposed to various concentrations (0, 0.5, 1 or and 3 mM) of 2-hydroxycinnamic acid or quinic acid were collect and crushed every 24 h. The pigment of chlorophyll a (chl a), chlorophyll b (chl b) and carotenoid of *M. nitidum* was extracted from the crushed thalli of *M. nitidum* using 5 mL 95% ethyl alcohol and analysed by the same ultraviolet spectrophotometry at 470, 649 and 665 nm according to the method described by Wang et al. [40]. The pigment content was calculated using the following equations:

$$chl\ a\left(mg \cdot g^{-1}\right) = (13.95 \times A_{665} - 6.88 \times A_{649})V/M/1000 \quad (5)$$

$$chl\ b\left(mg \cdot g^{-1}\right) = (24.96 \times A_{649} - 7.32\ \times\ A_{665})V/M/1000 \quad (6)$$

$$carotenoid\left(mg \cdot g^{-1}\right) = (4.08 \times A_{470} - 11.56 \times A_{649} + 3.29 \times A_{665})V/M/1000 \quad (7)$$

Here, A_{470}, A_{649} and A_{665} are the average optical density at 470, 649 and 665 nm, respectively, V is the volume of 95% ethyl alcohol (mL), M is the quality of *M. nitidum* (g), and the unit of pigment content is $mg \cdot g^{-1}$.

2.5 *Photosynthetic Fluorescence of N. closterium and M. nitidum Exposed to Various Concentrations of Potential Allelochemicals*

Photosynthetic efficiency represented by the maximum photochemical efficiency of photosystem II (PS II, Fv/Fm) and fluorescence transients of *N. closterium* were measured by a fluorimetry (AquaPen AP110, Czech Republic). After dark-acclimated for 15 min, 0.1 g *M. nitidum* were applied to determine values of F_v/F_m. 3 mL of *N. closterium* culture (during the growth experiment) were transferred to a reaction vessel and then dark-adapted for 15 min after shaking in triplicate. The fluorescence transients were recorded for up to 2 s on a logarithmic time scale. The fast phase gave rise to the OJIP fluorescence transients within the 2 s recording period. The O, J, I and P phases represented the fluorescence intensity at 20 μs, 2 ms, 30 ms and the maximum fluorescence, respectively. The fluorescence transients were applied to measure the photochemistry and the electron transport efficiency of PSII. The following JIP parameters were determined, based on the original results of the fluorescence transients: trapped energy/PSII unit reaction centre (TRo/RC), electron transport energy/RC (ETo/RC), dissipated energy/RC (DIo/RC), absorbed energy/RC (ABS/RC), trapped energy/excited cross-section (TRo/CSo), electron transport energy/CSo (ETo/CSo), dissipated energy/CSo (DIo/CSo), PSII active reaction centres/CSo (RC/CSo), absorbed energy/CSo (ABS/CSo), the probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_{A-} (ψo), and the quantum yield of electron transport at t = 0 (φEo) [28,41–45]. The JIP test was applied to analyse the change to the photosynthetic apparatus when exposed to stress; that is, fluorescence analysis based on the OJIP curve [46,47]. In addition, the values of all JIP indicators were normalized (each parameter was expressed as a fraction relative to the value of the control) [28]. Axess labels (0, 0.5, 1, 1.5 and 2) were 0, 0.5, 1, 1.5 and 2 value of control, respectively.

2.6 Statistical Analysis

The normality and homogeneity of variance of all data were determined using the Kolmogorov-Smirnov and Levene test, respectively. To determine the effect of concentration and exposure time on the algae cell density and inhibition rate of *N. closterium*, a two-way analysis of variance (ANOVA) was applied. Tukey's *post hoc* pairwise comparisons were used when all variances were equal; otherwise, logarithmic transformation was applied in advance. Statistical significance was determined using Student's *t*-test. Data comparisons were deemed to have a significant difference at an alpha value of $P < 0.05$. Data were visualised using GraphPad Prism 9 and Microsoft Excel 10.0. $IC_{50–120\ h}$ was estimated each day by probit analysis through the IR values and concentration logarithm [28].

3 Results

3.1 Effect of Potential Allelochemicals on the Growth of N. closterium and M. nitidum

Three concentrations of potential allelochemicals 2-hydroxycinnamic acid and quinic acid were applied to explore the effect on *N. closterium* and *M. nitidum* growth over 120 h (Figs. 1 and 2). Overall, both 2-hydroxycinnamic acid and quinic acid had inhibitory effects on *N. closterium* growth, with the degree of inhibition depending on the concentration ($P < 0.05$). Except for the 0.5 mM treatment, the growth of *N. closterium* was significantly suppressed at various concentrations of 2-hydroxycinnamic acid and quinic acid (Figs. 1A and 2A, 1B and 2B; $P < 0.05$). However, the half-maximal inhibitory concentration of 2-hydroxycinnamic acid was lower than quinic acid at 120 h ($IC_{50–120\ h}$ was 0.9000 and 1.278 mM, respectively) (Table 1). It is worth noting that the inhibition rates of all quinic acid treatment groups were slightly decreased from 96 to 120 h compared to when 2-hydroxycinnamic acid was used. 2-hydroxycinnamic acid and quinic acid had limited inhibitory effects on the growth of *M. nitidum* before 24 h (Figs. 1C and 1D; $P > 0.05$). Additionally, these potential allelochemicals had lower toxicity on the growth of *M. nitidum* compared to *N. closterium* (Fig. 1). Therefore, 2-hydroxycinnamic acid not only displayed a stronger allelopathic effect on the growth of *N. closterium* than quinic acid, but had limited inhibitory effects on the growth of *M. nitidum* before 24 h.

