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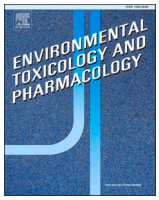

Beauvericin and enniatin B mycotoxins alter aquatic ecosystems: Effects on green algae

Ana Juan-García [a,b,*], Cristina Juan [a], Sami Taipale [b], Eeva-Riikka Vehniäinen [b]

[a] Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy and Food Science, University of Valencia, Av. Vicent Andrés Estellés s/n, València, Burjassot 46100, Spain
[b] Department of Biological and Environmental Science, University of Jyväskylä, Survontie 9C, Jyväskylä FI-40014, Finland

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ABSTRACT

Myxotoxins can contaminate algal-based products and arrive to the food chain to consumers producing chronic toxicity effects. Here, we studied phytotoxicity of mycotoxins, beauvericin (BEA) and ennaitin B (ENN B) in four phytoplankton strains: *Acutodesmus sp.*, *Chlamydomonas reinhardtii*, *Haematococcus pluvialis*, and *Monoraphidium griffithii*, which are all green algae. It was tested the capacity of clearing the media of BEA and ENN B at different concentrations by comparing nominal and measured quantifications. Results revealed that *Acutodesmus sp.* and *C. reinhardtii* tended to flow up and down growth rate without reaching values below 50% or 60%, respectively. On the other hand, for *H. pluvialis* and *M. griffith*, IC_{50} values were reached. Regarding the clearance of media, in individual treatment a decrease of the quantified mycotoxin between nominal and measured values was observed; while in binary treatment, differences among both values were higher and more noted for BEA than for ENN B.

1. Introduction

Detecting transformations in plant ecosystems at low exposure levels is challenging due to their high sensitivity to growth conditions. However, unicellular plants possess characteristics that make them a more accessible system for conducting various studies and monitoring ecosystem impacts. This is particularly true for algae species that have a well-defined genetic system and can be easily transformed (Alexander et al., 1999).

Aquaculture has emerged as a rapidly growing sector in global food production over the past few decades. However, little is known about food safety concerns related to potential toxigenic fungi in algal cultures (Viegas et al., 2019), and even less is understood about the environmental association between mycotoxins and aquatic organisms. Cereals and other plants are often utilized as components to supplement or enhance fish meal in aquaculture diets. This common practice is employed in intensive or semi-intensive aquaculture settings due to its advantages, including consistent quality, optimal nutrient balance for growth, easy administration, and lower overall cost (Viegas et al., 2019). However, this practice also carries the risk of introducing pollutants generated by fungi on cereals into the food chain. Moreover, cereal-based feeds have the potential to introduce toxigenic fungi into the aquaculture environment (Embaby et al., 2015; Viegas et al., 2019). While several researchers have documented the negative impact of mycotoxins on fish health (Pietsch et al., 2013; Tola et al., 2015), there is a lack of knowledge regarding the phytotoxic effects on unicellular plants such as algae, which are also the foundation of the aquatic trophic chain.

The presence of mycotoxins in feed, food, and the environment is a global problem of high significance that requires comprehensive study from various perspectives. It has been reported that exposure to these mycotoxins can lead to death and diseases in both animals and humans (Pietsch et al., 2013; Tola et al., 2015). Chronic exposure in humans can occur through the consumption of plant-based, fish-based, and meat-based foods. Food authorities have taken steps to regulate mycotoxins with well-known toxicities, as some have been classified by the International Association for Research in Cancer (IARC) (EFSA, 2014; EFSA, 2019; EC, 2006).

The impact of mycotoxins on algae is of particular interest and importance due to their role in the nutritional basis of both aquatic animals and the aquatic environment, especially in the context of cereal-based feed. Any changes or alterations in algal growth, nutritional

* Corresponding author at: Laboratory of Food Chemistry and Toxicology, Faculty of Pharmacy and Food Science, University of Valencia, Av. Vicent Andrés Estellés s/n, València, Burjassot 46100, Spain.
E-mail address: ana.juan@uv.es (A. Juan-García).

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properties, or oxygen accumulation can have ripple effects throughout the trophic chain, ultimately impacting a wide range of species, from small to large. Global warming has already brought about changes in phytoplankton, zooplankton, and fish communities, mainly through cyanobacteria blooms (Taipale et al., 2021). This, in turn, has affected the presence of certain mycotoxins, which were previously present at low levels but have now started to show a different profile in food (Juan et al., 2020; El Jai et al., 2021).

The literature highlights the vital role of microalgae in the global carbon cycle, production of bio-based compounds, water purification, and food production (Thornton, 2012; Devadas et al., 2021; Kusmayadi et al., 2021; Yadav et al., 2021). Accurate estimation of microalgae growth is crucial for environmental monitoring and microalgae biotechnology. However, the presence of contaminants can significantly impact their growth.

The presence of mycotoxins in water is often derived from leakage in plant tissue, which is carried into neighboring ponds through runoff water. This, as mentioned earlier, can affect aquatic species such as algae, fish, amphibians, and zooplankton (Goessens et al., 2021). Agricultural practices have been identified as the main source of mycotoxin contamination, as demonstrated in a study conducted by Schenzel et al. (2012), which detected various mycotoxins including 3-acetyl-deoxynivalenol, deoxynivalenol, fusarenone-X, nivalenol, HT-2 toxin, T-2 toxin, beauvericin, and zearalenone in water drainage.

From the group of emergent mycotoxins, beauvericin (BEA) and enniatin B (ENN B) have increased their presence in food and feed in the last decade (Krížová et al., 2021). Both have been mainly studied in different cell lines at quite low micromolar concentrations although revealing serious problems for consumers and animals. When assayed in mixtures synergistic and additive effects have been reported *in vitro* (Krížová et al., 2021; Agahi et al., 2020; Juan-García et al., 2019b; Juan-García et al., 2019a). BEA and ENN B work as ionophores incorporating into the cell membrane and creating pores that are specific for cations (Kamyar et al., 2004). Enniatins (ENs) inhibit enzymes like acyl-CoA, cholesterol acyl transferase (ACAT), and cause a decrease in triglyceride production and a smaller pool of free fatty acids in the cells arising a hypolipidemic impact (Kamyar et al., 2004). In addition, ROS production by both BEA and ENN B has been demonstrated in different studies (Juan-Garcia et al., 2020b; Prosperini et al., 2013).

This study aims to investigate the biological effects of mycotoxins in the aquaculture environment, specifically focusing on the potential exposure of various aquatic species to these toxins (Viegas et al., 2019). Understanding these effects is crucial as it allows us to assess the proximity of mycotoxins in edible tissues and marine food to consumers. Moreover, the impact of emergent mycotoxins and their combinations on algal species remains largely unknown, underscoring the necessity of comprehensively controlling the effects of these compounds. The co-occurrence of mycotoxins with other pollutants significantly increases the ecotoxicological risk. Therefore, investigating the effects of mycotoxins, both individually and in combination with other contaminants, is of utmost importance for holistic risk assessment and management in aquatic environments.

