hydrobiology

MDPI

Article

Inhibitory Effect of Aqueous Extracts from *Egeria densa* Planch. on Cyanobacteria *Microcystis aeruginosa* (Kützing) Lemmermann Growth

Ashika Wijesinghe, Mudalige Don Hiranya Jayasanka Senavirathna * and Takeshi Fujino

Graduate School of Science and Engineering, Saitama University, Saitama 338-8570, Japan; ashikawijesinghe23@gmail.com (A.W.); fujino@mail.saitama-u.ac.jp (T.F.)
* Correspondence: jayasanka@hotmail.com

Abstract: This study aimed to investigate the allelopathic effect of live *Egeria densa* Planch. and its aqueous extracts in inhibiting the cyanobacterium *Microcystis aeruginosa* (Kützing) Lemmermann through a possible growth inhibition pathway. Under coexistence, the presence of live *E. densa* reduced the growth of *M. aeruginosa* by 48% compared to when *M. aeruginosa* was alone. Consequently, we prepared two separate aqueous extracts with distilled water: one from *E. densa* plants collected from monocultures, and the other from *E. densa* plants collected from co-cultivation with *M. aeruginosa*. At a concentration of 0.5 g/L, both extracts successfully suppressed the growth of *M. aeruginosa* throughout the 5-day exposure period. The extracts obtained from *E. densa* plants grown in a combined culture with *M. aeruginosa* showed significant growth-inhibiting capabilities compared to the extracts obtained from *E. densa* monoculture ($p < 0.05$). They showed 22% more growth inhibition compared to cultures exposed to monoculture extracts at day 5, indicating that the production of allelochemicals in *E. densa* was induced during co-existence with cyanobacteria. However, the higher concentrations (2 and 4 g/L) of both extracts did not effectively exhibit a successful inhibitive ability, possibly due to the presence of high nutrient concentrations, specifically PO_4^{3-}, which may be potentially suppressing the activity of allelochemicals. Further studies are recommended in identifying the specific allelochemicals and exploring their practical implementation in the field.

Keywords: allelopathic suppression; biological control; cyanobacteria; macrophytes; plant extractions

check for updates

Citation: Wijesinghe, A.; Senavirathna, M.D.H.J.; Fujino, T. Inhibitory Effect of Aqueous Extracts from *Egeria densa* Planch. on Cyanobacteria *Microcystis aeruginosa* (Kützing) Lemmermann Growth. *Hydrobiology* **2023**, *2*, 491–506. https://doi.org/10.3390/hydrobiology2030033

Academic Editor: Cláudia Pascoal

Received: 3 August 2023
Revised: 6 September 2023
Accepted: 13 September 2023
Published: 15 September 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Cyanobacterial blooms are a significant global environmental threat worldwide [1–3]. The proliferation of cyanobacteria can be attributed to various factors, such as the influx of excessive nutrients from poor agricultural practices, the discharge of domestic and industrial solid and liquid waste, and changes in environmental parameters (i.e., temperature, light) [1,4,5]. They are responsible for various issues including eutrophication, hypoxia, and mortality of aquatic fauna [1,2] in both natural and man-made aquatic ecosystems. Furthermore, these blooms produce toxins, such as microcystins, which can cause severe health complications and even death in both animals and humans [5,6]. The problem has spread from temperate and tropical environments to natural and man-made aquatic environments. Therefore, the establishment of efficient methods to control harmful cyanobacteria blooms is essential for preserving water quality, protecting biodiversity, and ensuring the well-being of ecosystems and communities.

Numerous conventional in situ methods have been employed to control harmful cyanobacterial blooms. These methods include the application of synthetic halogenated algaecides, the utilization of chemicals and bio-coagulants, ultrasonic technology, sediment dredging, and the implementation of aeration towers [1,7,8]. However, these methods either adversely affect other aquatic organisms or impose significant initial investment and operational costs [1,9]. Consequently, there is a growing interest in natural control

methods for in situ biological cyanobacteria mitigation as a safer alternative. Phytoplanktivorous fishes, such as *Hypophthalmichthys nobilis* and *Oreochromis niloticus*, are commonly employed in the bio-manipulation of cyanobacteria. These fishes directly feed on cyanobacteria, including toxin-producing *Microcystis aeruginosa* [10]. Moreover, the utilization of macrophytes and their extracts as a biological control method for cyanobacteria has been widely adopted in previous studies due to their minimal impact on the ecosystem and human health [11,12]. A new generation of natural algaecides based on allelochemicals from aquatic plants increased the attention of researchers because of their low toxicity on other aquatic life [13].

Cyanobacteria growth is inhibited by the various mechanisms exhibited by aquatic macrophytes, including nutrient absorption, light reduction through shading, and the release of allelochemicals. Aquatic plants primarily release allelochemicals to compete for resources, prevent herbivores, and help their survival in their environment. These chemicals also influence ecosystems by affecting the growth of neighboring plants or cyanobacteria. According to the Maredová et al. [14], submerged plants and their extracts, including *Chara globularis*, *Elodea nuttallii*, *Ceratophyllum submersum*, *Hydrilla verticillata*, *Myriophyllum spicatum*, *Myriophyllum heterophyllum*, and *Vallisneria americana*, showed the inhibition of cyanobacterial growth. This phenomenon occurs because the formation of cyanobacterial bloom layer on the surface of the water reduces light penetration into the depths, potentially causing light scarcity for submerged macrophytes [15]. Consequently, these plants may utilize the production and release of compounds that inhibit cyanobacterial growth as a defensive approach in their competition for light resources [14].

Numerous studies have been conducted using different macrophyte species and cyanobacteria to specifically induce an allelopathic effect to reduce cyanobacterial growth [16–21]. However, the inhibitive mechanisms and the efficiency of cyanobacterial control vary with macrophyte species and the condition of the aquatic environment. Declerck et al. [22] reported that cyanobacteria combined with *E. nuttallii* in a mesocosm exhibited a clear decline in growth at high nutrient levels. They demonstrated that cyanobacterial growth inhibition in a combined culture with macrophytes is caused by many factors, such as the shading effect, nutrient competition, and the release of anti-cyanobacterial allelochemicals. Vanderstukken et al. [23] used two macrophytes, *E. densa* and *Potamogeton illinoensis*, to investigate the consequences of allelopathy, excluding nutrient competition and zooplankton grazing effects on cyanobacterial growth. Their study showed that both macrophytes inhibited the growth of cyanobacteria by allelopathic effects. Senavirathna et al. [24] also discussed the cyanobacterial inhibitory capacity of *E. densa* and the influence of light parameters on the allelopathic reaction. However, the practical application of live macrophytes for in situ control of cyanobacteria is a complex process due to various reasons, including environmental factors, species-specific interactions, and the potential for disrupting the ecological balance through invasive behavior. Therefore, research interest has grown in certain biologically derived substances (BDSs) found in various macrophytes, which may also have the potential to inhibit the growth of cyanobacteria. Pretreatments such as co-culturing, mechanical grinding, autoclaving during the extraction process, or the use of aqueous and organic solvents can enhance the inhibitory potential of the extracted compounds. Using aqueous solutions for extracting allelochemicals from macrophytes provides several advantages, such as being environmentally friendly, cost-effective, and safe, while preserving the biological activity of the compounds.

A few studies have investigated the application of powdered fresh macrophyte leaves and leaf extracts to obtain their natural allelochemical, thereby inhibiting cyanobacterial growth [14,25,26]. Gross et al. [27] and Mulderij et al. [28] demonstrated that extracts derived from *M. spicatum, Stratiotes aloides*, *Ceratophyllum demersum*, and *Najas marina* spp. exhibit significant inhibitory effects on cyanobacteria growth. These studies focused on highly invasive allelopathic macrophyte species as a potential inhibitor of cyanobacterial growth. By using rapidly growing invasive plants to produce extracts, this method becomes a sustainable and cost-effective approach to inhibit the growth of cyanobacteria.

Many invasive macrophyte species such as *M. spicatum*, *C. demersum,* and *E. densa* are widely distributed in many freshwater ecosystems and have shown higher allelopathic capacities [29–31]. Therefore, compounds extracted from such macrophytes can be used to suppress cyanobacteria in water bodies, particularly in cases where the introduction of live macrophytes is limited due to their invasive nature. This method is especially applicable to water bodies where the application of live macrophytes is impractical (i.e., boat yards, manmade shallow ponds in parks, or fountains with aesthetic value).