3.2 Effect of Potential Allelochemicals on the Pigment Content of N. closterium and M. nitidum

Three concentrations of potential allelochemicals 2-hydroxycinnamic acid and quinic acid were applied to explore their effect on the pigment content of *N. closterium* and *M. nitidum* over 120 h (Figs. 3 and 4). Overall, both 2-hydroxycinnamic acid and quinic acid decreased the pigment content of *N. closterium*, with the extent of the decrease depending on the concentration of 2-hydroxycinnamic acid or quinic acid ($P < 0.05$). The chl a and carotenoid pigment content of *N. closterium* was significantly decreased in a dose-dependent manner following 2-hydroxycinnamic acid exposure at concentrations above 0.5 mM (Figs. 3A and 3C; $P < 0.05$). However, only at concentrations of 3 mM did quinic acid significantly decrease the content of chl a and carotenoid from 48–72 h (Figs. 3D and 3F; $P < 0.05$). 0.5–1 mM quinic acid had a limited impact on chl c content on 120 h (Fig. 3E; $P > 0.05$), but 1 mM 2-hydroxycinnamic acid significantly decreased the chl c content (Fig. 3B; $P < 0.05$) on 120 h. Additionally, the pigment content of all quinic acid treatment groups gradually recovered from 72–120 h (Figs. 3D to 3F). 2-hydroxycinnamic acid and quinic acid had limited inhibitory effects on the pigment content of *M. nitidum* before 24 h (Figs. 4A to 4F; $P > 0.05$). Therefore, 2-hydroxycinnamic acid not only displayed a stronger allelopathic effect on the pigment content of *N. closterium* than quinic acid, but had limited inhibitory effects on the pigment content of *M. nitidum* before 24 h.

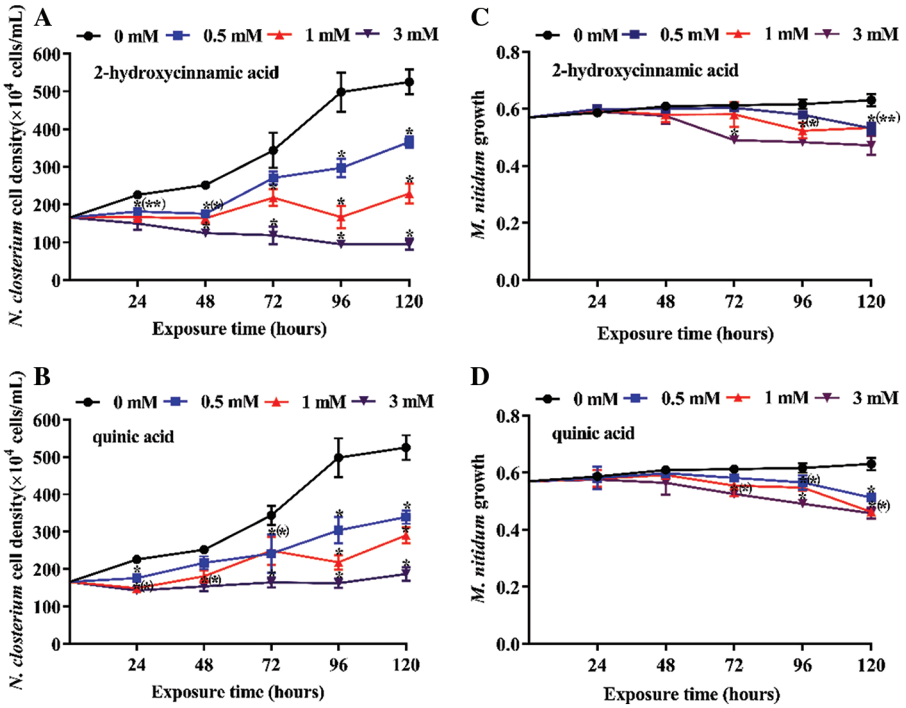

Figure 1: Inhibition effect of three concentrations of potential allelochemicals on the growth of *N. closterium* and *M. nitidum*: (A) *N. closterium* exposed to 2-hydroxycinnamic acid, (B) *N. closterium* exposed to quinic acid, (C) *M. nitidum* exposed to 2-hydroxycinnamic acid, (D) *M. nitidum* exposed to quinic acid. Unit: mM. The growth of *M. nitidum* is fresh wet (g). The change in *N. closterium* cell density indicates the inhibitory effect. The data are presented as mean ± standard deviation (n = 3). * indicates a significant difference compared with the controls, and * in brackets indicate a significant difference in overlapped plots (Student's test, $p < 0.05$)

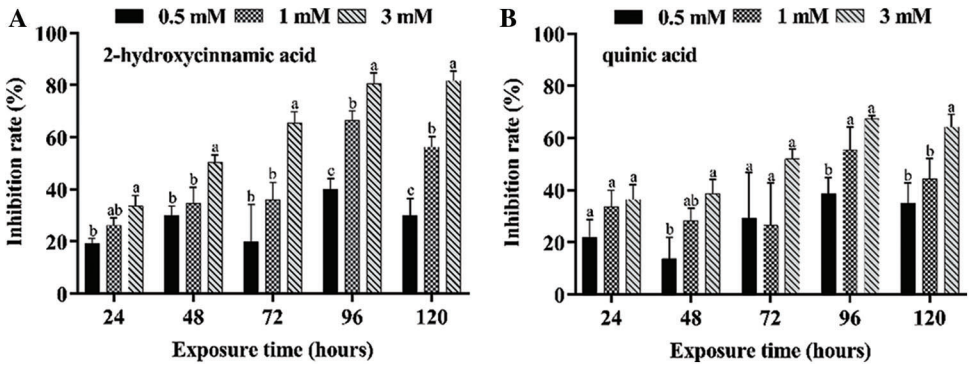

Figure 2: Inhibition rates of three concentrations of allelochemicals on *N. closterium*: (A) 2-hydroxycinnamic acid, (B) quinic acid. Unit: mM. The data are presented as the mean ± standard deviation (n = 3). Lowercase letters indicate significant differences (ANOVA/Tukey's ANOVA, $P < 0.05$)

Table 1: $IC_{50–120\ h}$, CI, regression equation, and R^2 of two potential allelochemicals against *N. closterium*

Material	$IC_{50–120\ h}$[a]	95% CI[b]	Regression equation[c]	R^2
2-hydroxycinnamic acid	0.9000 mM	0.7650–1.042	y = 1.83x + 0.07	0.950
Quinic acid	1.278 mM	0.9220–1.861	y = 0.98x − 0.11	0.811

Notes: [a]50% inhibition concentration. [b]95% confidence interval. [c]Correlation coefficient.

