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Research Article

Oxidative Stress and Activation of Enzymatic Antioxidative Defence Mechanism in *Tetraselmis chui*

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ABSTRACT

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and an organism's ability to detoxify these harmful intermediates, posing a significant challenge for aquatic organisms, including microalgae. *Tetraselmis chui* has developed defence mechanisms to manage oxidative stress, ensuring its survival and functionality in various ecosystems. This study explores the interactions between the growth, oxidative stress markers and the activation of enzymatic antioxidative defence systems in *T. chui* after 8 days of incubation. *T. chui* was cultured in F2 media and subjected to variations in media strength (F/2, F, F/4 and F/8), sucrose concentrations (0 to 50 g/L), pH (5 to 9), and light intensities (0 to 600 lux). Growth was highest in full-strength media and improved with 40 g/L sucrose addition. Growth was largely unaffected by pH, except at pH 5, which reduced it, while higher light intensities boosted fresh and dry weights. Full-strength media led to significant MDA and H_2O_2 accumulation, while F/2 media exhibited higher ion leakage. The absence of sucrose increased H_2O_2 and ion leakage but lowered MDA, whereas higher sucrose concentrations raised MDA while reducing ion leakage. pH 7 stimulated MDA production, with H_2O_2 concentrations largely unchanged except in controls. Cells grown in darkness showed elevated MDA and ion leakage but reduced H_2O_2. CAT and APX activities were significantly enhanced in F/8 media, while gPOD was highest in full-strength media. Sucrose-free media induced CAT and APX but reduced gPOD activity. All enzyme activities were most strongly induced at pH 9. Light intensity at 150 lux favoured CAT and gPOD, whereas APX was more active in dark conditions. These findings unveil the strategies employed by *T. chui* in response to oxidative stress, highlighting its ecological adaptability and contributing to its potential to produce antioxidants for various industrial applications.

Key words: Antioxidants, microalgae, oxidative stress, reactive oxygen species, *Tetraselmis chui*

INTRODUCTION

Global food security is predicted to worsen because the world's population is expected to surpass 10 billion by 2050. Dietary adjustments that decrease animal calorie intake and enhance consumption of calorie-efficient, nutrient-rich, and sustainably produced foods are recommended to prevent the worsening of this issue. The vast bioactive compounds in microalgae, including lipids, polysaccharides, carotenoids, vitamins, phenolics, phycobiliproteins, and various antioxidants, present a promising avenue for functional food development. Interest in their nutritional value and potential health benefits has surged globally. Notably, the quest for natural antioxidants as substitutes for synthetic ones has intensified, with microalgae showing significant variability in antioxidant activity across species and growth environments (Coulombier *et al*., 2021; Mavrommatis *et al*., 2023).

The diversity of microalgal species gives rise to various antioxidant molecules, which makes microalgae the richest natural resource for nutritional and bioactive components. Among these microalgae, *Tetraselmis chui*, a unicellular green alga, has garnered attention for its ecological significance and adaptability to diverse environmental conditions. *Tetraselmis* sp. are unicellular flagellates belonging to the Chlorodendraceae family within the Chlorodendrophyceae class. They typically feature nearly spherical, slightly flattened cells with an invagination at the anterior end, giving rise to four equal flagella in two opposing pairs. *Tetraselmis* sp. serve as valuable laboratory models for investigating the impacts of various conditions, such as saline or nitrogen-rich environments, on their growth, survival, and adaptive responses. These microalgae are widely utilised in aquaculture due to their rich nutritional profile, including proteins, fatty acids, and other essential biological compounds (Paterson *et al*., 2023). Their appeal for advancing biotechnological applications stems from their rapid growth rate, ease of cultivation, scalability in production, amenability to genetic modification, low maintenance costs, and adaptable metabolism, which can be tailored to produce desired compounds through manipulation of culture conditions (Daneshvar *et al*., 2021; Jareonsin & Pumas,

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2021). Owing to these factors, the demand for algal biomass will substantially rise in the forthcoming years. Projections indicate that the global algae market is poised to achieve a value of USD 22.64 billion by 2032, an increase from USD 12.75 billion in 2023 (Nandi, 2024).

The key factors affecting microalgal growth and biomass production are nutrient availability, carbon source and substrate concentrations, light intensities, light/dark cycle length, pH values, and cultivation temperature (Thepsuthammarat *et al*., 2023). Previous reports indicated that suitable carbon sources and mineral proportions could enhance microalgae growth and biomass production. Media strength determines the availability of essential nutrients, directly influencing growth rates, cellular division, and metabolite production. Full-strength media typically support rapid growth and higher biomass. In contrast, nutrient-limited conditions, such as diluted media strengths, may slow cell proliferation or induce stress responses, accumulating specific secondary metabolites (Udayan *et al*., 2023). Sucrose supplementation offers an additional carbon source for promoting energy availability beyond what is provided by photosynthesis. Optimal sucrose concentrations can boost growth and increase cellular energy reserves, yet excessive sucrose may inhibit photosynthetic activity and other metabolic processes (Bashir *et al*., 2019). The effects of pH and light intensity on microalgae growth are equally profound, as both influence enzyme activities, photosynthesis, and cellular stability (Kim *et al*., 2020). Light intensity is a key energy source for photosynthetic microalgae, with appropriate levels enhancing photosynthetic efficiency, growth, and biomass production. However, suboptimal light levels may limit growth, and excessively high intensities can lead to photoinhibition, damaging cellular components and reducing photosynthetic performance (Montoya-Vallejo *et al*., 2023).

Like all living organisms, *T. chui* faces the challenge of oxidative stress, a condition arising from the imbalance between the production of reactive oxygen species (ROS) and the organism's ability to detoxify them. ROS include both free radicals (superoxide radical, $O_2^{\cdot-}$; hydroxyl radical, $OH\cdot$; perhydroxyl radical, $HO_2^{\cdot}$; and alkoxy radicals, $RO^{\cdot)}$ and non-radical molecules (hydrogen peroxide, H_2O_2, and singlet oxygen, $^1O_2$) (Gupta *et al*., 2017). These ROS are natural byproducts of aerobic metabolism and are produced in various cellular compartments, including chloroplasts, peroxisomes, mitochondria, and the plasma membrane (Apel & Hirt, 2004). The accumulation of ROS is highly reactive and can disrupt numerous cellular processes, leading to damage in key cellular components. This includes the oxidation of carbohydrates, lipid peroxidation, protein denaturation, and the destruction of nucleic acids (DNA and RNA), as well as enzymes and pigments, ultimately affecting the overall physiological and biochemical functions of the cell (Xie *et al*., 2019). Oxidative stress constantly threatens cellular integrity, metabolic processes, and algal fitness. Lipid peroxidation, protein oxidation, and DNA damage are among the consequences that compromise the alga's structural integrity and functionality (Rezayian *et al*., 2019). Investigations into the cellular responses of *T. chui* to oxidative stress have highlighted disruptions in photosynthetic efficiency, growth inhibition, and alterations in biochemical pathways (Koletti *et al*., 2024). These consequences underscore the importance of elucidating the mechanisms by which *T. chui* mitigates oxidative stress to ensure its survival and ecological contributions.

