Removal of Cyantraniliprole from Aquatic Environments by *Chlamydomonas reinhardtii*

Raid Alrowais,[a,h] Eldesoky Sabri Ibrahim,[b] Dalia E. El-Hefny,[c] Rania M. A. Helmy,[c] Rania Saber Yousef,[d] Shady Abdel Mottaleb,[e] Mahmoud M. Abdel daiem,[f,g,*] Wassef Ounaies,[a] Bandar Alwushayh,[a] and Mohamed Mahmoud-Aly [e]

This paper reports the first study of phyco-remediation of cyantraniliprole, a second-generation diamide insecticide with high toxicity and persistence in aquatic environments, using the green microalga *Chlamydomonas reinhardtii*. Cultures of *C. reinhardtii* were treated with four concentrations of cyantraniliprole (0, 25, 50, and 100 ppm). The removal efficiency, antioxidant responses, and biomass composition of the microalga were measured after 1 h and one week of exposures. *C. reinhardtii* was able to remove cyantraniliprole from the medium by biodegradation, biotransformation, bioaccumulation, and bio-adsorption mechanisms, achieving up to 87.0% removal within 1 h and 84.5% after one week. The microalga also maintained acceptable levels of enzymatic and non-enzymatic antioxidants, indicating its tolerance to cyantraniliprole stress. Moreover, some treated cultures (especially those with 25 and 50 ppm cyantraniliprole) showed enhanced specific growth rate, and biomass productivity compared to control cultures. In addition, those with 50 and 100 ppm cyantraniliprole showed enhanced carbohydrate and lipid concentrations compared to the control cultures. These results suggest that *C. reinhardtii* is a promising candidate for bioremediation of cyantraniliprole-contaminated water and biofuel production.

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*Contact information: a: Department of Civil Engineering, College of Engineering, Jouf University, Sakakah 72388, Saudi Arabia; b: Department of Economic Entomology and Pesticides, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt; c: Pesticides Residues and Environmental Pollution Department, Central Agricultural Pesticide Laboratory, Agricultural Research Center, Giza, 12618, Egypt; d: Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt; e: Plant Physiology Division, Department of Agricultural Botany, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt; f: Environmental Engineering Department, Faculty of Engineering, Zagazig University, Zagazig, 44519, Egypt; g: Civil Engineering Department, College of Engineering, Shaqra University, 11911, Al-Duwadmi, Ar-Riyadh, Saudi Arabia; h: Sustainable Development Research and Innovation Center, Deanship of Graduate Studies and Scientific Research, Jouf University, Sakaka, Saudi Arabia; *Corresponding author: mmabdeldaiem@eng.zu.edu.eg*

INTRODUCTION

Cyantraniliprole, a member of the anthranilic diamide category of insecticides, has found widespread use in the agricultural industry for combating various insect pests, including *Coleoptera*, *Lepidoptera*, *Diptera*, *Homoptera*, and *Thysanoptera* (Dong *et al.* 2017; Ananthi *et al.* 2019; Yan *et al.* 2023). Its approval in various countries, such as the USA, select EU nations, and China underscores its significance (EFSA 2014; Wang *et al.*

2018; MDA 2024). However, despite its efficacy, cyantraniliprole poses a significant threat to aquatic ecosystems, particularly invertebrates. Both surface water bodies (such as rivers and lakes) and benthic habitats (inhabited by sediment-dwelling organisms) are at major risk (EFSA 2014; MDA 2014; Wang *et al.* 2018)

Phyco-remediation is a bioremediation technique that focuses on harnessing algal diversity to address hazardous contaminants such as hydrocarbons, pesticides, radioactive substances, and heavy metals (Alrowais *et al.* 2023a,b, 2024a,b). Simultaneously, it aims to utilize the treated algal biomass for creating value-added products such as fertilizers and biofuels. Thus, phyco-remediation is considered safe, cost-effective, and environment-friendly technology. Beyond acting as a carbon sink, algae possess a significant surface-to-volume ratio, enabling processes such as bio-adsorption (facilitated by metal-binding groups on cell surfaces), bioaccumulation (storing pollutants inside their cells' compartments), biotransformation, and biodegradation (facilitated by algal enzymes to transform and degrade pollutants to produce other metabolites) of pollutants (Méndez-Díaz *et al.* 2012; El-Shatoury *et al.* 2014; Abdel daiem *et al.* 2019; Kaloudas *et al.* 2021; Koul *et al.* 2022; Morais *et al.* 2022; Ummalyma and Singh 2022).

Chlamydomonas reinhardtii (hereafter Chlamy) is a model green microalga widely studied in several fields such as physiology, genetics, biochemistry, and environmental science (Sehrawat *et al.* 2021; Dupuis and Merchant 2023). While Chlamy is not traditionally associated with direct insecticide remediation, it possesses intriguing properties that could contribute to environmental detoxification. Notably, Chlamy demonstrates a remarkable ability to remediate water contaminated with phenols and pesticides (Nazos *et al.* 2017; Wan *et al.* 2020; Sehrawat *et al.* 2021).

Cyantraniliprole has been shown to increase the activities of several oxidative stress-related enzymes in *Procambarus clarkii*, as evidenced by the upregulation of related genes (Liang *et al.* 2020). Among these enzymes, catalase (CAT) and superoxide dismutase (SOD) play crucial roles in detoxification and antioxidant defense. They help metabolize toxic substances and protect living cells from oxidative damage in zebrafish, making them valuable markers of antioxidant response (Paravani *et al.* 2019).