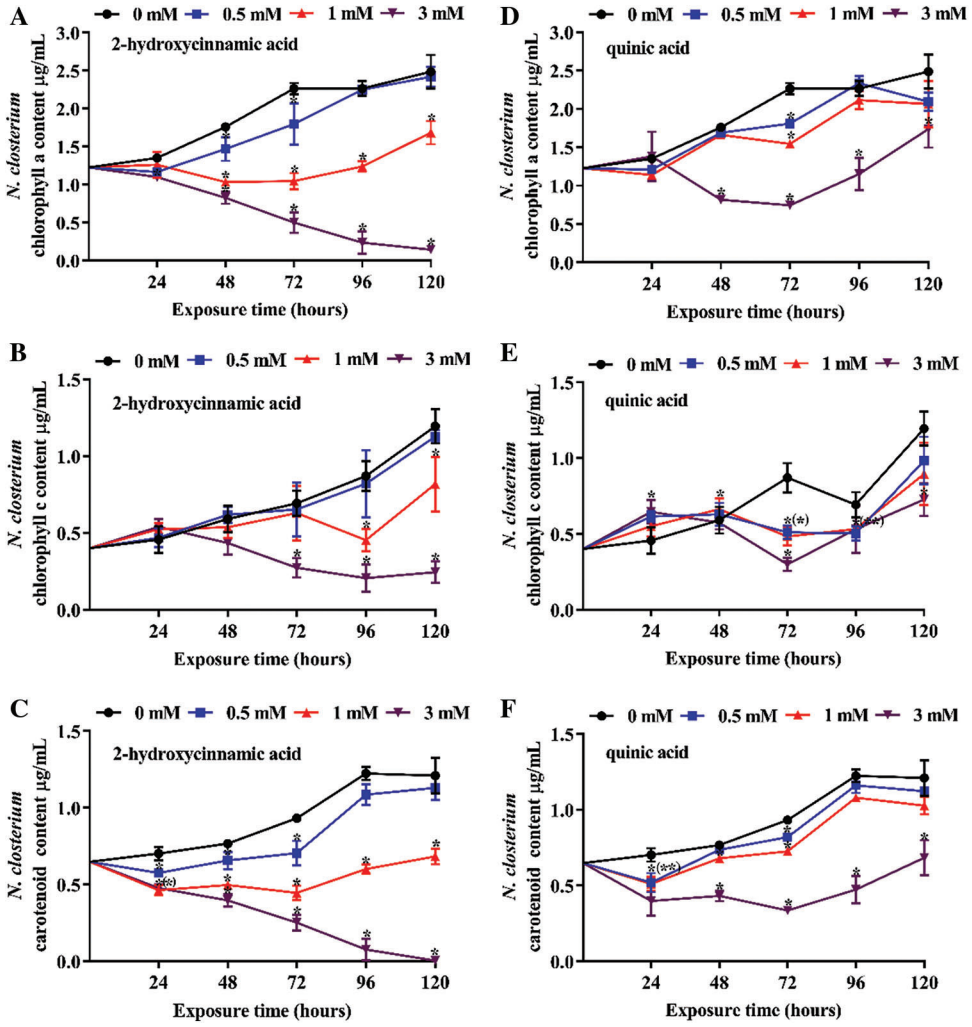

Figure 3: Pigment content of *N. closterium* exposed to three concentrations of the potential allelochemicals: (A) chl a exposed to 2-hydroxycinnamic acid, (B) chl c exposed to 2-hydroxycinnamic acid, (C) carotenoid exposed to 2-hydroxycinnamic acid, (D) chl a exposed to quinic acid, (E) chl c exposed to quinic acid, (F) carotenoid exposed to quinic acid. Unit: $\mu g \cdot mL^{-1}$. Changes in pigment content indicate changes in the physiology of *N. closterium*. The data are presented as the mean ± standard deviation (n = 3). * indicates a significant difference compared with the controls, and * in brackets indicate a significant difference in overlapped plots (Student's test, $P < 0.05$)