In response to oxidative stress, *T. chui* activates sophisticated antioxidative defence mechanism strategies to neutralise and detoxify ROS. The major group of antioxidants in microalgae is carotenoids (CAR). Still, there are also significant amounts of other antioxidants such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and glutathione reductase (GR) as well as low molecular weight antioxidants, i.e. ascorbate (ASC), tocopherols (TOC) and glutathione (GSH) (Hasanuzzaman *et al*., 2020; Sachdev *et al*., 2021). SOD is a primary defence antioxidant that catalyses the dismutation of $O_2^{\cdot-}$ to H_2O_2 and oxygen (O_2). H_2O_2 produced needs to be further detoxified by CAT and PODs to H_2O and O_2. ASC, GSH and TOC played interrelated roles in interacting and detoxifying oxygen-free radicals and thus contributed significantly to limiting ROS production (Zhu *et al*., 2023; Anwar *et al*., 2024). In addition, other types of antioxidants that have become increasingly explored in microalgae are polyphenol, astaxanthin and lutein, which are pivotal players in this defence system (Mishra & Jha, 2011; Lu *et al*., 2021). Research has indicated the upregulation of these enzymes as a protective response in *T. chui*, showcasing the alga's capacity to adapt to changing environmental conditions (Rahman *et al*., 2017; Koletti *et al*., 2024).

The susceptibility of *T. chui* to oxidative stress is closely linked to environmental factors. Fluctuations in temperature, medium pH, light intensity, and nutrient availability can disrupt cellular redox balance, exacerbating ROS production. Investigating the interplay between these environmental triggers and the alga's oxidative stress response is crucial for predicting *T. chui's* adaptability to changing ecosystems. Thus, this current study aims to explore the oxidative stress faced by *T. chui* by measuring its effect on growth, lipid peroxidation, hydrogen peroxide, and ion leakage in the cells. Moreover, enzymatic antioxidative responses (CAT, APX, and gPOD) were also assessed to counteract oxidative stress. This exploration contributes to the fundamental knowledge of algal adaptation to stresses and has practical implications for harnessing the potential of *T. chui* in various biotechnological applications, such as biofuel production and environmental remediation.

MATERIALS AND METHODS

Microalgae culture

Tetraselmis chui, obtained from The Institute of Marine Biotechnology at Universiti Malaysia Terengganu (UMT), was cultivated in an F2 medium (Guillard,1975; Guillard & Ryther, 1962) under conditions of 30 ppt salinity and pH 8.0 ± 0.2. Cultures were maintained at 24°C ± 2°C with continuous illumination at 300 lux provided by a 6,500 K daylight white light-emitting diode lamp (a type of LED light source with a colour temperature of 6,500 Kelvin (K). This colour temperature is often labelled as "daylight white" because it closely resembles the colour of natural daylight, making it ideal for applications where bright, clear illumination is needed for growth in the laboratory). A Hailea HAP-120 pump facilitated aeration (Hailea Group Co., China), delivering approximately 120 L/min of air at a pressure of 0.018 MPa, filtered through a 0.22 µm Minisart® Sartorius syringe filter to prevent contamination. Before experimental use, the stock culture was grown in a 500 mL liquid medium and harvested at a density of 1×10^6 cells/mL.

Microalga growth curve determination

The growth of *T. chui* was assessed by monitoring the cell density daily for 24 days. The growth curve was plotted by the total number of cell densities against cultivation time (days) to determine the growth phases. 200 µL of the cultures was diluted with 800 µL of Lugol solution (White *et al*., 2014). Ten µL of the diluted solution was transferred to the Neubauer hemocytometer and the cells were counted using a compound microscope (Leica CME, Leica Microsystems GmbH, Germany) (Sahastrabuddhe, 2016). Cell densities were determined using the following formula:

$$\text{Cell density}\left(\text{cells/mL}\right) = \frac{\text{Total cells number} \times \text{dilution factor}}{\text{Total squares number} \times \text{Square volume at 0.01 mm depth}}$$

Manipulation of culture growth conditions

Each treatment in this experiment was conducted by modifying specific conditions of the media strength, pH, sucrose concentration, and light intensity while keeping all other culture conditions constant to observe the isolated effects of each variable. For media strength, cultures were exposed to the varying strength of F2 medium, specifically full strength (F), half strength (F/2), quarter strength (F/4), and one-eighth strength (F/8), with half strength (F/2) serving as the control. The media strength in a culture medium represents the concentration of nutrients and minerals it provides to support *T. chui* growth. Full-strength (F) media contain the complete nutrient profile essential for optimal growth, including key macronutrients like nitrogen and phosphorus, along with micronutrients. In half-strength (F/2) media, nutrient concentrations are reduced to 50% of full strength, limiting nutrient availability. Quarter strength (F/4) media further reduces nutrient levels to 25% of the full concentration, and one-eighth strength (F/8), the nutrient concentration is just 12.5% of full strength. The cultures were then inoculated with algae and grown under these different nutrient levels to evaluate the effect of nutrient availability on growth and metabolism.

In assessing the effects of sucrose concentration, cultures were supplemented with sucrose at concentrations of 0, 10, 20, 30, 40, and 50 g/L, with 0 g/L as the control concentration. The effect of different initial media pH values was also examined, with initial pH levels ranging from 5 to 9, using pH 8 as the control. The pH was adjusted by adding either 0.1 N NaOH or 0.1 N HCl and measured with a Corning pH meter (USA). Additionally, cultures were exposed to varying light intensities (0, 150, 300, 450, and 600 lux) under cool white fluorescent lamps with constant illumination at 300 lux serving as the control light intensity. The light intensities in this experiment were carefully prepared by adjusting the distance between the algae cultures and cool white fluorescent lamps to achieve specific illumination levels. This range of light intensities was chosen to study the effect of varying light conditions on the algal cultures, particularly to observe how different levels of light influence growth and metabolic activities. The 300-lux intensity was chosen as the control because it represents an optimal, moderate light level suitable for algae growth without causing light stress or photoinhibition, which might occur at higher intensities (Nzayisenga *et al*., 2020). Light intensity was kept constant within each treatment group to observe how changes in illumination affect growth and other physiological parameters. For the other experimental treatments (media strength, pH, and sucrose concentration), the light intensity was kept consistently at 300 lux to eliminate light as a variable.

The cells were harvested on day 8 (at the end of the exponential and early stationary phase) to determine fresh and dry weights, oxidative stress early markers, and enzymatic antioxidant assays. All results are presented as means and standard error (n = 5).

Fresh and dry weight determination

The cell culture was centrifuged for 10 minutes at 10,000 rpm. Fresh microalgae paste was rinsed twice with distilled water after discarding the supernatant. For the dry weight, the fresh paste was dried in an oven at 70±2°C for 24 h until it obtained the persistent weight to give the dry weight (g/L) (Kong *et al*., 2011).

Oxidative stress early markers determination

Lipid peroxidation

The lipid peroxidation was measured based on the malondialdehyde (MDA) produced by Thiobarbituric acid (TBA) (Heath & Packer, 1968). Using the sonicator, the fresh sample of *T. chui* (0.15 g) was homogenised for 2 minutes with 6.0 mL of 0.1% (w/v) Trichloroacetic acid (TCA) at 0-4°C. Then, the homogenate was centrifuged for 5 minutes at 10,000 rpm, 4°C. Next, 3.0 mL of supernatant obtained was added with 9.0 mL of Thiobarbituric acid (TBA) reagent. The mixture was left in an ice bath for 15 minutes after being heated at 95°C for 30 minutes. The absorbance of the supernatant was taken at 532 nm, with the value of non-specific absorption at 600 nm deducted from the absorbance results. By applying its extinction coefficient at 155 $mM^{-1}cm^{-1}$, the MDA was calculated and given as nmol MDA/g fresh weight of the sample.

$$A = \in bl$$

A = 532 nm absorbance - 600 nm absorbance

b = [X] concentration

l = cuvette length

$\in$ = extinction coefficient [155 mM^{-1} cm^{-1}]

Hydrogen peroxide

The H_2O_2 was measured using Velikova *et al*. (2000) and Alexieva *et al*. (2001) with minor modifications. 0.20 g of the fresh sample was sonicated using the sonicator for 2 minutes at 4°C with 4.0 mL of 0.1% (w/v) TCA. Next, the mixture was centrifuged for 10 minutes at 10,000 rpm and 4°C. The reaction mixture consists of 2.0 mL supernatant and 2.0 mL 10 mM potassium phosphate buffer (pH 7.0). After that, 4.0 mL of 0.1 mM potassium iodide (KI) was added. Then, the mixtures were left under dim illumination for 10 minutes. After that, the absorbance reading was taken at 390 nm using a spectrophotometer (Shimadzu UV-1601).

