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Research Article

Growth induction in *Spirulina platensis* under SiO_2 nanoparticles: Function of antioxidant enzymes, phycobiliproteins, and secondary metabolites

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Abstract

In the current study, the effect of different *silicon dioxide nanoparticles* (SiO_2 NPs) concentrations was investigated on biomass yield, chlorophyll (Chl *a*) pigment, antioxidant enzymes, and defence metabolites of *Spirulina platensis* alga. The alga sample was cultured in the Zarrouk medium supplemented with various SiO_2 NPS concentrations (0, 50, 100, and 150 mg L^{-1}) for two weeks. Results showed that SiO_2 NPs at 100 mg L^{-1} increased significantly dry weight, specific growth rate, and Chl pigment, possibly due to the induction of protein content and antioxidant enzyme activities of catalase and peroxidase. Secondary metabolites such as phycobiliprotein, phycocyanin, allophycocyanin, phycoerythrin, carotenoids, and extracellular polymeric substances increased upon all concentrations of SiO_2 NP, although their contents were more increased under 100 mg L^{-1} treatment. Treatment of SiO_2 NP at 150 mg L^{-1} induced toxicity in the algal growth along with the accumulation of H_2O_2, inhibition of antioxidant enzyme activities, and decline in the content of secondary metabolites. The findings suggest that 100 mg L^{-1} SiO_2NPs is an optimum concentration for sustainable production of *S. platensis*, and may act as an elicitor to stimulate antioxidant metabolites for suppressing oxidative injuries.

Keywords: Carotenoid, Chlorophyll, SiO_2 NPs, Phycobiliprotein, *Spirulina platensis*

Introduction

Today, microalgae cultivation has paid more attention due to their value biomass, which is a rich source of biologically active compounds and macromolecules such as fatty acids, carbohydrates, proteins, carotenoids (Car), and antioxidants (Khorshidi *et al*., 2022). *Arthrospira platensis* (commonly called *Spirulina platensis*) is a multicellular, filamentous, and blue-green microalga belonging to the Oscillatoriaceae family and widely found in tropical alkaline lakes (Gershwin and Belay, 2007; Gupta *et al*., 2013). *Spirulina* is a valuable source of protein (approximately 60-70% of dry weight) and also contains Chl pigment, carbohydrates, fatty acids, and high-valued secondary metabolites, including carotenoids, phycobiliproteins, and phenolic compounds, which can be used in pharmacological, cosmetological, and nutraceutical industries (Markou *et al*., 2012; Beheshtipour *et al*., 2012; Spolaore *et al*., 2006). It has been reported that *S. platensis* extract has various biological activities, including anti-inflammatory, antioxidant, anticarcinogenic (Tajvidi *et al*., 2021; Konickova *et al*., 2014), and neuroprotective effects (Haider *et al*., 2021). Moreover, *Spirulina* produces extracellular polymeric substances (EPSs), and its polysaccharide moiety has biological activities such as antibacterial, antiviral, and anticoagulant agents (Challouf *et al*., 2011; Rechter *et al*., 2006). Several studies have reported that various environmental factors and the composition of the cultural medium can influence the production of algal biomass and bioactive compounds (Khorshidi *et al*., 2022; Zhang *et al*., 2019). Therefore, obtaining the optimal vegetative-growth condition will be so valuable to access sustainable production of *spirulina*.

Nanoparticles (NPs) are particle materials with size ranges between 1 and 100 nm. NPs contain unique physicochemical properties such as a high surface-to-volume ratio, small size, and specific physical and chemical properties, which cause them to be widely applied in industry, medicine, plant biology, agriculture, etc. (Khan *et al*., 2019). Silicon (Si) is not an essential nutrient for alga and/or plant culture, but several studies have reported that it has beneficial impacts on many species by increasing nutrient availability (Pavlovic *et al*., 2021). It has many biotechnological applications such as cancer therapy, DNA transfection, drug delivery, and enzyme immobilization (Santos *et al*., 2014; Slowing *et al*., 2008; Fenollosa *et al*., 2014). Researchers have reported that metal-based NPs can transport from the cell wall and plasma membrane into the cells and cause various physiological and biochemical responses, which depend on several factors