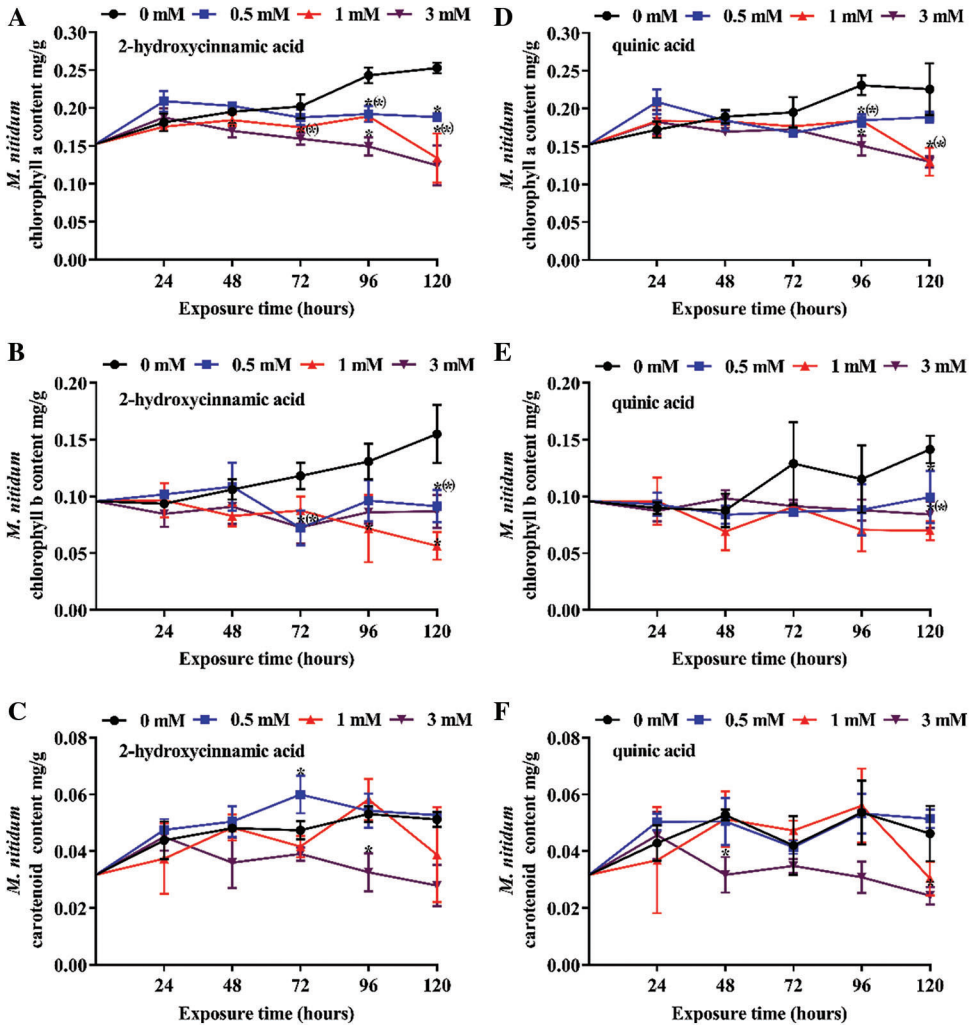

Figure 4: Pigment content of *M. nitidum* exposed to three concentrations of the potential allelochemicals: (A) chl a exposed to 2-hydroxycinnamic acid, (B) chl b exposed to 2-hydroxycinnamic acid, (C) carotenoid exposed to 2-hydroxycinnamic acid, (D) chl a exposed to quinic acid, (E) chl b exposed to quinic acid, (F) carotenoid exposed to quinic acid. Unit: $mg \cdot g^{-1}$. Changes in pigment content indicate changes in the physiology of *M. nitidum*. The data are presented as the mean ± standard deviation ($n = 3$). * indicates a significant difference compared with the controls, and * in brackets indicate a significant difference in overlapped plots (Student's test, $P < 0.05$)

3.3 Effect of Potential Allelochemicals on Photosynthetic Fluorescence of N. closterium and M. nitidum

Three concentrations of potential allelochemicals 2-hydroxycinnamic acid and quinic acid were applied to explore their effect on the photosynthetic efficiency of *N. closterium* and *M. nitidum* for 120 h (Fig. 5). Overall, these potential allelochemicals had relatively limited effect on the photosynthetic efficiency of *N. closterium* and *M. nitidum* excepted for 3 mM 2-hydroxycinnamic acid treatment groups of *N. closterium* after 24 h. The photosynthetic efficiency of 3 mM 2-hydroxycinnamic acid treatment groups of *N. closterium* almost disappeared before 24 h and was irreversible during this experiment (Fig. 5A). The photosynthetic efficiency of 3 mM 2-hydroxycinnamic acid treatment groups of *M. nitidum* was stable compared to the control before 24 h (Fig. 5C). To further investigate the

allelopathic effect of 2-hydroxycinnamic acid and quinic acid, OJIP fluorescence transient of *N. closterium* were measured for 120 h (Figs. 6 and 7). Overall, both 2-hydroxycinnamic acid and quinic acid could reduce the OJIP fluorescence induction curves of *N. closterium*, with the extent of the reduction depending on the concentration of 2-hydroxycinnamic acid and quinic acid (Figs. 6 and 7; $P < 0.05$). Figs. 6 and 7 shows that 0.5−1 mM of potential allelochemicals 2-hydroxycinnamic acid and quinic acid resulted in typical fluctuating OJIP fluorescence induction curves. However, when the concentration reached 3 mM, the fluorescence transient of *N. closterium* decreased significantly. Moreover, the fluorescence transient of *N. closterium* almost disappeared before 24 h in the 3 mM 2-hydroxycinnamic acid treatment group; additionally, the heterogeneous behaviour of the OJIP fluorescence induction curves for the 3 mM 2-hydroxycinnamic acid treatment groups was irreversible during this experiment. In contrast, although the OJIP fluorescence induction curves for the 3 mM quinic acid treatment group were significantly lower in intensity than that of the control, the intensity gradually recovered before 24 h.

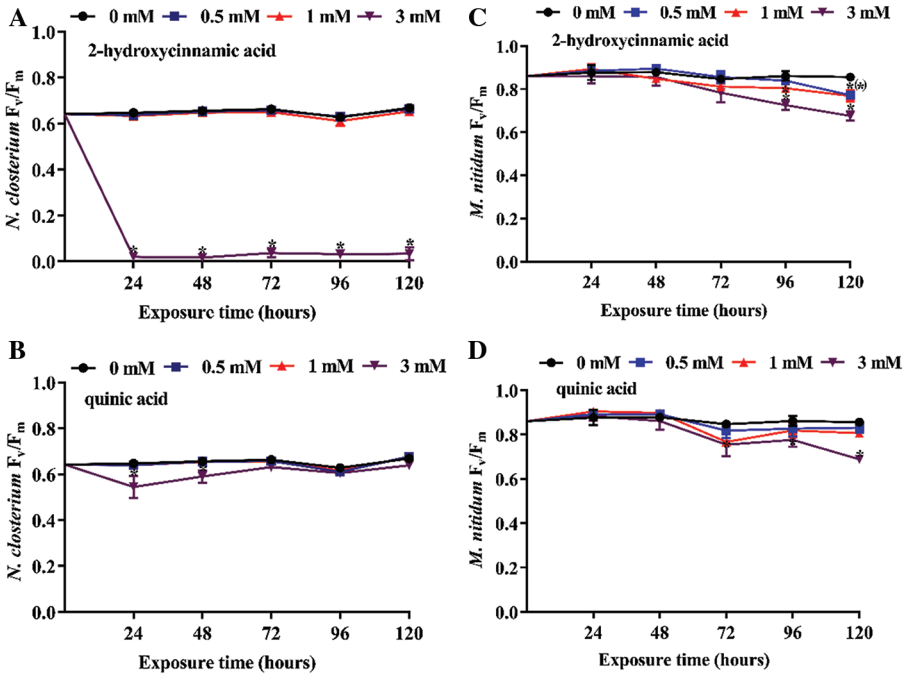

Figure 5: Photosynthetic efficiency (Fv/Fm, the maximum quantum yield of photosystem II) of *N. closterium* and *M. nitidum* exposed to three concentrations of the potential allelochemicals: (A) *N. closterium* exposed to 2-hydroxycinnamic acid, (B) *N. closterium* exposed to quinic acid, (C) *M. nitidum* exposed to 2-hydroxycinnamic acid, (D) *M. nitidum* exposed to quinic acid. The data are presented as the mean ± standard deviation ($n = 3$)