For the first time, it has been attempted to understand the relationship between mycotoxins in aquatic settings and their effects on algae. Here it is examined the effects of mycotoxins in growth algae, their accumulation in algae, and how algae remove such contaminants from the media by clearing. This has been carried out by exposure of beauvericin (BEA) and ennaitin B in four phytoplankton green algal strains, including *Acutodesmus* sp., *Chlamydomonas reinhardtii*, *Haematococcus pluvialis*, and *Monoraphidium griffithii* (ENN B). Assays were performed by individual and mixture exposure. This last reports a real scenario as exposure to contaminants does not occur usually one by one compound. The study brought us to highlight the negative implication that mycotoxins can have in the environment and in invertebrates that constitute the nutritional basis of most aquatic animals.

2. Material and methods

2.1. Reagents

The mycotoxins used were purchased from Sigma Aldrich: beauvericin (BEA, MW: 783.95 g/mol) and enniatin B (ENN B, MW: 639.82 g/mol). Methanol (MeOH) was obtained from Fisher Scientific (Helsinki, Finland), LC–MS solvent grade. Deionized water (<18 MV cm resistivity) was obtained from milli-Q water purification system (Millipore, Bedford, MA, USA). Stock solutions of mycotoxins were prepared in MeOH and maintained at −20ºC in the dark. The final concentration of MeOH in the medium was ≤ 0.5% (v/v) as previously reported (Dom et al., 2012).

2.2. Algae culture maintenance

The algae spp. *Acutodesmus spp.* (University of Basel), *Chlamydomonas rehniadrti* (UWCC), *Haematoccocus pluvialis,* and *Monoraphidium griffthii* (NIVA-CHL 8) were grown in MWC-medium (Modified Wright Chu-medium) (Guillard et al., 1972) in 600 mL plastic tissue culture flasks. Each strain was kept at +18ºC and under a light:dark cycle of 16:8 h. The intensity of the illumination was selected based on known preferences which oscillated between 10 and 80 μmol quanta s^{-1} m^{-2} (lums) in order to ensure homogeneous growth conditions. Plates were prepared by taking aliquots from exponential growing stocks.

2.3. Mycotoxin exposure

The concentration of the mycotoxins and exposure time are two factors that were considered in this study. The algae *spp*. were exposed to BEA and ENN B mycotoxins individually for 24, 48, 72 and 96 h at a concentration in the ranges of 0.1–64 μM for BEA and 0.05–25 μM for ENN B, all with 1:2 dilution. Concentrations were chosen based on studies for aquatic *spp* previously reported (Juan-García et al., 2020a, 2021). Algae were also exposed to mycotoxins in a binary mixture [BEA + ENN B] at a concentration range from [8 + 1.6] μM to [0.06 + 0.01] μM, at four exposure times (24, 48, 72 and 96 h) and including eight dilutions of each mycotoxin concentration. The dilution ratios of the concentrations for the binary combinations were 5:1. Solvent control used was MeOH and maintained at ≤ 0.5% for all assays.

2.4. Phytotoxicity of BEA and ENN B in Acutodesmus spp., Chlamidomonas reinhardtii, Haematoccocus pluvialis, and Monoraphidium griffithii

Culture of algae strains was maintained in MWC media as indicated in Section 2.2. Sub-culture of algae was prepared previous to experiments with dilutions maintained on an orbital shaker at 120 rpm in Erlenmeyers with constant illumination (80 lums) and temperature (24 ºC). Only when growth culture was exponential (observed by up-growth in cell density) 96-well/plates were prepared at the following density according to the algae *spp*.: *Acutodesmus spp* and *Chlamidomonas reinardtii* at 5×10^4 cells/mL, *Haematoccocus pluvialis* at 3×10^5 cells/mL and *Monoraphidium griffthii* at 1×10^5 cells/mL. A total of 6 replicates per test solution were used.

The phytotoxicity assay was performed by measuring the chlorophyll fluorescence parameter using a Fluoroskan Ascent (Labsystems). Briefly, 96 well/plates were algae seeded at densities reported above (100 μL/well) jointly to mycotoxińs dilutions. Plates were placed at 18 ºC and 80 lums and fluorescent measurement at 670 nm took place after 24, 48, 72, and 96 h.

2.5. Quantification procedure of mycotoxins by LC-MS/MS in aquatic media from algae exposure

For quantifying mycotoxins in the aquatic media of algae species a

long of the exposure time (24–96 h), a total of 0.6 mL of media collected from six repeats from the 96-well plate was allowed to proceed. Mycotoxins were contained at different concentrations (Section 2.3).

Chromatographic separation was done by using an Agilent 1290 Infinity UHPLC system coupled to a 6460 Triple Quadrupole mass spectrometer (Agilent Technologies, Waldbronn, Germany). Separation was performed by using a column Agilent InfinityLab Poroshell 120 EC-C18 100 mm × 2.1 mm (inside diameter), 2.7 μm, the temperature was maintained at 30 °C. Mobile phases were water containing 0.1% formic acid and 5 mM ammonium formate (phase A), and methanol (phase B) with 0.1% formic acid and 5 mM ammonium formate. An elution gradient of 15 min was made keeping the flow rate of the mobile phase at 0.3 mL/min, The following gradient was used: equilibration for 1 min at 70% A, 70–10% A in 10 min, 10% A for 2 min, 10–70% A in 0.5 min, 70% A for 4.5 min. and the injection volume was set at 3 μL.

The analysis was performed with a triple quadrupole mass spectrometer Agilent 6460 equipped with an electrospray ionization source (ESI) using Agilent Jet Stream Technology. The ion source parameters were set as follows: sheath gas temperature, 340 °C; sheath gas flow, 10 L/min, gas temperature, 200 °C; gas flow, 8 L/min; nebulizer, 40 psi; capillary voltage, 3500 V; and nozzle voltage 500 V. The fragment voltage (FV), cell accelerator voltage (CAV), collision energy (CE) and mass transitions were optimized for each metabolite using MassHunter Optimizer software (Table 1). In all cases, the CAV parameter was set at 7. Analysis was done in dynamic multiple reaction monitoring (dMRM) mode and two mass transitions were monitored for each toxin, one for quantification and another for confirmation. The quantification was done with MassHunter Quantitate Software.