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such as NPs size and concentration, solubility, kind of species, etc. (Khan *et al*., 2019; Hassanpour *et al*., 2021). The toxicity impact of these particles can result from their ability to induce oxidative stress and damage to the living tissues (Min *et al*., 2023). For example, in *Chlorella kessleri*, SiO_2 NPs decreased the Chl content and cell proliferation compared to the control group (Fujiwara *et al*., 2008). Phenolic metabolites secreted to the extracellular medium induced in *A. platensis* and *Haematococcus pluvialis* after treatment with TiO_2NPs (Comotto *et al*., 2014). Artemisinin content changed significantly in *Artemisia annua* L. hairy root cultures after AgNPs application for 20 days (Zhang *et al*., 2013). H_2O_2 production and lipid peroxidation stimulated in *Matricaria chamomilla* seedlings under Fe_2O_3 NPs (Rastegaran *et al*., 2022). Moreover, antioxidant enzyme activities revealed different responses under NPs in a dose-dependent manner (Torabzadeh *et al*., 2019; Ahmadi *et al*., 2020). There is no data about the impact of SiO_2 NPs on the growth responses and secondary metabolites in *S. platensis* cyanobacteria, and their mechanisms are not clear. Thus, the purpose of this study was to investigate the effect of SiO_2 NPs at different concentrations on biomass production, Chl pigment, and secondary metabolites of Car and EPS in *S. platensis* alga. Data from this study will aid us in elevating our understanding of the underlying physiological and biochemical mechanisms of the toxic and suitable concentrations of nanoparticles in algae.

Material and methods

Materials: All chemicals, solvents, and standards used in this study were purchased from Merck and Sigma-Aldrich Chemical Companies.

Microalga growth conditions and NPs treatment: *S. platensis* MGH-1 was obtained from the Iranian Research Organization for Science and Technology (accession no. of MW628543) and grown in Zarrouk's medium (1966). The initial biomass concentration of *S. platensis* MGH-1 (0.1 g L^{-1}) in the logarithmic growth phase was inoculated in the Zarrouk medium supplemented with various SiO_2 NPS concentrations (0, 50, 100, and 150 mg L^{-1}) and afterward placed under the condition of 25 ± 2 °C, pH 9, 70 µmol photons m^{-2} s^{-1} white LED-light intensity, and 200 mL min^{-1} airflow (Iwaki filter, 0.2 µm pore size). The NPs were homogenized in deionized water and dimethyl sulfoxide 0.05% with an IKA ULTRA-TURRAX Disperser (T18, German) at 20000 rpm for 30s and then kept in water in an ultrasonic bath for 25 min. After the preparation of Zarrouk medium, different NPs concentrations were added to the medium at 40-45 ºC. After 15 days of cultivation, the samples were harvested at three replications by centrifugation (8,000 × g for 5 min), washed twice with the deionized water, and then the pellets were stored at -80 ºC for the biochemical analyses.

Growth parameter: For determination of the algal growth, based on the time and NPs concentration, *S. platensis* MGH-1 was inoculated in Zarrouk medium. The alga growth curve was evaluated by weighing the biomass every three days for 18 days. After centrifugation (8,000 × g for 5 min) of alga suspension, fresh pellets were washed twice with the distilled water to remove the salt and then dried overnight at 70 °C to access the dry weight. The optical density of each sample was also obtained at 750 nm and the following formula is used to calculate the maximum specific growth rate (µ) Costa *et al*. (2002):

$\mu = (LnN_2 - LnN_1)/(t_2 - t_1)$.

Where N_2 was the dry weight measured at day t_2, N_1 was the dry weight measured at day t_1.

Photosynthetic pigment measurement: For determination of Chl *a*, 10 mL of algal suspension of control and each treatment was centrifuged at 10,000 × *g* for 5 min, washed twice with distilled water, and pellets (ca. 50 mg) were extracted with 5 mL of 80% (v/v) acetone for 24 h at 4.0 °C (Marker, 1972). After the removal of the cell debris by centrifugation, the absorbance of the supernatant was recorded at 461 and 664 nm, and the Chl and Car concentrations were determined using the following equations (Chamovitz *et al*., 1993).

Chl a (µg mL^{-1}) = 13.14 × OD664

Car (µg mL^{-1}) = [OD461 − (0.046 × OD664)] × 4

Phycobiliprotein assay: The frozen samples (100 mg) were homogenized in 50 mM phosphate buffer (10 mL, pH 6.8) and then centrifuged at 8000 × g for 10 min. The absorption spectrum was measured with a spectrophotometer (UNICO, 2802, USA) at wavelengths of 620, 650, and 565 nm. The concentration of phycobilins was calculated according to the formula given by Bryant *et al*. (1976).