In this study, we selected *E. densa* plant, which is an invasive perennial macrophyte known to produce allelochemicals that inhibit cyanobacteria, as indicated in previous studies [11,23,24,32]. Although research has confirmed the applicability of live plants and plant extracts to suppress cyanobacteria, the enhanced allelochemical content in extracts of macrophytes that co-cultured with cyanobacteria has not been studied. Therefore, in this study, we hypothesize that co-cultivation of *E. densa* with cyanobacteria induces the production of allelochemicals in *E. densa*, resulting in an enhanced inhibitory effect of plant extracts on the growth of *M. aeruginosa*.

2. Materials and Methods

2.1. Preparation of Stock Cultures

2.1.1. *Microcystis aeruginosa* (Kützing) Lemmermann Culture

M. aeruginosa was selected for this study because it is known to be the most common bloom-forming cyanobacterial species worldwide [3]. *M. aeruginosa* strains (NIES-111) were obtained from the National Institute of Environmental Studies (NIES), Japan. The cells were cultured in disinfected 500 mL Erlenmeyer flasks using the BG-11 medium [33] and maintained at a controlled temperature of 20 °C. A photoperiod of 12 h darkness and 12 h light was maintained using an automatic setup device (REVEX PT7, Saitama, Japan) with a light intensity of 30 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) using LED lamps inside the incubators. The flasks were closed using porous stoppers (SILICOSEN, Shin-Etsu Polymer Co., Ltd., Tokyo, Japan). The cultures were gently shaken daily during acclimatization, and cultures in the exponential stage were used for experiments.

2.1.2. *Egeria densa* Planch. Culture

In this study, agrochemicals and chemical-free *E. densa* were commercially purchased and cultured in 20-litre (25 cm × 24 cm × 40 cm, height × width × length) glass tanks at 25 ± 2 °C in a temperature-controlled room. A layer of nutrient-removed river sand was used as the substrate at the bottom of the glass tank. Nutrients were provided by adding 5 ppm of commercial aquaponic solution (Hyponex concentrated nutrient solution, Hyponex, Osaka, Japan). Plants were exposed to a 12 h/12 h light/dark photoperiod with a light intensity of 80–85 µmol photons m^{-2} s^{-1} PAR using LED lamps at plant height. Water levels were kept constant by replenishing with distilled water.

E. densa Monoculture and Combined Culture with *M. aeruginosa*

We maintained two types of cultures: monocultures of *E. densa* and combined cultures of *E. densa* with *M. aeruginosa*, both under the same conditions. To establish these cultures, six healthy *E. densa* cuttings with an approximate length of 10 cm were planted in six 1 L Pyrex glass beakers by fixing them to half-cylindrical rubber sponges (Carboy Inc. Chiba, Japan). All beakers were filled with 1 L of 10% Hoagland nutrient solution (HNS). For the combined cultures, three of the *E. densa* beakers were inoculated with *M. aeruginosa* cells at a cell density of 2.1 ± 0.2 × 10^4 cells/mL.

The cultures were allowed to acclimate for 5 days under controlled conditions within incubators, which maintained a light intensity of 80 µmol photons m^{-2} s^{-1} PAR, a temperature of 25 ± 1 °C, and a light duration of 12 h/12 h. After acclimatization, treatment was continued for 7 days under the same conditions. The water levels were kept constant throughout the experiment. After 7 days, macrophyte samples were collected and rinsed before plant samples were stored in a −80 °C freezer until the extraction process.

2.2. E. densa Fresh Tissue Extraction Processes

Fresh plant tissue extract was prepared according to Zhang et al. [34] with modifications using distilled water. Approximately 200 mg of fresh plant samples, separately collected from both *E. densa* monocultures and *E. densa* grown with *M. aeruginosa*, were finely crushed using a cold ceramic mortar and pestle in the presence of liquid nitrogen, utilizing 5 mL of distilled water. The extraction mixtures were transferred into 15 mL centrifuge tubes and centrifuged at 1400× *g* (TOMY MX-105, Tomy Seiko Co., Ltd., Tokyo, Japan) at 4 °C for 10 min separately. The extracts were stored at −80 °C for further experiments.

2.3. Experimental Design

2.3.1. Application of *E. densa* Live Plants

An experiment including *M. aeruginosa* monocultures and combined cultures (2×10^4 cells/mL) with *E. densa* was conducted to confirm the inhibition of *M. aeruginosa* growth in the presence of *E. densa* live plants. Artificial plants were used in the *M. aeruginosa* monoculture to eliminate the potential shading effect of *E. densa* plants. The cultures were temperature-controlled at 25 ± 1 °C and subjected to a 12 h/12 h light/dark photoperiod using an automatic setup device (REVEX PT7) with a light intensity of 80 µmol photons m^{-2} s^{-1} PAR, achieved through LED lamps inside the incubators. The cultures were incubated for 7 days, with triplicates maintained for both mono and combined cultures.

2.3.2. Application of *E. densa* Extracts

Figure 1 represents the experimental procedure of *E. densa* aqueous extracts on *M. aeruginosa* inhibition. A cell suspension of cyanobacterium *M. aeruginosa*, with a cell density of 1×10^5 cells/mL and a Chl-a concentration of 0.178 ± 0.005 µg/mL in BG-11 medium, was prepared for the experiment in accordance with the guidance levels of cyanobacteria that may potentially trigger restrictions of site use, as published by WHO [9,35]. This served as the initial cell suspension. The experiment was conducted in autoclaved 100 mL conical flasks filled to 50 mL with the *M. aeruginosa* suspensions. We used cultures in the exponential growth stage for the experiments. The openings of the flasks were secured with porous stoppers (SILICOSEN). The samples were acclimatized for 3 days before introducing the extracts. All experiments were conducted under controlled laboratory conditions. The cultures were temperature-controlled at 25 ± 1 °C and subjected to a 12 h/12 h light/dark photoperiod using an automatic setup device (REVEX PT7) with a light intensity of 80 µmol photons m^{-2} s^{-1} PAR and LED lamps inside of the incubators (Figure 1). The extracts were introduced after 4 days of acclimatization. The *M. aeruginosa* cultures were exposed to two different *E. densa* extracts at concentrations of 0, 0.5, 1, 2, and 4 g/L. Each flask was gently shaken twice daily during the incubation. Triplicates were maintained for each extract concentration. The experiment was conducted for 5 days, during which we regularly monitored growth parameters and collected samples every 24 h. The collected samples were centrifuged at 10,000× *g* for 15 min at 4 °C (TOMY MX-105) in 1.5 mL microcentrifuge tubes. The supernatant was removed, and the microcentrifuge tubes containing *M. aeruginosa* cell pellets were stored at −80 °C for further analysis.

2.4. Analytical Methods

The growth parameters of both *M. aeruginosa* monoculture and the combined culture with *E. densa* were measured to investigate the influence of different types and concentrations of *E. densa* extract on *M. aeruginosa* growth. Growth parameters of *M. aeruginosa* cultures were analyzed daily until the 5-day exposure period. To estimate *M. aeruginosa* growth, cell density, inhibition rate (IR), mean growth rate (µ), and Chl-a concentrations were analyzed.

