Hindawi
Aquaculture Research
Volume 2023, Article ID 5967771, 8 pages
https://doi.org/10.1155/2023/5967771

Research Article

Effect of Phytohormone Kinetin on Cell Density, Photosynthetic Pigments, Antioxidant Enzymes, and Fatty Acid Composition of the Microalgae *Tetraselmis suecica*

Tahereh Asghari,[1] Nasrollah Ahmadifard,[1] and Mehdi Nikoo[2]

[1]*Department of Fisheries, Faculty of Natural Resources, Urmia University, Urmia, West Azerbaijan 5756151818, Iran*
[2]*Department of Pathobiology and Quality Control, Artemia and Aquaculture Research Institute, Urmia University, Urmia, West Azerbaijan 5717944514, Iran*

Correspondence should be addressed to Nasrollah Ahmadifard; n.ahmadifard@urmia.ac.ir

Received 14 January 2023; Revised 23 April 2023; Accepted 29 June 2023; Published 22 July 2023

Academic Editor: Femi Fawole

The effects of phytohormone Kinetin on cell density, photosynthetic pigments, antioxidant enzymes, and fatty acids composition of the microalgae *Tetraselmis suecica* were investigated. *T. suecica* was treated with different concentrations (0, 5, 10, 15, 20, and 25 mg/L) of Kinetin at optimal growth conditions (salinity 28 g/L, temperature 25°C, and 24 hr photoperiod). Results indicated an increase in cell density in all treatments compared to the control; however, it was not significant, except for the treatment, 15 mg/L ($p < 0.05$). The content of photosynthetic pigments and the activities of catalase and superoxide dismutase, measured after 10 days, significantly increased in the presence of Kinetin ($p < 0.05$). Total *n*-3 fatty acid was significantly higher at 25 mg/L of Kinetin. At 15 mg/L, arachidonic acid (ARA) and eicosapentaenoic acid (EPA) were significantly higher compared to the control ($p < 0.05$). Therefore, Kinetin could be useful for the production of the microalgae *Tetraselmis suecica* to increase growth, chemical composition, and antioxidant enzyme activity.

1. Introduction

The microalgae, *T. suecica* is a saltwater microscopic plant widely studied for its applications in aquaculture for larval culture, especially as live food [1]. *T. suecica* is rich in some valuable components such as lipids and proteins, which make it beneficial to fish nutrition. Dietary *T. suecica* decreased pathogenic bacteria in the intestine of *Fenneropenaeus indicus* shrimp [2]. Seaweed species have been used as food additives to increase growth and survival and disease resistance in *Nile tilapia* [3], *rabbit fish* [4], and *red tilapia* [5]. *T. suecica* contains 22.4% carbohydrate, 8% lipids, 48.7% proteins, 0.4% pigments (including lutein), vitamins A, C, E, and minerals including N, P, and K [6]. Lutein from *T. suecica* showed several health benefits including antioxidant activity and anti-inflammatory activities and protection against vascular and amnesia diseases [7]. Recently, it is shown that *T. suecica* can improve the quality of aquaculture discharged wastewater by absorbing soluble nutrients [8, 9].

Phytohormones are shown to increase the microalgae growth mainly through stimulating cell division and intensifying algae photosynthetic processes, increasing the number of cells and cell masses [7]. Phytohormones are a group of natural organic substances that increase growth and development of plants at low concentrations. There are different types of phytohormones, including auxins, cytokinins, abscisic acid, ethylene, and gibberellin [10]. Physiological functions of phytohormones have been studied widely in green algae and cyanobacteria [11–13]. Addition of phytohormone cytokinins reduced some of the adverse effects of microalgae culture under the conditions caused by nitrogen limitation [14] and increased growth rate and tolerance of algae under harsh environmental conditions [15–17]. Phytohormones generally prevent the formation of reactive oxygen species (ROS) by activating antioxidant enzymes, followed by