In this study, the authors explore the potential of Chlamy for safeguarding aquatic environments from leaching toxic insecticides, specifically cyantraniliprole. This research is the first reported instance of Chlamy's involvement in cyantraniliprole removal. Additionally, various remediation strategies were discussed, including the roles of enzymatic and non-enzymatic antioxidants. Through bridging the gap between insecticide science and algal physiology, this study opens new avenues for sustainable environmental protection.

EXPERIMENTAL

Materials and Methods

Chemical and biological materials

The source of cyantraniliprole used in this study was Benevia® (10.26% cyantraniliprole) purchased from FMC India Pvt. Ltd (India). The green microalga used in this study was *Chlamydomonas reinhardtii* CC1690 (Chlamy) purchased from the Chlamydomonas resource center (CRC, University of Minnesota, Minneapolis, MN, USA).

Experimental setup and growth conditions

Seed culture was grown mixotrophically in tris-acetate-phosphate medium (TAP) prepared based on a standard recipe (Gorman and Levine 1965) for 4 days and then inoculated into fresh media after harvesting and washing twice with TAP as described in (Mahmoud-Aly *et al*. 2018).

Two separate experiments were applied. First, a preliminary drop-test experiment on TAP-agar plates containing (0, 25, 50, and 100 ppm cyantraniliprole) was executed. Nine separate 5 µL drops (OD_{750} = 0.39) of Chlamy inoculum were cultured in each plate, then the plates were incubated under low light intensity 32 ± 2 µmol photons m^{-2} s^{-1} and 16:8 h light: dark cycles at 20 ± 1 °C for 96 h.

Second, the main experiment, Fig. 1a, involved growing Chlamy in 1-L bioreactors containing (0, 25, 50, and 100 ppm cyantraniliprole; green reactors) and cyantraniliprole in TAP medium without Chlamy (25, 50, and 100 ppm; white reactors). All experiments were executed in three separate biological replicates following completely randomized design (CRD).

The initial cell density after inoculation was nearly 1.23 ± 0.001 × 10^6 cells/mL and OD_{750} = 0.078 ± 0.002. Under fully aseptic conditions, cultures were mixotrophically grown 500 mL each in 1-L bioreactor units, as shown in Figs. 1a and 1b, under low light intensity 32 ± 2 µmol photons m^{-2} s^{-1} and 16:8 h light: dark cycles at 20 ± 1 °C. All reactors containing Chlamy cultures (green reactors) or containing cyantraniliprole in TAP medium without Chlamy (white reactors) were handled at the exact same conditions under continuous atmospheric air pumping 1.75 L/min through 0.22-µm filters.

Algal growth analyses and pH determination

The growth was monitored in three different ways; determination of cell density (CD) using a hemocytometer (Hausser Scientific, Horsham, PA, USA), measuring optical density at 750 nm (OD_{750}) with a Helios gamma spectrophotometer (ThermoSpectronic, Allentown, PA , USA), and cell dry weight (CDW) by weighing dried 8 mL-containing cells harvested through 0.45 µm cellulose nitrate filters (Sartorius, Göttingen, Germany). All the following parameters, specific growth rate (SGR), division time (DivT), generation time (GenT), and biomass productivity (BP), were calculated as mentioned before by Morgan *et al.* (2023). The pH values were determined in algal cultures and cyantraniliprole-TAP by HANNA® pH meter (Padova, Italy).

Chemical and biochemical analyses

For quantification of photosynthetic pigments (Chlorophyll a, Chlorophyll b, and total carotenoids), 1 mL from each algal culture was used (at zero time, after 1 h, and 7 days of cyantraniliprole treatment). For all other parameters (algal main chemical components, oxidative stress indicators, and cyantraniliprole residues), 10 mL of each culture (at zero time, after 1 h, and 7 days of cyantraniliprole treatment) were centrifuged at 1500 ×g for 5 min at 20 °C, then supernatants were used for cyantraniliprole residual quantification.

Photosynthetic pigments

Chlorophyll a (Chl-a), Chlorophyll b (Chl-b), total Chlorophylls, Chl-a/Chl-b ratio, and total Carotenoids were determined in a 1.0 mL algal culture sample using dimethyl sulfoxide (DMSO) according to Wellburn (1994) following Eqs. 1 through 4,