To further explore the effect of potential allelochemicals 2-hydroxycinnamic acid and quinic acid on *N. closterium*, the relevant parameters obtained from the OJIP transients were applied to analyse the JIP test. The following results were obtained (Figs. 8 and 9). The phenomenological energy flux indices (TRo/CSo, ETo/CSo, DIo/CSo and ABS/CSo) and the density of the RC index (RC/CSo) fluctuated for all treatment groups but were always lower than for the control group. Regarding 0.5–1 mM 2-hydroxycinnamic acid treatment groups, except for the above-mentioned changes in phenomenological energy flux indices and the density of the RC index, there were limited changes in the specific energy flux indices (TRo/RC, ETo/RC, DIo/RC and ABS/RC) and yield or flux ratio indices (ψo and φEo) of

N. closterium. Additionally, when the concentration of 2-hydroxycinnamic acid reached 3 mM, most relevant JIP test indicators were close to zero before 24 h. The quinic acid treatment groups displayed similar results for the change in TRo/CSo, ETo/CSo, DIo/CSo, ABS/CSo, RC/CSo, ψo and φEo, but quinic acid gradually increased the ABS/RC and DIo/RC in a dose-dependent manner. Additionally, although 3 mM quinic acid significantly decreased the TRo/CSo, ETo/CSo, DIo/CSo, ABS/CSo and RC/CSo, the ψo, φEo, TRo/RC, ETo/RC, DIo/RC and ABS/RC were similar to the control.

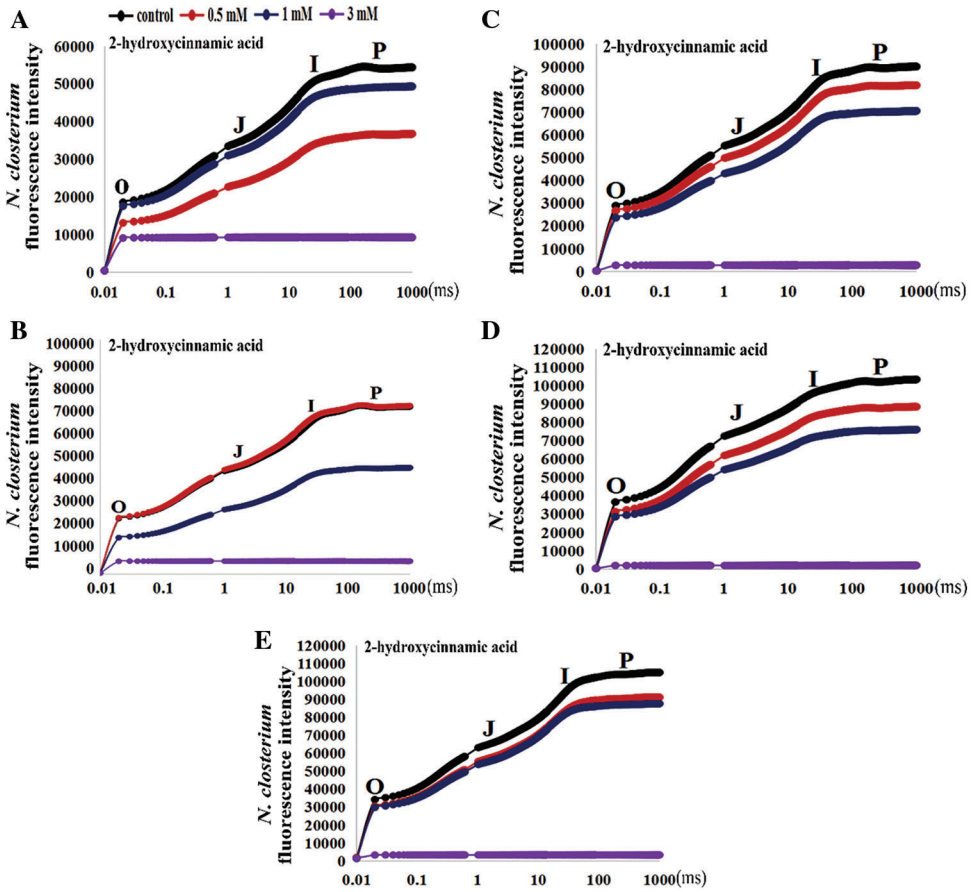

Figure 6: Increase in fluorescence transients for *N. closterium* exposed to 2-hydroxycinnamic acid. A, B, C, D, and E represent *N. closterium* exposed to 2-hydroxycinnamic acid for 24, 48, 72, 96 and 120 h, respectively. O: initial fluorescence, J: fluorescence at 2 ms, I: fluorescence at 30 ms, P: maximum fluorescence. Unit: ms

4 Discussion

Several previous studies have reported similar allelopathic effects of botanical allelochemicals, the major components of which are phenolic acids, organic acids, alkaloids, terpenoids and flavonoids [38]. For example, phenolic acids 2-phenylphenol, *p*-hydroxybenzoic acid, protocatechuic acid, ellagic acid, gallic acid, *p*-coumaric acid and vanillic acid have effective allelopathic effects on the growth of harmful algae [48–53]. Additionally, some organic acids such as quinic acid, hexanedioic acid, linoleic acid, α-linoleic acid, palmitic acid and azelaic acid also exhibit strong allelopathic potential on controlling harmful algae [54–58]. However, Nakai et al. found that quinic acid had no algal suppression effect [19]. This phenomenon might be due to allelochemicals showing high species specificity in suppressing algae

[59–61]. Furthermore, allelochemicals that contain hydroxyl groups and benzene rings may exert more effective allelopathic effects on algal growth [28]. Changes to the pigment of algae indicate that the photosynthetic activity of algal cells is affected. For example, a decrease in pigment content means that cellular resistance has decreased; an increase in pigment content is indicative of the amelioration of photochemical loss [62]. In the present study, 2-hydroxycinnamic acid—which contains hydroxyl groups and benzene rings—showed a better allelopathic effect on *N. closterium* than quinic acid—which contains no benzene rings (Figs. 1A and 1B and 2 and 3A to 3F)—with $IC_{50–120\ h}$ values of 0.9000 and 1.278 mM, respectively (Table 1). Additionally, these potential allelochemicals had limited inhibitory effects on *M. nitidum* (Figs. 1C and 1D and 4A to 4F). Hence, 2-hydroxycinnamic acid not only displayed a stronger allelopathic potential on the growth of *N. closterium* than quinic acid, but had relatively little effect on *M. nitidum*.