Phycobiliproteins (total phycobilins) = phycocyanin (PC) + allophycocyanin (APC) + phycoerythrin (PE)

PC = (OD_{620}-0.72 OD_{650}) / 6.29 (mg mL^{-1} of extract)

APC = (OD_{650}-0.191 OD_{620}) / 5.79 (mg mL^{-1} of extract)

PE = (OD_{565}-2.41 (PC) -1.4 (APC) / 13.02 (mg mL^{-1} of extract)

H_2O_2 content: The H_2O_2 evaluation was quantified by the procedure described by Velikova *et al*. (2000). The extraction of fresh algae cells (15 mg) was conducted in 2 mL trichloroacetic acid 0.1% (w/v) in an ice bath. After centrifugation at 12,000 × g for 15 min, the extract (0.5 mL) was added to 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1 M KI. The absorbance of the supernatant was measured at 390 nm and H_2O_2 content was calculated using a standard curve.

Protein content and antioxidant enzyme activities: The frozen samples (0.1 g) were homogenized in 2.5 mL cold Tris-HCl (1 M, pH 6.8), as an extraction buffer and after centrifugation (12000 × g, 4 °C for 10 min), the extracts were used for protein and antioxidant enzymes assays. Total protein content was evaluated by Bradford's procedure (1976), using bovine serum albumin (BSA) as standard. The absorbance was

measured at 595 nm.

Peroxidase (POX) activity assays were evaluated spectrophotometrically (UNICO, UV/VIS, 4802, USA) based on the procedure of Abeles and Biles (1991). The reaction mixture was 2 mL of acetate buffer (0.2 M, pH 4.8), 0.2 mL H_2O_2 (3%), 0.2 ml benzidine (20 mM), and enzyme extract (30 µL). The absorbance of the reaction solution was measured using a spectrophotometer (UNICO UV/Vis, 4802, USA) at 530 nm. The POX activity was specified as 1 µmol of benzidine oxidized per min per mg protein. Catalase activity assay was measured as the rate of H_2O_2 decomposition, which was measured by decreasing the absorbance at 240 nm (Aebi, 1984). The reaction mixture contained 0.625 mL of sodium phosphate buffer (50 mM, pH 7.0), 0.075 mL H_2O_2 (3%), and 5 µL enzyme extract. The enzyme activity was defined as 1 µmol of H_2O_2 oxidized per min per mg protein.

Extracellular polymeric substance (EPS): EPS content was determined using the method described by Lewin (1956). Briefly, the total recovered filtrates of each experimental culture were used for EPS content determination by evaporation of water in open crucibles in a forced-air oven for 24 h at 105 °C. The obtained dried matter was incinerated in a muffle furnace at 550 ± 5 °C until white ash remains. The change in the weight of the crucibles, containing EPS-free water and -free ashes, was considered as the EPS content.

Experiment analyses were performed by the One-way ANOVA (SPSS 21.0). The analyses were conducted with five replications for growth and three replications for the biochemical analyses. Comparisons of the data were performed with the Duncan test at the level of $P \leq 0.05$. Principal component analysis (PCA) was carried out through the XLSTAT 2021.2.2 software.

Results

Alga growth rate and Chl pigment: The growth curve of *S. platensis* showed that there was no actual lag phase at the initial days of cultivation (Fig. 1a). Cells were in the logarithmic phase until the 12th day with the highest dry weight (0.76 ± 0.028 g L^{-1}). Then cells entered the stationary phase until the 18th day and their growth did not change considerably compared to the 12th day. Moreover, SiO_2 NPs application at 50 and 100 mg L^{-1} increased significantly the dry weight and specific growth rate (SGR) of *S. platensis* as compared to the control ($P \leq 0.05$), while at higher concentration (150 mg L^{-1}) it decreased the algal growth (Fig. 1b, Table 1).

Chl *a* content increased at 100 mg L^{-1} of SiO_2 NPs with a 51.7 % rise as compared to the control, while its content decreased under the higher concentration of SiO_2 NPs (150 mg L^{-1}) (Fig. 1c). Car content increased markedly at 150 mg L^{-1} SiO_2 NPs with a 82.6% rise as compared to the control (Fig. 1d).

Phycobiliproteins: Phycobiliproteins displayed an increasing trend compared to the control, especially at treatments of 50 and 100 mg L^{-1} SiO_2 NPs (Fig. 2). As the amount of phycocyanin showed a rise of 3.96 and 3.56-folds at 50 and 100 mg L^{-1} SiO_2 NPs as compared to the control (Fig. 2a). The highest content of phycoerythrin, allophycocyanin, and total phycobilins was observed at the treatment of 100 mg L^{-1} SiO_2 NPs with the 5.4, 1.7, and 2.1-fold rise compared to the control, respectively (Fig. 2 b, c, and d).

