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Allelopathic effects of the aquatic macrophyte *Ceratophyllum demersum* L. on phytoplankton species: contrasting effects between cyanobacteria and chlorophytes

Efeitos alelopáticos da macrófita aquática *Ceratophyllum demersum* L. sobre espécies fitoplanctônicas: efeitos contrastantes entre cianobactérias e clorófitas

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Abstract: Aim: To assess the allelopathic effects of the submerged macrophyte *Ceratophyllum demersum* on four strains of phytoplankton species: two cyanobacteria (*Microcystis aeruginosa* - microcystin producing and *M. panniformis* - non-microcystin producing), and two chlorophytes (*Ankistrodesmus falcatus* and *Raphidocelis subcapitata*). **Methods:** A coexistence experiment between *C. demersum* and the four strains was carried out for six days, with eight treatments and three replicates. The strains were cultivated in ASM1 culture medium, under controlled laboratory conditions. Two treatments were assigned for each strain, one with 6 $g.L^{-1}$ of the macrophyte, and the control without the plant. Biomasses and growth rates of the strains were evaluated every two days, which were compared through the T-test and two-way ANOVA, respectively. **Results:** The results varied among the strains, with toxic *M. aeruginosa* being intensely inhibited by *C. demersum*, with a decrease of 99.5% in its biomass ($p<0.001$), while non-toxic *M. panniformis* was less affected by the allelochemicals, with a reduction of 86.2% ($p<0.001$). *Ankistrodesmus falcatus* delayed its growth when in coexistence with the macrophyte, decreasing its biomass in 50.4% ($p<0.01$), while *R. subcapitata* was not altered ($p>0.05$). In coexistence with *C. demersum*, *M. aeruginosa* exhibited the lowest growth rates (-0.65 d^{-1}), followed by *M. panniformis* (-0.15 d^{-1}), *A. falcatus* (0.19 d^{-1}), and *R. subcapitata* (0.34 d^{-1}), with significant differences between all strains ($p<0.001$). *Microcystis aeruginosa* presented higher inhibition rates than *M. panniformis* ($p<0.001$), as well as, *A. falcatus* was more inhibited than *R. subcapitata* ($p<0.05$). **Conclusions:** The presence of microcystins could influence the allelopathic responses of *C. demersum*, that may release more allelochemicals in coexistence with toxic strains of *M. aeruginosa*. Accordingly, *C. demersum* can be used in biomanipulation strategies to control toxic and non-toxic cyanobacterial blooms, without damaging other phytoplankton species, like chlorophytes.

Keywords: allelopathy; biomanipulation; coexistence experiments; control of cyanobacteria; submerged macrophytes.

Resumo: Objetivo: Avaliar os efeitos alelopáticos da macrófita submersa *Ceratophyllum demersum* sobre quatro cepas de espécies fitoplanctônicas: duas cianobactérias (*Microcystis aeruginosa* - produtora de microcistinas e *M. panniformis* - não produtora) e duas clorófitas (*Ankistrodesmus*

falcatus e *Raphidocelis subcapitata*). **Métodos:** Foi realizado um experimento de coexistência entre *C. demersum* e as quatro cepas, durante seis dias, com oito tratamentos e três réplicas. As cepas foram cultivadas em meio ASM1, sob condições laboratoriais controladas. Foram designados dois tratamentos para cada cepa, um com 6 $g.L^{-1}$ da macrófita, e um controle sem a planta. Biomassas e taxas de crescimento das cepas foram avaliadas a cada dois dias e comparadas pelo teste-T e ANOVA two-way, respectivamente. **Resultados:** Os resultados variaram entre as cepas, sendo a cepa tóxica de *M. aeruginosa* intensamente inibida por *C. demersum*, com uma redução de 99,5% na sua biomassa (p<0,001), enquanto a cepa não tóxica de *M. panniformis* foi menos afetada pelos aleloquímicos, com uma redução de 86,2% (p<0,001). *Ankistrodesmus falcatus* retardou seu crescimento em coexistência com a macrófita, reduzindo sua biomassa em 50,4% (p<0,01), enquanto que *R. subcapitata* não foi afetada (p>0,05). Em coexistência com *C. demersum*, *M. aeruginosa* apresentou as menores taxas de crescimento (-0,65 d^{-1}), seguida de *M. panniformis* (-0,15 d^{-1}), *A. falcatus* (0,19 d^{-1}) e *R. subcapitata* (0,34 d^{-1}), com diferenças significativas entre todas as cepas (p<0,001). *Microcystis aeruginosa* apresentou maiores taxas de inibição que *Microcystis panniformis* (p<0,001), bem como, *A. falcatus* foi mais inibida que *R. subcapitata* (p<0,05). **Conclusões:** A presença de microcistinas pode afetar as respostas alelopáticas de *C. demersum*, que pode liberar mais aleloquímicos em coexistência com cepas tóxicas de *M. aeruginosa*. Portanto, *C. demersum* pode ser utilizada em estratégias de biomanipulação para controle de florações de cianobactérias tóxicas e não tóxicas, sem causar danos às demais espécies fitoplanctônicas, como as clorófitas.