lowering cell damages induced by oxidative stress [18]. Particularly, cytokinins have been shown to enhance biogenesis and differentiation of chloroplasts, prevent leaf aging, while increasing nutrient absorption [19]. It increased cytokinins division and photosynthetic pigments [20]. Moreover, cytokinins prevent the destruction of chlorophyll and delay the decomposition of chlorophyll, thus enhancing amino acid absorption and protein storage in plants. Phytohormone, Kinetin belongs to the group of cytokinins, with a small molecule that can regulate cell division and growth in microalgae via N6 furfuryladenine [21–23]. Cytokinin is a purine product and its main form is Zeatin, which has two structures, trans and cis. In microalgae, it is mainly as cis structure [24]. By adding foreign phytohormones, the spread of ROS can be prevented by activating antioxidant enzymes and antioxidants. Therefore, it can limit cell damage caused by abiotic stress and increase the efficient storage of biochemical compounds in microalgae. So, by using plant hormones, abiotic stresses, growth, and metabolism of microalgae can be regulated. Owning to this work, not only the improvement of photosynthesis efficiency and fat density can be increased but also the stressful environmental conditions can be combated [25]. Therefore, the aims of this study investigated the effects of Kinetin on growth, photosynthetic pigment, antioxidant enzymes, and fatty acid composition of microalgae *Tetraselmis suecica*.

2. Materials and Methods

2.1. Chemicals and Reagents. The stock of *T. suecica* was kindly donated from Shrimp Research Institute (Bushehr, Iran). To cultivate this microalga, the Walne [26] culture medium was used. Stock solution of Kinetin (powder, 26-79-1, Sigma–Aldrich Chemical Co., St. Louis, MO, USA) was prepared by dissolve 21.5 mg of Kinetin in 1 mL of 1 N NaOH. Then, the volume was brought to 10 mL with molecular biology-grade water to get a final solution of 10 mM. Catalase and superoxide dismutase (SOD) kits were obtained from Navand Salamat Co. (Urmia, Iran).

2.2. Experimental Design. Phytohormone Kinetin at the concentrations of 0, 5, 10, 15, 20, and 25 mg/L in each of three replicates were added to the cell culture medium. The culture medium without the addition of Kinetin was used as the control. Algae cultivation was done in a medium made with 28 g/L salinity, temperature 25°C, and using a 24 hr photoperiod with continuous aeration. Growth was monitored daily for up to 10 days using a spectrophotometer (BioTek, Synergy HT, China) at 680 nm [27]. Algae were harvested when they reached logarithmic growth.

2.3. Measurement of Pigments. To measure the content of photosynthetic pigments, 5 mL of algal suspension (2.5×10^6 cell/mL) was taken from each treatment and centrifuged (SIGMA, 1-15PK, GERMANY) for 10 min at 8,000 × rpm and 4°C to obtain biomass. Then, 5 mL of 96% methanol was mixed with the obtained biomass, and homogenized for 5 min at 2,000 × rpm and 4°C. After the homogenizing, stored for 1 hr in darkness at refrigerator temperature. The amount of chlorophyll *a*, chlorophyll *b*, and total carotenoid was calculated based on the following formula [28]:

$$\begin{aligned}\text{Chlorophyll } a\ (\mu\text{g/mL}) &= \text{Chl} - \text{a}\ (\text{mg L}^{-1}) \\ &= 16.72\text{A}665.2 - 9.16\text{A}652.4,\end{aligned} \tag{1}$$

$$\begin{aligned}\text{Chlorophyll } b\ (\mu\text{g/mL}) &= \text{Chl} - \text{b}\ (\text{mg L}^{-1}) \\ &= 34.09\text{A}652.4 - 15.28\text{A}665.2,\end{aligned} \tag{2}$$

$$\begin{aligned}\text{Total carotenoids}\ (\mu\text{g/mL}) = (1,000 \times \text{A}\,470 - 1.63\,\text{Chl} \\ -\text{a} - 104.9\,\text{Chl} - \text{b})/221.\end{aligned} \tag{3}$$

2.4. Fatty Acid Composition. The composition of fatty acids from *T. suecica* was measured using a gas chromatography (GC) system (Agilent Technologies 7890A, Santa Clara, CA, USA) coupled to a DB225MS (30 m × 0.25 mm) column according to the method of Li-Beisson et al. [29]. The content of each fatty acid was expressed as a percentage of the total of the peaks obtained [29].

2.5. Antioxidant Enzymes. Antioxidant enzyme (catalase and superoxide dismutase) activities were determined using commercial kits (Navand Salamat Co., Urmia, Iran) according to the manufacturer's protocols. For the catalase activity assay, briefly, algae cell walls were destructed using frozen mortars with nitrogen, and the extract was obtained using extraction buffer. One-hundred microliter of assay buffer with 30 μL of R1 (the solution in the assay kit) was poured into the wells of the microplate and 20 μL of the extract biological sample (homogenized tissue) was added. To start the reaction, another 20 μL of R2 (the solution in the assay kit) was added and stored for 20 min at ~20°C. The reaction was then terminated by adding 30 μL of R3 (the solution in the assay kit) and R4 (chromogen), respectively. After 10 min, 10 μL of R5 was added and the absorbance read at 550 nm. Catalase activity was calculated using the following equation:

$$\text{Formaldehyde}\ (\mu\text{M}) = \frac{\text{OD sample} - \text{B}}{\text{A}} \times \frac{0.17\ \text{mL}}{0.02\ \text{mL}}, \tag{4}$$

$$\text{Catalase activity} = \frac{\text{sample}\ (\mu\text{M})}{20\ \text{min}}. \tag{5}$$