H_2O_2 and protein contents: The H_2O_2 level showed a considerable alteration at 100 and 150 mg L^{-1} SiO_2 NPs treatments with 46.1 and 109.2% enhancement compared to the control, respectively (Table 1). However, there was no significant change in H_2O_2 content between SiO_2 NPs at 50 mg L^{-1} and control conditions.

Protein content changed variously under SiO_2 NPs treatments. Its content didn't change under 50 mg L^{-1} SiO_2 NPs, increased significantly (*ca*. 41.8%, $P \leq 0.05$) under 100 mg L^{-1} SiO_2 NPs, and decreased slightly under 150 mg L^{-1} SiO_2 NPs.

Antioxidant enzyme activities: Antioxidant enzyme activities of CAT and POX showed an increasing trend under 50 and 100 mg L^{-1} SiO_2 NPs (Table 1). As the highest CAT and POX activities were observed at 100 mg L^{-1} SiO_2 NPs with 91.7 and 54.1% increase compared to the control. However, enzyme activities didn't change significantly under the higher concentration of SiO_2 NPs 150 mg L^{-1} compared to the control.

Extracellular polymeric substances (EPS): EPS content increased slightly (17.1%) at 150 mg L^{-1} SiO_2 NPs, and augmented under 50 and 100 mg L^{-1} SiO_2 NPs with 24.3 and 53.6% enhancement compared to the control (Fig. 3).

Discussion

The present study was performed to clear the underlying mechanisms of potential and toxicity impacts of different SiO_2 NPs concentrations on the *S. platensis* growth and defense metabolites. Cell growth (biomass concentration curve) of *S. platensis* in the Zarrouk medium showed that alga cells from 1 to 12 days of culture are in the logarithmic phase, and then cells enter the stationary phase (Fig. 1a). The largest cellular growth was obtained at day of 12 of culture (0.76 g DW L^{-1}), so this time was selected to harvest the samples after application of SiO_2 NPs treatments. As shown in Fig. 1b and Table 1, SiO_2 NPs at 100 mg L^{-1} enhanced markedly *S. platensis* dry weight and specific growth rate and this concentration can be considered a suitable concentration to improve the alga growth. The PCA graph showed the results from Chl *a*, protein, antioxidant enzymes, SGR, and growth are closely loaded to F1 axis and are positively related together (Fig. 4). Algae typically have glycoproteins and polysaccharides in their cell walls. *S. platensis* cell wall is composed of multilayers of glucan and peptidoglycan polymers covered with a sheath of polysaccharides on the outside (Vaneykelenburg, 1977). These components can act as binding sites to promote the adsorption of