Ion leakage

The fresh sample (0.15 g) and 40 mL of double distilled water were added into a 100 mL conical flask and heated at 40°C for 30 minutes. The conductivity (C1) was then determined. Next, the mixture was heated up to 100°C for 10 minutes before cooling it at room temperature before the conductivity (C2) was measured. The percentage of ion leakage was calculated using the formula by Correia *et al*. (2013).

Relative leakage (% leakage) = [1-(C1/C2)] × 100
C1 = Conductivity before boiling to 100°C
C2 = Conductivity after boiling to 100°C

Enzymatic antioxidants assays

CAT-specific activity

The CAT-specific activity was assessed following the method outlined by Claiborne (1985). 0.15 g of samples were sonicated with 1.0 mL of 50 mM phosphate buffer (pH 7.4) in a prechilled beaker at 0-4 °C for 10 minutes. Then, the mixture was centrifuged at 10,000 rpm and 4°C for 10 minutes. For the assay, the reaction was started by adding 100 µL of enzyme extract into 3 mL of reaction buffer (19 mM hydrogen peroxide in 50 mM phosphate buffer, pH 7.0). The absorbance was monitored at 240 nm for 3 minutes.

APX-specific activity

The APX-specific activity was determined following the procedure outlined by Nakano and Asada (1980). 0.15 g of sample was sonicated with 1.0 mL of 1 mM ascorbic acid in 100 mM phosphate buffer (pH 7.0) at 0-4°C for 10 minutes. The enzyme extract was obtained by centrifuging the homogenate at 10,000 rpm at 4°C for 10 minutes. The reaction mixture comprised 1.5 mL of 100 mM phosphate buffer (pH 7.0), 0.5 mL of 3 mM ascorbic acid, 0.1 mL of 3 mM EDTA, 0.3 mL distilled water, and 0.2 mL of 1.5 mM H_2O_2, added to 200 µL of enzyme extract. Absorbance was monitored at 290 nm for 3 minutes.

gPOD-specific activity

The gPOD-specific activity was determined according to the method described by Agrawal and Patwardhan (1993). 0.15 g of sample was sonicated with 0.9 mL of 100 mM phosphate buffer (pH 7.0) at 0-4°C for 10 minutes. Subsequently, the homogenate was centrifuged (Eppendorf 5810) at 10,000 rpm at 4°C for 10 minutes to obtain the enzyme extract. The reaction mixture comprised 1 mL of 50 mM phosphate buffer (pH 7.5), 1 mL of 20 mM guaiacol, 1 mL of 30 mM H_2O_2, and 200 µL of enzyme extract. Absorbance changes were monitored at 470 nm for 3 minutes.

Protein content

The protein content was determined using the Bradford method (1976). Bradford reagent was prepared by adding 100 mg of Coomassie Brilliant Blue G-250 and dissolved in 50 mL of 95% ethanol. To this, 100 mL of phosphoric acid was added, and the mixture was diluted to a final volume of 1.0 L with distilled water. The solution was filtered through filter paper and stored in light-proof bottles at 2-4°C. For the assay, 100 µL of enzyme extract was added to 3 mL of Bradford reagent. Absorbance was measured at 595 nm after a 10-minute incubation. Protein concentration was determined using a standard curve prepared with Bovine Serum Albumin (BSA) ranging from 20 to 100 mg/mL.

Statistical analysis

The experiment was conducted in five biological replicates (n=5), where each replicate represents an independently prepared culture of algae subjected to the same treatment conditions. Data were presented as mean ± standard error. The significant differences were determined using a one-way analysis of variance (ANOVA) and tested by Duncan's Multiple Range Test (DMRT) to compare the differences between treatments at a 0.05% significance level.

RESULTS AND DISCUSSION

Microalgae growth phases

The growth curve pattern observed in this study can be divided into four main phases: lag, log, stationary, and death. These phases align with those reported by Kasan *et al*. (2020) in species such as *Chlorella sp.*, *Nannochloropsis sp.*, and *Desmodesmus sp.*. Accordingly, the growth curve for *T. chui* in this study was characterized as follows: lag phase (I) on day 1, log phase (II) on day 4, early stationary phase (III) on day 8, mid-stationary phase (IV) on day 16, and death phase (V) on day 24 (Figure 1). Based on this growth phase, samples were collected on day 8 for the growth, early oxidative stress markers and enzymatic antioxidant analysis.

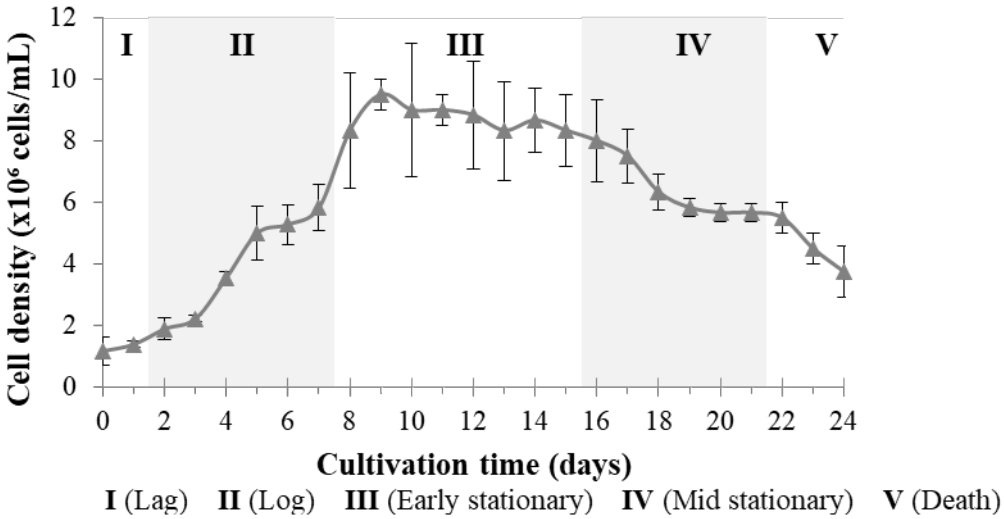

Fig. 1. Growth curve of *Tetraselmis chui* throughout 24 days of cultivation. Capital Roman numerals I, II, III, IV, and V correspondingly represent lag, log, early stationary, mid stationary, and death phase, respectively. Data presented are means ± SE