2.6. Statistical analysis

Mean inhibition concentration (IC_{50}) values were estimated by lineal interpolation. Statistical analysis of data were carried out using SPSS version 13 (SPSS, Chicago, IL, USA) statistical software package. Data were expressed as mean ± SD of three independent experiments. The statistical analysis of the results was per-formed by student's T-test for paired samples. Differences between different types of cells were analyzed statistically with ANOVA followed by the Tukey HDS posthoc test for multiple comparisons. The level of $p \leq 0.05$ was considered statistically significant.

3. Results

3.1. Phytotoxicity of BEA, ENN B and their mixtures in Acutodesmus spp.,

The effect of BEA, ENN B, and their mixtures on *Acutodesmus spp*., is reported in Fig. 1. Results revealed a strong decrease of growth rate at all times assayed for BEA and at concentrations >8 μM ranging from 50% to 11% for 24 h and 96 h, respectively respect to control(Fig. 1a). Similarly, this happened for ENN B at concentrations >6.25 μM with growth rate ranging from 92% to 56% for 72 h and 96 h, respectively (Fig. 1b). IC_{50} values were reached for the following conditions: i) for BEA: 18.0± 0.3 μM, 9.5 ± 0.3 μM, 14.0 ± 0.7 μM and, 13.1 ± 0.5 μM for 24 h, 48 h, 72 h, and 96 h, respectively (Table 2); and ii) for ENN B: 12.0± 0.5 μM, 10.6 ± 0.6 μM, 5 ± 0.4 μM for 48 h, 72 h, and 96 h, respectively (Table 2). However, when mixtures were assayed at several concentration (< 8 μM for BEA and < 1.6 μM for ENN B) there was a decrease in growth rate when concentrations increased but there was not a clear tendency observed. Such decreases were above 60% and oscillations for all times, concentrations described the profile of this assay (Fig. 1c), and no IC_{50} values were obtained. In summary, while in individual exposure the IC_{50} values were reached for almost all times assayed, in binary mixture number of algae cells were never above 50% of control (Fig. 1).

Table 1
IC_{50} values reached for algae *spp* at different exposure times.

	IC_{50} (μM) - Exposure time			
Algae spp.	**24 h**	**48 h**	**72 h**	**96 h**
Accutodesmus spp.				
BEA	18 ± 0.3	9.5 ± 0.3	14.0 ± 0.7	13.1 ± 0.5
ENN B	n.r.	12.0 ± 0.5	10.6 ± 0.6	5.0 ± 0.4
BEA + ENN B	n.r.	n.r.	n.r.	n.r.
Haematococcu pluvialis				
BEA	15.0 ± 0.5	22.5 ± 0.8	22.5 ± 0.8	15 ± 0.2
ENN B	21.5 ± 0.5	18 ± 0.3	16 ± 0.3	8 ± 0.5
BEA + ENN B	[8 + 1.6]	n.r.	n.r.	n.r.
Monoraphidium griffthii				
BEA	14.0 ± 0.7	n.r.	n.r.	n.r.
ENN B	5.0 ± 0.5	9.0 ± 0.3	8 ± 0.5	8 ± 0.5
BEA + ENN B	[4 + 0.8]	n.r.	n.r.	n.r.

n.r.: not reached

3.2. Phytotoxicity of BEA, ENN B and their mixtures in Chlamydomonas reinhardtii

Fig. 2 reports the effect on *Chlamydomonas reinhardtii* caused by mycotoxins studied here. Effect of BEA resulted in a decrease of growth rate after 8 μM for 24 h and 48 h in 14% and 22%, respectively; while at 72 h and 96 h an increase of growth rate was observed reaching maximum values of 163% (32 μM) and 134% (64 μM), respectively (Fig. 2a). For ENN B increases of growth rate were less noticeable although it was kept for all times above 80% and almost a straight line defines the profile for all times assayed (Fig. 2b). At the highest concentration assayed (25 μM) the following percentages of growth rate were reported 105%, 96%, 126% and, 149%, for 24 h, 48 h, 72 h, and 96 h respectively. Lastly, for BEA + ENN B different tendency was observed with the exposure time and the growth rate was above 60% (Fig. 2b). At 24 h a decrease in growth rate was observed (40%) at the highest concentration tested [8 + 1.6] μM; at 48 h and 96 h, it was observed slight recovery of growth rate with oscillation ranges of 95–78% and 102–123%, respectively; while at 72 h growth rate ranged from 89% to 134% at [0.12 + 0.02] μM and [8 + 1.6] μM, respectively (Fig. 2c). In summary and in general terms for *Chlamydomonas reinhardtii* either for individual or binary treatment, there effects on growth rate were mild, without getting values below 60% (Fig. 2), so that no IC_{50} values were reached.

3.3. Phytotoxicity of BEA, ENN B and their mixtures in Haematoccocus pluvialis

The profile effect of mycotoxins in algae *Haematoccocus pluvialis* reported in Fig. 3 is similar to *Accutodesmus spp*. (Fig. 1) for both scenarios studied, individually and combined. For BEA, at the two highest concentrations studied (64 μM and 32 μM), growth rate was below 37% for all times studied; however, at lower concentrations growth rate was above 41% except for 24 h which at 8 μM growth rate decreased until 39% and for the next concentration, 4 μM, growth rate increased until 60% (Fig. 3a). IC_{50} values were reached for all times as follows: 15.0 ±0.5 μM for 24 h and 22.5±0.8 μM for 48 h and 72 h, and 15.0 ±0.2 for 96 h (Table 2). For ENN B, decreases in growth occurred in a concentration-dependent manner with decreases at the highest concentration (25 μM) of 60%, 73%, 85%, and 89% for 24 h, 48 h, 72 h, and 96 h, respectively (Fig. 3b). IC_{50} values were reached for all times as follows: 21.5 ± 0.5 μM, 18.0 ± 0.3 μM, 16 ± 0.3 μM and, 8 ± 0.5 μM for 24 h, 48 h, 72 h, and 96 h, respectively (Table 2). For mixtures of BEA + ENN B decreases respect to the control went from 2% to 52%, from 1% to 34%, from 1% to 12%, and from 21% to 35% for 24 h, 48 h, 72 h, and 96 h, respectively (Fig. 3c). It is important to remark that growth rate in [BEA + ENN B] for 24 h and 72 h was above 100% in all concentrations studied except for [4 + 0.8] and [8 + 1.6] μM (Fig. 3c). IC_{50} values were reached only for 24 h at [8 + 1.6] μM (Table 2).