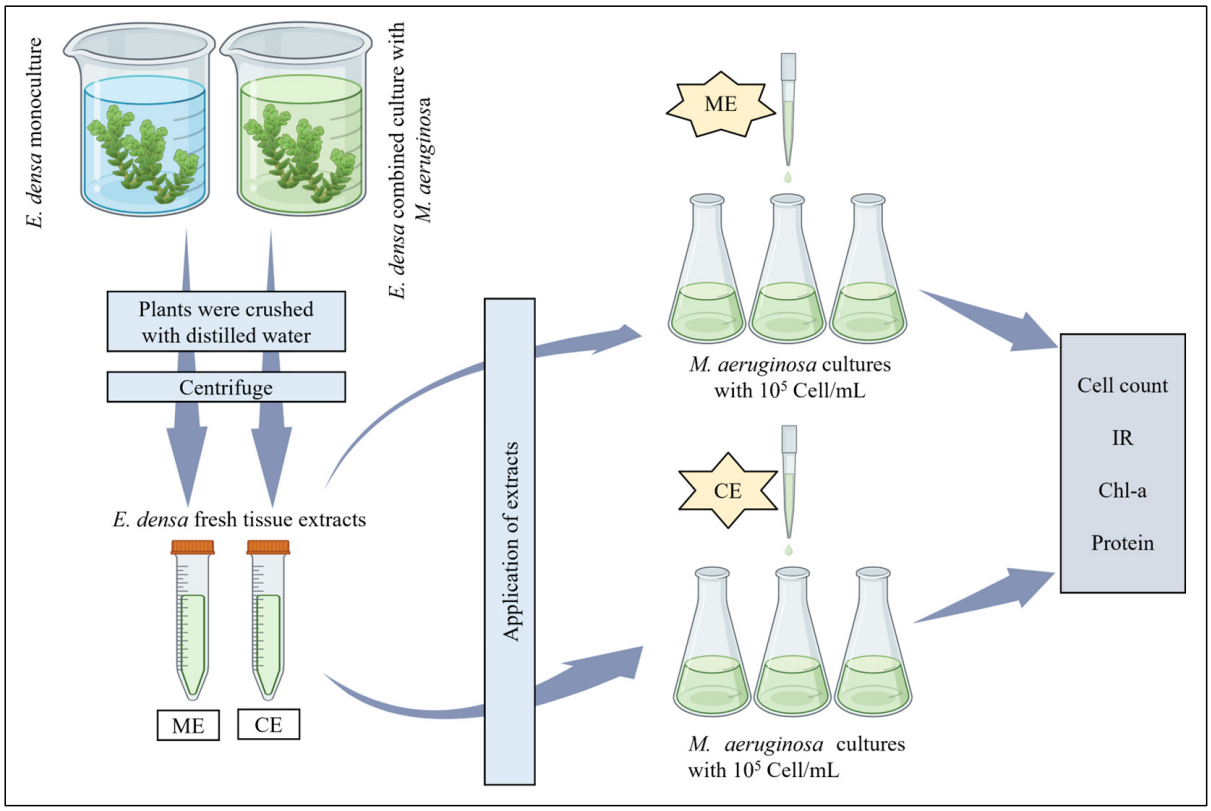

Figure 1. Experimental procedure of *E. densa* aqueous extracts on *M. aeruginosa* inhibition. ME and CE refer to monoculture extract and combined culture extract, respectively. IR and Chl-a refer to the inhibition rate and chlorophyll-a of the treated *M. aeruginosa* cultures, respectively (created with BioRender.com).

2.4.1. Cell Density

Cell densities were determined by manual counting using a hemocytometer and light microscope (Zeiss Axiolab 5, Carl Zeiss, ZEISS, Tokyo, Japan) and Luna Automated Cell Counter (L10001, Logos Biosystems, Anyang, Republic of Korea) in a hemocytometer. The cell density was expressed as cells/mL. Detailed features of *M. aeruginosa* cells, including their shape, the presence of mucus, and the number of cells per group were observed using a light microscope (Zeiss Axiolab 5) at a magnification of 400× before and after treatment with different concentrations of *E. densa* extracts.

Inhibition Rate (IR)

The percentage of growth inhibition by each extract at different extract concentrations was calculated compared to the control group. The IR of the extracts on cyanobacteria growth was calculated according to Equation (1).

$$IR\% = \left[1 - \left(\frac{N}{N_o}\right)\right] \times 100 \quad (1)$$

N and N_o stand for the cell density of the treated culture and cell density of the control culture, respectively.

2.4.2. Chlorophyll-A

The *M. aeruginosa* suspension (1 mL) was collected and centrifuged at 10,000× g for 10 min at 4 °C (TOMY MX-105); the supernatant was removed, before storing the cell pellets at −80 °C until analysis. The concentration of Chl-a was measured by homogenizing the cell pellets in 1 mL of 95% ethanol. The samples were vibrated and incubated under dark

conditions overnight at room temperature (25 ± 2 °C). The samples were then centrifuged at 10,000× *g* for 10 min at 4 °C (TOMY MX-105), and the supernatant was used to measure the absorbance spectrophotometrically (UV-1280) at 665 and 649 nm wavelengths. Chl-a concentration was calculated as µg/mL using Equation (2) described by Liu et al. [36].

$$Chl\ a = (13.95 \times A_{665}) - (6.88 \times A_{649}) \quad (2)$$

A_{665} and A_{649} are the absorbances at 665 nm and 649 nm, respectively, and the Chl-a concentration per protein was expressed.

2.4.3. Nitrate (NO_3^-) and Phosphate (PO_4^{3-})

The NO_3^- and PO_4^{3-} concentrations of *E. densa* extracts were evaluated using PACKTEST Nitrate (range 1–45 mg/L) and PACKTEST Phosphate (range 2–100 mg/L), respectively, with the Digital pack test meter (DPM-MTSP, KYORITSU CHEMICAL Corp, Japan). The extracts were filtered through 0.45 µm before the measurements.

2.4.4. Three-Dimensional Excitation–Emission Matrix (3D EEM)

The compounds present in two *E. densa* aqueous extracts were investigated using a 3D EEM created using a fluorescence spectrophotometer (LS 45, Perkin Elmer, Waltham, MA, USA). The principle of a fluorescence spectrophotometer is that the molecules in the sample absorb light energy at an extreme speed (~10^{-8} s) at certain wavelengths and produce emission light with greater wavelengths than the excitation wavelengths [37]. Since the excitation and emission wavelengths of every compound are unique and fixed [38], this method is widely utilized to identify the various compounds in solutions. The excitation and emission wavelengths were changed from 200 to 600 nm and 300 to 625 nm, respectively, at a scanning speed of 1500 nm/min.

2.5. Data Analysis

A statistical analysis was carried out statistical software (20.0, IBM SPSS) with a significance level of 5% ($p \leq 0.05$) to evaluate the efficacy of two *E. densa* extracts, one from monoculture and the other from coexistence with *M. aeruginosa*, in inhibiting *M. aeruginosa* growth. Daily measurements of growth parameters over 5 days were obtained across varying extract concentrations (0, 0.5, 1, 2, and 4 g/L). The statistical analysis included a one-way analysis of variance (ANOVA) to identify differences in cell densities and Chl-a concentrations among the cultures exposed to two different extracts and the control, followed by post hoc tests. The two-sample t-test was carried out to compare the initial and final cell density, protein concentration, and Chl-a concentration of *M. aeruginosa* mono and combined culture with *E. densa* live plant. Each experiment was performed in triplicate. All of the graphs were created using OriginLab software 2022 (OriginLab Corporation, Northampton, MA, USA). All graphical images were created with BioRender.com.

3. Results

3.1. Effect of E. densa Live Plants on M. aeruginosa Growth during Coexistence

3.1.1. Cell Density

The initial cell density of *M. aeruginosa* culture was 0.024 ± 0.003 × 10^6 cells/mL. The *M. aeruginosa* monoculture resulted a cell density of 0.09 ± 0.005 × 10^6 cells/mL after 7 days, whereas the coculture of *M. aeruginosa* with *E. densa* showed a significantly lower cell density of 0.046 ± 0.005 × 10^6 cells/mL ($t(2) = 11.2366$, $p < 0.001$) (Figure 2).

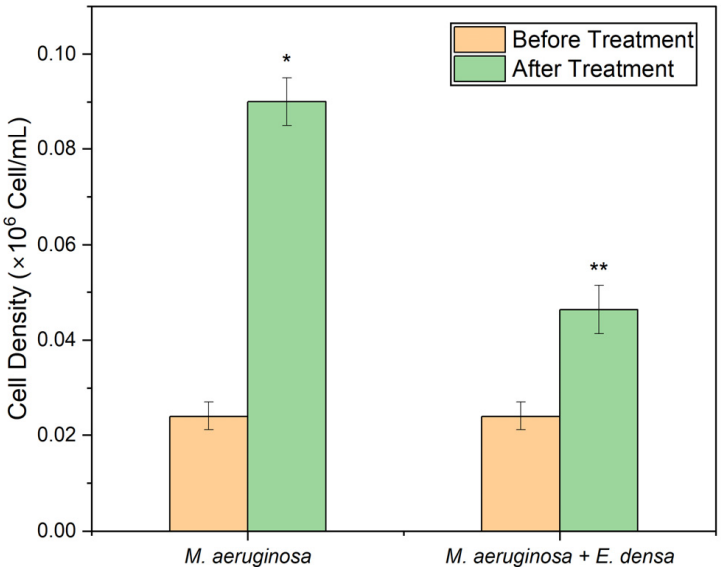

Figure 2. Cell density of the *M. aeruginosa* monoculture and combined culture with *E. densa* fresh plant before and after the experiment. Error bars represent the standard deviation (n = 3). Different superscripts (* and **) in the bars indicate significant differences at $p \leq 0.05$ for the cell density of *M. aeruginosa* monoculture and combined culture with *E. densa* after treatment.

3.1.2. Protein Concentration

The initial protein concentration of the *M. aeruginosa* culture was 68.8 ± 8.1 µg/mL. The *M. aeruginosa* monoculture showed a protein concentration of 94.41 ± 8 µg/mL after 7 days of exposure, whereas the *M. aeruginosa* cultured with *E. densa* showed a significantly lower protein concentration of 78.42 ± 6.6 µg/mL after exposure ($t(2) = 3.217$, $p = 0.03$) (Figure 3), consistent with the cell density results (Figure 2).