Changes in fresh and dry weights

The full-strength medium yielded the highest fresh and dry weights of *T. chui*, followed by F/2 and F/4, with the lowest growth observed in the F/8 medium (Figure 2a). These results indicate that adjusting nutrient concentrations can significantly enhance growth performance in *T. chui*. Algal growth relies mostly on two nutrients: nitrogen and phosphorus (Nzayisenga et al., 2020). In this study, culturing cells in a full-strength medium notably increased the specific growth rate, achieving a 3.5-fold increase compared to the F/8 medium by supplying higher concentrations of essential components like nitrogen and phosphorus. Nitrogen and phosphorus regulate metabolic activities if supplied in an acceptable form. Both nutrients are essential for nucleic acids, proteins, ATP synthesis, and enzymes involved in cell proliferation (Yaakob *et al*., 2021). Nitrogen supports critical cellular functions, while phosphorus is fundamental for energy storage and transfer, information processing, and serves as a vital structural component within the cell (Solovchenko *et al*., 2024) Together, these nutrients promote optimal photosynthetic activity, resulting in higher growth in *T. chui* cultured in full-strength medium. Low nutrient concentrations can restrict growth, while excessive nutrient levels may lead to imbalances or toxicity, disrupting cellular processes and reducing growth yield (Singh *et al*., 2016). Studies have shown a clear relationship between media strength and microalgal biomass production, demonstrating that adjusting nutrient concentrations in growth media can optimize conditions for maximum growth.

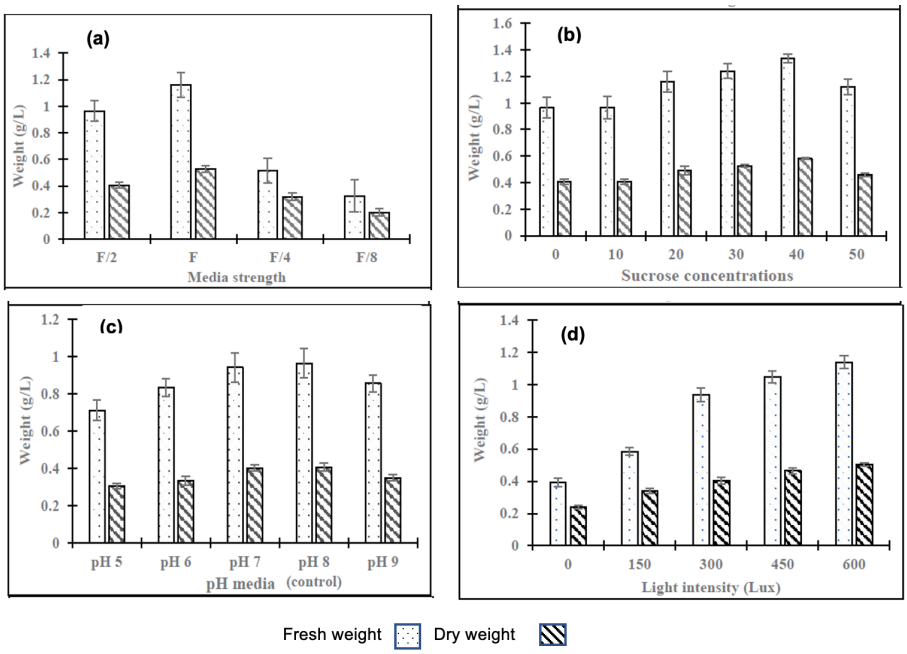

Fig. 2. Fresh and dry weights of *Tetraselmis chui* in different a) media strength, b) sucrose concentrations, c) pH of the media and d) light intensities. Data are mean ± SE, n=5.

The addition of sucrose led to a gradual increase in fresh and dry weights of *T. chui*, reaching a maximum at 40 g/L, but significantly declined at a higher concentration of 50 g/L. However, sucrose concentrations ranging from 0 to 40 g/L did not significantly impact growth (Figure 2b). The gradual rise in fresh and dry weights observed at 0-40 g/L sucrose can be attributed to sucrose's role as an energy source during photosynthesis. Adding sucrose to the growth media promotes carbon assimilation, providing readily available energy and enabling microalgae to allocate more resources toward growth and biomass production as observed in *Chlorella vulgaris* (Rajivgandhi *et al*., 2022). Moderate sucrose levels often boost biomass production by supplying an extra carbon source for metabolism and synthesis. However, excessively high concentrations, such as 50 g/L, may inhibit growth due to potential metabolic imbalances or osmotic stress. The results were consistent with the study of Ren *et al*. (2013). They found that a glucose concentration of 10 g/L resulted in the highest biomass production, while lower or higher concentrations led to reduced growth and biomass yields of *Scenedesmus* sp. strain R-16.

As shown in Figure 2c, *T. chui* was able to grow across a broad range of initial pH values, from 5.0 to 9.0. The highest fresh and dry weights were observed at initial pH values between 7.0 and 8.0. At other pH values outside this range, the production of the fresh and dry weight was lower. At slightly alkaline pH between 7.0 and 8.0, critical nutrients like nitrogen, phosphorus, and trace metals are often more bioavailable, promoting better growth. Conversely, at lower (acidic) or higher (alkaline) pH values, nutrient availability may be compromised, resulting in reduced biomass production. In addition, it helps to maintain optimal conditions for enzyme activity, photosynthesis, and cellular metabolism, thereby supporting efficient metabolic processes and biomass production (Qiu *et al*., 2017). The results from this current study were supported by Patrinou *et al*. (2022). They reported that marine microalgae like *Tetraselmis* strains tend to thrive within a slightly alkaline pH range of around 7.5 to 8.5. A similar observation was also found in *C. vulgaris* and *Scenedesmus* sp. (Wu *et al*., 2021).

Light intensity is a critical factor influencing microalgal growth and biomass production. In this study, an increase in the fresh and dry weights of microalgae was observed with rising irradiance, up to 600 lux (Figure 2d). Adequate light intensity enhances the utilization of CO_2 by microalgae and boosts energy production, resulting in increased cell growth. Generally, increases in light intensity increase microalgal growth up to a photo-inhibitory threshold, but both the strength of this effect and the threshold vary among species (Chia *et al*., 2018). This phenomenon leads to an overload of the photosystem, causing pigment bleaching and damage to the photosystems, ultimately resulting in decreased cell growth (Montoya-Vallejo *et al*., 2023).

Changes in the early oxidative stress markers

Microalgae, being photosynthetic organisms, play a pivotal role in aquatic ecosystems and offer immense potential in various biotechnological applications. Understanding how environmental factors affect crucial physiological parameters like lipid peroxidation, H_2O_2 accumulation, and ion leakage in microalgae is essential for optimising their growth and productivity. The effect of media composition on early oxidative stress responses in T. chui sheds light on the intricate interplay between nutrient availability and cellular redox balance. This study explores how media component variations influence the alga's susceptibility to oxidative stress and its subsequent antioxidative defence mechanisms.