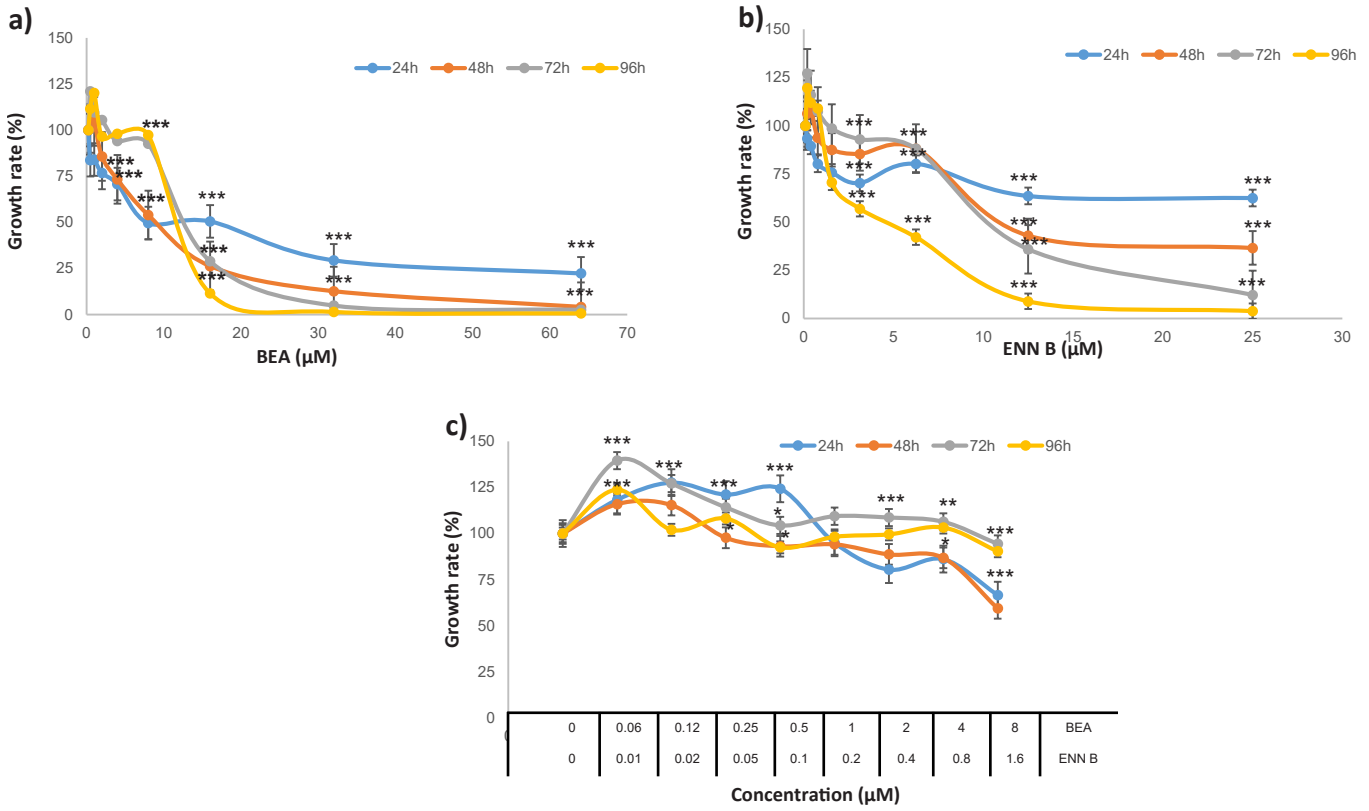

Fig. 1. -Effectof BEA (a), ENN B (b) and their mixtures (c) in the growth of *Accutodesmus spp*. at 24 h, 48 h, 72 h and 96 h. Data corresponds to n=3 and eight independent replicates. *$p \leq 0.05$, **$p \leq 0.01$ and***$p \leq 0.001$ respect to the control.

Table 2
UHPLC-MS/MS conditions used for the determination of BEA and ENN B remaining in algae *spp* media.

Mycotoxin	Prec Ion MS1	Prod Ion MS2	Frag (V)	CE (V)	Ret Time (min)
BEA	801.5	262 244	180	32 36	10
ENNB	657.3	214 196	160	32	9.6

CE: collision energy; Frag: fragmentation; Prec: precursor; Prod: product; V: voltage

3.4. Phytotoxicity of BEA, ENN B and their mixtures in Monoraphidium griffthii

Results for *Monorpahidium griffthii* are reported in Fig. 4. At 24 h both in individual and combined assays, the decrease in growth rate was close to 50% for all concentrations; while for 48 h, 72 h, and 96 h the profile described is very similar among concentrations assayed (Fig. 4). For BEA the highest decrease of growth rate was detected at 64 μM: 24%, 34%, and 39% for 48 h, 72 h, and 96 h, respectively (Fig. 4a) IC_{50} value was calculated only for 24 h and it was 14.0±0.7 μM (Table 2). For ENN B, there was a valley effect at 12.5 μM for exposure times of 48 h, 72 h, and 96 h, to increase afterward at 25 μM and reaching values of 62% and 71% of growth rate for 72 h and 96 h, and 48 h, respectively (Fig. 3b). IC_{50} values were reached for all times as follows: 5.0±0.5 μM, 9.0 ±0.3 μM, 8±0.5 μM for 24 h, 48 h, and both 72 h and 96 h, respectively (Table 2). For BEA + ENN B, the profile described for 48 h, 72 h, and 96 has an up and down profile shape although not below 80% and with decreases of 2–39% (Fig. 4c). IC_{50} value could be calculated only for 24 h at [BEA + ENN B] [4 + 0.8] μM (Table 2).

3.5. Quantification of mycotoxins in algae spp

Mycotoxin concentrations were tested at the beginning and the end of studies with mean concentrations for the exposure period calculated, where studies of 24 h in length concentrations were based upon start concentrations only due to the small volume of solution used across the replicates.

The quantified concentration of BEA and ENN B found in the media after the phytotoxicity assay is reported in Fig. 5. It is represented the nominal concentration and the measured concentrations that were able to quantify after the exposure. Graphics collect mean values of either individually and mixture scenarios at different conditions tested. It is also revealing the capacity that the algae *spp*. had in clearing the aquatic media of bot BEA and ENN B mycotoxin.

In individual treatment exposures (Figs. 5a and 5b), BEA showed decrease of mycotoxins quantified in 0.5 μM (decrease of 36% respect to the control) to 16 μM (decrease of 25% respect to the control), for the lowest and the highest concentrations assayed, respectively (Fig. 5a); while for ENN B, these decrease were from 0.2 μM (48%) to 2.5 μM (20%) corresponding to lowest and highest concentrations assayed as well (Fig. 5b). On the other hand, when binary treatment was assayed the differences of nominal and measured concentrations quantified reduction of BEA 0.04 μM (40%) to 4 μM (50%) corresponding to only BEA in the mixture BEA + ENN B at [0.12 + 0.02] μM and [8 + 1.6] μM, respectively (Fig. 5c). For ENN B levels quantified were 0.006 μM (32%) to 0.4 μM (50%) corresponding to only ENN B in the mixture BEA + ENN B at [0.12 + 0.02] μM and [4 + 0.8] μM, respectively (Fig. 5d). In summary, in mixture assays, the decrease of mycotoxins were higher compared to individual treatment and more noted for BEA than for ENN B (Fig. 5).