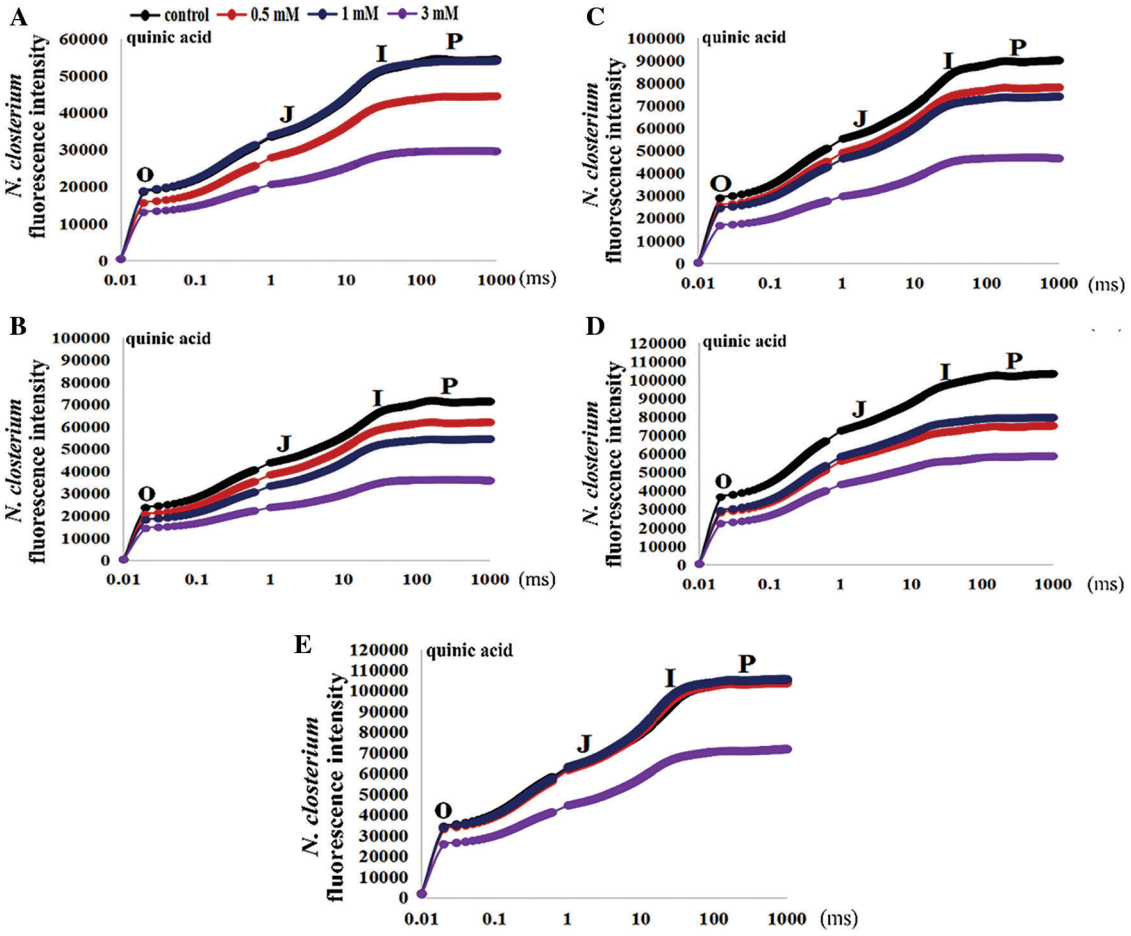

Figure 7: Increase in fluorescence transients for *N. closterium* exposed to quinic acid. A, B, C, D, and E represent *N. closterium* exposed to quinic acid for 24, 48, 72, 96 and 120 h, respectively. O: initial fluorescence, J: fluorescence at 2 ms, I: fluorescence at 30 ms, P: maximum fluorescence. Unit: ms