Palavras-chave: alelopatia; biomanipulação; experimentos de coexistência; controle de cianobactérias; macrófitas submersas.

1. Introduction

In the last few decades, the rising temperatures linked to an excessive input of nutrients in the water bodies have supported the occurrence of cyanobacterial blooms (Kosten et al., 2012; Paerl & Otten, 2013). These blooms have become a frequent global problem for the public supply reservoirs, which can be composed of species that produce cyanotoxins, such as hepatotoxins, neurotoxins, and dermatotoxins (Wiegand & Pflugmacher, 2005). Among the most frequent and harmful cyanobacteria, the genus *Microcystis* presents bloom records in 108 countries, 79 of which report the production of microcystin (Harke et al., 2016).

Microcystins are the most extensively studied cyanotoxins in the world, which are considered the most frequent and lethal. Therefore, the World Health Organization (WHO) and the Brazilian Ministry of Health established a tolerable limit of 1.0 $\mu g.L^{-1}$ of microcystins in waters destined for public supply (Chorus & Bartram, 1999; Brasil, 2011). These regulations were created after the "Tragedy of Caruaru", known worldwide as the largest case of human poisoning by microcystins, in which 76 renal patients died after using water contaminated with microcystins at a hemodialysis clinic in Caruaru, Pernambuco, in 1996 (Carmichael et al., 2001). In the Brazilian Semiarid region, the occurrence of microcystin-containing cyanobacteria blooms is still more recurrent (Bittencourt-Oliveira et al., 2014; Lorenzi et al., 2018), which is certainly due to the climatic and eutrophication conditions of the water bodies in this region that favor the occurrence and establishment of these blooms (Moura et al., 2018).

One potential solution for the control of cyanobacterial blooms includes the use of submerged aquatic macrophytes as a biomanipulation alternative since they can efficiently decrease phytoplankton growth (Zuo et al., 2012). These plants help to maintain clear conditions in shallow lakes (Scheffer et al., 1993, 2003; Hilt & Gross, 2008). Their mechanisms of action are related to the reduction of nutrient concentrations in the water column, which is essential for phytoplankton growth; reduction of sediment resuspension; and supply of refuge to zooplankton and macroinvertebrates (Scheffer et al., 1993; Mulderij et al., 2007), which are efficient phytoplankton consumers (Amorim et al., 2019). In addition, these plants can also release allelochemicals in the water, acting on the inhibition of planktonic and epiphytic algae (Erhard & Gross, 2006; Hilt & Gross, 2008).

Several studies have shown the allelopathic potential of aquatic macrophytes on cyanobacteria and microalgae in laboratory studies, with submerged macrophytes being more efficient (Mohamed, 2017). In this context, cyanobacteria are more sensitive to allelochemicals when compared to chlorophytes (Körner & Nicklisch, 2002; Erhard & Gross, 2006; Zhu et al., 2010). However, Chang et al. (2012) pointed out that *Myriophyllum verticillatum* L. is able to inhibit the growth of *Microcystis aeruginosa* (Kützing) Kützing in pure cultures, however, this cyanobacterium is stimulated when co-cultivated with the green

algae *Desmodesmus armatus* (Chodat) E.Hegewald. Besides, Švanys et al. (2016) showed that non-toxic strains of *M. aeruginosa* are more sensitive to tannic acid, an allelochemical isolated from aquatic macrophytes. However, Amorim (2017) found that when coexisting, toxic strains of *Microcystis* are more affected by submerged macrophytes, as the stress caused by the microcystins promotes a greater release of allelochemicals by aquatic plants.