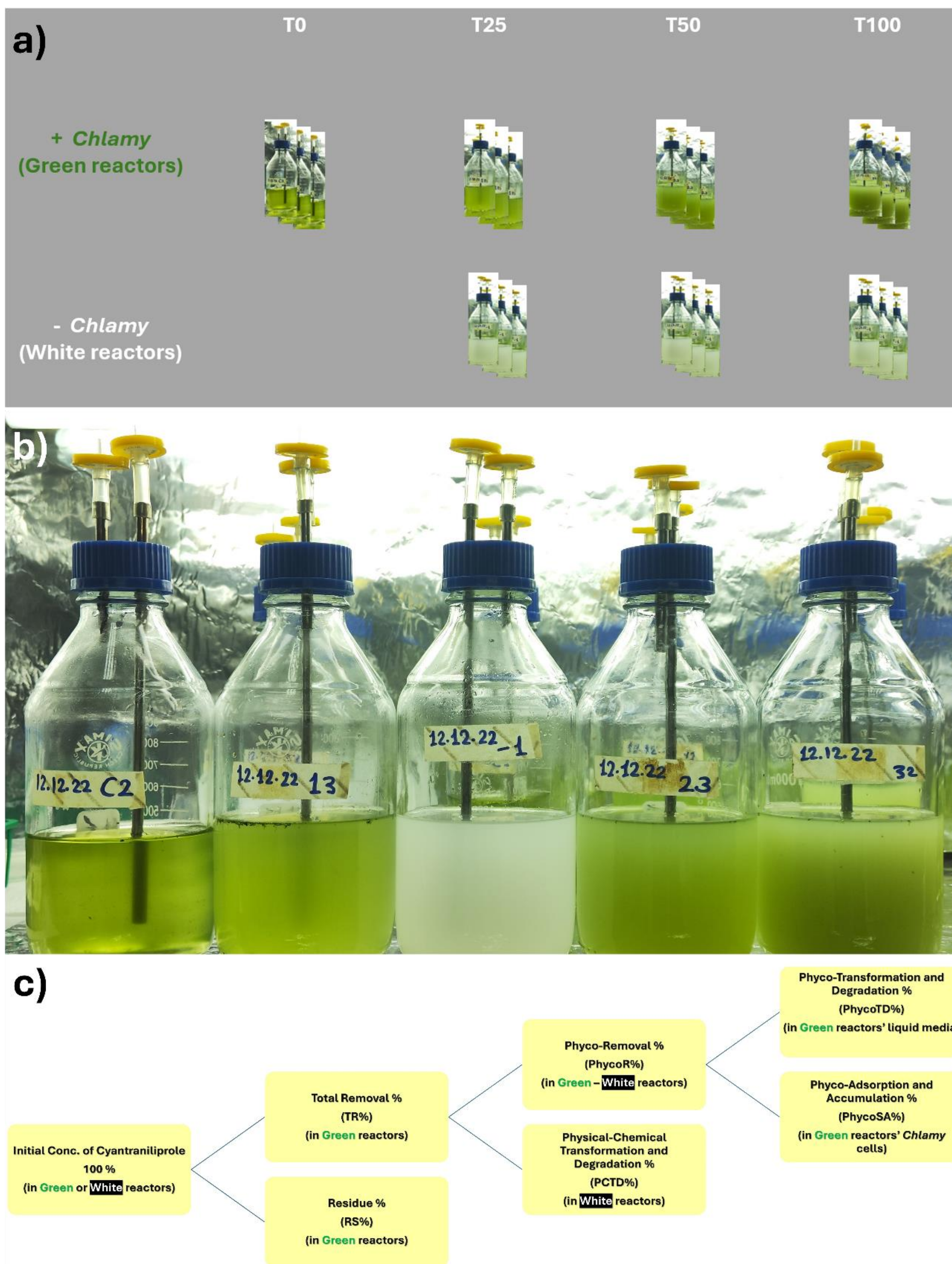

Fig. 1. a) The experimental setup of the main experiment where TAP medium treated with cyantraniliprole 0, 25, 50, and 100 ppm (T0, T25, T50, and T100, respectively) in 1-L bioreactors in triplicates divided into *C. reinhardtii* containing reactors (+ Chlamy = green reactors) and no Chlamy containing reactors (Chlamy = White reactors); b) The fully aseptic system of bioreactors used in this study. It was composed of separate 1-L bioreactors, each bioreactor contained submerged inlet stainless-steel pipe for continuous atmospheric air pumping through 0.22-µm filters and outlet short stainless-steel pipe connected with 0.22-µm filters for gas exhausts; and c) Hierarchy diagram shows removal fate of Cyantraniliprole

$$\text{Chl-a} = (12.47 * A_{665}) - (3.62 * A_{649}) \quad (1)$$

$$\text{Chl-b} = (25.06 * A_{649}) - (6.5 * A_{665}) \quad (2)$$

$$\text{Total chlorophylls} = \text{Chl-a} + \text{Chl-b} \quad (3)$$

$$\text{Carotenoids} = ((1000 * A_{480}) - (1.29 * \text{Chl-a}) - (53.78 * \text{Chl-b})) / 220 \quad (4)$$

where A_{665}, A_{649}, and A_{480} stand for absorption at 665, 649, and 480 nm respectively. All equations were calculated as mg/L considering the dilution factor.

Chemical composition of algal cells

Using a modified micro-Kjeldahl method as described in Latimer (2023), total nitrogen was determined; then the nitrogen percentage was multiplied by 6.25 for crude protein estimation. For extraction of total lipids, the rapid chloroform: methanol (2:1) method (Bligh and Dyer 1959) was used then total lipids were quantified by dry lipid weighing method as mentioned in Abomohra *et al.* (2018). Total carbohydrates were quantified using phenol-sulfuric method (Dubois *et al.* 1956).

Some oxidative stress indicators and enzymatic and non-enzymatic antioxidants

As an indicator of lipid peroxidation, malondialdehyde (MDA) levels were determined according to Senthilkumar *et al.* (2021). Ascorbic acid and tocopherols were quantified by colorimetric methods in Chlamy cells as detailed in Tütem *et al*. (1997) and Gómez Ruiz *et al.* (2016), respectively. The total phenolic contents in samples were quantified using Folin-Ciocalteau method at 765 nm compared to a standard curve generated with gallic acid standard solutions (Kupina *et al.* 2019). Total flavonoids content was quantified as described by Ayele *et al.* (2022). Both CAT and SOD were determined according to Cohen *et al*. (1970) and Levine *et al*. (1994), respectively.