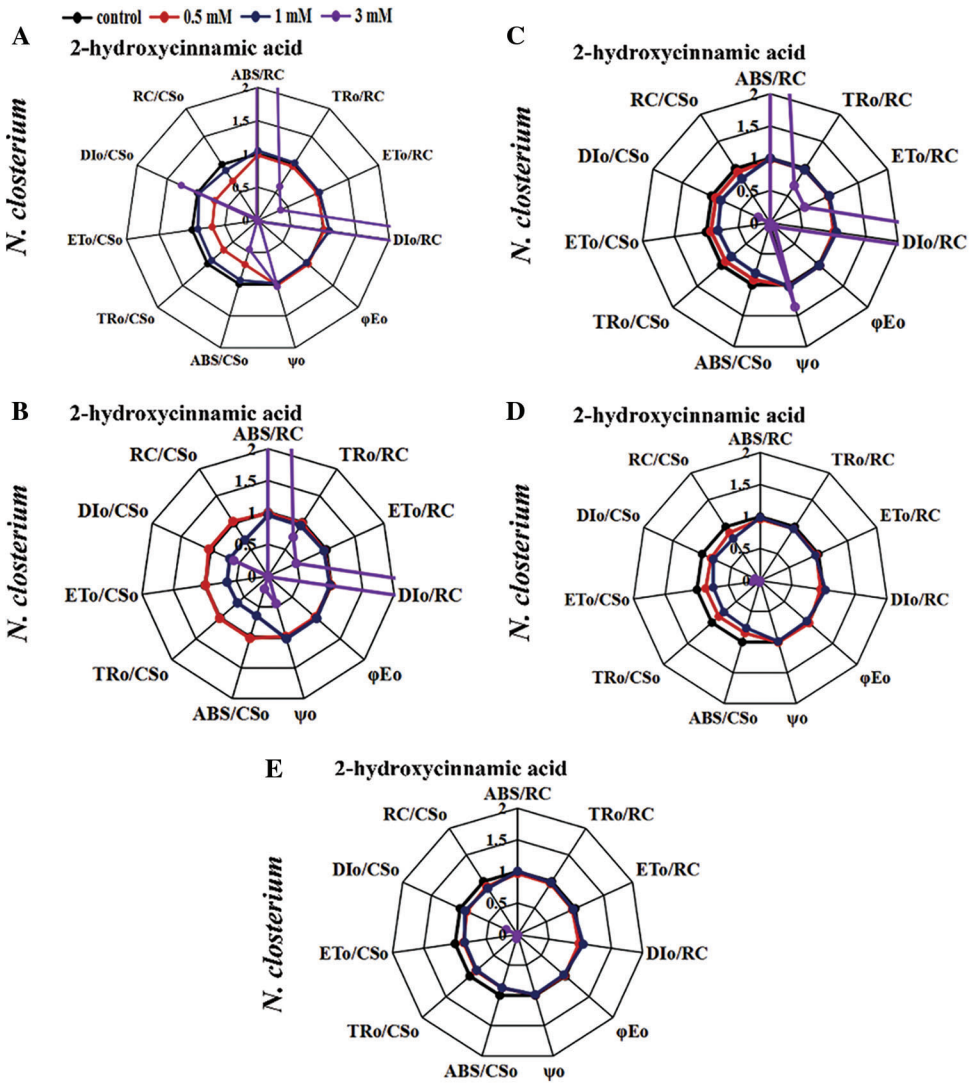

Figure 8: Effect of 2-hydroxycinnamic acid on chlorophyll fluorescence transient JIP parameters in *N. closterium*. A, B, C, D and E represent *N. closterium* exposed to 2-hydroxycinnamic acid for 24, 48, 72, 96 and 120 h, respectively. Each parameter is expressed as a fraction relative to the value of the control (regular circle with 100% = 1). Detailed physiological interpretations of each parameter are shown in Table 2

A decrease in photosynthetic efficiency and kinetics and magnitude of fluorescence intensity can be indicative of a decrease in algal state or biomass [41,45,63]. Additionally, a decrease in the kinetics and magnitude of fluorescence intensity (reduction in the O-point, J-point, I-point and P-point) can indicate an increase in the number of non-Q_B acceptors in PS II, which blocks electron transport from plastoquinone combined with D_1 protein (Q_A) to plastoquinone combined with D_2 protein (Q_B) [42,43,64–69]. In the present study, 3 mM 2-hydroxycinnamic acid showed a better allelopathic effect on the photosynthetic efficiency of *N. closterium* than quinic acid before 24 h (Figs. 5A and 5B). Additionally, both 3 mM 2-hydroxycinnamic and quinic acid had relatively little effect on the photosynthetic efficiency of *M. nitidum* before 24 h (Figs. 5C and 5D). Hence, 3 mM 2-hydroxycinnamic acid could not only more effectively reduce the photosynthetic efficiency of *N. closterium* than quinic acid, but also had minimal effect on *M. nitidum* before 24 h. Further studies revealed that both 3 mM 2-hydroxycinnamic and quinic

acid significantly decreased the OJIP kinetics and magnitude (Figs. 6 and 7). In especially, 3 mM 2-hydroxycinnamic showed a better allelopathic effect on the OJIP kinetics and magnitude of *N. closterium* than quinic acid. Hence, both 3 mM 2-hydroxycinnamic acid and quinic acid could block electron transport from Q_A to Q_B in *N. closterium*. In which, 3 mM 2-hydroxycinnamic showed more effective block on electron transport of *N. closterium* than quinic acid.