The extracts obtained from cell wall destruction were used for measuring superoxide dismutase (SOD) activity using R1 and R2 (assay kit solutions). After 5 min of incubation at room temperature in darkness, absorbance was read at 405 nm using a microplate reader (BioTek, Synergy HT, China). SOD was calculated using the following equation:

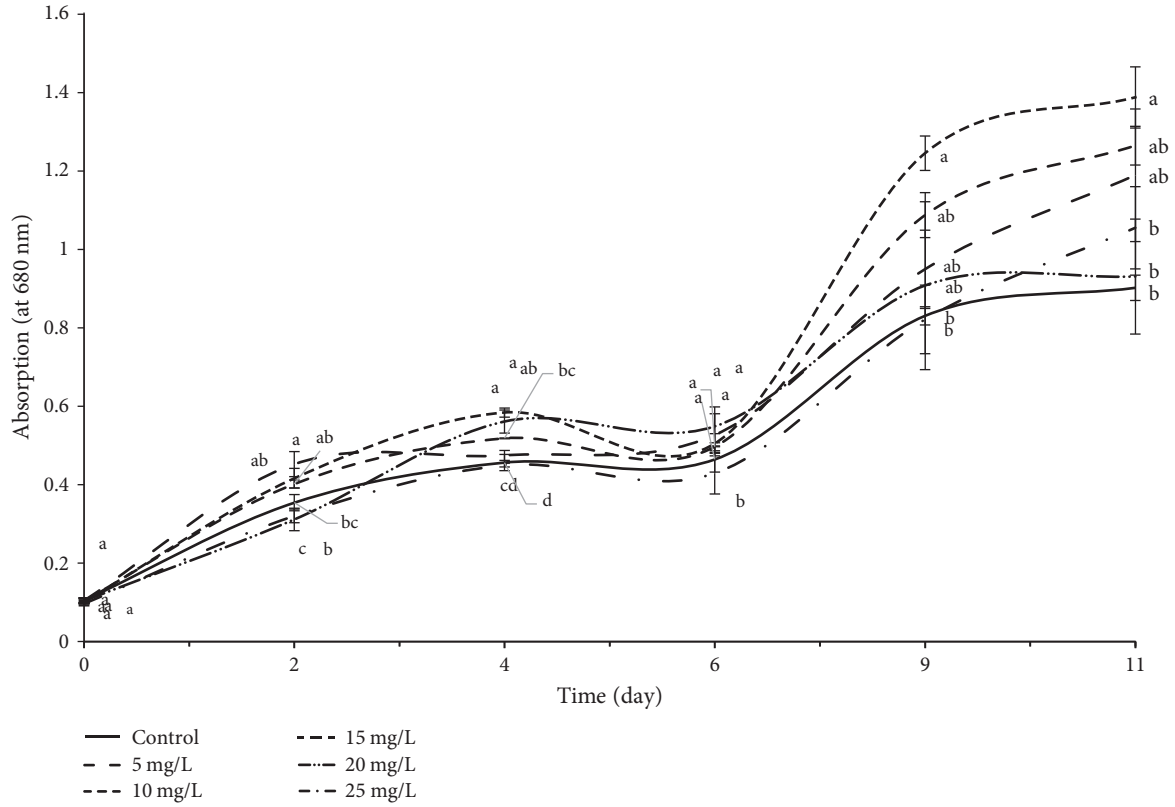

FIGURE 1: The cell density of microalgae *T. suecica* in the presence of 0, 5, 10, 15, 20, and 25 mg/L of phytohormone Kinetin during the experimental period.

$$\text{SOD activity (U/mL or mg protein)} = \frac{\text{OD test}}{\text{OD control}} \times 200. \quad (6)$$

2.6. Statistical Analysis. Statistical analysis was performed with the SPSS software, Version 21. Using Levene's and Shapiro–Wilk's tests, the data were analyzed for homogeneity of variances and normality, respectively ($P<0.05$). The comparisons among means were done by an analysis of variance (ANOVA), followed by Tukey–Kramer HSD for post hoc multiple comparisons. Differences among the means were considered at $P<0.05$. Algae cell densities compared and analyzed in all days. The rest of the data (photosynthetic pigments, antioxidant enzyme, and fatty acid profile) was analyzed at the end of the experimental period. The data of six experimental groups are displayed as means of three replicates ± standard deviation (SD).