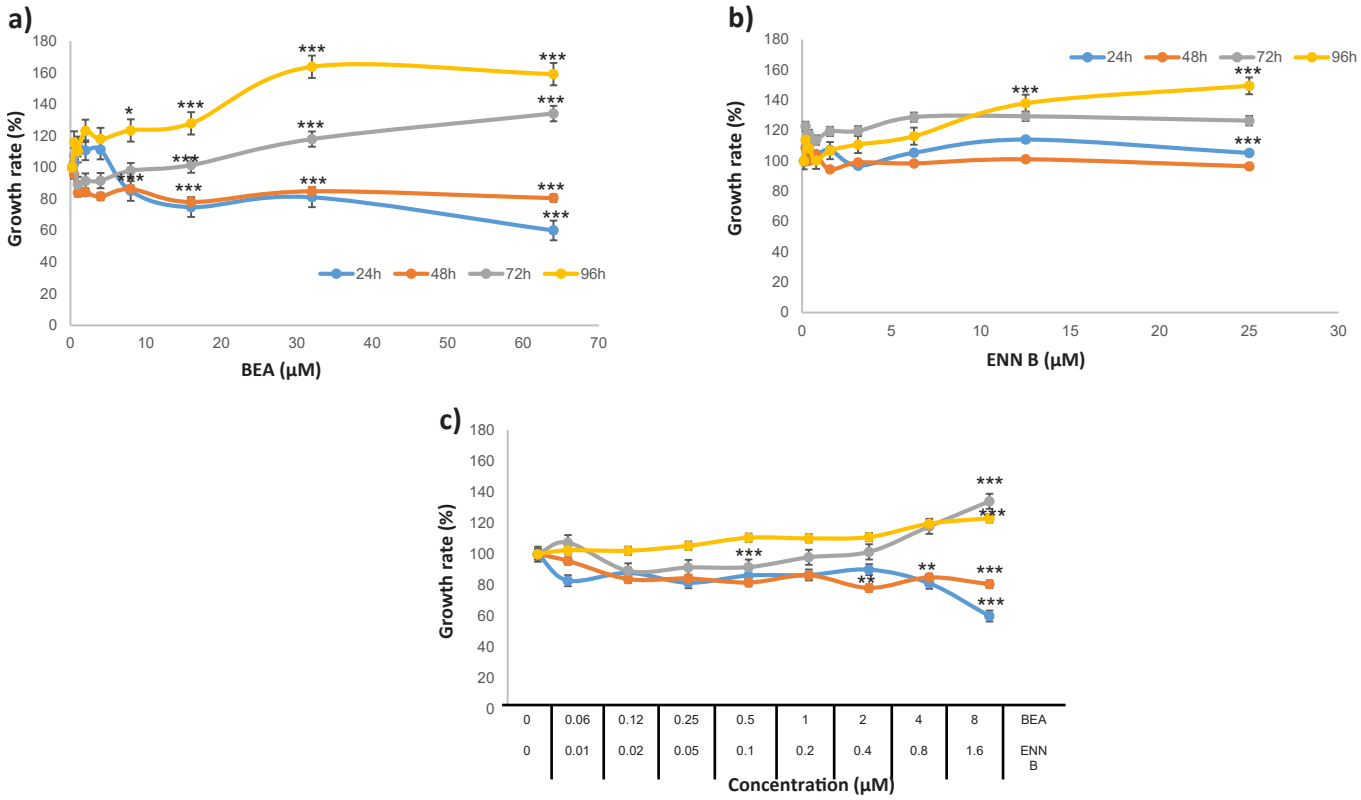

Fig. 2. -Effectof BEA (a), ENN B (b) and their mixtures (c) in the growth of *Chlamydomonas reinardthii*at 24 h, 48 h, 72 h and 96 h. Data corresponds to n=3 and eight independent replicates. *$p \leq 0.05$, **$p \leq 0.01$ and***$p \leq 0.001$ respect to the control.