Media strength plays a significant role in determining nutrient availability for physiological processes in microalgae. This study has shown that full-strength media (F) leads to a notable accumulation of malondialdehyde (MDA) and H_2O_2 (Table 1), indicative of oxidative harm to *T. chui*. This accumulation suggests that higher media strength, characterised by elevated nutrients such as nitrogen and phosphorus, can lead to increased lipid peroxidation and H_2O_2 in the cells. Full-strength media provide an abundance of nutrients, which can significantly enhance cell metabolic activity. This increased activity promotes rapid growth and an increase in cell fresh and dry weights (Figure 2a). However, this boost in growth is accompanied by an increase in oxidative stress, as indicated by the elevated MDA and H_2O_2 concentrations. This phenomenon is also attributed to stimulating metabolic processes under nutrient-rich conditions, resulting in higher rates of ROS generation and subsequent oxidative damage to lipids within the cell membranes (Fal *et al*., 2022). Lower MDA concentrations were observed in cells under F/4 and F/8 compared to F media strengths, thus indicating that reducing media strength or culturing microalgae in nutrient-deficient conditions may alleviate oxidative stress by limiting metabolic activity and ROS production. Interestingly, the lowest MDA concentration was produced in cells grown in F/2 media (control) and comparable amount of H_2O_2 was in F/2 and F/4 media. F/2 media maintains a balance of essential nutrients that may still support some level of metabolic activity while reducing oxidative stress compared to F/4 and F/8. Although it is less concentrated than full-strength media, it may provide enough nutrients to support cellular processes without pushing the cells into states of severe oxidative stress. In contrast, F/4 and F/8 media might limit nutrients more significantly, resulting in a stress response that could involve an increase in ROS production and lipid peroxidation, thus leading to higher MDA levels (Widjaja *et al*., 2009; Reshma & Arumugam, 2022). The impact of media strength on lipid peroxidation and H_2O_2 has significant implications for microalgal physiology and cellular homeostasis. Elevated ROS levels can disrupt membrane integrity, impair cellular functions, and compromise microalgal viability and productivity (Ben Ouada *et al*., 2018). Conversely, minimising lipid peroxidation by optimising media strength can enhance cellular resilience to oxidative stress and promote overall microalgal health and performance (Ayala *et al*., 2014). Moreover, the modulation of lipid peroxidation in response to varying media strength reflects the metabolic plasticity of microalgae. This species exhibits remarkable adaptability to fluctuating environmental conditions, allowing them to adjust their biochemical and physiological responses to optimise growth and survival in diverse habitats (Aratboni *et al*., 2019).

Another stress marker, ion leakage, also known as electrolyte leakage, is a phenomenon commonly observed under stress conditions, indicating damage to cellular membranes, particularly the plasma membrane, which plays a crucial role in maintaining cell integrity and regulating the passage of ions and molecules into and out of the cell. In this study, higher ion leakage was found in F/2 media, and the lowest was in F/8 media, in contrast to the results obtained for MDA and H_2O_2 concentration (Table 1). Higher ion leakage in F/2 media in *T. chui* cells may be related to higher cell growth and cell density in the media (Figure 2a). Cells compete for nutrient resources, which can lead to stress conditions, as some cells may experience limitations in essential resources. As a result, cells may undergo physiological changes that affect membrane function, potentially leading to increased ion leakage (Guo *et al*., 2023).

Table 1. Malondialdehyde (MDA), hydrogen peroxide (H_2O_2) concentrations and percentage of ion leakage of *Tetraselmis chui* in different media strength, sucrose concentrations, pH of the media and light intensities

Treatments		Oxidative stress markers		
		MDA (nmole/g.fwt)	H_2O_2 (mM/g.fwt)	Ion leakage (%)
Media strength	F/2 (control)	0.083 ± 0.003^{c}	4.885 ± 0.492^{c}	53.658 ± 2.442^{a}
	F	0.110 ± 0.006^{a}	0.072 ± 0.292^{a}	49.906 ± 1.484^{b}
	F/4	0.101 ± 0.002^{b}	4.978 ± 0.132^{c}	47.113 ± 1.437^{b}
	F/8	0.097 ± 0.004^{b}	5.435 ± 0.091^{b}	23.472 ± 1.181^{c}
Sucrose (g/L)	0 (control)	0.083 ± 0.003^{e}	4.885 ± 0.492^{a}	53.658 ± 2.442^{a}
	10	0.110 ± 0.003^{d}	3.907 ± 0.229^{c,d}	51.461 ± 1.383^{ab}
	20	0.107 ± 0.002^{d}	3.477 ± 0.280^{e}	51.888 ± 1.650^{ab}
	30	0.124 ± 0.005^{c}	4.050 ± 0.092^{c,d}	50.080 ± 2.455^{b}
	40	0.131 ± 0.002^{b}	4.665 ± 0.310^{a,b}	41.655 ± 1.885^{c}
	50	0.153 ± 0.004^{a}	4.410 ± 0.205^{b,c}	32.484 ± 1.885^{d}
pH	5	0.058 ± 0.002^{d}	7.397 ± 0.496^{a}	64.401 ± 1.060^{a}
	6	0.082 ± 0.005^{c}	6.875 ± 0.319^{a}	58.956 ± 1.950^{b}
	7	0.123 ± 0.001^{a}	7.026 ± 0.356^{a}	60.243 ± 2.761^{b}
	8 (control)	0.083 ± 0.003^{c}	4.885 ± 0.492^{b}	53.658 ± 2.442^{c}
	9	0.104 ± 0.002^{b}	6.917 ± 0.116^{a}	53.889 ± 0.306^{c}
Light intensity (lux)	0	0.150 ± 0.004^{a}	2.822 ± 0.238^{d}	72.923 ± 2.229^{a}
	150	0.090 ± 0.005^{c}	5.140 ± 0.127^{b}	72.227 ± 2.554^{a,b}
	300 (control)	0.083 ± 0.003^{d}	4.885 ± 0.492^{b}	53.658 ± 2.442^{c}
	450	0.107 ± 0.003^{b}	4.399 ± 0.342^{c}	69.682 ± 1.043^{b}
	600	0.045 ± 0.004^{e}	7.086 ± 0.154^{a}	55.904 ±1.608^{c}

Data are mean ± SE with different superscript letters in a column are significantly different (P<0.05).

The addition of exogenous sugar to microalgae cultures can act as a carbon source for these organisms. This supplementation often leads to increased growth rates and biomass production, as microalgae can allocate energy from sugar metabolism to various other metabolic pathways. However, the impact of sugar supplementation can vary depending on factors such as microalgae species, sugar concentration and type, as well as cultivation conditions. Glucose stands out as the most commonly utilized organic carbon source due to its superior performance in culture settings compared to other substrates (Perez-Garcia *et al*., 2011). Nevertheless, the significant expense associated with glucose, sometimes accounting for up to 80% of the total medium cost, renders microalgae cultivation economically challenging (Li *et al*., 2007). Studies have shown that many microorganisms thrive when provided with sucrose as a carbon source (Wang *et al*., 2016). Among the most studied microalgae for the supplementation of external sucrose are *C. vulgaris*, *Dunaliella salina* and *Arthrospira platensis* (Andreeva *et al*., 2021). In this study, the addition of sucrose slowly increased the MDA concentration in *T. chui* to a maximum value at a high sucrose concentration (50 g/L). The results were supported by Wang et al. (2016). They found that high sucrose levels induced oxidative stress in *C. pyrenoidosa*, leading to elevated MDA production due to lipid peroxidation. This suggests that sucrose feeding may exacerbate oxidative stress and subsequently increase MDA levels in microalgae under certain conditions. Higher sucrose concentration may lead to metabolic imbalances and stress responses in microalgae. This can result in reduced growth rates, impaired photosynthesis, and changes in the composition and quality of the biomass. Furthermore, the impact of sucrose on MDA production may vary depending on the metabolic status of the microalgae. For instance, under nutrient limitation or other stress conditions, sucrose feeding may provide additional energy and carbon sources, which could either alleviate or exacerbate oxidative stress and MDA production (Tan *et al*., 2018).