3. Results

3.1. The Cell Density of Microalgae. Based on Figure 1, the cell density of microalgae *T. suecica* was investigated during the experimental period from the first day to the last day. Results indicated most differences for Kinetin on the cell density after day 6 to the last day ($P<0.05$). The highest cell density of microalgae on the last day was significantly obtained at concentrations of 5, 10, and 15 mg/L of Kinetin compared to the control; however, a decrease of algae cell density was not significantly observed in the treatments 20 and 25 mg/L of Kinetin.

3.2. Photosynthetic Pigments. According to the results, the content of photosynthetic pigments of microalgae *T. suecica*, measured after 10 days, was significantly affected in the presence of Kinetin ($P<0.05$). The highest content of chlorophyll *a* was significantly observed at concentrations of 20 mg/L. However, there was no significant difference at concentrations of 10, 15, and 25 mg/L of phytohormone.

The content of chlorophyll *b* is significantly affected by concentrations of 15, 20, and 25 mg/L of Kinetin. However, other concentrations had no significant effect on chlorophyll *b* content (Figure 2(b)). The highest content of chlorophyll *b* was observed at a concentration of 15 mg/L of Kinetin. According to Figure 2(c), the total pigment of treated microalgae was not significantly affected by Kinetin concentration and the content of total pigments showed a relative increase with phytohormone content in the algae medium culture.

3.3. Antioxidant Enzymes. The activity of the catalase enzyme was significantly increased at concentrations of 5 and 25 mg/L of Kinetin compared to the control ($P<0.05$, Figure 3(a)). However, there was no significant effect at a concentration of 20 mg/L of Kinetin on catalase enzyme activity. Moreover, at concentrations of 10 and 15 mg/L, the catalase enzyme activity had no significant differences compared to the control ($P<0.05$). The highest amount of SOD enzyme activity was significantly observed at concentrations of 5 and 25 mg/L of