Considering the high occurrence of cyanobacterial blooms in the Brazilian Semiarid region, in addition to the expectation of increasing of the blooms for the coming years due to climate change and eutrophication, more studies aiming to control cyanobacterial blooms using aquatic macrophytes are needed. Thus, the aim of this study was to evaluate the allelopathic potential of the aquatic macrophyte *Ceratophyllum demersum* L. on the growth of cyanobacteria species, with one toxic and another non-toxic strain, and chlorophytes to understand the role of allelochemicals on different phytoplankton species.

2. Material and Methods

2.1. Phytoplankton organisms, submerged macrophyte and culture conditions

During the experiments, four strains of phytoplankton species were used: two cyanobacteria and two chlorophytes. The strain of *M. aeruginosa* (NPLJ-4) was obtained from the cyanobacteria culture collection at the Laboratory of Ecophysiology and Toxicology of Cyanobacteria, Federal University of Rio de Janeiro. In previous experiments with the same conditions of the present study, this strain was found to produce four variants of microcystins, mainly [D-Leu^{-1}] microcystin-LR, with about 90% of the total microcystins, in addition to three other unknown variants (for details of toxin detection by High-Performance Liquid Chromatography see Amorim et al., 2017). A strain of *M. panniformis* Komárek et al. (BCCUSP29) was provided by the Brazilian Cyanobacteria Collection at the University of São Paulo and does not produce microcystins (Bittencourt-Oliveira, 2003). The chlorophyte strains, *Ankistrodesmus falcatus* (Corda) Ralfs (BMIUFRPE-01) and *Raphidocelis subcapitata* (Korshikov) Nygaard et al. (BMIUFRPE-02), were obtained from the Culture Collection of Microalgae from the Federal Rural University of Pernambuco – BMIUFRPE.

These strains were cultivated in ASM1 nutrient medium (Gorham et al., 1964), in a climatic chamber with controlled temperature (25 °C ± 1.5), light intensity (40 µmol photons $m^{-2}.s^{-1}$), pH (7.5), photoperiod (12 h), and the cultures were homogenized three times a day. The cultivations were kept until a biomass of approximately 50 $mg.L^{-1}$ for cyanobacterial strains and 5 $mg.L^{-1}$ for chlorophyte strains, during the exponential growth phase. These culture conditions were tested at the laboratory with higher growth for all tested phytoplankton species (e.g. Amorim, 2017; Amorim et al., 2017, 2019).

Young and photosynthetically active plants of *C. demersum* were collected from the Carpina Reservoir (latitude 7°53'08" S, and longitude 35°20'42" W), municipality of Lagoa do Carro, Pernambuco, Brazil. After collection, the plants were washed several times with a soft brush and distilled water jets to remove sediment, epiphyte microalgae, and zooplankton/zoobenthos, and cultivated in 8 L aquaria containing tap water, which was renewed weekly. Subsequently, they were cultivated in an aseptic and climatized room under the same conditions described for the culture of the strains, with constant aeration.

2.2. Experimental design

The coexistence experiment was carried out in an aseptic climatized room with the same conditions previously described for the culture of the strains. Eight treatments were used, each one with three replicates, totaling 24 experimental units, consisting of 1,000 mL Erlenmeyer flasks filled with 500 ml of ASM1 cultivation medium. The coexistence treatments consisted of the cultivation of each strain with the addition of a young and apical branch of *C. demersum* to achieve biomass of 6.0 $gFW.L^{-1}$ (g of fresh weight per liter). Three days before the beginning of the experiment, the plants were washed five times with ultrapure water to remove algae and adhered animals and were subsequently kept in ASM1 medium for acclimatization. The control treatments consisted of the cultivation of each strain without *C. demersum* branches.

The experiment lasted six days and samples were taken to quantify the biomass of the strains every two days. For each sampling day, 2 mL aliquots were taken, which were fixed with 4% formalin for further density determination ($cells.mL^{-1}$), by counting cells in a Fuchs-Rosenthal chamber. At least 400 cells per sample were counted to obtain a 90% reliability degree (Lund et al., 1958). Then, the biomass ($mg.L^{-1}$) was determined by multiplying the density by the average biovolume of the strains, using geometric formulas proposed by Sun & Liu (2003).