However, the 10-20 g/L sucrose concentrations reduced the MDA concentration and the lowest was in media without sucrose (Table 1). The reduction in MDA concentrations at 10-20 g/L sucrose suggests that moderate sucrose levels may reduce oxidative stress in *T. chui*. Sucrose provides an alternative carbon source, reducing reliance on photosynthesis as the principal energy pathway and thereby lessening the risk of ROS formation associated with high photosynthetic activity. In this way, modest sucrose supplementation may help balance the energy demands of cells, reducing oxidative stress and lowering MDA, a lipid peroxidation marker typically associated with cellular damage caused by oxidative stress (Foyer & Shigeoka, 2014). In contrast, the lowest MDA observed in the media without sucrose may indicate that, in the absence of an external carbon source, *T. chui* cells are under minimal oxidative stress. This could be because cells in this condition rely entirely on basal photosynthesis and adapt by adjusting metabolic pathways to survive in carbon-limited conditions, potentially reducing ROS formation as an adaptation to low-energy environments.

H_2O_2 has been identified as the primary ROS in algae (Abdel-Kader *et al*., 2023). In this study, it was observed that media without sucrose accumulated significantly higher H_2O_2 concentrations. On the other hand, sucrose at 10, 20 and 30 g/L produced lower H_2O_2 concentrations than at 40 and 50 g/L in *T. chui* cells. H_2O_2 is known to be generated widely under both stresses and normal conditions. Depending on its concentration, it can function either as a harmful molecule causing irreversible damage or as a secondary messenger regulating antioxidative defense mechanisms (Asaeda *et al*., 2020). At lower sucrose concentrations, the presence of H_2O_2 is thought to act as a secondary messenger that activates the cell's antioxidant defense system rather than causing toxicity. However, at higher sucrose levels, such as 40 and 50 g/L, *T. chui* may experience intensified energy and

biomass production processes, including photosynthesis and respiration (Bashir *et al*., 2019). These heightened metabolic activities can contribute to increased H_2O_2 production as a byproduct. Additionally, high sucrose levels may trigger a stress response, activating signaling pathways that inherently use H_2O_2 as a stress signal. This oxidative signaling helps the cells adapt to the abundant carbon but also leads to elevated H_2O_2 concentrations.

In the absence of sucrose (0 g/L, control), microalgae rely solely on photosynthesis to meet their energy and carbon demands, which amplifies photosynthetic activity and raises ROS generation, including H_2O_2. Furthermore, the lack of an external carbon source weakens the cell's antioxidant defences, reducing its capacity to counteract oxidative stress effectively. As a result, H_2O_2 accumulates due to intensified photosynthetic activity, photorespiration, and stress signaling associated with carbon deprivation (Zhu *et al*., 2023).

A similar trend was observed in the percentage of ion leakage at 0 mg/L sucrose (control). Interestingly, the percentage of ion leakage in control was comparable to lower sucrose feeding (10-30 mg/L) which resulted in higher ion leakage compared to higher sucrose concentrations (40-50 mg/L) (Table 1). This observed trend can be explained by the role of sucrose in stabilizing cell membranes and reducing oxidative stress. When sucrose availability is low or absent (0 mg/L), microalgae rely solely on photosynthesis for energy, leading to increased H_2O_2 production. This accumulation of ROS can compromise cellular membranes, resulting in elevated ion leakage due to oxidative damage. Conversely, at higher sucrose levels, the additional carbon source reduces reliance on photosynthesis, thereby lowering ROS production and oxidative stress. As sucrose concentrations increase, sucrose also acts as an osmoprotectant, stabilizing cell membranes and reducing ion leakage by mitigating cellular stress responses. This is consistent with findings from other studies, which show reduced ion leakage and improved cell membrane stability at increased carbon availability (Zhu *et al*., 2023). By supplying an extra carbon source, higher sucrose concentrations support cellular integrity, minimizing oxidative damage and ion leakage. Further research supports this mechanism. Deng *et al*. (2017) reported that sugars function as osmotic molecules, enhancing water absorbency and regulating water metabolism in algae. Additionally, sugars can act as signaling molecules, activating the production of specific ROS scavengers or directly neutralizing ROS. This effect has been observed in *C. vulgaris* and *Scenedesmus quadricauda* cells exposed to various stresses (Mohamed, 2008; Bolouri-Moghaddam *et al*., 2010).

The development of microalgae is significantly influenced by pH due to its direct effect on the NH_3/NH_4^+ balance and nitrogen availability (Mathew *et al*., 2021). Different microalgae species display varying degrees of tolerance to pH levels, and fluctuations in ambient pH affect the functionality of microorganisms, particularly the activation of enzymes and proteins (Li *et al*., 2020). While the majority of microalgae prefer neutral pH conditions, certain species have been observed to thrive in highly acidic (pH < 5) or alkaline (pH > 9) environments (Yu *et al*., 2022). In *T. chui*, pH 7 significantly increased the MDA concentration compared to higher (pH 9) or lower pH media (pH 5-6) (Table 1). Accumulation of MDA in cells under pH 7 can be attributed to the higher cell growth rate as observed in Figure 2c, thus increasing the cell activities, adjustments in membrane permeability and ion balance, ultimately impacting cellular redox equilibrium and oxidative stress vulnerability. Comparable outcomes were also documented in *S. obliquus* (pH 7.1) and *Haematococcus pluvialis* (pH 7.3) (Dolganyuk *et al*., 2020) and *Scenedesmus* sp. BHU1 (Singh *et al*., 2023). Qiu *et al*. (2017) have shown that extreme pH values, both acidic and alkaline, can induce oxidative stress and elevate MDA concentration in microalgal cells. At acidic pH levels, lipids' protonation and membrane integrity disruption can occur, promoting lipid peroxidation and MDA accumulation. Conversely, alkaline pH conditions can stimulate ROS production and oxidative damage, increasing lipid peroxidation and MDA levels in microalgae. This is well correlated with the percentage of ion leakage obtained in this study, where the highest ion leakage was noticed in cells grown at acidic pH (pH 5.0) (Fig. 3c). On the other hand, varying the pH did not significantly alter the H_2O_2 concentrations in *T. chui* except in control (pH 8). Microalgae, like most cells, are sensitive to pH changes, as pH directly influences enzyme function and metabolic reactions. When pH deviates from the optimal/control value (pH 8), enzymes involved in vital cellular processes may become less efficient or even inactivated. This inefficiency can stress the cell, causing a metabolic imbalance that increases the generation of H_2O_2 and alters metabolic pathways (Sies, 2017).

Microalgae require light for photosynthesis, which converts light energy into chemical energy, contributing to the cellular energy supply and cell structure formation (Chen *et al*., 2023). However, excessive light can lead to photo-oxidative stress, producing ROS, which can damage cellular components, including lipids (Nzayisenga *et al*., 2020). Conversely, insufficient light can limit photosynthesis and growth. Therefore, an optimal light intensity range exists for each microalgal species, controlling the formation of ROS production and regulating the normal physiological process and cell structure (Gorelova *et al*., 2019). This current study proposed that MDA and ion leakage were significantly accumulated in the dark condition at 1.9 and 1.4-fold higher than the control, respectively. It is postulated that when *T. chui* is grown in the dark (Figure 2d), photosynthesis does not occur, so the generation of ROS via light-driven processes is reduced. However, other metabolic pathways, including respiration and cellular processes, continue to produce ROS (Toro & Pinto, 2015). This metabolic shift can result in the accumulation of metabolic intermediates and by-products, including ROS, which can induce lipid peroxidation. Another possible fact is that the cells adjust their lipid metabolism in response to light availability. They may undergo changes in lipid composition, such as an increase in unsaturated fatty acids, which are more susceptible to peroxidation, thus inducing higher MDA accumulation and membrane permeability in dark-grown microalgae (Fu *et al*., 2022). The absence of light also means that the microalgae are unable to activate their light-dependent antioxidant systems efficiently. As a result, ROS can accumulate, leading to lipid peroxidation and higher levels of MDA. The lack of light-driven electron transport reduces the capacity to detoxify ROS, leading to more significant oxidative damage. Ion leakage is a direct consequence of membrane damage, and it is often used as an indicator of membrane integrity. In the dark, increased oxidative stress leads to compromised cell membranes, causing ions to leak out of the cells. Therefore, both MDA and ion leakage are high under dark conditions due to the inability to mitigate oxidative stress effectively.