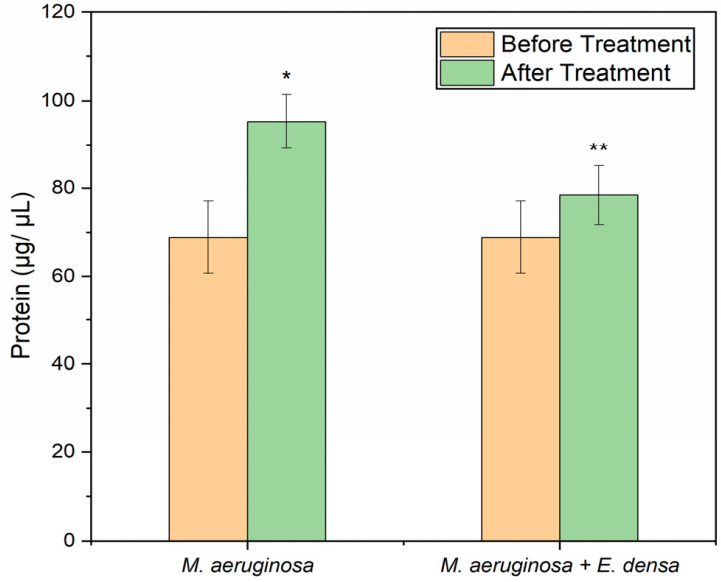

Figure 3. Changes in protein concentration of the *M. aeruginosa* monoculture and combined culture with *E. densa* fresh plant before and after the experiment. Error bars represent the standard deviation (n = 3). Different superscripts (* and **) in the bars indicate significant differences in protein concentrations at $p \leq 0.05$ for *M. aeruginosa* monoculture and combined culture with *E. densa* after treatment.

3.1.3. Chl-A Concentration

The *M. aeruginosa* monoculture with artificial plants showed an increase in Chl-a concentration per protein compared to the culture combined with *E. densa* (Figure 4). In contrast, when *M. aeruginosa* cultures were combined with *E. densa*, they exhibited a significantly lower Chl-a concentration compared to the monoculture ($t(2) = 5.606$, $p = 0.004$) after 7 days and appeared to be more controlled than the *M. aeruginosa* monoculture. The Chl-a concentration in the combined culture of *M. aeruginosa* was measured at $2.3 \pm 0.5 \times 10^{-4}$ µg/µg protein.

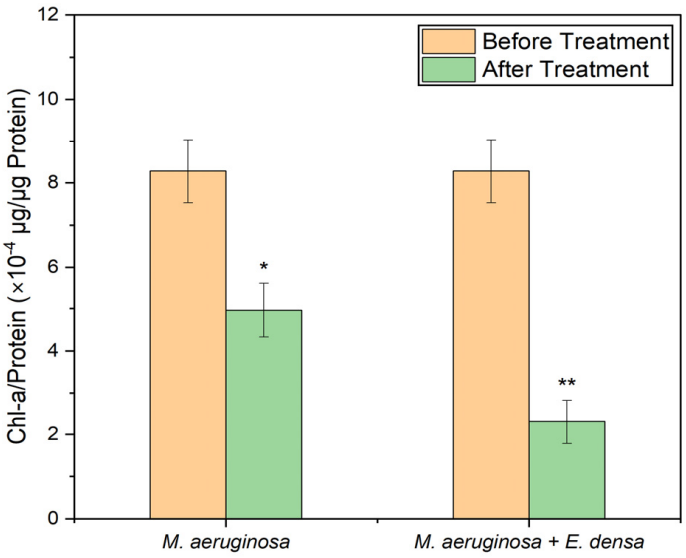

Figure 4. Chlorophyll-a concentration per µg protein in *M. aeruginosa* monoculture and combined culture with *E. densa* fresh plant before and after the experiment. Error bars represent the standard deviation (n = 3). Different superscripts (* and **) in the bars indicate significant differences in Chl-a per protein concentration at $p \leq 0.05$ for *M. aeruginosa* monoculture and combined culture with *E. densa* after treatment.

3.2. Effect of Different E. densa Extracts on Cyanobacteria Growth

3.2.1. Cell Density

Figure 5 shows the variation in the cell density of *M. aeruginosa* cultures under the effect of both *E. densa* extract gradients (0.5, 1, 2, and 4 g/L). At the end of the exposure period, the culture treated with a concentration of 0.5 g/L of the combined *E. densa* extract reported the lowest cell density (2.44 ± 0.1 cells/mL). A significantly lower cell density was observed in the culture treated with 0.5 g/L combined extract compared to the cell culture treated with 0.5 g/L *E. densa* monoculture extract and the control culture ($F(2, 6) = 55.818$, $p < 0.001$) after 5 days of exposure. Cultures treated with higher concentrations (2 and 4 g/L) of both extracts exhibited significantly increased growth of *M. aeruginosa* after day 3 compared with the control culture ($p < 0.05$). The highest cell density, 8.36 ± 0.1 cells/mL, was observed in cultures treated with 4 g/L of monoculture extract on day 5. *M. aeruginosa* cell cultures treated with both extracts showed high cell densities, especially after day 3, with high extract concentrations (2 and 4 g/L) compared to control cultures. However, cultures treated with *E. densa* combined extracts showed lower cell density when compared to cultures treated with extracts from *E. densa* monocultures.

The highest IR among cell cultures treated with the different concentrations of *E. densa* monoculture extracts was achieved by the 0.5 g/L concentration group, reaching 23.5% on day 2 (Figure 6a). Throughout the exposure period, the 0.5 g/L concentration group maintained a higher IR compared to all other concentration groups. The highest IR in the extracts obtained from *E. densa* combined culture was 31%, also observed in the 0.5 g/L concentration group on day 3 (Figure 6b). The 0.5 g/L concentration group of combined

E. densa extracts consistently showed a high IR throughout the exposure period compared to concentration groups. Higher concentrations of both *E. densa* extracts did not exhibit any inhibition towards the end of the exposure period. However, the 1 g/L concentration group of combined *E. densa* extract did exhibit an increased IR only up to the third day.

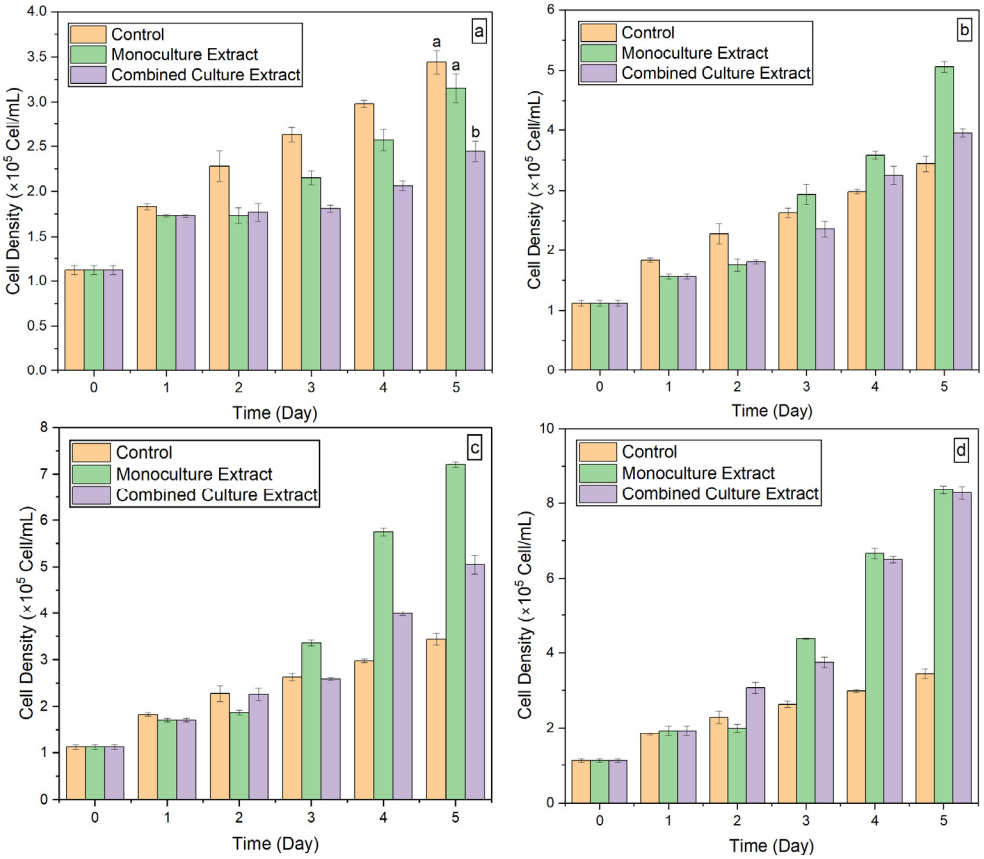

Figure 5. *M. aeruginosa* cell density in the cultures under the presence of both *E. densa* extract gradient 0.5 (**a**), 1 (**b**), 2 (**c**), and 4 g/L (**d**) compared to the control sample. Error bars represent the standard deviation (n = 3). Different lowercase letters in the bars indicate significant differences at $p \leq 0.05$ for different treatments.