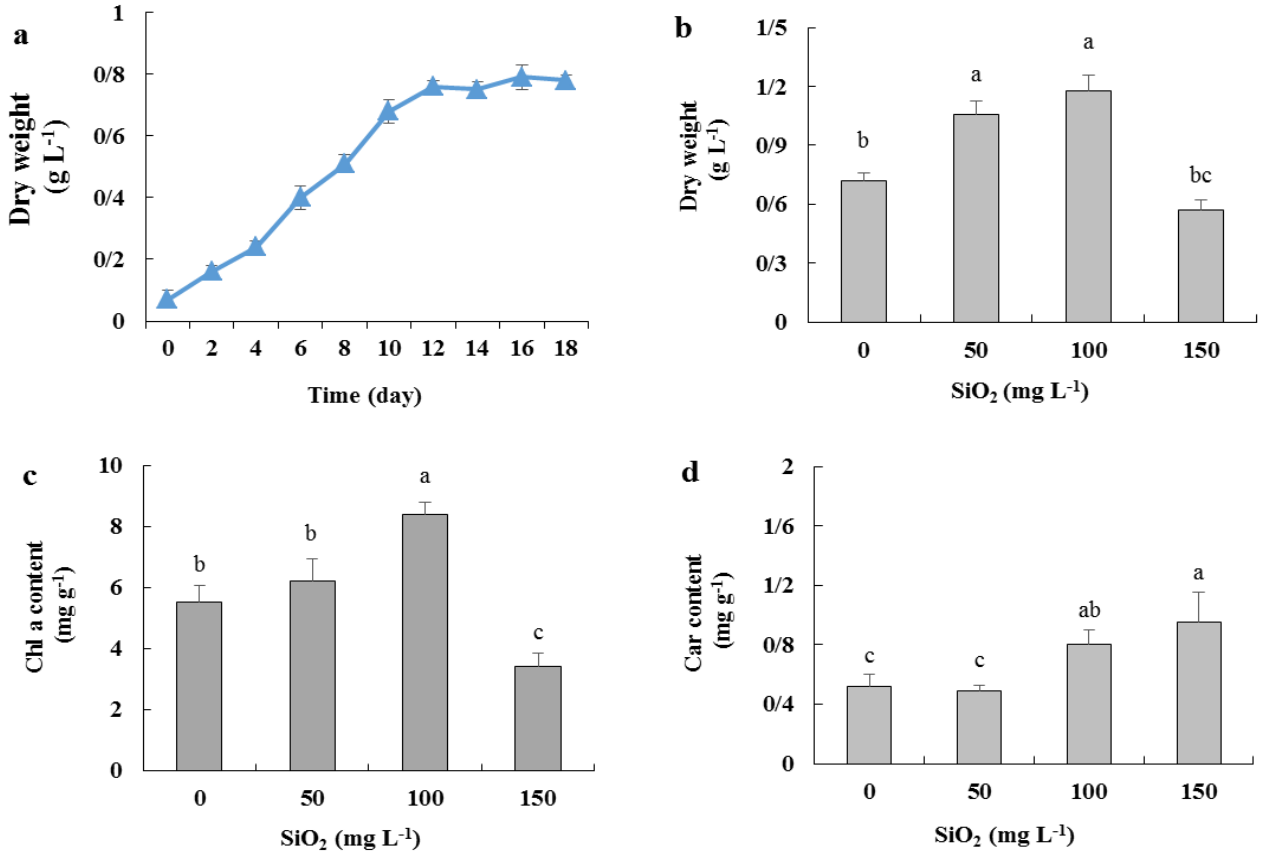

Figure 1. Growth curve after 18 days (a) and effect of different SiO_2 NPs concentrations on the biomass concentration (b), Chl *a* (c), and Car (d) in *S. platensis* alga. Values are given as mean ± SE (*n* = 5 for biomass and *n*=3 for pigments) in each group. Different letters present significant differences at P ≤ 0.05 (Duncan test).

Table 1. The effects of different SiO_2 NPs concentrations on the specific growth rate (SGR), H_2O_2 and protein contents, and enzyme activities of catalase (CAT) and peroxidase (POX) in *S. platensis*.

SiO_2 NPs (mg L^{-1})	SGR (day^{-1})	H_2O_2 (µmol g^{-1} FW)	Protein (mg g^{-1} FW)	CAT (µmol H_2O_2 min^{-1} mg^{-1} protein)	POX (µmol benzidine min^{-1} mg^{-1} protein)
0	0.073 ± 0.0021 ^{b}	179.8 ± 18.26 ^{c}	52.49 ± 2.28 ^{b}	3.26 ± 0.49 ^{c}	1.09 ± 0.06 ^{c}
50	0.094 ± 0.0038 ^{a}	188.4 ± 11.62 ^{c}	55.35 ± 3.92 ^{b}	5.08 ± 0.61 ^{b}	1.38 ± 0.091 ^{b}
100	0.098 ± 0.0044 ^{a}	252.6 ± 14.56 ^{b}	74.42 ± 3.85 ^{a}	6.25 ± 0.26 ^{a}	1.68 ± 0.106 ^{a}
150	0.065 ± 0.0026 ^{bc}	376.1 ± 15.29 ^{a}	43.61 ± 2.79 ^{bc}	3.48 ± 0.35 ^{c}	1.12 ± 0.072 ^{c}

Values are means ± SE of three replicates. Different letters indicate significant differences (P≤0.05) (Duncan test) and the same letters indicate no differences.

NPs (Navarro *et al*., 2008). After NPs entrance into the cytoplasm, they can contact with organelles such as chloroplasts, vacuoles, endoplasmic reticulum, golgi apparatus, and mitochondria, and significantly damage or alter their function and structures (Zhao *et al*., 2016), which may associate with alga species and/ or NPs size and concentrations. On the other hand, they can aggregate in the cytoplasm, influence the metabolism and reproductive function of the algal cell, and/ or transport to the DNA and cause upregulating or downregulating specific genes linked to the growth (Wu *et al*., 2018). In the present study, the higher concentration of SiO_2 NPs decreased the algal growth, which may be related to the low absorption of essential macro-and microelements, NPs aggregation in the cytoplasm, or injury in the organelle structures under toxicity NPs concentration (Tian *et al*., 2018; Hassanpour *et al*., 2021). Fujiwara *et al*. (2008) revealed the morphological changes and cellular deformation resulting from a collision with silica NPs at high concentrations.