The lowest MDA concentration and ion leakage were observed in cells under the highest light intensity (600 lux) in contrast to the results obtained in *Chlorella* sp. HL studied by Liu *et al*. (2022). Under high light intensities (600 lux), photosynthesis is more active, which leads to an increased production of ROS. However, in contrast to dark conditions, the increased light intensity might also activate *T. chui* antioxidant defense systems, which reduce ROS levels, thus preventing extensive membrane damage and lipid peroxidation. As a result, MDA concentrations and ion leakage are lower in high light intensity because the microalgae

can effectively neutralize ROS and protect the cell membranes from oxidative damage.

H_2O_2 production, however, increased in higher light intensity and peaked at 600 lux. The results were supported by Córdova *et al*. (2018). They reported an increase in light intensity during *C. sorokiniana* growth may promote photo-inhibition and allow the synthesis of specific compounds that change the overall efficiency of the organism (Pagels *et al*., 2021). H_2O_2 is a type of ROS that accumulates when oxidative stress exceeds the cell's ability to neutralize it. In high-light conditions, while more ROS are generated due to increased photosynthetic activity, microalgae often activate antioxidant mechanisms to keep H_2O_2 concentrations in check. However, if light intensity is too high, or if antioxidant capacity is overwhelmed, H_2O_2 concentrations can rise. In the dark, the lack of photosynthesis means that H_2O_2 production from light-driven processes is minimized. This leads to contrasting results for H_2O_2 where dark conditions result in lower production of H_2O_2.

Changes in the enzymatic antioxidant activities

Microalgae are recognised as promising sources of antioxidants due to their ability to produce compounds that scavenge ROS. These compounds include phenols, vitamins (primarily vitamin C), and pigments such as carotenoids and chlorophylls, along with enzymes like SOD, GR, CAT, glutathione peroxidase (GSH-Px), and APX (López-Hernandez *et al*., 2020). These antioxidants play crucial roles in protecting cells from oxidative damage (Pagels *et al*., 2021). For instance, SOD acts as the first line of defence by converting superoxide anions ($O_2^{\bullet-}$) into H_2O_2 and O_2. At the same time, other enzymes like CAT and peroxidases neutralise H_2O_2 produced during various metabolic processes (Noctor & Foyer, 2016). This antioxidant defence system helps microalgae mitigate oxidative stress and maintain cellular homeostasis.

Alterations in the nutrient composition of the growth medium can trigger the generation ROS within the cell, prompting an antioxidant response to restore balance (Gauthier *et al*., 2020). *T. chui* exhibited higher CAT and APX-specific activities in F/8 strength media than in other media (Table 2). The activities were at 1.5 and 1.7-fold higher compared to their respective controls. Higher CAT and APX produced might enhance the ability of this species to mitigate organelle damage instigated by ROS, as observed in (Table 1). However, high CAT and APX in F/8 do not correspond to a lower H_2O_2 obtained in this study. Under nutrient-limited conditions, *T. chui* may undergo stress-related metabolic shifts, such as increased photorespiration or the activation of alternative respiratory pathways, both of which can inadvertently lead to higher H_2O_2 production. This surge in ROS generation serves as a stress signal, prompting the upregulation of CAT and APX (Hasanuzzaman *et al*., 2020). However, if the ROS signaling remains consistently elevated due to prolonged nutrient deficiency, the cells may experience sustained oxidative stress. Consequently, H_2O_2 levels can remain high, even with increased CAT and APX activity, as the antioxidant defences may struggle to counterbalance the ongoing ROS production (Sachdev *et al*., 2021). CAT and APX catalyse the conversion of H_2O_2 produced by the action of SOD into H_2O and O_2. One CAT molecule can catalyze the conversion of millions of H_2O_2 molecules in less than a minute. Moreover, APX exhibits a greater affinity for H_2O_2 than CAT, underscoring its significance in scavenging H_2O_2 molecules. Together, these enzymes function cooperatively to mitigate lipid peroxidation and protect against membrane damage (Nimse & Pal, 2015; Ighodaro & Akinloye, 2018). Ruiz-Domínguez *et al*. (2015) and Proch´azkov´a *et al*. (2014) also demonstrated that nutrient limitation generally induced the activities of CAT and APX in eukaryotic green microalgae, *Acutodesmus dimorphus* and *Coccomyxa* sp. indicating the connection between nutrient deprivation and oxidative stress in microalgae. Interestingly, gPOD was 9-fold higher in full-strength media than the control. It was observed that full-strength media could induce a higher growth rate in *T. chui*, thus increasing cell density (Norhayati *et al*., 2023). Cell activities and cellular processes include photosynthesis, enzymatic reactions, and other reactions caused by cellular compounds, thus inducing higher MDA, H_2O_2, and membrane leakage in the cells and triggering higher gPOD activities to protect the cells.

Adding sugars to the culture medium has been shown to enhance microalgae growth. Typically, disaccharides like sucrose are not readily utilized by green microalgae. However, sucrose serves as an effective carbon source for the growth of *T. chui*, indicating that *T. chui* possesses efficient cellular mechanisms for metabolizing sucrose to support its growth and metabolic activities (Norhayati *et al*., 2023). Similar findings have been reported in certain green microalgae species such as *Tretadesmus dimorphus* (Bajwa *et al*., 2018), *Haematococcus lacustris* (Du *et al*., 2021) and *C. vulgaris* (Rajivgandhi *et al*., 2022). In this study, higher H_2O_2 and ion leakage led to higher CAT and APX-specific activities in sucrose starvation media. Higher H_2O_2 levels and ion leakage might serve as signals for *T. chui* to activate their antioxidant defence mechanisms, including the upregulation of CAT and APX activities. By increasing the activities of these enzymes, the cells can effectively detoxify H_2O_2 and mitigate oxidative damage, thereby enhancing their tolerance to oxidative stress conditions. Alternatively, gPOD was activated at low sucrose concentration (10 g/L) and was significantly suppressed at other sucrose concentrations as well as in cells without sucrose supplementation. Higher gPOD activity under low sucrose concentration can be attributed to the plant's response to oxidative stress, activation of defence mechanisms, redox signalling, and metabolic regulation in this species. An emerging concept suggests that sucrose plays a role in classic antioxidative mechanisms indirectly and is also implicated in directly scavenging ROS within various organelles, thereby aiding in abiotic stress tolerance. Additionally, it is anticipated that there is an interaction between ROS and sugar signaling pathways, indicating that sugars operate within an integrated cellular redox network (Keunen *et al*., 2013).