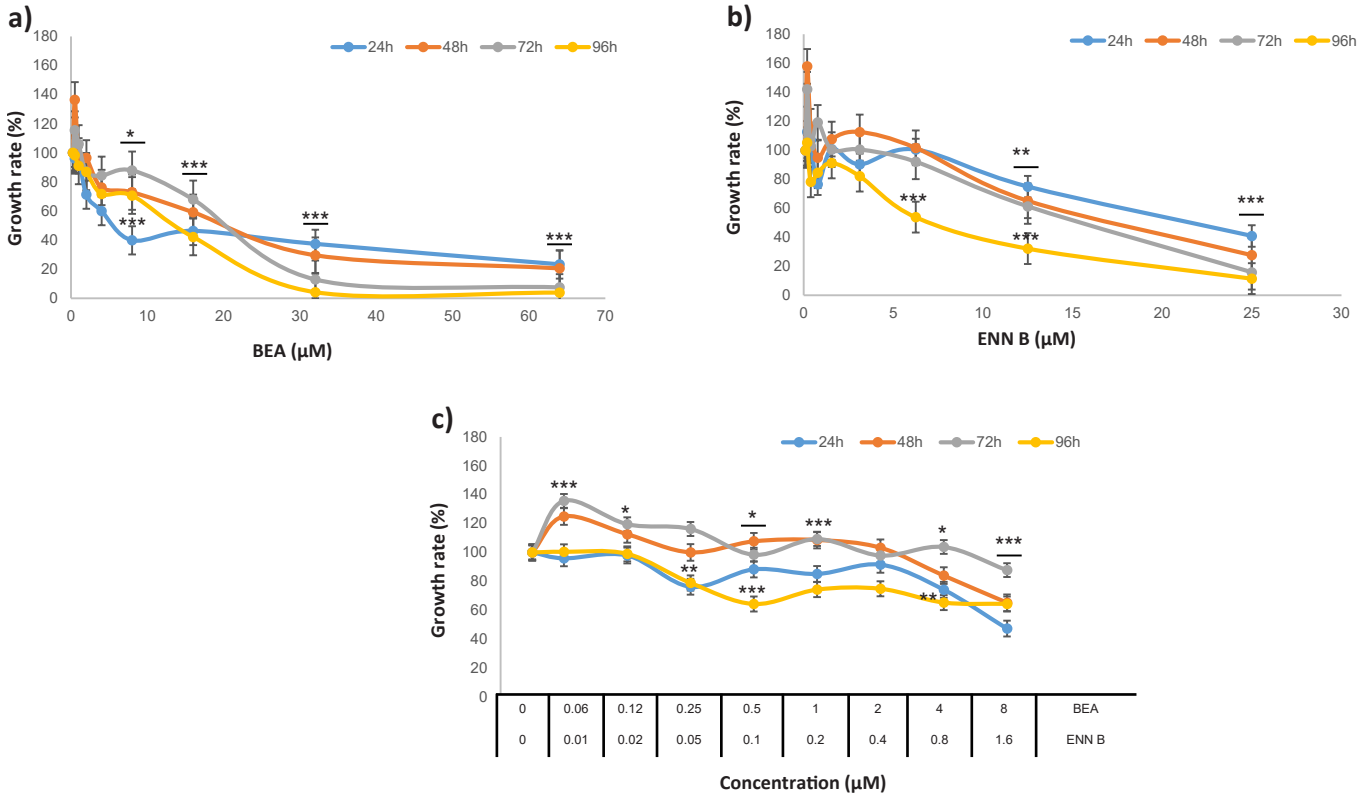

Fig. 3. -Effectof BEA (a), ENN B (b) and their mixtures (c) in the growth of *Haematoccocus pluvialis*at 24 h, 48 h, 72 h and 96 h. Data corresponds to n=3 and eight independent replicates. *$p \leq 0.05$, **$p \leq 0.01$ and***$p \leq 0.001$ respect to the control.

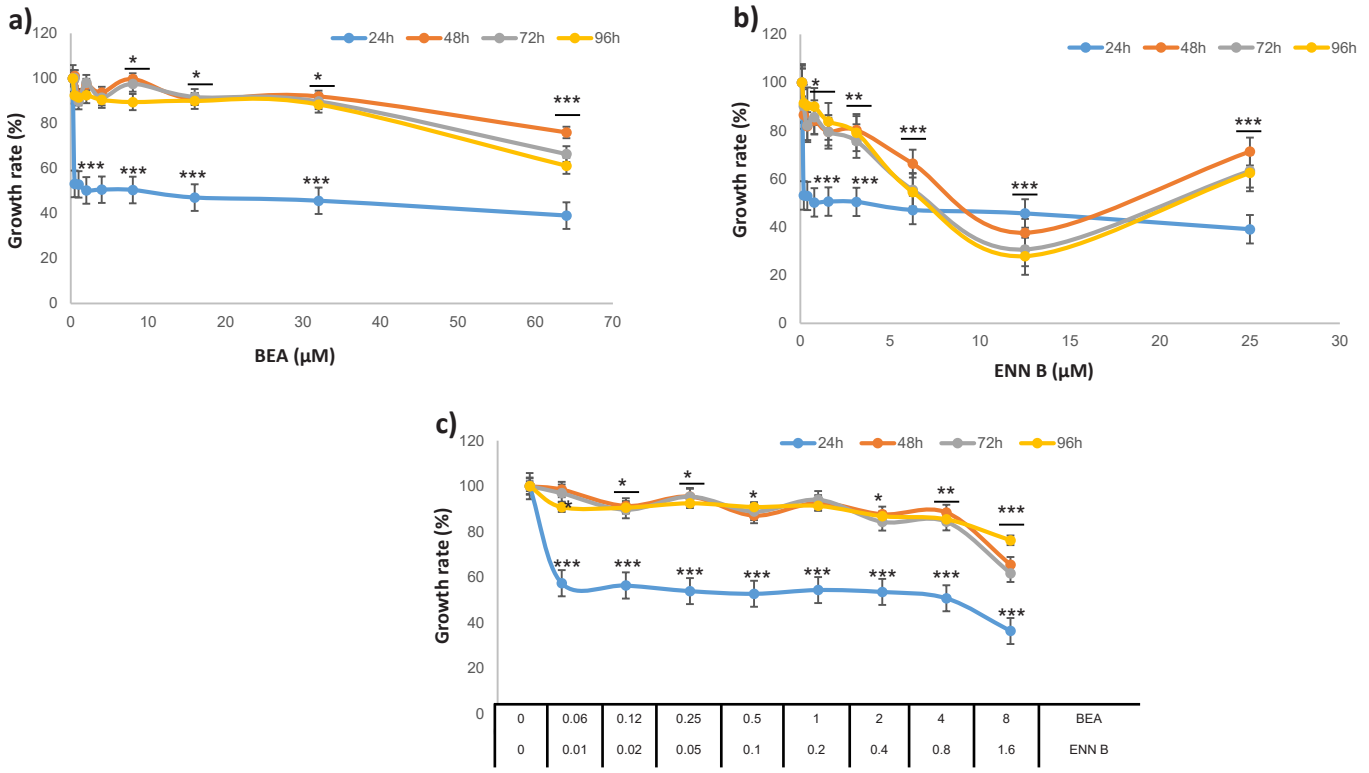

Fig. 4. -Effectof BEA (a), ENN B (b) and their mixtures (c) in the growth of *Monoraphidium griffthii*at 24 h, 48 h, 72 h and 96 h. Data corresponds to n=3 and eight independent replicates. *$p \leq 0.05$, **$p \leq 0.01$ and***$p \leq 0.001$ respect to the control.