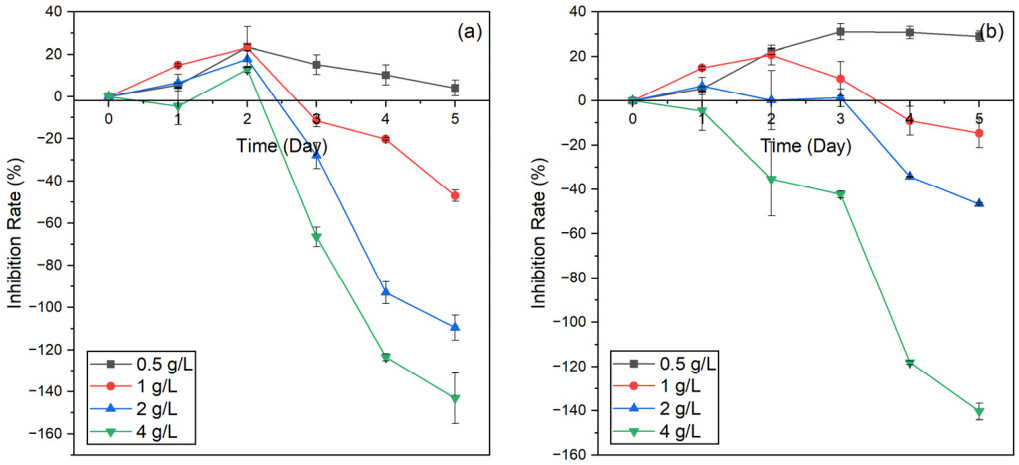

Figure 6. Inhibition rate (IR) of *M. aeruginosa* cultures exposed to (**a**) *E. densa* monoculture extract and (**b**) combined culture extract gradient throughout the experiment period. Error bars represent the standard deviation (n = 3).

3.2.2. Chl-A Concentration

The Chl-a concentration in the treated *M. aeruginosa* cell cultures exhibited changes that were comparable to the variations in cell densities. All cultures treated with a 0.5 g/L concentration of both types of extracts showed lower Chl-a concentrations compared to the control culture (Figure 7). The Chl-a concentration of the cultures treated with the combined *E. densa* extract (0.5 g/L) was significantly lower than that of cultures treated with *E. densa* monoculture extracts ($F(2, 6) = 118.457$, $p < 0.001$) and the control ($F(2, 6) = 118.457$, $p = 0.004$) after day 5 of the exposure period. The lowest Chl-a concentration was observed in the culture treated with 0.5 g/L concentration of *E. densa* combined culture extract, measuring $3.2 \pm 0.1 \times 10^{-3}$ µg/µg protein on day 5. *M. aeruginosa* cultures treated with high concentrations (1, 2, and 4 g/L) of both types of *E. densa* extracts showed an increase in Chl-a concentration over time compared to control cultures. The highest Chl-a concentration was observed in the culture treated with 4 g/L *E. densa* monoculture extract on day 5 (131×10^{-3} µg/µg protein). However, cultures treated with the *E. densa* combined extracts showed lower Chl-a concentration than cultures treated with the monoculture extracts at all concentrations throughout the exposure period.

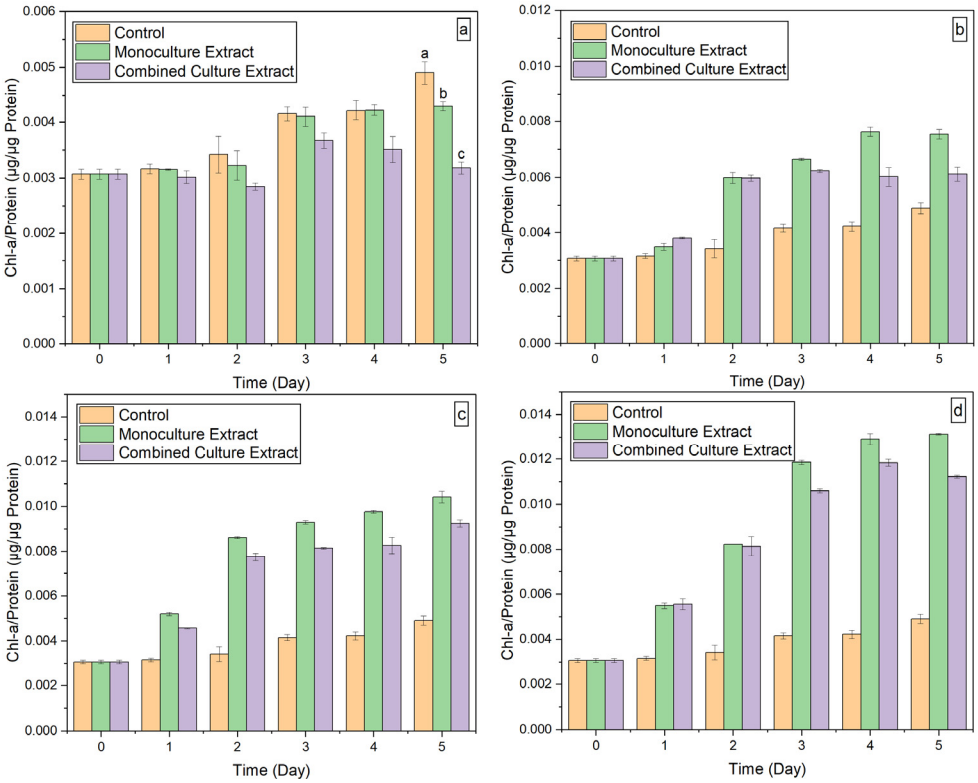

Figure 7. Chlorophyll-a concentration per protein in *E. densa* monoculture and combined culture extract gradient (**a**) 0.5, (**b**) 1, (**c**) 2, and (**d**) 4 g/L compared to the control sample. Error bars represent the standard deviation (n = 3). Different lowercase letters in the bars indicate significant differences at $p \leq 0.05$ for different treatments.

3.3. E. densa Extracts Analysis

The analysis of NO_3^- and PO_4^{3-} concentrations in both *E. densa* monoculture and combined culture extract gradients showed that the NO_3^- concentration in both extracts is negligible and below the detection level of PACKTEST (<1 mg/L). Both extracts exhibited the availability of PO_4^{3-} at higher concentrations (Figure 8). However, no PO_4^{3-} was detected at a concentration of 0.5 g/L in either of the extracts (<2 mg/L). The extracts obtained from the *E. densa* monoculture exhibited a PO_4^{3-} concentration of 7.5 ± 0.2 mg/L at 4 g/L, which is slightly higher than the concentration showed by the combined culture extract (7.4 ± 0.2 mg/L) at 4 g/L.

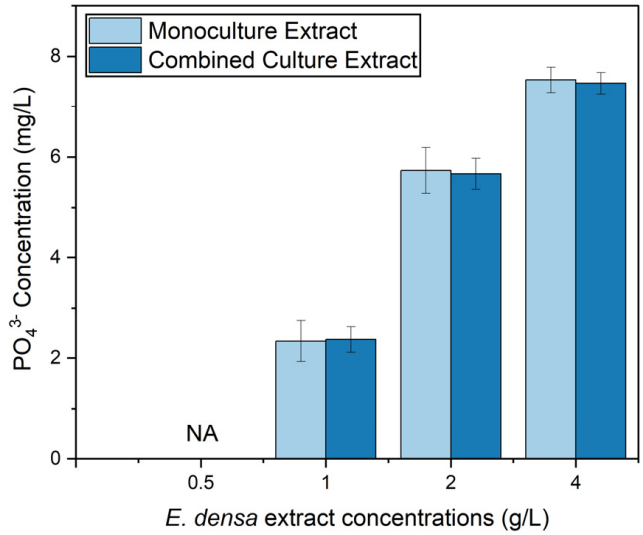

Figure 8. Variation of phosphate concentrations in *E. densa* mono and combined culture with *M. aeruginosa* extracts. Error bars represent the standard deviation (n = 3). "ND" refers to "not detected", and the limit of detection (LOD) for phosphate was set at 1 mg/L.