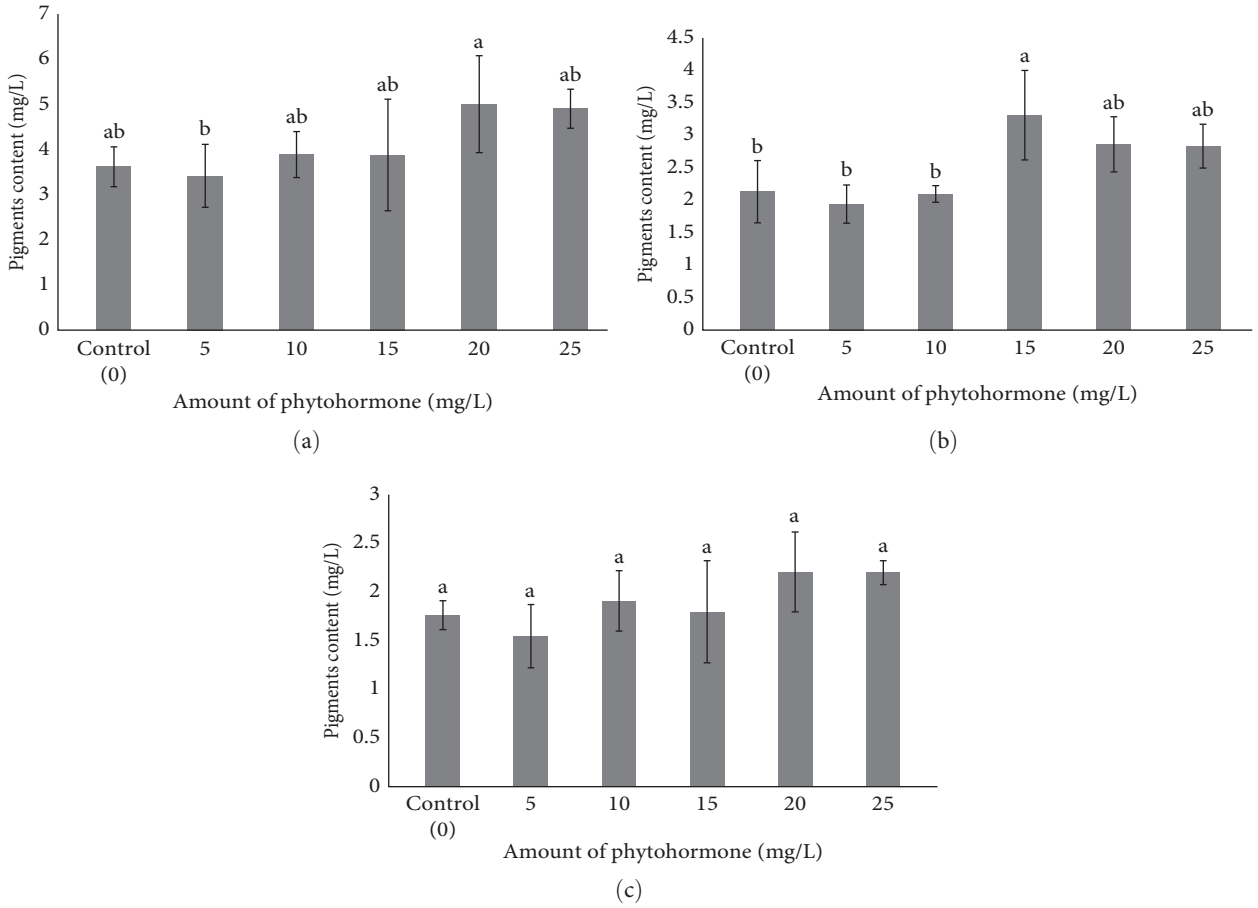

Figure 2: The content of chlorophyll *a* (a), chlorophyll *b* (b), and total pigments (c) in microalgae *T. suecica* in the presence of 0, 5, 10, 15, 20, and 25 mg/L of phytohormone Kinetin during the experiment trial.

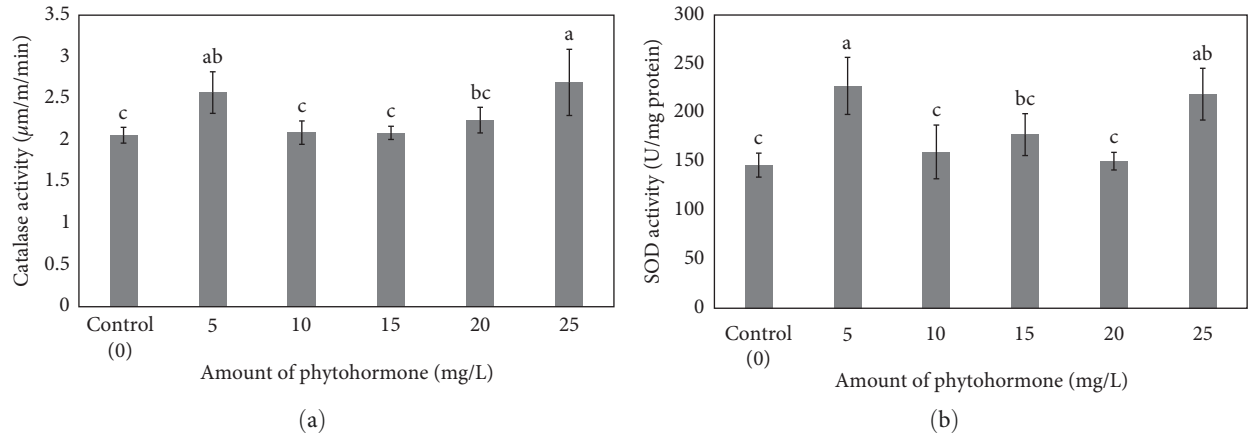

Figure 3: Catalase (a) and SOD (b) activity in microalgae *T. suecica* in the presence of 0, 5, 10, 15, 20, and 25 mg/L of phytohormone Kinetin during the experiment trial.

Kinetin, 227 ± 29, and 219 ± 26 U/mg of protein, respectively, ($P < 0.05$, Figure 3(b)).

3.4. Fatty Acid Composition. The fatty acid profiles of treated *T. suecica* were affected by Kinetin (Tables 1 and 2). In treated algae cells, the content of saturated (SFA) and monounsaturated fatty acid (MUFA) insignificantly increased ($P < 0.05$, Table 1). Besides, in treated algae cells, the amount of n6-PUFA and n6-HUFA insignificantly decreased, except at a concentration of 15 mg/L of Kinetin (Table 1, $P < 0.05$). The n3-PUFA fatty acids of treated algae cells were significantly affected by phytohormone and the lowest and highest amount, respectively, were observed at 10 and 25 mg/L of Kinetin ($P < 0.05$). Besides, the n3-HUFA fatty acids content

Table 1: Fatty acids composition of microalgae *T. suecica* in the presence of 0, 5, 10, 15, 20, and 25 mg/L of phytohormone Kinetin during the experiment time (10 days, mean ± SD).