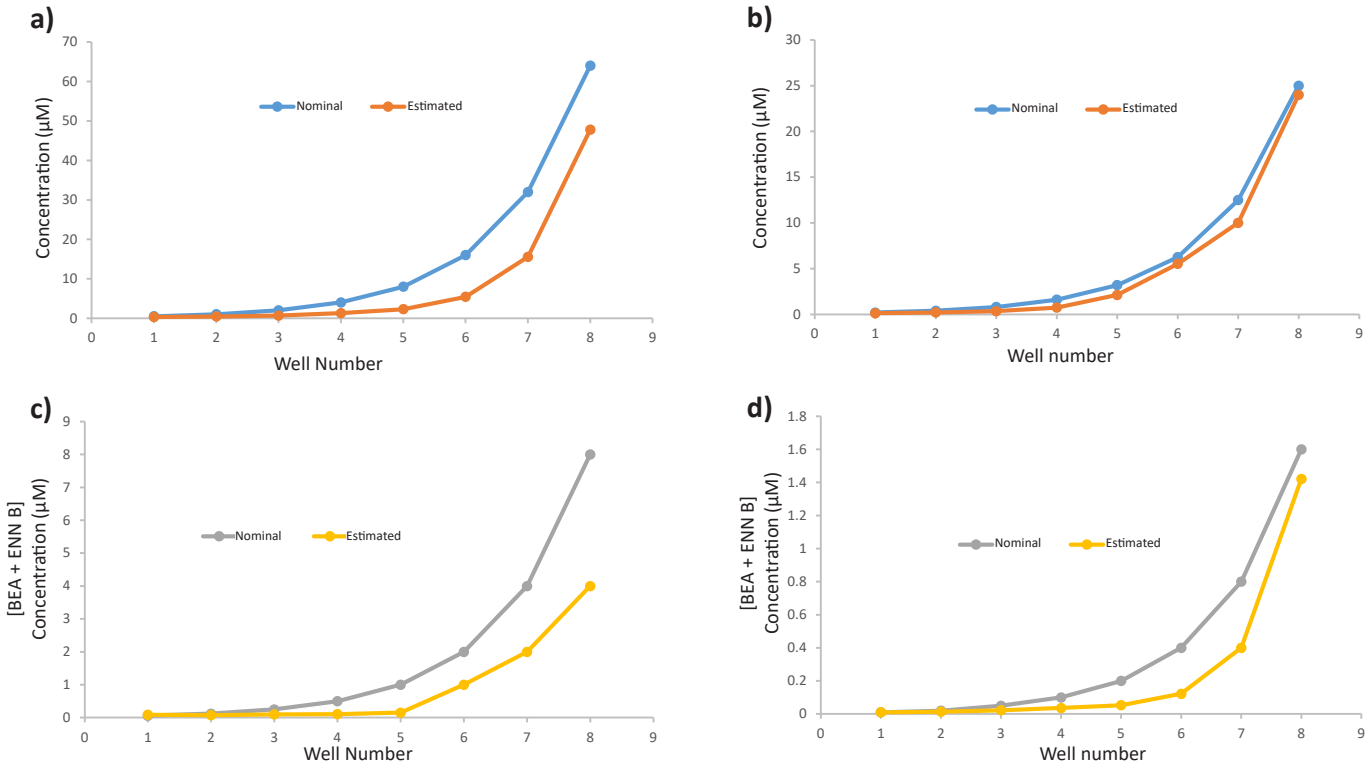

Fig. 5. -Theoretical and estimated concentrations of BEA (a), ENN B (b) and their mixtures (BEA in c and ENN B in d) quantified by LC-MS.

4. Discussion

The importance in monitoring contaminants in the environment and the implications of mycotoxins in aquatic ecosystems has become a trend for regulatory authorities. From our results, it can be observed how growth effects change according to mycotoxin, and the algae strain;

which denotes a different sensibility with the characteristics of each *spp*. tested (Figs. 1 to 4). In fact, effects were observed at very low concentrations, which denotes the interaction of mycotoxins in nature and specifically in green algae. This reveals the necessity of exploring these compounds and spread the toxicity studies.

According to literature, some studies have performed the exposure of algae to mycotoxins (Alexander et al., 1999; Guo et al., Suzuki et al., 2014 and 2015) although it is scarce and none can be found for three of the *spp*. studied in here. In fact, there are only results in literature for *C. reinardhtii* of studying mycotoxins. *C. reinhardtii* is characterized by having flagella movement and was shown to be a promising plant trichothecene bioassay system when a total of 14 mycotoxins of trichothecene family were tested at 80 μM (including DAS (4,15-diacetoxyscirpenol), T-2 toxin, DON (deoxynivalenol), 3-ADON (3-acetyldeoxynivalenol), 15-ADON (15-acetyldeoxynivalenol), FusX (fusarenon-X), NIV (nivalenol) and, TAS (3,4,15-triacetoxyscirpenol)) (Alexander et al., 1999; Suzuki et al., 2015). It was demonstrated that the chemical structure of mycotoxins determines the potential of phytotoxicity against *C. reinhardtii* and loss of movement happened. This coincided with what it was reported earlier related to the presence of a hydroxyl group in C-3 of trichothecenes chemical structure which presented higher toxicity than those with an acetylated group (Alexander et al., 1999); and noticing that the glycosylation of mycotoxins contributes to attenuate the toxicity potential, as demonstrated with DON-3 G (Deoxynivalenol-3-glucoside) (Suzuki et al., 2015).

In another study carried out on *C. reinhardtii* with different concentrations of mycotoxins from 0.1 to 25 ppm including DON, 3-ADON, 15-ADON, NIV and FusX (Suzuki et al., 2014), it was shown that the growth profile was very similar for all tested concentrations. However, differences were observed for 10 and 25 ppm on which growth decreased, considerably and most markedly for FusX, NIV and 15-ADON (Suzuki et al., 2014). This fact coincides with the differences of toxicity reported between FusX and NIV in cellular test conditions resulting higher for FusX than for NIV but without having clear its mechanism (Sundtol et al., 2004; Suzuki et al., 2012). In the study on *C. reinhardtii* by Suzuki et al. (2014), the effect of DON and NIV was marked but when compared between mycotoxins, NIV had higher toxicity. Similar results for NIV happened when compared with the study of glucoside form of DON (DON3G) (Suzuki et al., 2015). With these studies, it was set the best concentration for testing Type B trichothecenes in *C. reinhardtii* at 25 ppm for future research (Suzuki et al., 2014; Alexander et al., 1999).

On the other hand, Vargas et al., (2015) studied the effect on the average of the culture on *C. reinhardtii* after 4 days exposed at 100 μM individually to 3-ADON, DON, NX-2 and NX-3 with a decrease on its growth and ranging between 6.25% and 96% respect to the control (Vargas et al., 2015). Concentrations in our study did not follow the limit reported in previous publication because there are no studies of BEA and ENN B in this meaning to compare directly our results with the algae *spp*. studied. However, growth rates for *C. reinardthii* in individual and combined treatment were higher for BEA than for ENN B. The slight tendency and oscillations observed can be associated with the ionophoric properties of these compounds. Thus, ion pump mechanisms as flagella mobility or enzyme activation for clearing the media of toxicants are being affected by the properties of these compounds, and at different concentration and time point, alterations in growth rate are observed; nevertheless, no IC_{50} values were reached (Fig. 2). According to literature, only specific dose points have been studied in *C. reinardhtii* algae (Suzuki et al., 2014 and 2015). Comparison with our IC_{50} values is not possible to be associated; however, for NIV, 15-ADON, 3-ADON and DON 3 G inhibitory growth rate below 50% has been reported at 80 μM for *C. reinardhtii* (Suzuki et al., 2015).