Chl *a* is an important photosynthetic pigment responsible to absorb light energy and is located in the reaction center. Chl pigments can absorb light energy between 400 and 500 nm in the blue zone and 650 and 720 nm in the red zone (Chen and Blankenship, 2011). In this research, Chl *a* content increased considerably at 100 mg L^{-1} of SiO_2 NPs. It has been reported that Si elements can interact with the macro and micro-nutrient elements and affect the uptake and transport of nutrient elements, including magnesium, iron, nitrogen, calcium, etc. from the medium culture into cells (Pavlovic *et al*.,

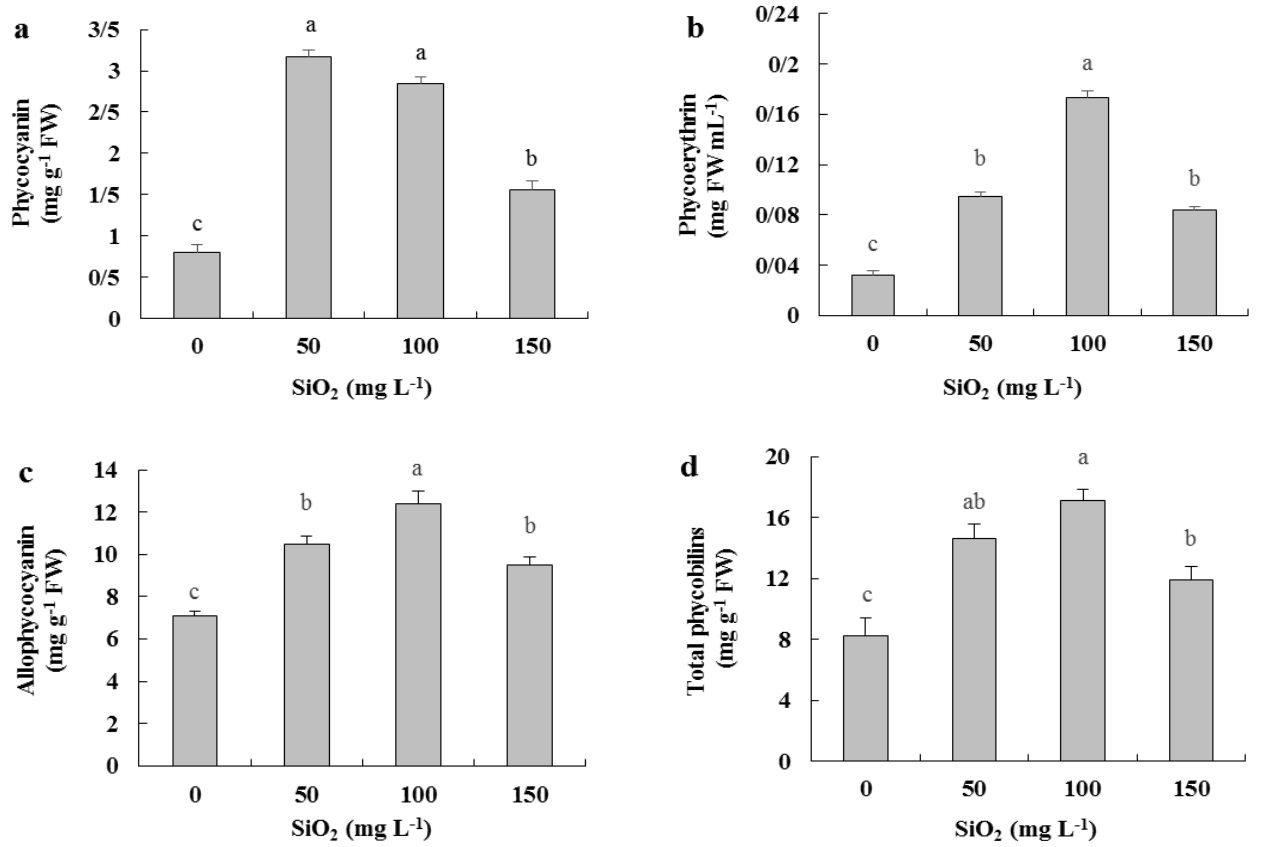

Figure 2. Effect of different SiO_2 NPs concentrations on the phycocyanin (a), phycoerythrin (b) allophycocyanin (c), and total phycobilin contents (d) in *S. platensis* alga. Values are given as mean ± SE (*n*=3) in each group. Different letters present significant differences at P ≤ 0.05 (Duncan test).