Calculations of Cyantraniliprole total removal percentage (TR%) and its divisions as shown in Fig. 1c are given below:

Residue in water (RS%) = Residual quantity of Cyantraniliprole in water (ppm) ×100 / initial quantity of Cyantraniliprole (ppm) in zero time (5)

Total removal % from water (TR%) = 100 - RS% [in green bioreactors containing cyantraniliprole in TAP media with algae] (6)

Physical-chemical transformation and degradation % (PCTD) = 100 - RS% [in white bioreactors containing cyantraniliprole in TAP media without algae] (7)

Phyco-removal % (Phyco R%) = TR% - PCTD% (8)

Phyco-adsorption and accumulation % (Phyco SA%) = Residual quantity of Cyantraniliprole in the whole algal biomass (ppm) ×100 / initial quantity of Cyantraniliprole (ppm) in zero time (9)

Phyco-transformation and degradation % (Phyco TD%) = Phyco R% - Phyco SA% (10)

Statistical analyses

The reported data are mathematical means of three separate biological repeats with standard deviations (SD) as error values. Significant differences among treatments were analyzed by one way analysis of variance (ANOVA) at probability level $P \leq 0.05$. , Duncan's multiple range *post hoc* tests were applied. All data were analyzed and plotted using MS Excel 365 (Microsoft Corp., Redmond, WA, USA), IBM SPSS package 27.0.1.1 (IBM Corp., Armonk, NY, USA), and GraphPad Prism 9.0.2 (University of California San Diego, San Diego, CA, USA).

RESULTS AND DISCUSSION

Preliminary Experiment

Cyantraniliprole, known for its high toxicity to aquatic invertebrates, poses an environmental threat (EFSA 2014). Remarkably, Chlamy is a model green microalga that thrives naturally in freshwater and soil and exhibits potential for bioremediation of xenobiotics-contaminated water (Nazos *et al.* 2017; Sehrawat *et al.* 2021). Inspired by this, the authors formulated a hypothesis: could Chlamy cells be harnessed for bioremediating agricultural wastewater and aquatic environments?

To test the hypothesis, two experiments were conducted. The first one was a drop test experiment using Chlamy cells on TAP-agar plates. These plates contained varying concentrations of cyantraniliprole: T0 (0 ppm), T25 (25 ppm), T50 (50 ppm), and T100 (100 ppm) (Fig. 2). After 96 h of inoculation, the results revealed important information about the effects of various cyantraniliprole concentrations on cellular growth. T0 drops were coherent, dark, and uniformly colored. T25 drops were slightly less dense, with a green hue showing a moderate impact on chlorophyll content. T50 drops had an even lighter green color, indicating further cyantraniliprole effect. Finally, T100 drops were very light in color and highly dispersed, showing a significant impact from that high concentration of cyantraniliprole.

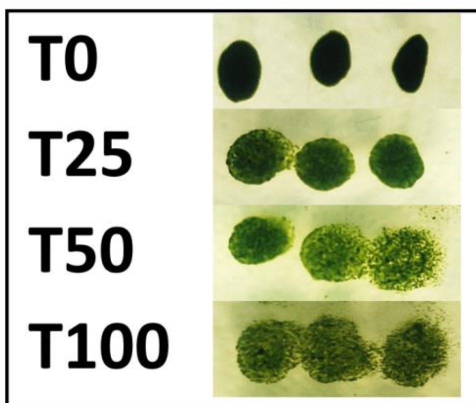

Fig. 2. The drop test of *C. reinhardtii* grown for 96 h on TAP agar medium contains 0, 25, 50, and 100 ppm Cyantraniliprole (T0, T25, T50, and T100), respectively

These observations suggest that cyantraniliprole affects Chlamy growth, chlorophyll content, and cell coherence. Interestingly, previous study on Chlamy CC125 cells (similar to Chlamy CC1690 cells used in this study) revealed that smaller, slower-growing cells tend to aggregate (Sathe and Durand 2016). The current findings align with

this; high concentration of cyantraniliprole (*i.e.*, 100 ppm) appeared to hinder cell division and promote larger dispersed cells. Intrigued by these preliminary results, the authors then aimed to delve deeper into cyantraniliprole's ecological implications for Chlamy cell physiology in the second experiment using liquid culture bioreactors.

Cyantraniliprole Removal Strategies by Chlamy

It could be inferred from Wan *et al.* (2020) that Chlamy cells, like other microalgal cells, have different ways to deal with water chemical-contaminants. In this study (Fig. 1c) when Chlamy cells were grown in a cyantraniliprole contaminated medium, part of the insecticide will be removed (Total removal; TR) by algal cells (Phyco-removal; PhycoR), and the other part by physical and chemical factors (Physical-chemical transformation and degradation; PCTD). PhycoR of the contaminant can happen by two main processes: 1) bioadsorption and bioaccumulation (Phyco-adsorption and -accumulation; PhycoSA) as well as 2) biotransformation and biodegradation (Phyco-transformation and -degradation; PhycoTD).

Table 1 describes the behavior of cyantraniliprole when exposed to green microalga Chlamy. Cyantraniliprole was effectively removed from water in Chlamy green reactors, with higher removal percentages observed at longer exposure time (7 days), where Chlamy absorbed and accumulated cyantraniliprole. Physical and chemical transformations contribute to the overall removal process as well because some degradation of cyantraniliprole occurred within the microalgal cells and the surrounding water.

These results provide valuable insights into the interactions between cyantraniliprole and Chlamy, which can have implications for environmental remediation and risk assessment.