Regarding *H. pluvialis*, it is characterized by having a strong natural carotenoid antioxidant, astaxanthin (Mularczyk et al., 2020); in fact, most of the applications of *H. pluvialis* are associated to nutrition and health as well as low cost of its production. Applications in the aquaculture industry of *H. pluvialis* (rich in astaxanthin) related to the pigmentation of fish have been also reported; as it improves the coloration of many aquatic animal *spp* (lobsters, shrimps, salmon, etc) and the properties associated to antioxidants as tolerance to stress and growth performance (Shah et al., 2016; Mularczyk et al., 2020; Lim et al., 2018). The effect of other carotenoids (lutein and zeaxanthin) have been studied *in vitro* in Caco-2 cells and in SH-SY5Y cells in alleviating mycotoxins effects of BEA (Juan-García et al., 2019b; Montesano et al., 2020). Astaxanthin from *H. pluvialis* has demonstrated to protect mesenchymal stem cells form oxidative stress and it has been postulated as a good candidate in controlling the harmful impacts of oxidative stress (Mohammadi et al., 2021), reducing TNF-α or exerting neuroprotective effects (Pereira et al., 2021).

The effects reported in here for *H. pluvialis* at the lowest concentration of mycotoxins tested could be related to the protective effect that this source of antioxidant exerts which ends up reaching IC_{50} values. A fact that has been demonstrated as both BEA and ENN B promote the production of ROS in different *in vitro* cell lines (Juan-García et al., 2020a; Prosperini et al., 2013). The results of phytotoxicity were stronger for BEA than for ENN B in individual treatment reaching IC_{50} values; while in combination and for the concentrations studied, the activation of mechanism of resistance to mycotoxins seems to be avoiding the inhibitory concentration effect.

Besides the results of phytotoxic effect of mycotoxins in the four algae strains, it took us to go a step further and explore the function of protection associated to some of them. In fact a distinguish function of some microalgae is to remove contaminants of the environment. The capacity of microalgae to remove such contaminants may depend on both their individual qualities and how they are organized in the environment, as reported for *M. griffithii* (de Bashan and Bashan, 2010). For instance, because unicellular forms have larger surface for the adsorption process, they should potentially have better removal abilities. However, they frequently organize into cell aggregates that produce biofilms, which may cover portions of the surface regions (De Philippis et al., 2011; Lakmali et al., 2022). The ability in removing metals or tolerance to metals in water with algae has been the focus of several years of study. For some algae *spp* it is known that metal ions removed serve as nutrients in their metabolic processes, as it is the case of *M. griffithii* (de Bashan and Bashan, 2010). To mention that *M. griffithii* removes zinc from the environment by binding significant amounts (Bacsi et al., 2015; Brinza et al., 2007; Chong et al., 2000) similarly as it happens for other microalgae for chromium (VI) (Takami et al., 2012), for thorium (de Queiroz et al., 2012) or for nickel (Chong et al., 2000). In most cases, low levels of cleansers suppose a widespread worldwide problem as it can accumulate in living organisms as do not biodegrade (Long et al., 2014). Related to this function of clearance, the use of *M. griffithii* for evaluating ecosystem health in wastewater with pesticides in lakes from Rumania (region of Cluj-Napoca) was carried out due to its characterization as dominant phytoplankton in this region and giving acceptable results (Lakmali et al., 2022). There are no studies of BEA and ENN B carried out in this direction although it has been observed some resistance of this algae *spp*. against some compounds, which will affect the ecosystems and the nutritional basis of the food chain and those located above (Houssou et al., 2020). Studies of the removal of contaminants conclude that algae *spp*. present in surface waters can help to clear, eliminate and be feasible in the treatment of wastewaters (Bacsi et al., 2015). Checking our results, it is denoted that *M. griffithii* was the algae *spp*. with the highest capability in clearing the media of mycotoxins as after the strong reduction of growth rate at 24 h, in the following exposure times it increases and was kept to slightly decrease at the highest concentrations tested (Fig. 4). For all four algae, the reduction of mycotoxins in the media was observed at very low doses; this could be associated to the fact that at the highest concentrations the algae cells die or losses the flagella mobility (as for *C. reinardthii*) so the clearance function does not take place (Fig. 5).

Lastly, *Accutodesmus spp*. have garnered attention as a potential strain for biofuel production due to their rapid growth and high

photosynthetic capacity compared to plants (Chisti, 2007). These algae are known for their high content of saturated fatty acids, which is essential for this application (Piligaev et al., 2015). However, they also possess the unique ability to remove zinc from the environment (Güçlü and Ertan, 2012) and phenolic compounds (Lindner et al., 2022). Similar to other unicellular algae, *Accutodesmus spp.* serve as excellent subjects for studying the direct effects of external growth agents (Talarek-Karwel et al., 2020).

The influence of various cultivation factors, such as nutrients, temperature, light spectra, and nitrogen, has been investigated to enhance fatty acid content and improve industrial and food-related properties (Helamieh et al., 2021; Breuer et al., 2012; Mandotra et al., 2016). It has been observed that cultivating microalgae under low temperatures and green light spectra can yield a substantial biomass and fatty acid output suitable for the food industry (Helamieh et al., 2021).

Phytohormones play a significant role in biological processes, affecting growth, stress response, and physiological functions. In this context, mycotoxin exposure, particularly the impact of BEA and ENN B as reported in previous studies, may create stress conditions that lead to lower levels of endogenous phytohormones. Insufficient amounts of these hormones may contribute to the inability to mitigate the negative effects of mycotoxin-induced stress (Fig. 1), as evidenced by the IC_{50} values being reached only after surpassing a certain concentration threshold. The observed strong effects of BEA and ENN B on *Accutodesmus* could potentially be attributed to the decline and reduced effectiveness of endogenous phytohormones crucial for stress adaptation (Talarek-Karwel et al., 2020).

5. Conclusions

The evidence of phytotoxicity of BEA and ENN B highlights the negative implications that mycotoxins can have on the growth of algae, which are crucial nutritional components for many aquatic animals. The introduction of these compounds into the food chain results in an increase in chronic exposure to consumers, even at very low levels. This emphasizes the importance of food authorities ensuring food safety and paying attention to food contaminants that are not yet regulated.

Individual exposure of algae to BEA and ENN B demonstrated significant phytotoxicity in *Accutodesmus spp* and *Haematococcus pluvialis*, as indicated by the IC_{50} values. However, when these mycotoxins were combined, phytotoxicity was observed only at very specific concentrations of BEA and ENN B. The properties associated with the different algae species, such as their cleaning abilities or antioxidant richness, were not sufficient to alleviate the effects of mycotoxin exposure. Consequently, the presence of mycotoxins in the environment, even at low levels, can disrupt ecosystems. Special attention should be given to preserving and addressing the changes presented by the changing climate and the introduction of mycotoxins into aquatic environments.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Data Availability

The data that has been used is confidential.

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