2.3. Growth and inhibition rates

Growth rates (μ) were calculated according to Wood et al. (2005): $\mu\ (d^{-1}) = (\ln(N_t) - \ln(N_{t0})) / t - t0$, where N represents the biomass values on the sixth day of the experiment (t) and at the initial time (t0). The inhibition rate (IR%) was calculated as follows: IR% = ((Nm – Nc) / Nc) × 100; where N_m represents the biomass of the strains in the coexistence treatments with the macrophyte in each replicate, and N_c represents the mean biomass of the strains in the controls.

2.4. Statistical analyses

Significant differences in the biomasses of the strains were verified by the T-test between the treatments of coexistence and controls, for each strain and day of the experiment. Before the analyses, the data were tested for normality by the Shapiro test. A two-way ANOVA was performed to compare the differences in the growth rates between the species and the treatments. For that, the data were tested for normality and homoscedasticity using the Kolmogorov-Smirnov and Bartlett tests, respectively. The inhibition rates were compared between the strains separately for cyanobacteria and for chlorophytes by the T-test. Statistical analyses were performed in the R program, with a level of significance set at $p<0.05$ (R Core Team, 2016).

3. Results

3.1. Effects of C. demersum on the biomass of the strains

The submerged macrophyte *C. demersum* induced varied responses between the strains, both for the coexistence treatments with the cyanobacteria strains (producer and non-producer of microcystins), as well as with the chlorophytes. The biomass of the toxic *M. aeruginosa* strain was inhibited from the second day of coexistence with *C. demersum* to the end of the experiment, reaching a biomass close to zero on the sixth day (Figure 1a). However, the non-toxic strain of *M. panniformis*

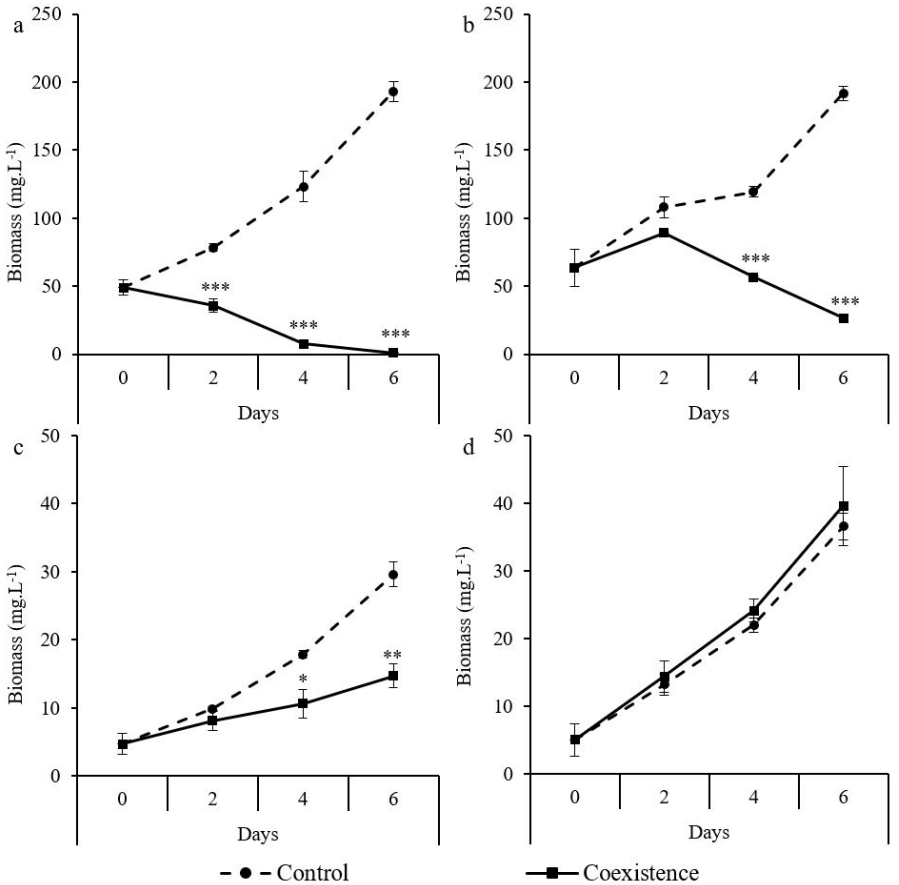

Figure 1. Biomasses of the strains of toxic *Microcystis aeruginosa* (a), non-toxic *M. panniformis* (b), *Ankistrodesmus falcatus* (c), and *Raphidocelis subcapitata* (d), submitted to the treatments of coexistence with *Ceratophyllum demersum* and control. Lines are the means and standard error. Significant differences between the coexistence and control treatments for each day are represented by asterisks (T-test, *$p<0.05$; **$p<0.01$; ***$p<0.001$).