Table 1. Percentages of Cyantraniliprole's Removal, Transformation, Bio-adsorption, and Bioaccumulation by *C. reinhardtii* Grown for 1 h and 7 days on TAP Medium Treated with Various Cyantraniliprole Concentrations

Treatment		C_i (ppm)	TR (%)	RS (%)	PCTD (%)	PhycoR (%)	PhycoSA (%)	PhycoTD (%)
1 h	T25	25	**74.17**^{c} ± 3.267	**25.83**^{a} ± 3.267	**4.36**^{bc} ± 0.880	**69.81**^{d} ± 3.620	**0.19**^{d} ± 0.020	**69.62**^{d} ± 3.600
	T50	50	**88.29**^{ab} ± 1.198	**11.71**^{bc} ± 1.198	**6.24**^{b} ± 1.970	**82.05**^{b} ± 0.980	**0.27**^{d} ± 0.050	**81.79**^{b} ± 0.990
	T100	100	**89.90**^{a} ± 0.152	**10.10**^{c} ± 0.152	**2.94**^{c} ± 1.280	**86.96**^{a} ± 1.350	**0.24**^{d} ± 0.020	**86.72**^{a} ± 1.350
7 days	T25	25	**88.08**^{ab} ± 1.441	**11.92**^{bc} ± 1.441	**11.44**^{a} ± 0.884	**76.64**^{c} ± 2.325	**1.52**^{c} ± 0.156	**75.12**^{c} ± 2.326
	T50	50	**85.77**^{b} ± 0.692	**14.23**^{b} ± 0.692	**9.37**^{a} ± 0.278	**76.40**^{c} ± 0.968	**3.15**^{a} ± 0.377	**73.25**^{cd} ± 0.730
	T100	100	**89.45**^{ab} ± 0.343	**10.55**^{bc} ± 0.343	**4.94**^{bc} ± .806	**84.51**^{ab} ± 0.952	**2.55**^{b} ± 0.225	**81.96**^{b} ± 0.749

C_i: Initial concentration; TR%: Cyantraniliprole total removal % from water; RS%: Residue % in water; PCTD%: Physical-chemical transformation and degradation %; PhycoR%: Phyco-removal % from water; PhycoSA%: Phyco-adsorption and accumulation%; PhycoTD: Phyco-transformation and degradation in (cells and water) %; SD: standard deviation. Data are presented as means of three biological replicates ± SD. The same letter in the same column indicates insignificant differences at P ≤ 0.05

Chlamy Growth and Photosynthetic Patterns Under Cyantraniliprole Treatments

Table 2 and Fig. 3 present growth parameters for Chlamy algal cells grown under different cyantraniliprole concentrations for 7 days. In T0, the algae exhibited a specific growth rate of 0.187 d^{-1}, a division number of 0.270 div.d^{-1}, and a generation time of 3.709 days. The biomass productivity was 38.7 mg CDW.L^{-1}.d^{-1}. Interestingly, in T25 and T50, they showed significantly higher growth parameters compared to T0. In T100, their specific growth rate was lower (0.110 d^{-1}), division number was less (0.159 div.d^{-1}), and generation time was longer (6.286 days). In addition, their biomass productivity was significantly lower (16.667 mg CDW.L^{-1}.d^{-1}).

Table 2. Percentages of Cyantraniliprole's Removal, Transformation, Bio-adsorption, and Bioaccumulation by *C. reinhardtii* Grown for 1 h and 7 days on TAP Medium Treated with Various Cyantraniliprole Concentrations

Treatments	SGR (d^{-1})	DivT (div.d^{-1})	GenT (d)	BP (mg CDW.L^{-1}.d^{-1})
T0	$0.187^{b} \pm 0.006$	$0.270^{b} \pm 0.009$	$3.709^{b} \pm 0.122$	$38.690^{b} \pm 2.227$
T25	$0.195^{ab} \pm 0.008$	$0.281^{ab} \pm 0.012$	$3.565^{b} \pm 0.150$	$41.667^{ab} \pm 3.367$
T50	$0.204^{a} \pm 0.005$	$0.294^{a} \pm 0.008$	$3.404^{b} \pm 0.088$	$45.238^{a} \pm 2.227$
T100	$0.110^{c} \pm 0.004$	$0.159^{c} \pm 0.006$	$6.286^{a} \pm 0.214$	$16.667^{c} \pm 0.842$

SGR: Specific growth rate; DivT: Cell division time; GenT: Generation time; BP: Biomass productivity; CDW: Cell dry weight and SD: Standard deviation. Data are presented as means of three biological replicates ± SD. The same letter in the same column indicates insignificant differences at P ≤ 0.05.

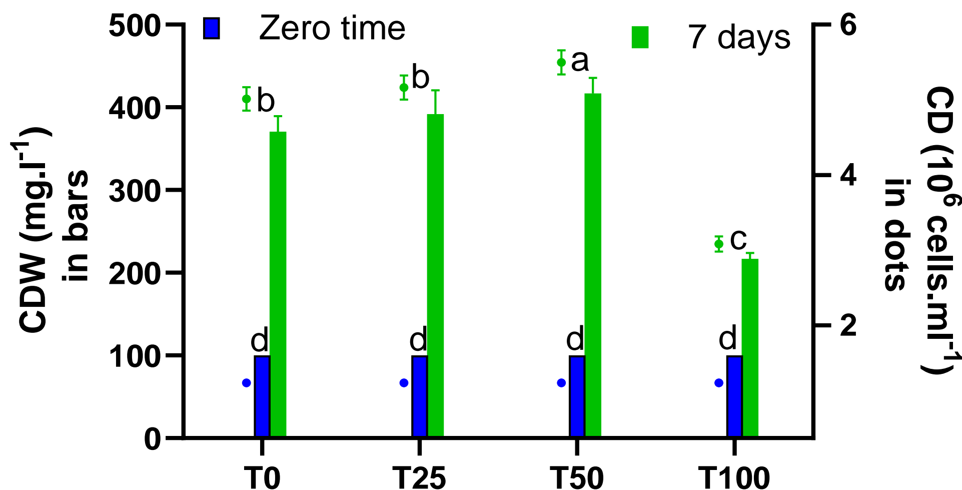

Fig. 3. Growth monitoring as cell dry weight (CDW), and cell density (CD) of *C. reinhardtii* cells grown for 7 days on TAP medium treated with cyantraniliprole at T0, T25, T50, and T100 in 1-L photobioreactors. Data are presented as means of three biological replicates ± standard deviation (SD). The same letter in the same data series indicates insignificant differences at P ≤ 0.05.