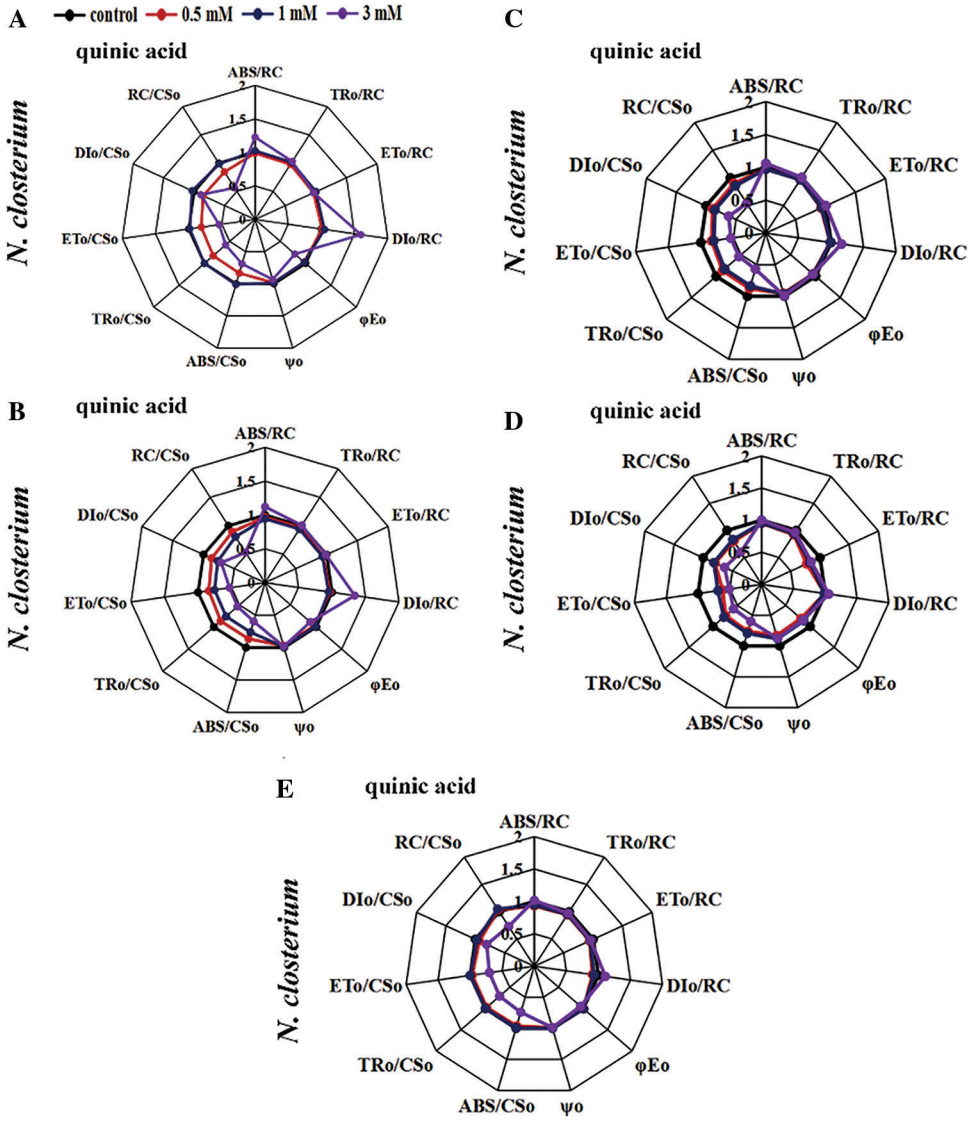

Figure 9: Effect of quinic acid on chlorophyll fluorescence transient JIP parameters in *N. closterium*. A, B, C, D and E represent *N. closterium* exposed to quinic acid for 24, 48, 72, 96 and 120 h, respectively. Each parameter is expressed as a fraction relative to the value of the control (regular circle with 100% = 1). Detailed physiological interpretations of each parameter are shown in Table 2

Table 2: Parameters and formulas derived from OJIP

JIP test parameter	Physiological description
Specific energy flux indexes	
$ABS/RC = M_o/V_J/\varphi P_o$	Absorption flux per RC
$TR_o/RC = M_o/V_J$	Trapped energy flux per RC at t = 0
$ET_o/RC = (M_o/V_J) \cdot \psi o$	Electron transport flux per RC at t = 0
$DI_o/RC = ABS/RC - TR_o/RC$	Dissipated energy flux per RC at t = 0
Yield or flux ratio indexes	
$\psi o = ET_o/TR_o = 1 - V_J$	Probability (at t = 0) that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-
$\varphi E_o = ET_o/ABS = (Fv/Fm)\cdot\psi o$	Quantum yield for electron transport at t = 0
Phenomenological energy flux indexes per excited cross-section (CS)	
$ABS/CS_o \approx F_o$	Absorption flux per CS at t = 0
$TR_o/CS_o = (ABS/CS_o) \cdot \varphi P_o$	Trapped energy flux per CS at t = 0
$ET_o/CS_o = (ABS/CS_o) \cdot \varphi E_o$	Electron transport flux per CS at t = 0
$DI_o/CS_o = ABS/CS_o - TR_o/CS_o$	Dissipated energy flux per CS at t = 0
Density of reaction centres	
$RC/CS_o = \varphi P_o\cdot(ABS/CS_o) \cdot (V_J/M_o)$	Number of active PSII RCs per CS at t = 0

Notes: V_J–relative variable fluorescence at the J-step (2 ms). Mo–approximated initial slope (in ms-1) of the fluorescence transient. φPo (Fv/Fm)–maximum quantum yield of primary photochemistry at t = 0. Fo-Minimum fluorescence, when all PSII RCs are open. Fm-Maximum fluorescence, when all PSII RCs are closed.

The decrease in phenomenological energy flux indexes (TRo/CSo, ETo/CSo, DIo/CSo and ABS/CSo) and the density of the RC index (RC/CSo) could be indicative that excessive excitation energy accumulated in the RCs. This would convert active centres into inactive centres (RC/CSo), impeding energy absorption efficiency (ABS/CSo) and decreasing the photon trapping rate (TRo/CSo), electron transport (ETo/CSo) and energy dissipation (DIo/CSo) in PSII [28,36,70,71]. The increase in ABS/RC represented an enhancement in the effective antenna size of active unit RC, which meant that some active RCs in PSII converted to inactive RCs (dissipation sinks) and that part of the unit RC enhanced energy absorption to offset the reduction in electron transport [72–75]. Additionally, changes in TRo/RC, ETo/RC and DIo/RC are usually attributed to changes in ABS/RC. Reports have shown that PSII downregulates the RCs and upregulates dissipation (DIo/RC) to maintain absorption (ABS/RC), capture (TRo/RC) and the electron transport balance in response to stress [63,76–78]. In the present study, 0.5–1 mM 2-hydroxycinnamic decreased the phenomenological energy flux indexes and density of the RC index, but had limited effect on the specific energy flux and yield or flux ratio indices. 3 mM 2-hydroxycinnamic acid let most of relevant JIP test indicators close to zero before 24 h. In contrast, all concentrations of quinic acid not only decreased the phenomenological energy flux indexes and density of the RC index, but increased the specific energy flux indices. Therefore, according to the OJIP and JIP results in the present study (Figs. 6 to 9), it was believed that 2-hydroxycinnamic acid impacted the photosynthetic efficiency of *N. closterium* mainly by downregulating the PSII RC density to reduce the Q_A photochemical redox reaction and decrease electron transport capacity. When the 2-hydroxycinnamic acid concentration reached 3 mM, the whole photosynthetic system was destroyed before 24 h. Compared to 2-hydroxycinnamic acid, quinic acid impacted the photosynthetic efficiency of *N. closterium* by changing the effective antenna size of unit RCs and downregulating the PSII RC density. Even at a quinic acid concentration of 3 mM,

N. closterium still could enhance the dissipation and efficiency of unit RCs to offset overall photosynthetic efficiency declines.

5 Conclusions

The present study showed 2-hydroxycinnamic acid to decrease the photosynthetic efficiency of *N. closterium* by destroying the PSII reaction centre, thus inhibiting its growth. Quinic acid changed the effective antenna size of unit reaction centres and downregulated the PSII reaction centre density, thereby impacting the photosynthetic efficiency and growth of *N. closterium*. 3 mM 2-hydroxycinnamic acid displayed stronger allelopathic potential on the PSII reaction centres compared to quinic acid. Additionally, 3 mM 2-hydroxycinnamic acid had minimal effects on *M. nitidum* before 24 h. Thus, short-term treatment of 2-hydroxycinnamic acid maybe consider as a new strategy to control diatom felt bloom the high-density seaweed farming areas.

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