The pH of the culture medium significantly impacts the activity of the studied antioxidant enzymes. CAT, APX, and gPOD activities varied across different pH levels. CAT activity peaked at alkaline pH (pH 8.0-9.0) and reached its lowest levels between pH 5 and 7. Conversely, APX activity was highest at pH 6, 7, and 9, while it was lowest at pH 5 and 8. gPOD activity showed its highest level at pH 9, with considerably lower activity observed at lower pH values (Table 2). These enzymes, CAT, APX, and gPOD, play crucial roles in protecting algal cells against peroxidation and maintaining cellular redox balance. The concurrent elevation in their production suggests a collaborative function in ROS detoxification at alkaline pH. These findings are consistent with the study conducted by Ismaiel *et al*. (2016) on the blue-green alga *Spirulina (Arthrospira) platensis*. The reduced activity at lower pH levels may be attributed to cells primarily relying on other cellular antioxidants for ROS detoxification rather than these enzymatic antioxidants (Sakihama *et al*., 2002).

Table 2. Catalase (CAT), ascorbate peroxidase (APX) and guaiacol peroxidase (gPOD) specific activities of *Tetraselmis chui* in different media strength, sucrose concentrations, pH of the media and light intensities

Treatments		Enzymatic antioxidants (units/mg protein)		
		CAT	APX	gPOD
Media strength	F/2 (control)	1.525 ± 0.099^{b}	0.155 ± 0.010^{c}	0.0011 ± 0.0001^{b}
	F	0.709 ± 0.061^{c}	0.098 ± 0.003^{d}	0.0093 ± 0.0007^{a}
	F/4	0.241 ± 0.053^{d}	0.177 ± 0.011^{b}	0.0008 ± 0.0001^{b}
	F/8	2.245 ± 0.070^{a}	0.266 ± 0.008^{a}	0.0008 ± 0.0001^{b}
Sucrose (g/L)	0 (control)	1.525 ± 0.099^{a}	0.155± 0.010a	0.0011 ± 0.0001^{c,d}
	10	1.237 ± 0.067^{a}	0.108 ± 0.013^{b}	0.0063 ± 0.0007^{a}
	20	1.208 ± 0.106^{b,c}	0.073 ± 0.010^{c}	0.0006 ± 0.0001^{e}
	30	1.135 ± 0.059^{c}	0.021 ± 0.007^{d}	0.0014 ± 0.0001^{c,d}
	40	0.847 ± 0.050^{d}	0.013 ± 0.004^{d}	0.0008 ± 0.0001^{d,e}
	50	0.873 ± 0.035^{d}	0.018 ± 0.005^{d}	0.0024 ± 0.0003^{b}
pH	5	0.151 ± 0.031^{c}	0.141 ± 0.011^{b}	0.0028 ± 0.0003^{b}
	6	0.146 ± 0.014^{c}	0.239 ± 0.011^{a}	0.0032 ± 0.0002^{b}
	7	0.149 ± 0.015^{c}	0.221 ± 0.017^{a}	0.0023 ± 0.0002^{b}
	8 (control)	1.525 ± 0.099^{b}	0.155 ± 0.010^{b}	0.0011 ± 0.0001^{c}
	9	2.142 ± 0.119^{a}	0.233 ± 0.020^{a}	0.0454 ± 0.0017^{a}
Light intensity (lux)	0	0.514 ± 0.034^{c}	0.159 ± 0.009^{a}	0.0003 ± 0.0000^{c}
	150	3.093 ± 0.160^{a}	0.092 ± 0.004^{c}	0.2071 ± 0.0110^{a}
	300 (control)	1.525 ± 0.099^{b}	0.155 ± 0.010^{a}	0.0011 ± 0.0001^{c}
	450	0.418 ± 0.048^{c}	0.027 ± 0.002^{d}	0.0034 ± 0.0005^{c}
	600	1.453 ± 0.085^{b}	0.108 ± 0.009^{b}	0.0468 ± 0.0026^{b}

Data are mean ± SE with different superscript letters in a column are significantly different (P<0.05).

Photosynthetic organisms, such as green microalgae, depend on light to facilitate carbon fixation through photosynthesis and to produce biomass. It's widely recognized that excessive light intensities can induce harm to both plants and animals, leading to the formation of photochemical smog, bleaching of vegetation, and cellular photodamage (Singh *et al*., 2019). To counteract such damage, cells employ various defensive mechanisms, among which is an antioxidant defense system (Rastogi *et al*., 2019). In *T. chui*, enzymatic antioxidants CAT, APX, and gPOD all had differing outcomes due to the different light intensities. CAT and gPOD activities were significantly higher in cells grown under 150 lux, 2.1-fold and 210-fold higher than the control. At 150 lux, CAT rapidly converts high H_2O_2 concentrations into H_2O and O_2, offering a quick response without requiring reducing agents like ascorbate, which APX depends on (Anwar *et al*., 2024). gPOD complements this by detoxifying other peroxide forms, potentially working alongside CAT to effectively manage ROS at this light intensity. This strategy enables *T. chui* to conserve energy, as APX operates at a low, steady-state without requiring further upregulation. This reflects a coordinated effort among the antioxidant enzymes, where APX, CAT, and gPOD work together synergistically to detoxify ROS. By effectively managing the levels of different ROS, these enzymes optimize the cellular response to oxidative stress, ensuring a balanced and efficient detoxification process tailored to the specific oxidative challenges encountered by the cells.

Conversely, APX activities were enhanced in both the control and cells grown in darkness. In both conditions, *T. chui* cells appear to favour APX to neutralize ROS, indicating a shift in their antioxidant strategy to prioritize this enzyme for managing ROS. In the darkness, the reduction in photosynthetic activity diminishes the typical ROS generation, altering the cellular redox state and allowing APX to play a more prominent role in scavenging H_2O_2 using ascorbate as an electron donor (Rezayian *et al*., 2019). This preference for APX suggests that the cells aim to maintain redox homeostasis and prevent oxidative damage, even under low-light conditions. CAT and gPOD are also effective in neutralizing ROS (Anwar *et al*., 2024), the reliance on APX in these scenarios highlights its importance in managing oxidative stress when other sources of reducing power are limited.

This observation suggests that variations in light intensity, whether lower or higher, could disrupt the balance of the ROS metabolism system in *T. chui*, potentially leading to damage to cell membranes, proteins, and lipids. The increase in CAT and gPOD activities corresponded to changes in MDA and ion leakage production under different light intensities, indicating that inadequate or excessive light intensity could trigger the activation of *T. chui*'s antioxidant enzyme system to counteract oxidative stress (Ranjan *et al*., 2021). Lower activities observed under certain light intensities might be compensated for by other non-enzymatic antioxidant defences, including glutathione (GSH), tocopherols, ascorbate (AsA), ascorbic acid (AA), flavonoids, polyphenols, carotenoids, as well as the production of mycosporine amino acids (MAA) and proline in response to stress conditions (Suh *et al*., 2014; Hamed *et al*., 2017; Chokshi *et al*., 2017). It can be inferred that both enzymatic and non-enzymatic antioxidant mechanisms collaborate in microalgae to mitigate ROS levels, while elevated levels of ROS and MDA content indicate an imbalance in antioxidant systems under conditions of high light intensity.

CONCLUSION

The present study shows that *T. chui* exhibited different sensitivity to the manipulation of cultural growth conditions through the responses in MDA concentration, H_2O_2, and ion leakage produced by the cells. A unique cellular defensive antioxidant response includes the CAT, APX, and gPOD produced by this species, varied depending on the stressors. It can be seen that all enzymatic antioxidants studied work in a coordinated fashion by balancing their roles for eliminating the oxidative stress markers in their cells.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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