showed a less pronounced inhibition, with reductions in growth from the fourth to the sixth day of the experiment (Figure 1b). Both chlorophyte strains were less affected in relation to cyanobacteria. The strain of *A. falcatus* presented a delay in its growth, showing biomass lower than the control from the fourth day (Figure 1c). On the other hand, the strain of *R. subcapitata* was not affected by *C. demersum* in coexistence, with biomass similar to the control during all experiment (Figure 1d).

3.2. Effects of C. demersum on the growth and inhibition rates of strains

Both strains of *Microcystis* showed similar growth rates in the control treatment, with the non-toxic strain of *M. panniformis* presenting lower values (0.18 d^{-1}) in relation to the toxic *M. aeruginosa* (0.23 d^{-1}). In coexistence with *C. demersum*, both strains presented negative values, and showed a significant reduction in growth, being more pronounced in the toxic strain (-0.65 d^{-1}) than in the non-toxic strain (-0.15 d^{-1}). Both strains showed significant differences in the growth rates for the coexistence and control treatments ($p < 0.001$) (Figure 2).

Both chlorophyte strains showed positive growth rates when co-cultivated with *C. demersum* and in the control. The strain of *A. falcatus* presented a significantly lower growth rate in the coexistence (0.19 d^{-1}), when compared to the control (0.31 d^{-1}) ($p < 0.05$). In contrast, *R. subcapitata* did not show significant differences in the growth rates between the control (0.33 d^{-1}) and coexistence (0.34 d^{-1}) treatments ($p > 0.05$) (Figure 2).

The growth rates of the strains showed significant differences between the treatments ($F = 569.7$, $p < 0.001$) and the species ($F = 316.7$, $p < 0.001$). In the control, both strains of cyanobacteria showed similar growth, as well as both chlorophyte strains. All tested strains presented different growth rates when in coexistence with *C. demersum*, with *M. aeruginosa* presenting lower values, followed by *M. panniformis*, *A. falcatus*, and *R. subcapitata* (Figure 2).

When evaluating the inhibition rates, the high sensitivity of the tested cyanobacteria to allelochemicals of *C. demersum*, especially the toxic strain of *M. aeruginosa*, is evident, while the strains of chlorophytes were less sensitive. The toxic strain of *M. aeruginosa* was inhibited in 99.5%, reaching biomass close to zero on the sixth day of the experiment in the coexistence treatment with *C. demersum*, while the non-toxic strain of *M. panniformis* was inhibited in 86.2%. The chlorophyte strains were less affected by the allelochemicals of *C. demersum*. *Ankistrodesmus falcatus* was inhibited only in 50.4% when in coexistence with the macrophyte, while *R. subcapitata* was stimulated in 8.3%. The inhibition rates of the strains showed significant differences between the strains for both cyanobacteria ($t = 24.89$, $p < 0.001$) and chlorophytes ($t = 3.43$, $p < 0.05$) (Figure 3).

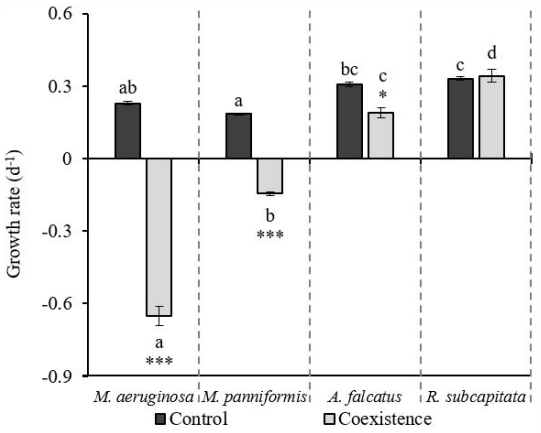

Figure 2. Growth rates of the strains of toxic *Microcystis aeruginosa*, non-toxic *M. panniformis*, *Ankistrodesmus falcatus*, and *Raphidocelis subcapitata*, submitted to the treatments of coexistence with *Ceratophyllum demersum* and control. Bars are the means and standard error. Different letters represent significant differences between the strains for each treatment (two-way ANOVA, $p < 0.05$). Significant differences between the coexistence and control treatments for each strain are represented by asterisks (two-way ANOVA, *$p<0.05$; ***$p<0.001$).