The 3D EEM obtained from both *E. densa* extracts displayed similar patterns with four distinct peaks (Figure 9). However, despite the similarity in the presence of these four peaks in both extracts, there were differences in their intensities. Specifically, the EEM of the extract obtained from *E. densa* combined culture showed higher intensities. These peaks represent the available groups of compounds and their respective intensities in the extracts. Peaks B, C, X, and Y, which are responsible for protein-like compounds, were observed in the emission wavelengths ranging from 300 to 345 nm, while peaks labeled A, D, W, and Z occurred in emission wavelengths ranging from 435 to 480 nm in the EEM, indicating their association with humic-like substances.

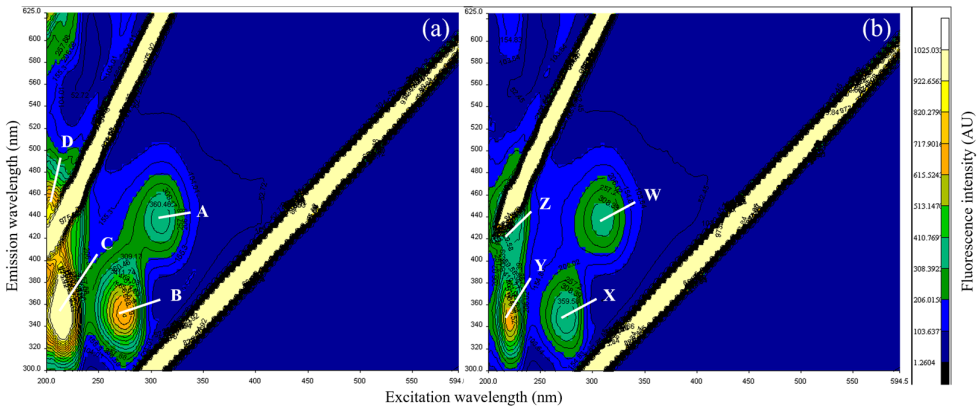

Figure 9. The 3D EEM of (**a**) *E. densa* combined culture extract and (**b**) monoculture extract. A–D and W–Z indicate the different peaks produced by the combined culture and monoculture extracts, respectively.

4. Discussion

The coculturing experiment of *M. aeruginosa* and *E. densa* was carried out to confirm the inhibitive ability of live *E. densa* plants on *M. aeruginosa* growth, before obtaining *E. densa* extracts. The combined culture of *M. aeruginosa* with *E. densa* showed suppression in cell density, protein concentration, and Chl-a concentration compared to the monoculture of *M. aeruginosa* after 7 days of exposure. The concentration of Chl-a in the culture serves as a direct indicator for determining the biomass of cyanobacteria [9]. The Chl-a concentration in the combined culture also showed a similar trend as the cell density and the protein

concentration. However, after 7 days, the monoculture showed a lower Chl-a concentration per protein than at the beginning (Figure 4). This could have been a result of the shading effect of artificial plants or a result of *M. aeruginosa* cells being in different growth phases. Previous studies confirmed the inhibitory effects of *E. densa* on cyanobacteria [11], alga *Scenedesmus acutus* [31], and phytoplanktons [23] during co-culture. Therefore, the inhibition of *M. aeruginosa* growth in the combined culture could have been due to the presence of live *E. densa*, potentially resulting from nutrient competition or allelochemicals, as the shading effect was eliminated by using artificial plants in the *M. aeruginosa* monoculture. To investigate the inhibitory effect caused by allelochemicals produced by *E. densa*, plant extracts were obtained and utilized to treat *M. aeruginosa* instead of live plants.

Numerous studies have also reported the release of allelochemical induced by various species of live macrophytes when they coexisted with cyanobacteria [17,19,23,39]. However, only a few studies have investigated the effects of allelochemicals obtained from *E. densa* on cyanobacteria inhibition, despite its known success as a live plant in suppressing cyanobacteria growth. After confirming the ability of live *E. densa* plants to suppress *M. aeruginosa* growth, we investigated the inhibitive ability of the extracts obtained from *E. densa*. Therefore, we selected *E. densa* grown as monocultures and combined cultures with *M. aeruginosa* to obtain extracts to investigate the effect of coexistence on allelochemical production. The *M. aeruginosa* cultures exposed to 0.5 g/L concentrations of both extracts exhibited inhibition in growth compared to the control group during the 5-day exposure period (Figure 5). However, the extracts obtained from the combined culture showed a more successful inhibitory effect than the extract from *E. densa* monoculture, resulting in the lowest cell density and Chl-a concentration of the *M. aeruginosa* cultures treated with it on day 5. Cyanobacteria possess intracellular thylakoid membranes that contain chlorophyll, which is used for oxygenic photosynthesis [40]. Chlorophyll synthesis tends to decrease under certain stress conditions due to the downregulation of pigment genes in cyanobacteria [41]. Therefore, Chl-a concentration is considered a direct measure to evaluate the cyanobacteria growth. At the end of the exposure period (5 days), the 0.5 g/L concentration of combined extract suppressed *M. aeruginosa* growth by 22% compared to the monoculture (Figure 5). The mechanisms of the effect of allelochemicals on *M. aeruginosa* suppression can vary. Some allelochemicals, such as Tellimagrandin II, (+)-catechin, polyphenols pyrogallic acids, gallic, nonanoic acid, ellagic, cis-6-octadecenoic acid, and cis-9-octadecenoic acid, which were extracted from *M. spicatum*, have been shown to contribute to 53% of the inhibition of *M. aeruginosa* by decreasing the activity of photosystem II through interference with electron transfer [14]. In addition, reduction in algal photosynthesis may also be mediated by oxidative stress. This phenomenon occurs because of the excessive production of O_2^- (superoxide ions), which initiates free-radical reactions, leading to the peroxidation of lipids in the cell membranes. As a result, the permeability of these membranes is changed. Allelochemicals such as ethyl 2-methyl acetoacetate (EMA), produced by *Phragmites communis*, were found to impose significant oxidative stress, ultimately leading to the inactivation of the antioxidant defense system of *M. aeruginosa* [42].

In this study, a 3D EEM analysis was conducted to investigate the compounds present in the extracts. The EEM analysis revealed four similar peaks in both extract types. However, the EEM of the extract obtained from *E. densa* combined culture showed higher intensities. Increased intensity corresponds to higher compound concentrations. Peaks B and X (Figure 9a,b) belong to the aromatic protein region, containing amino acids such as tyrosine and tryptophan [37]. Peaks B, C, X, and Y (Figure 9a,b) observed in the emission wavelengths 300 to 345 nm are related to protein-like compounds. According to Hu and Yin [38] and Yin et al. [43], peaks labeled A, D, W, and Z (Figure 9a,b), found in the emission wavelengths of 435 to 480 nm, are associated with humic-acid-like compounds. According to Traversa et al. [44], peaks A and W (Figure 9a,b) represent phenolic compounds in the extracts. When *E. densa* coexists with cyanobacteria, it may induce the synthesis of phenolic compounds that exhibit allelopathic properties. These compounds can be responsible for *M. aeruginosa* inhibition [14]. Therefore, the high-intensity peaks observed in the combined

culture could be attributed to the higher concentrations of the allelopathic compounds available in extracts obtained from the *E. densa* combined culture with *M. aeruginosa*. Moreover, the low nutrient availability in 0.5 g/L concentrations of the extract may have enhanced the effect of allelochemicals, thereby promoting the suppression of *M. aeruginosa* (Figure 8).

When considering the morphological variations of *M. aeruginosa*, cells generally exhibit a unicellular, spherical-shaped morphology. However, we observed that some of these cells produced a protective mucilaginous sheath, especially when exposed to 0.5 g/L *E. densa* extract concentrations. According to Rossi et al. [45], cyanobacteria were observed to produce gelatinous envelop or a sheath around the cell at unfavorable environmental conditions. In addition, no significant changes were observed in the number of cells per group at low *E. densa* extract concentrations, typically ranging from four to six cells per group. Cell density increased when cell suspensions were exposed to high extract concentrations, resulting in larger cell groups. Previous studies have also reported morphological changes, such as yellowing, depigmentation [46], and cell damage [43], in response to allelochemicals from macrophytes in cyanobacteria cells. The high concentrations of both *E. densa* extracts did not exhibit considerable growth suppression in *M. aeruginosa* over the exposure period. Instead, the high concentrations (1, 2, and 4 g/L) of the *E. densa* extracts promoted the *M. aeruginosa* growth towards the end of exposure time. Higher values of PO_4^{3-} concentrations were observed in both cultures as the concentrations of extracts increased (Figure 8). Even though the combined extract contained effective allelochemicals, the high concentrations of nutrients may have suppressed their inhibitory effects, resulting in increased cell density in the cultures treated with high concentrations of *E. densa* extracts. However, the *M. aeruginosa* cultures treated with combined *E. densa* extract consistently exhibited lower cell density and Chl-a concentrations compared to the cultures exposed to monoculture extracts, confirming the presence of a high concentration of allelochemicals in the *E. densa* combined culture extract than in the monoculture extracts [14]. Accordingly, results suggest that the application of extracts of *E. densa* could be an eco-friendly potential approach for small-scale water bodies in controlling cyanobacteria at low cell densities.