Fatty acids	Treatments					
	Control (0 mg/L)	5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
SFA	21.84 ± 2.25	25.86 ± 1.94	25.07 ± 2.76	24.58 ± 1.05	22.33 ± 3.78	23.82 ± 0.63
MUFA	11.63 ± 0.54	13.32 ± 1.18	13.60 ± 2.10	12.28 ± 0.54	13.10 ± 2.25	13.51 ± 0.49
PUFA	44.33 ± 5.19	40.50 ± 2.09	40.44 ± 1.15	39.15 ± 3.36	41.27 ± 1.60	41.95 ± 0.76
Detected FA	77.79 ± 3.20	79.68 ± 1.04	79.11 ± 3.71	76.00 ± 2.78	76.70 ± 4.43	79.28 ± 1.17
n3-PUFA	19.76 ± 2.01^{ab}	18.74 ± 1.69^{ab}	16.89 ± 2.79^{b}	19.37 ± 1.45^{ab}	19.65 ± 2.91^{ab}	21.26 ± 0.46^{a}
n6-PUFA	24.56 ± 3.29^{a}	21.76 ± 0.39^{ab}	23.56 ± 1.64^{ab}	19.78 ± 1.96^{b}	21.62 ± 1.32^{ab}	20.69 ± 0.51^{ab}
n3/n6	0.81 ± 0.05^{bc}	0.86 ± 0.06^{abc}	0.72 ± 0.17^{c}	0.98 ± 0.04^{ab}	0.91 ± 0.19^{abc}	1.03 ± 0.03^{a}
n3-HUFA	2.92 ± 0.28^{ab}	3.77 ± 0.14^{a}	3.41 ± 0.15^{a}	3.16 ± 0.60^{a}	2.03 ± 1.12^{b}	3.02 ± 0.11^{ab}
n6-HUFA	24.56 ± 3.29^{a}	21.76 ± 0.39^{ab}	23.56 ± 1.64^{ab}	19.78 ± 1.96^{b}	21.62 ± 1.32^{ab}	20.69 ± 0.51^{ab}

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. $^{a,b,c,ab,bc,\ abc}$Different letters on the each row indicate significant differences by Tukey's test ($P < 0.05$).

Table 2: Profile of fatty acids microalgae *T. suecica* in the presence of 0, 5, 10, 15, 20, and 25 mg/L of phytohormone Kinetin during the experiment time (10 days, mean ± SD).

Fatty acids	Treatments					
	Control (0 mg/L)	5 mg/L	10 mg/L	15 mg/L	20 mg/L	25 mg/L
C14 : 0	0.57 ± 0.25	0.70 ± 0.15	0.47 ± 0.16	0.71 ± 0.19	0.42 ± 0.13	0.48 ± 0.04
C16 : 0	20.25 ± 1.97	23.80 ± 0.74	22.62 ± 3.63	21.91 ± 1.71	21.15 ± 3.81	22.20 ± 0.97
C16 : 1n7	2.70 ± 0.09	1.99 ± 0.66	2.19 ± 0.27	2.68 ± 0.37	2.90 ± 0.00	2.80 ± 0.11
C18 : 0	0.51 ± 0.16	0.56 ± 0.05	0.54 ± 0.15	0.54 ± 0.09	0.47 ± 0.05	0.40 ± 0.06
C18 : 1n9	5.04 ± 0.32^{b}	7.29 ± 0.13^{a}	7.14 ± 1.52^{a}	6.09 ± 0.56^{ab}	5.72 ± 0.96^{b}	6.55 ± 0.50^{ab}
C18 : 1n7	3.49 ± 0.18	3.65 ± 0.39	3.75 ± 0.70	3.15 ± 0.27	4.01 ± 1.09	3.78 ± 0.07
C18 : 2n6	21.32 ± 2.83	17.71 ± 1.04	19.52 ± 0.74	17.10 ± 1.56	18.75 ± 0.50	18.21 ± 0.39
C18 : 3n3	16.84 ± 1.78^{ab}	14.97 ± 1.56^{b}	13.47 ± 2.64^{b}	16.20 ± 0.86^{ab}	17.62 ± 1.80^{a}	18.24 ± 0.57^{a}
C20 : 1n9	0.40 ± 0.07	0.38 ± 0.00	0.52 ± 0.14	0.35 ± 0.07	0.47 ± 0.20	0.39 ± 0.07
C20 : 2n6	0.37 ± 0.20	0.55 ± 0.33	0.35 ± 0.05	0.32 ± 0.04	0.60 ± 0.57	0.20 ± 0.07
C20 : 4n6	2.87 ± 0.51^{ab}	3.50 ± 0.31^{a}	3.68 ± 0.85^{a}	2.36 ± 0.44^{b}	2.27 ± 0.24^{b}	2.29 ± 0.11^{b}
C20 : 5n3	2.92 ± 0.28^{ab}	3.77 ± 0.14^{a}	3.41 ± 0.15^{a}	3.16 ± 0.60^{a}	2.03 ± 1.12^{b}	3.02 ± 0.11^{ab}
C24 : 0	0.51 ± 0.36	0.81 ± 1.00	1.43 ± 0.87	1.43 ± 1.00	0.29 ± 0.05	0.74 ± 0.70