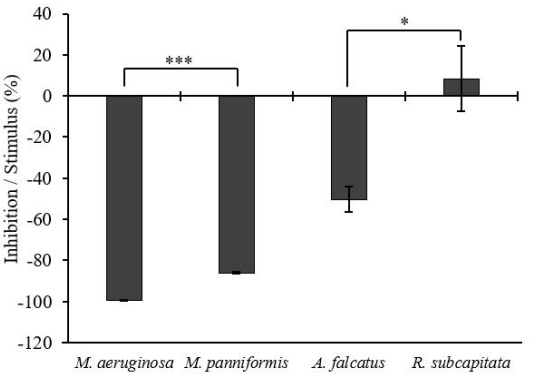

Figure 3. Inhibition rates of the strains of toxic *Microcystis aeruginosa*, non-toxic *M. panniformis*, *Ankistrodesmus falcatus*, and *Raphidocelis subcapitata*, submitted to the treatments of coexistence with *Ceratophyllum demersum* and control after six days. Bars are the means and standard error. Significant differences between the inhibition rates for both cyanobacteria strains and both chlorophyte strains are represented by asterisks (T-test, *$p<0.05$; ***$p<0.001$).

4. Discussion

The macrophyte *C. demersum* was able to allelopathically inhibit the growth of both cyanobacterial strains, more specifically the toxic *M. aeruginosa*, in relation to the non-toxic *M. panniformis*. In contrast, chlorophyte strains were less affected in coexistence with *C. demersum*, demonstrating lower sensitivity to the allelochemicals of this macrophyte. Nakai et al. (1999) also demonstrated the potential of this macrophyte in inhibiting allelopathically species of cyanobacteria, including *M. aeruginosa*. In addition, Dong et al. (2014) showed that *C. demersum* can alter the structure of phytoplankton community from a eutrophic lake, promoting the dominance of chlorophytes, as well as inhibiting the growth of *M. aeruginosa* and benefiting the restoration of water quality for the lake.

Allelopathy can be observed in both terrestrial and aquatic plants. In aquatic ecosystems, submerged and floating plants, in addition to algae, play a primordial role in the local dynamics (Pflugmacher, 2002). In these environments, allelopathy occurs in all groups of macrophytes and algae, including cyanobacteria, and its effects are usually negative for other living organisms, commonly inhibiting the growth and photosynthesis of their competitors (Żak et al., 2012). However, Li et al. (2016) emphasize that, in addition to allelopathy, other factors, such as competition for light and nutrients, can give to the macrophytes a greater advantage in relation to cyanobacteria.

Allelopathically active macrophytes can be used in the restoration process of eutrophic aquatic environments, since these plants can control algal growth, especially of cyanobacteria (Gross et al., 1996; Ghobrial et al., 2015). Recently, many studies aiming to restore the water quality in eutrophic environments have been performed, especially using submerged macrophytes as a biomanipulation strategy (e.g. Dong et al., 2014; Vanderstukken et al., 2014; Yu et al., 2016; Liu et al., 2018). These plants have various mechanisms of action in these environments, particularly through the release of allelopathic compounds (Scheffer et al., 1993; Gross et al., 1996).

However, most studies conducted in laboratory use extracts of aquatic plants or purified allelochemicals (e.g. Li et al., 2016; Gao et al., 2017; Švanys et al., 2016), which often exceed the concentrations released by aquatic macrophytes in natural environments (Nakai et al., 1999). Therefore, studies about coexistence between aquatic macrophytes and phytoplankton more accurately reflect the reality of aquatic environments, as they elucidate other mechanisms involved in the inhibition of phytoplankton, such as competition for nutrients, light, or mechanical interference of plants.

According to Mohamed (2017), more than 40 aquatic macrophytes can inhibit phytoplankton species, and most studies have been developed using cyanobacterial strains, in particular, *M. aeruginosa* (e.g. Chen et al., 2012; Zhu et al., 2014; Gao et al., 2017). Other studies show that cyanobacteria are more sensitive to allelochemicals from aquatic macrophytes, followed by chlorophytes (Körner & Nicklisch, 2002; Erhard & Gross, 2006), as verified in the present study. The main mode of action of allelochemicals on cyanobacteria is the inhibition of photosystem II, through damage caused to the electron transport chain during photosynthesis, in addition to oxidative stress (Leu et al., 2002; Zhu et al., 2010; Gao et al., 2017).