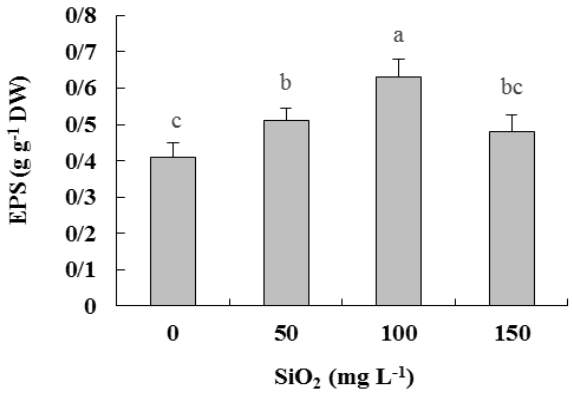

Figure 3. Effect of different SiO_2 NPs concentrations on the extracellular polymeric substance (EPS) in *S. platensis* alga. Values are means ± SE (n = 3) in each group. Different letters present significant differences at P ≤ 0.05) (Duncan test).

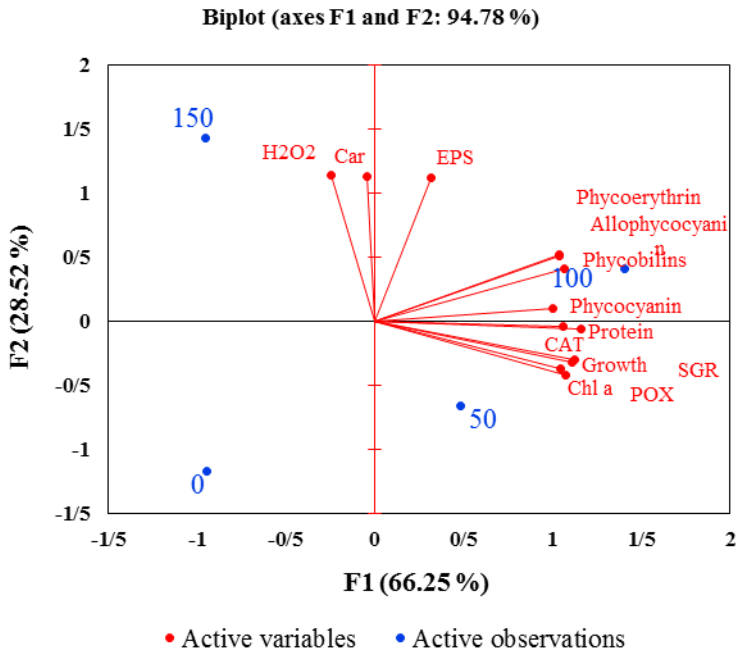

Figure 4. PCA analysis of the investigated parameters of *S. platensis* under SiO_2 NPs. Car-carotenoid, Chl-chlorophyll, EPS- extracellular polymeric substance, CAT-catalase, POX-peroxidase, SGR-specific growth rate

2021). Buchelt *et al*. (2020) stated that the impact of Si on the alleviation of Mg stress is mediated through the enhancement of Mg use efficiency. It seems that increased Chl content under SiO_2 NPs may be associated with more access to elements linked to Chl biosynthesis in *S. platentis*. On the other hand, the content of Chl *a*, as a main pigment in the reaction center decreased slightly under higher concentrations (150 mg L^{-1}). Ko *et al*. (2018) reported that the Chl content and alga growth declined considerably under ZnO NPs, which may be related to the chemical properties of NPs (such as the size, surface chemistry, dissolution of ions, etc.) or oxidative stress induced by these particles.

Phycobilisome (PBS) is a multiprotein complex in cyanobacteria and red algae, which efficiently captures light energy in the wavelength range of 450– 650 nm at green and yellow zones, and transfers them to the Chl molecules in the photosystem. The major component of PBS is phycobiliproteins (PBPs), which are divided into three main groups, including allophycocyanins, phycocyanins, and phycoerythrins (David *et al*., 2014). Besides its auxiliary function in light absorption of cyanobacterial cells, PBPs have several biological activities such as inhibition of oxidative stress, anti-inflammatory (Prabakaran *et al*., 2020), and anti-cancer activities (Kaur *et al*., 2020). In the present study, the content of allophycocyanin, phycocyanin, and phycoerythrin increased under SiO_2 NPs, especially at 100 mg L^{-1} treatment, and total PBPs reached the highest content (17.1 mg g^{-1} FW) in this treatment comparing to control (8.22 mg g^{-1} FW). PBPs content decreased at the treatment of 150 mg L^{-1} (11.9 mg g^{-1} FW) compared to 100 mg L^{-1}, although its content was higher than the control condition. Similarly, Cepoi *et al*. (2020) reported that the application of AgNPs (0.05 µM) increased the content of phycobilins in *S. platensis.* Phycocyanin isolated from *S. platensis* inhibited oxidative stress through the inhibition of NAD(P)H oxidase activity and radical superoxide production (Zheng *et al*., 2013). In this study, the enhancement of PBPs was most likely due to the protective function of PBPs on photosystems via reactive oxygen species (ROS) detoxification and/or its impact on the absorption of light energy under stress conditions.