In summary, cyantraniliprole affects Chlamy growth parameters, with higher concentrations leading to altered growth rates and biomass productivity. These findings are crucial for understanding the impact of cyantraniliprole on algal populations and ecosystem dynamics.

Figure 4 shows the status of the photosynthetic pigments of Chlamy cells grown under different cyantraniliprole concentrations. It is well known that Chlorophyll a (Chl-a) is the primary pigment involved in capturing light energy during photosynthesis, while Chlorophyll b (Chl-b) is an accessory pigment that complements Chl-a in light absorption. The sum of both Chl-a and Chl-b form the total Chlorophylls (Total Chls). In contrast, total carotenoids are pigments that assist in light harvesting and photoprotection. It is clear from the results that the concentrations of Chl-a and Chl-b decreased when the concentrations of cyantraniliprole were increased.

Nevertheless, carotenoids concentrations were positively correlated with cyantraniliprole concentrations until T50 only. Early reports document that biochemical reactions displayed by the photosynthetic system can differ depending on the insecticide (Haile *et al.* 1999). This is also the case between insecticides of the same family such as diamides. For instance, Vukovic *et al.* (2021) reported a high reduction in Chl-a relative percentage caused by chlorantraniliprole (compared to control) to 58.7% in the leaves of peach trees. In contrast, a smaller decrease to only 81.2% was caused by cyantraniliprole. Moreover, the effect of one pesticide has distinct impacts between different photosynthetic microalgae genera, such as *Chlorella* green alga and *Anabaena* cyanobacterium (Mostafa and Helling 2002).

The specific causes of these differences are still unclear. However, in addition to chlorophyll degradation, it is assumed that insecticides decrease photosynthetic rates in microalgae by affecting Photosystem II (PSII) machinery in terms of PSII quantum yields, photochemical quenching, and electron transport rate (Chalifour *et al.* 2009). Based on chlorophyll fluorescence methodology, the authors also speculate that a blocking in the flow of electrons occurs at the QB binding site, a plastoquinone molecule that plays a crucial role in the electron transport chain of photosynthesis. All these factors lead to a significant decrease in photosynthetic rates.

In contrast, the same holds true regarding the differential response of carotenoids content to different types of insecticides, where it increases with increasing levels of insecticides, but with different percentages. For chlorantraniliprole, it had a higher stimulatory effect and led to a higher relative accumulation of carotenoids than cyantraniliprole, being 170% and 153%, respectively (Vukovic *et al.* 2021). This is expected, as carotenoids increase to high levels when the stressor is more harmful. In the case of the authors' experiments, increases were detected between T25 and T50, but higher levels (*e.g.*, T100 in this study) become detrimental and carotenoids levels drop below control levels.

Carotenoids' primary antioxidant protective function is to shield membranes from damage caused by reactive oxygen species (ROS). Peroxyl (ROO·), alkoxyl (RO·), hydroxyl (·OH), and superoxide anion radicals ($O_2^{\cdot -}$) can all be effectively scavenged by carotenoids. Conjugated double bonds, which can accept unpaired electrons and widely delocalize them over the conjugated system of double bonds, are linked to the antioxidant qualities of carotenoids (Mortensen *et al*. 2001).

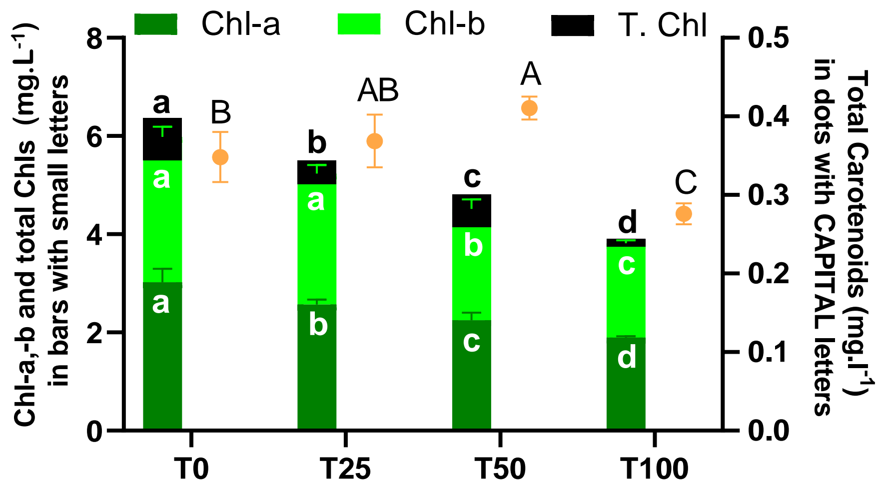

Fig. 4. Photosynthetic pigments (Chl-a, Chl-b, Total Chls, and total Carotenoids) of *C. reinhardtii* cells grown for 7 days on TAP medium treated with cyantraniliprole (T0, T25, T50, and T100 respectively): Chl-a: Chlorophyll a; Chl-b: Chlorophyll b; Total Chls: total Chlorophylls and Chl-a/b: Chlorophyll a/ Chlorophyll b. All values are presented as means of three biological replicates ± standard deviation (SD) except the values of total chlorophylls are presented as minimum to maximum values. The same letter in the same data series indicates insignificant differences at $P \leq 0.05$.