During the experiments, the toxic strain of *M. aeruginosa* presented a higher inhibition by *C. demersum* when compared to the non-toxic strain of *M. panniformis*. Few studies show the allelopathic effects of submerged macrophytes on toxic and non-toxic strains, for example, Mulderij et al. (2005) showed that a toxic strain of *M. aeruginosa* was more sensitive to the exudates of *Stratioides aloides* L. than the non-toxic lineage. However, when evaluating the effects of tannic acid on several toxic and non-toxic strains of *M. aeruginosa*, Švanys et al. (2016) verified that non-toxic strains are more sensitive to this allelochemical, showing that something related to the synthesis of microcystins confers higher tolerance to toxic strains.

However, these studies were carried out by using purified exudates or allelochemicals, and in coexistence, it is possible that other factors contribute to the allelopathic responses of aquatic macrophytes over toxic and non-toxic strains. For example, when studying the allelopathic effects of *Egeria densa* Planch. on the same strains of *Microcystis* used in the present study, Amorim (2017) found that the microcystin-producing strain was inhibited by macrophyte allelochemicals, while the non-toxic strain was stimulated. Therefore, the presence of microcystins may act as a stress factor for aquatic macrophytes, which are stimulated to release a greater amount of allelochemicals in the medium that are toxic to *Microcystis* (Amorim, 2017). This justifies the greater sensitivity of the toxic strain of *M. aeruginosa* in the present study, which may have

been exposed to a greater amount of allelochemicals than the non-toxic strain.

Several studies have shown the adverse effects of microcystins on submerged macrophytes. For example, Amorim et al. (2017) showed that the co-cultivation of *E. densa* with the same strains of *Microcystis* from the present study caused serious damage to the plant when exposed to toxic strains. This damages caused by the toxic strain was the reduction of the plant length and biomass, inhibition of the emission of shoots and roots, alteration in the content of photosynthetic pigments and oxidative stress, with a higher production of malondialdehyde and greater activity of enzymes catalase, superoxide dismutase and ascorbate peroxidase, while none of these alterations were verified in the co-cultivation with the non-toxic strain of *M. panniformis* and in the control (Amorim et al., 2017). Other studies also showed the adverse effects of microcystins or extracts of cyanobacterial blooms containing these toxins on the growth and physiological performance of *C. demersum* (e.g. Pflugmacher, 2004; Romero-Oliva et al., 2014, 2015a, b).

Unlike in Amorim (2017), the non-toxic strain of *M. panniformis* was not stimulated but showed a delay in growth during the initial days of the experiment. However, the reduction in biomass was less pronounced than in the toxic strain of *M. aeruginosa*, since the absence of microcystins did not stimulate the plant to release allelochemicals. However, at the end of the experiment, this strain was inhibited, with a negative growth rate, since the plant may have started releasing allelochemicals in response to nutrient limitation caused by the non-toxic strain, or the presence of other compounds such as lipopolysaccharide.

Both strains of chlorophytes were less affected from the beginning of the experiment by *C. demersum*. This fact can be justified by the absence of toxins, which did not stimulate the plant to release potentially toxic allelochemicals. In addition, some studies report the lower sensitivity of chlorophytes to allelochemicals of aquatic macrophytes, correlated to their physiology and adaptations to these compounds (Hilt & Gross, 2008; Zhu et al., 2010).

In conclusion, the submerged macrophyte *C. demersum* inhibited both strains of cyanobacteria tested, being more markedly for the toxic *M. aeruginosa* than the non-toxic *M. panniformis*. Certainly, the greatest inhibition activity in the toxic strain was due to an intense release of allelochemicals by *C. demersum* in the treatment with this strain, since the presence of microcystins may have stressed the plant, resulting in higher production of allelochemicals. However, the chlorophyte strains were less affected in relation to cyanobacteria, showing delays in growth and demonstrating low sensitivity to the allelochemicals of aquatic macrophytes. In this sense, *C. demersum* could efficiently control toxic and non-toxic cyanobacterial blooms, without causing adverse effects to other phytoplankton organisms, such as chlorophytes.

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