Evaluations of the feasibility, environmental impacts, and cost-effectiveness are necessary steps to be considered by managers when implementing bio-derived materials for cyanobacteria control. In addition, effective communication and collaboration among managers, researchers, and policymakers can facilitate the successful implementation of bio-derived solutions into practical use. Moreover, it is important to conduct economic evaluations that compare the application of bio-derived materials for cyanobacteria control with conventional methods. For further research, monitoring the production of soluble toxins by harmful cyanobacteria is essential. It provides a more comprehensive understanding of the effectiveness of bio-derived materials in successfully inhibiting cyanobacterial growth while minimizing toxin production. Furthermore, the long-term effects of repeated use of bio-derived substances on aquatic ecosystems should be studied.

5. Conclusions

In conclusion, live *E. densa* plants have been found to successfully inhibit the growth of *M. aeruginosa* during coexistence. When applying aqueous extracts of *E. densa*, it was observed that a lower concentration of extracts (0.5 g/L) obtained from *E. densa* plants co-cultured with *M. aeruginosa* exhibited a stronger inhibitory capacity compared to extracts obtained from *E. densa* monocultures. This suggests that the production of allelochemicals in *E. densa* seems to be induced when coexisting with *M. aeruginosa*. However, higher extract concentrations with elevated nutrient levels promote the growth of *M. aeruginosa* rather than inhibition. For future research, we recommend considering different types of macrophytes to obtain natural allelochemicals to suppress cyanobacteria and investigation and identification of available cyanobacteria inhibitive allelochemicals.

Author Contributions: Conceptualization, A.W. and M.D.H.J.S.; data curation, A.W.; formal analysis, A.W.; funding acquisition, M.D.H.J.S.; investigation, A.W.; methodology, A.W. and M.D.H.J.S.; resources, T.F.; supervision, M.D.H.J.S. and T.F.; validation, M.D.H.J.S.; writing—original draft, A.W.;

writing—review and editing, M.D.H.J.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Japanese Society for the Promotion of Science (JSPS), JSPS KAKENHI, grant number 21K14248 and the APC was funded by the authors.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data and materials are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Paerl, H.W.; Otten, T.G. Harmful Cyanobacterial Blooms: Causes, Consequences, and Controls. *Microb. Ecol.* **2013**, *65*, 995–1010. [CrossRef] [PubMed]
2. Paerl, H.W. Mitigating Harmful Cyanobacterial Blooms in a Human- and Climatically-Impacted World. *Life* **2014**, *4*, 988–1012. [CrossRef]
3. Harke, M.J.; Steffen, M.M.; Gobler, C.J.; Otten, T.G.; Wilhelm, S.W.; Wood, S.A.; Paerl, H.W. A Review of the Global Ecology, Genomics, and Biogeography of the Toxic Cyanobacterium, *Microcystis* Spp. *Harmful Algae* **2016**, *54*, 4–20. [CrossRef] [PubMed]
4. House, W.A. Geochemical Cycling of Phosphorus in Rivers. *Appl. Geochem.* **2003**, *18*, 739–748. [CrossRef]
5. United States Environmental Protection Agency Health Effects from Cyanotoxins. Available online: https://www.epa.gov/cyanohabs/health-effects-cyanotoxins (accessed on 21 November 2022).
6. Dolman, A.M.; Rücker, J.; Pick, F.R.; Fastner, J.; Rohrlack, T.; Mischke, U.; Wiedner, C. Cyanobacteria and Cyanotoxins: The Influence of Nitrogen versus Phosphorus. *PLoS ONE* **2012**, *7*, e38757. [CrossRef]
7. Pokrzywinski, K.L.; Bishop, W.M.; Grasso, C.R.; Fernando, B.M.; Sperry, B.P.; Berthold, D.E.; Laughinghouse, H.D.; Van Goethem, E.M.; Volk, K.; Heilman, M.; et al. Evaluation of a Peroxide-Based Algaecide for Cyanobacteria Control: A Mesocosm Trial in Lake Okeechobee, FL, USA. *Water* **2022**, *14*, 169. [CrossRef]
8. Sample, D.J.; Fox, L.J. Innovative Best Management Fact Sheet No.1: Floating Treatment Wetlands. *Va. Polytech. Inst. State Univ.* **2013**, 1–5.
9. Chorus, I.; Welker, M. *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*; Taylor & Francis: Abingdon, UK, 2021; ISBN 9780367533311.
10. Chen, L.; Giesy, J.P.; Adamovsky, O.; Svirčev, Z.; Meriluoto, J.; Codd, G.A.; Mijovic, B.; Shi, T.; Tuo, X.; Li, S.C.; et al. Challenges of Using Blooms of *Microcystis* Spp. in Animal Feeds: A Comprehensive Review of Nutritional, Toxicological and Microbial Health Evaluation. *Sci. Total Environ.* **2021**, *764*, 142319. [CrossRef]
11. Nakai, S.; Inoue, Y.; Hosomi, M.; Murakami, A. Growth Inhibition of Blue-Green Algae by Allelopathic Effects of Macrophytes. *Water Sci. Technol.* **1999**, *39*, 47–53. [CrossRef]
12. Tazart, Z.; Manganelli, M.; Scardala, S.; Buratti, F.M.; Di Gregorio, F.N.; Douma, M.; Mouhri, K.; Testai, E.; Loudiki, M. Remediation Strategies to Control Toxic Cyanobacterial Blooms: Effects of Macrophyte Aqueous Extracts on *Microcystis aeruginosa* (Growth, Toxin Production and Oxidative Stress Response) and on Bacterial Ectoenzymatic Activities. *Microorganisms* **2021**, *9*, 1782. [CrossRef]
13. Kurashov, E.; Krylova, J.; Protopopova, E. The Use of Allelochemicals of Aquatic Macrophytes to Suppress the Development of Cyanobacterial "Blooms". In *Plankton Communities*; Pereira, L., Gonçalves, A.M., Eds.; Intech Open: London, UK, 2021. [CrossRef]
14. Maredová, N.; Altman, J.; Kaštovský, J. The Effects of Macrophytes on the Growth of Bloom-Forming Cyanobacteria: Systematic Review and Experiment. *Sci. Total Environ.* **2021**, *792*, 148413. [CrossRef] [PubMed]
15. Zhang, H.; Shang, Y.; Lyu, T.; Chen, J.; Pan, G. Switching Harmful Algal Blooms to Submerged Macrophytes in Shallow Waters Using Geo-Engineering Methods: Evidence from a 15N Tracing Study. *Environ. Sci. Technol.* **2018**, *52*, 11778–11785. [CrossRef] [PubMed]
16. Zhang, S.H.; Guo, L.; Cao, J.Y.; Chang, J.J. Allelopathic Activities of Three Emergent Macrophytes on Several Monospecific Cyanobacterial Species and Natural Phytoplankton Assemblages. *Polish J. Environ. Stud.* **2015**, *24*, 397–402. [CrossRef] [PubMed]
17. Amorim, C.A.; de Moura-Falcão, R.H.; Valença, C.R.; de Souza, V.R.; Moura, A.D.N. Allelopathic Effects of the Aquatic Macrophyte *Ceratophyllum demersum* L. on Phytoplankton Species: Contrasting Effects between Cyanobacteria and Chlorophytes. *Acta Limnol. Bras.* **2019**, *31*, e21. [CrossRef]
18. Zhu, J.; Liu, B.; Wang, J.; Gao, Y.; Wu, Z. Study on the Mechanism of Allelopathic Influence on Cyanobacteria and Chlorophytes by Submerged Macrophyte (*Myriophyllum spicatum*) and Its Secretion. *Aquat. Toxicol.* **2010**, *98*, 196–203. [CrossRef]