^{a,b,ab}Different letters on the each row indicate significant differences by Tukey's test ($P < 0.05$).

increased at concentrations 5 and 10 mg/L of Kinetin. Inversely, the amount of n6-HUFA fatty acids significantly decreased in all treated algae cell groups (Table 1, $P < 0.05$). The linoleic acid content of treated algae cells insignificantly decreased compared to the untreated group, but linolenic acid content at 20 and 25 mg/L of Kinetin significantly increased compared to the control (Table 2, $P < 0.05$).

According to Table 2, the amount of eicosapentaenoic fatty acid (EPA) and arachidonic acid (ARA) in the treated algae cells significantly increased at concentrations of 5 and 10 mg/L of Kinetin. The amount of EPA in the treatment of 5, 10, and 15 mg/L significantly increased compared to the untreated algae cell, but decreased at concentrations of 20 and 25 mg/L of phytohormone. The ratio of n3/n6 in the treated algae cell was affected by a concentration of 25 mg/L Kinetin; however, other treatments had insignificant effects.

4. Discussion

Phytohormones in microalgae by stimulating cell division activate the growth and intensify some photosynthetic processes, but the pathways in microalgae are still unknown. The growth of microalgae includes increases in cell division and the number of cells, resulting in more cell biomass. Besides, the significant increase in photosynthetic pigments content is due to the increase in the number of cells and the hormone-related prevention of the destruction of chlorophyll and protein. In the current study, an increase in cell density and photosynthetic pigments was observed, which is in line with the results of a combination of auxin and cytokinin [23, 30]. Kinetin or cytokinin compounds stimulate growth and increase the activity of cell division in plants [30]. The amount of chlorophyll *a* and *b* and carotenoids was increased per unit volume of culture medium under the influence of phytohormone cytokinin, which was due to the positive effects of cytokinins on the biosynthesis and development of the photosynthetic apparatus of microalgae *T. suecica*. Mousavi et al. [31] also pointed to a similar result, regarding the effects of cytokinins on the carotenoids of microalgae *Dunaliella salina*. Cytokinins play a role in chloroplast differentiation and cause the synthesis of chlorophyll and photosynthetic enzymes [32]. In plants, cytokinins induced more chlorophyll biosynthesis

and also coordinated increases of auxiliary pigments (carotenoids) and primary photosynthetic pigments (chlorophyll) [33]. Moreover, Renuka et al. [34] indicated that cytokinin by enhancing the activity of enzymes involved in photosynthesis probably could increase in photosynthetic performance.

In the present study, the activity of two antioxidant enzymes, SOD, and catalase, was affected by the concentration of phytohormone Kinetin. Based on our findings, the phytohormone effects showed a concentration-dependent trend. So at low concentration it had an increasing effect on the activity of antioxidant enzymes, at a medium concentration showed an effect equivalent to the control treatment, while after that, a sudden increase in the activity of antioxidant enzymes was observed at the maximum concentration of the Kinetin (25 mg/L hormones). Based on our findings, the addition of exogenous phytohormones revealed differential responses at different growth phases. Until day 4 (at lag phase) due to the low cell density, there was not a big difference, however, after that, at mid log phase there was a little decrease in cell density probably due to the oxidative stress and then at late log phase resulted in the highest activity of antioxidant enzymes. Similarly, in the research of Renuka et al. [34], the effect of exogenous cytokinin on photosynthetic efficiency was more remarkable in the late log phase, as compared to the initial stages of growth. In line with our research, Piotrowska-Niczyporuk et al. [35] showed the effects of the phytohormones, auxin, and cytokinin on the activity of antioxidant enzymes, and pigments in microalgae *Acutodesmus obliquus* under potassium stress. Also, based on their results, cytokinins were more effective in reducing hydrogen peroxide than auxins [35].