Implications of Cyantraniliprole in Changing Biochemical Composition and Oxidative Stress Response of Chlamy Cells

Metabolism plays a crucial role in sustaining biological activities within organisms. It enables growth, reproduction, and interaction with the external environment. Researchers have found that pollutants can inhibit sensitive microorganisms, while, interestingly, the metabolic capacity of degrading microorganisms can be enhanced (Huang *et al*. 2022; Chen *et al.* 2023).

The results of chemical composition are presented in Table 3 for Chlamy biomass grown under different cyantraniliprole concentrations. At zero time, the initial composition of the algal biomass showed 15.1% proteins, 31.7% carbohydrates, and 11.8% lipids. After 7 days, in T0 cells, the concentrations of proteins and carbohydrates decreased slightly to 13.6% and 30.1%, respectively. The concentration of lipids increased to 12.9% in T25, T50, and T100. Similar trends are observed with a decrease in the protein concentrations (13.2%, 12.7%, and 9.2%, respectively). However, gradual increases in concentrations of carbohydrates (33%, 38.4%, and 43.8%, respectively) and lipids (15.5%, 16.4%, and 20.1%, respectively) were observed. In summary, cyantraniliprole exposure affected the algal biomass composition, with alterations in protein, carbohydrate, and lipid levels. These changes may have implications for algal growth, metabolism, and overall health.

As the concentrations and exposure time of cyantraniliprole are increased, all studied oxidative parameters and antioxidative parameters (MDA, total phenolic compounds, total flavonoids, SOD, CAT, and Tocopherols) increased, as shown in Fig. 5, except for ascorbic acid (Vit C). There is a negative correlation usually between Vit C and Tocopherol (Vit E) as shown in a previous study (Morgan *et al*. 2023). Normal levels of ROS play a crucial role in various cellular activities, including enhancing enzyme function and boosting immunity (Li and Kataoka 2020). However, exposure to pesticides can lead to toxic effects by elevating ROS levels (Moniruzzaman *et al*. 2020).

Table 3. The Chemical Composition at Zero Time and after 7 days of *C. reinhardtii* Biomass Grown on TAP Medium Treated with Cyantraniliprole 0, 25, 50, and 100 ppm

Treatments		Proteins (%)	Carbohydrates (%)	Lipids (%)
At zero time		**15.107**^{a} ± 0.109	**31.680**^{d} ± 0.230	**11.813**^{e} ± 0.247
7 days	T0	**13.583**^{b} ± 0.175	**30.130**^{e} ± 0.144	**12.940**^{d} ± 0.188
	T25	**13.213**^{b} ± 0.231	**33.000**^{c} ± 0.179	**15.533**^{c} ± 0.392
	T50	**12.727**^{c} ± 0.301	**38.410**^{b} ± 0.577	**16.383**^{b} ± 0.323
	T100	**9.157**^{d} ± 0.202	**43.827**^{a} ± 0.212	**20.080**^{a} ± 0.206

Data are presented as means of three biological replicates ± standard deviation (SD). The same letter in the same column indicates insignificant differences at $P \leq 0.05$.

In this study, the authors observed an upward trend in ROS levels after exposure to cyantraniliprole throughout the exposure period. Elevated ROS levels can harm cells and macromolecules such as DNA and lipids (Song *et al.* 2019). Notably, significant increases in MDA contents were found (Fig. 5a) after 1 h and seven days of exposure to all concentrations of cyantraniliprole. These findings align with Yan *et al.* (2023)'s work, which demonstrated increased MDA content during the late stage of insecticide exposure.

To counter excess ROS, cells rely on both enzymatic and non-enzymatic antioxidant defenses. Catalase (CAT) and SOD activities must maintain a dynamic balance with ROS levels to support organismal survival and manage environmental stress. Additionally, Ca^{2+} directly activates antioxidant enzymes like glutathione reductase, CAT, and SOD, facilitating ROS clearance (Thompson *et al.* 2014).

In the current study, CAT and SOD activities were enhanced in cyantraniliprole-exposed algae cells from time zero to the seventh day compared to the control group (T0). These results confirm that cyantraniliprole induces oxidative stress and detoxification effects. Furthermore, Yan *et al.* (2023) reported a positive correlation between the SOD gene and SOD activity, suggesting that cyantraniliprole has subchronic toxic impacts related to oxidative stress and DNA changes in the earthworm *Eisenia fetida*.

An essential line of defense for cells against ROS is represented by low molecular weight antioxidants. A subset of water-soluble low molecular weight antioxidants exists within the cytoplasmic matrix or cytosol. Another class of lipid-soluble antioxidants functions within the membranes. The most prominent instances of these antioxidants include flavonoids, carotenoids, glutathione, and vitamins C, E, and A (Pinchuk *et al.* 2021).

Vitamin C's pro-oxidant properties in the presence of oxygen and redox-active metal ions have primarily been demonstrated *in vitro*; evidence for their occurrence *in vivo* has not been sufficiently demonstrated (Halliwell 1996). This could be because redox metal homeostasis is strictly regulated and metal sequestration results in minimal amounts of free metals. *In vitro*, ascorbate can regenerate vitamin E from its radical tocopheryl form. Fat-soluble vitamin E, also known as α-tocopherol, shields cellular membranes from ROS-caused lipid peroxidation (NIH 2021). The hydroxyl group on vitamin E's six-membered ring is outside the membrane and faces the aqueous environment, where it scavenges ROS. Vitamin E is anchored in a lipid membrane. α-Tocopheryl radical (α-TO·) is created when α-Tocopherol (α-TOH) reacts with radicals, such as·OH.