19. Wang, H.; Liu, F.; Luo, P.; Li, Z.; Zheng, L.; Wang, H.; Zou, D.; Wu, J. Allelopathic Effects of *Myriophyllum aquaticum* on Two Cyanobacteria of *Anabaena flos-aquae* and *Microcystis aeruginosa*. *Bull. Environ. Contam. Toxicol.* **2017**, *98*, 556–561. [CrossRef]
20. Park, M.H.; Chung, I.M.; Ahmad, A.; Kim, B.H.; Hwang, S.J. Growth Inhibition of Unicellular and Colonial *Microcystis* Strains (*Cyanophyceae*) by Compounds Isolated from Rice (*Oryza sativa*) Hulls. *Aquat. Bot.* **2009**, *90*, 309–314. [CrossRef]
21. Kim, J.S.; Kim, J.C.; Lee, S.; Lee, B.H.; Cho, K.Y. Biological Activity of L-2-Azetidinecarboxylic Acid, Isolated from *Polygonatum odoratum* Var. Pluriflorum, against Several Algae. *Aquat. Bot.* **2006**, *85*, 1–6. [CrossRef]
22. Declerck, S.; Vanderstukken, M.; Pals, A.; Muylaert, K.; De Meester, L. Plankton Biodiversity along a Gradient of Productivity and Its Mediation by Macrophytes. *Ecology* **2007**, *88*, 2199–2210. [CrossRef]
23. Vanderstukken, M.; Mazzeo, N.; van Colen, W.; Declerck, S.A.J.; Muylaert, K. Biological Control of Phytoplankton by the Subtropical Submerged Macrophytes *Egeria densa* and *Potamogeton illinoensis*: A Mesocosm Study. *Freshw. Biol.* **2011**, *56*, 1837–1849. [CrossRef]
24. Senavirathna, M.D.H.J.; Muhetaer, G.; Atapaththu, K.S.S.; Fujino, T. *Egeria densa* Allelopathy on *Microcystis aeruginosa* Under Different Light Intensities and Preliminary Insight into Inter-Parameter Relationships. *Water. Air. Soil Pollut.* **2021**, *232*, 135. [CrossRef]
25. Chen, J.; Zhang, H.; Han, Z.; Ye, J.; Liu, Z. The Influence of Aquatic Macrophytes on *Microcystis aeruginosa* Growth. *Ecol. Eng.* **2012**, *42*, 130–133. [CrossRef]
26. Ghobrial, M.G.; Nassr, H.S.; Kamil, A.W. Bioactivity Effect of Two Macrophyte Extracts on Growth Performance of Two Bloom-Forming Cyanophytes. *Egypt. J. Aquat. Res.* **2015**, *41*, 69–81. [CrossRef]
27. Gross, E.M.; Erhard, D.; Iványi, E. Allelopathic Activity of *Ceratophyllum demersum* L. and *Najas marina* Ssp. Intermedia (Wolfgang) Casper. *Hydrobiologia* **2003**, *506–509*, 583–589. [CrossRef]
28. Mulderij, G.; Smolders, A.J.P.; Van Donk, E. Allelopathic Effect of the Aquatic Macrophyte, *Stratiotes aloides*, on Natural Phytoplankton. *Freshw. Biol.* **2006**, *51*, 554–561. [CrossRef]
29. Senavirathna, M.D.H.J.; Wijesinghe, N.A.; Liu, Z.; Fujino, T. Effects of Short-Term Exposure to Different Salinity Levels on *Myriophyllum spicatum* and *Ceratophyllum demersum* and Suitability of Biomarkers to Evaluate Macrophyte Responses to Salinity Stress. *Ann. Limnol.* **2020**, *56*, 23. [CrossRef]
30. Hu, H.; Hong, Y. Algal-Bloom Control by Allelopathy of Aquatic Macrophytes—A Review. *Front. Environ. Sci. Eng. China* **2008**, *2*, 421–438. [CrossRef]
31. Espinosa-Rodríguez, C.A.; Rivera-De La Parra, L.; Martínez-Téllez, A.; Gómez-Cabral, G.C.; Sarma, S.S.S.; Nandini, S. Allelopathic Interactions between the Macrophyte *Egeria densa* and Plankton (Alga, *Scenedesmus acutus* and Cladocerans, *Simocephalus* Spp.): A Laboratory Study. *J. Limnol.* **2016**, *75*, 151–160. [CrossRef]
32. Virginia, V.; Ranielle, B.; Silva, S.; Severiano, S. Can the Presence of Green Microalgae Reverse the Allelopathic Effects of the Submerged Macrophyte *Egeria densa* on the Toxin-Producing Cyanobacterium *Raphidiopsis raciborskii*? *Hydrobiologia* **2022**, *849*, 4391–4406. [CrossRef]
33. Rippka, R.; Deruelles, J.; Waterbury, J.B. Generic Assignments, Strain Histories and Properties of Pure Cultures of Cyanobacteria. *J. Gen. Microbiol.* **1979**, *111*, 1–61. [CrossRef]
34. Zhang, H.; Ye, Y.K.; Wang, S.H.; Luo, J.P.; Tang, J.; Ma, D.F. Hydrogen Sulfide Counteracts Chlorophyll Loss in Sweetpotato Seedling Leaves and Alleviates Oxidative Damage against Osmotic Stress. *Plant Growth Regul.* **2009**, *58*, 243–250. [CrossRef]
35. Pilotto, L.S.; Douglas, R.M.; Burch, M.D.; Cameron, S.; Beers, M.; Rouch, G.J.; Robinson, P.; Kirk, M.; Cowie, C.T.; Hardiman, S.; et al. Health Effects of Exposure to Cyanobacteria (Blue-Green Algae) during Recreational Water-Related Activities. *Aust. N. Z. J. Public Health* **1997**, *21*, 562–566. [CrossRef] [PubMed]
36. Hua, Q.; Liu, Y.G.; Yan, Z.L.; Zeng, G.M.; Liu, S.B.; Wang, W.J.; Tan, X.F.; Deng, J.Q.; Tang, X.; Wang, Q.P. Allelopathic Effect of the Rice Straw Aqueous Extract on the Growth of *Microcystis aeruginosa*. *Ecotoxicol. Environ. Saf.* **2018**, *148*, 953–959. [CrossRef]
37. Zhang, H.; Zhao, Y.; Yin, H.; Wang, Y.; Li, H.; Wang, Z.; Geng, Y.; Liang, W.; Wang, H. Effect of Aquatic Macrophyte Growth on Landscape Water Quality Improvement. *Environ. Sci. Pollut. Res.* **2019**, *26*, 33791–33803. [CrossRef]
38. Hu, L.; Yin, C. Development of a New Three-Dimensional Fluorescence Spectroscopy Method Coupling with Multilinear Pattern Recognition to Discriminate the Variety and Grade of Green Tea. *Food Anal. Methods* **2017**, *10*, 2281–2292. [CrossRef]
39. Körner, S.; Nicklisch, A. Allelopathic Growth Inhibition of Selected Phytoplankton Species by Submerged Macrophytes. *J. Phycol.* **2002**, *871*, 862–871. [CrossRef]
40. Norling, B.; Zak, E.; Andersson, B.; Pakrasi, H. 2D-Isolation of Pure Plasma and Thylakoid Membranes from the Cyanobacterium *Synechocystis* Sp. PCC 6803. *Fed. Eur. Biochem. Soc. Lett.* **1998**, *436*, 189–192. [CrossRef]
41. Li, H.; Singh, A.K.; McIntyre, L.M.; Sherman, L.A. Differential Gene Expression in Response to Hydrogen Peroxide and the Putative PerR Regulon of *Synechocystis* Sp. Strain PCC 6803. *J. Bacteriol.* **2004**, *186*, 3331–3345. [CrossRef]
42. Mohamed, Z.A. Macrophytes-Cyanobacteria Allelopathic Interactions and Their Implications for Water Resources Management—A Review. *Limnologica* **2017**, *63*, 122–132. [CrossRef]
43. Wang, F.; Zhao, W.; Chen, J.; Zhou, Y. Allelopathic Inhibitory Effect on the Growth of *Microcystis aeruginosa* by Improved Ultrasonic-Cellulase Extract of Vallisneria. *Chemosphere* **2022**, *298*, 134245. [CrossRef]
44. Traversa, A.; Loffredo, E.; Gattullo, C.E.; Senesi, N. Water-Extractable Organic Matter of Different Composts: A Comparative Study of Properties and Allelochemical Effects on Horticultural Plants. *Geoderma* **2010**, *156*, 287–292. [CrossRef]

45. Rossi, F.; De Philippis, R. Role of Cyanobacterial Exopolysaccharides in Phototrophic Biofilms and in Complex Microbial Mats. *Life* **2015**, *5*, 1218–1238. [CrossRef] [PubMed]
46. Tazart, Z.; Douma, M.; Tebaa, L.; Loudiki, M. Use of Macrophytes Allelopathy in the Biocontrol of Harmful *Microcystis aeruginosa* Blooms. *Water Sci. Technol. Water Supply* **2019**, *19*, 245–253. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.