H_2O_2 content increased at all concentrations of SiO_2 NPs in *S. platensis*, and treatment of 150 mg L^{-1} showed the highest level of this parameter. ROS are natural by-products of normal cell activity and participate in cellular signaling. ROS accumulation at a high level can induce deleterious impacts on cell division and growth, cell hemostasis, and finally, it results in oxidative stress (Ghalkhani *et al*., 2020). Sosan *et al*. (2016) stated that the induction of Ca^{2+} and ROS signaling by NPs can be conducted via the enhancement of Ca^{2+} permeable pores and oxidation of apoplastic L-ascorbic acid. In *Arabidopsis thaliana*, Mittler (2017) reported that the accumulation of cellular ROS is mediated through plasma membrane-bound NADPH oxidase enzymes. To minimize the oxidative damage, enzymatic and non-enzymatic defense machinery are activated in algal and plant cells to scavenge the excessive ROS (Khorshidi *et al*., 2022; Hassanpour and Ghanbarzadeh, 2021), which is in agreement with the results of the increase in antioxidant enzyme activities in this research. As the activity of POX and CAT enzymes increased under SiO_2 NPs, especially at 50 and 100 mg L^{-1} (Table 1). CAT activity was significantly elevated upon treatment

of wheat roots with CuO NPs (Dimkpa *et al*., 2012), which was related to cell membrane protection from lipid peroxidation. In this research, SiO_2 NP_S induced Car and EPS to mitigate the oxidative damage in *S. platensis*, and their content decreased with enhancing NPs concentrations (Fig. 3). In the PCA graph, the results of Car and EPS were highly closed F2 axis, and showed a positive relation with H_2O_2 level (Fig. 4). Car pigments are involved in preventing the photodamage of the photosynthetic machinery from excess light energy and also can act as an antioxidant metabolite to prevent the oxidative injuries. Moreover, the sulfated polysaccharide moiety of EPS has antioxidant activity (Zhang and Yi, 2022). EPSs have strong reductive groups (OH, C=C, C=O C–O–C groups) and also complex structure containing α-pyranose, which able them to scavenge superoxide and hydroxyl free radicals, and finally prevent oxidative damage (Zhang and Yi, 2022). In *Dunaliella*, Car content decreased with enhancing concentrations of silica NPs (Shariati and Shirazi, 2019), which was related to NPs agglomeration and reducing surface area to volume ratio.

However, increased content of Car and EPS under the suitable concentration of SiO_2 NPs in this study may state the induction of ROS signaling, antioxidant machinery, mitogen-activated protein kinase (MAPK) cascades, etc., which could lead to transcriptional reprogramming of secondary metabolism, including phycobilins, carotenoids, etc. (El-Saadony *et al*., 2022). MAPK cascade regulates the gene expression of antioxidative response and increases antioxidant enzyme activities to detoxify ROS and sustain ROS homeostasis under stress conditions (Nawaz *et al*., 2010).

Conclusion

The present study showed that the different SiO_2 NPs concentrations affected the growth and defense metabolites in a dose-dependent method. According to the obtained results, 100 mg L^{-1} of SiO_2 NPs promoted the algal growth and Chl *a* pigment by activation of antioxidant enzyme activities (CAT and POX) and protein contents. Also, defense metabolites, including phycobiliproteins, Car, and EPS contents induced in this treatment. Indeed, the antioxidant functions are induced under optimal concentrations of silica NPs. However, SiO_2 NPs at higher content (150 mg L^{-1}) induced toxicity (H_2O_2 as a marker of cellular damage), and accumulation of EPS and Car metabolites. The oxidative damage resulted in a decline in algal growth. Nevertheless, the underlying molecular mechanism of defense metabolites is unclear in *S. platensis* alga and needs to more investigate in the future.

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