Phenolic compounds and flavonoids have antioxidant properties that are able to terminate free radicals. Additionally, they can stimulate the production of antioxidant

enzymes and regenerate certain vitamins, such vitamin E, that have a higher electron reduction potential (Simunkova *et al.* 2019). Hydroxyl groups of flavonoids are directly involved in the scavenging activity of radicals, forming flavonoid radicals that can be regenerated by Glutathione (GSH).

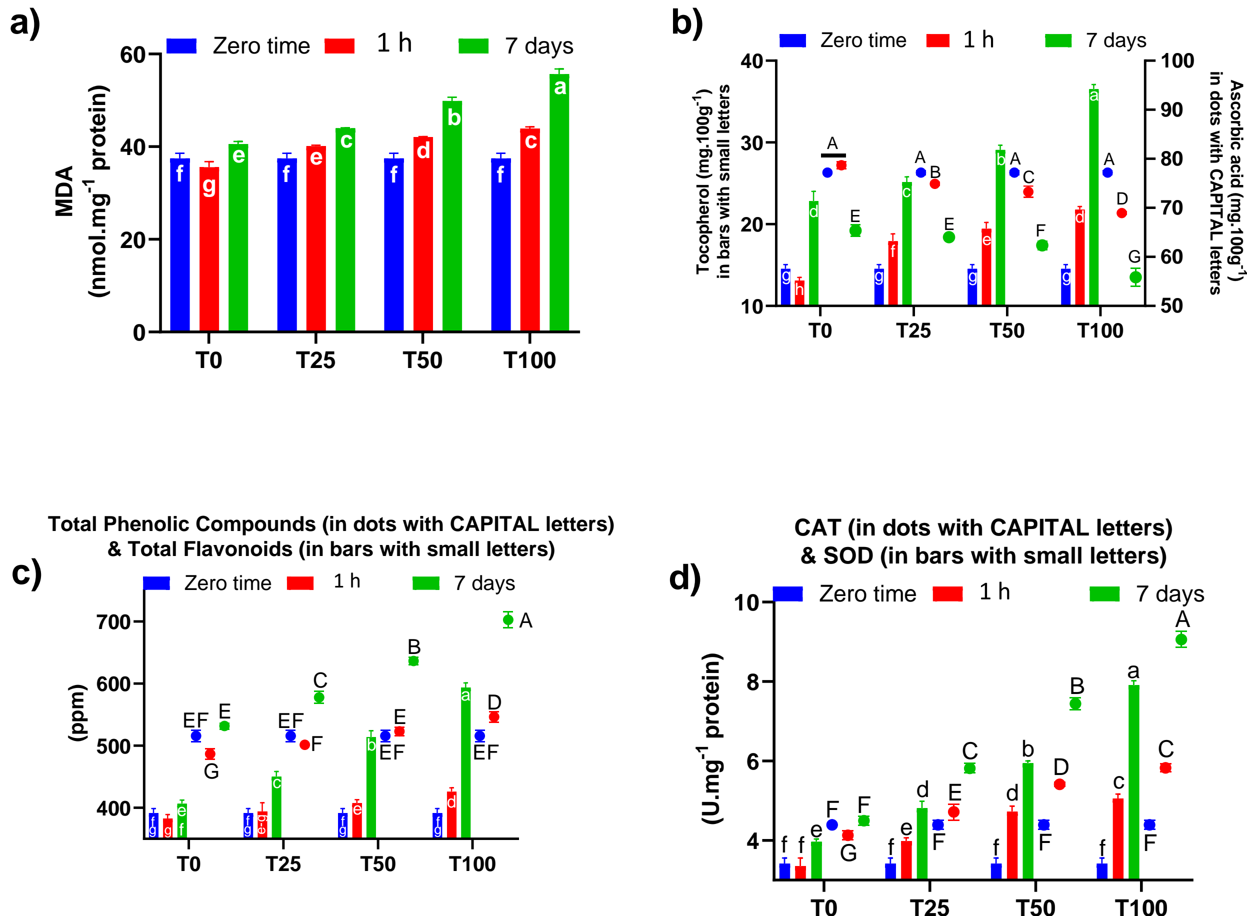

Fig. 5. Oxidative stress indicators and certain defense factors in *C. reinhardtii* cells grown for 7 days on TAP medium treated with cyantraniliprole at 0, 25, 50, and 100 ppm: MDA: Malondialdehyde; CAT: Catalase enzyme and SOD: Superoxide dismutase enzyme. Data are presented as means of three biological replicates ± standard deviation (SD). The same letter in the same data series indicates insignificant differences at P ≤ 0.05.

CONCLUSION

1. This study demonstrated the potential of *Chlamydomonas reinhardtii* in the bioremediation of cyantraniliprole-contaminated water, achieving up to 87.0% removal within 1 h.
2. The microalga exhibited resilience to cyantraniliprole stress, maintaining antioxidant levels.

3. Remarkably, certain treated cultures showed enhanced growth rate, biomass productivity, and increased carbohydrate and lipid concentrations, suggesting potential for biofuel production.
4. Therefore, *C. reinhardtii* not only offers a solution for cyantraniliprole pollution, but also contributes to sustainable bioenergy production. Further research could explore optimizing these processes for large-scale applications.

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