It has been reported that phytohormones increase the amount of lipid of microalgae *A. obliquus* under stress conditions by increasing the activity of critical enzymes such as ACCase in the lipid substrate, [36]. Sivaramakrishnan and Incharoensakdi [37] indicated that Omega-3 fatty acids can be considerably elevated with the addition of phytohormone indole acetic acid in microalgae *Chlorella sp*. They indicated that oxidative stress caused by the phytohormones could increase the amount of fatty acids. The mechanism of fatty acid biosynthesis in microalgae includes the activating of acetyl-coenzyme carboxylase, as increased in phytohormone treatments. In addition to ACC, expression levels of other genes involved in the fatty acid synthesis, including (ACP) Acyl carrier protein and (Mctk) malonyl-Coa transacylase were observed after treatment with phytohormones [37]. Udayan et al. [38] indicated that phytohormones Kinetin and gibberellin had an increasing effect on unsaturated fatty acids of *Nannochloropsis oceanica*. They also stated that a culture media containing phytohormone can significantly accumulate SFAs in microalgae. The combination of two hormones, Kinetin and Gibberellin, decreased the amount of SFAs and increased the content of MUFAs. The addition of Kinetin increased the percentage of EPA 4 times as compared to the control. In addition, an increasing of PUFA amount was observed in cultures with in response to 0.86% Kinetin compared to control [38]. In the study of Renuka et al. [34], the highest lipid content (39.05% and 36.34%) of microalgae *A. obliquus* was obtained in presence of Kinetin and Zeatin, respectively. Moreover, the amount of SFA and C16 : 0 fatty acids showed an increase in presence of phytohormones under nitrogen limitation and standard control conditions. Therefore, phytohormones supplements may induce fatty acids synthesis to improve fluidity and permeability of cell membrane facilitate increased absorption of plant hormones [34].

5. Conclusion

The present study demonstrate that phytohormone Kinetin had promoting effect on cell density and resulted in improvement in photosynthetic pigments, antioxidant enzyme, and fatty acid composition of microalgae *T. suiciceca*. The concentration of 15 mg/L of phytohormone Kinetin resulted in 49% increase in cell density. Therefore, this concentration of Kinetin can be used for cultivation and biomass production of microalgae *T. suiciceca*. Besides, the concentration of 5 mg/L of Kinetin exhibited an increase in antioxidant enzyme such as superoxide dismutase and catalase activity and production of n3HUFA and saturated fatty acid as compared to control. The funding of the current study could provide a basis for the development of scalable strategies for microalgae *T. suiciceca* based on phytohormone Kinetin for biomass and biofuels production.

Data Availability

The data sets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Tahereh Asghari, Nasrollah Ahmadifard, and Mehdi Nikoo designed the study. Tahereh Asghari and Nasrollah Ahmadifard participate in curation and analysis of data and drafting the manuscript. Mehdi Nikoo helped in methods of instrumental analysis. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the staff at the Department of Fisheries, Urmia University and Artemia and Aquaculture Research Institute for their cooperation and for providing necessary facilities and conditions are concerned.

References

[1] B. Profotová, L. Burketová, Z. Novotná, J. Martinec, and O. Valentová, "Involvement of phospholipases C and D in early response to SAR and ISR inducers in *Brassica* napus plants," *Plant Physiology and Biochemistry*, vol. 44, no. 2-3, pp. 143–151, 2006.

[2] Z. Z. Sharawy, M. Ashour, E. Abbas et al., "Effects of dietary marine microalgae, *Tetraselmis suecica*, on production, gene

expression, protein markers and bacterial count of Pacific *white shrimp Litopenaeus vannamei*," *Aquaculture Research*, vol. 51, no. 6, pp. 2216–2228, 2020.

[3] E. EL-Sayed M Hussein, "Effect of seaweed supplemented diets on Nile tilapia, *Oreochromis niloticus* performance," *International Journal of Fisheries and Aquatic Studies*, vol. 5, no. 2, pp. 205–210, 2017.

[4] M. F. A. Abdel-Aziz and M. A. Ragab, "Effect of use fresh macro algae (seaweed) *Ulva fasciata* and *Enteromorpha flaxusa* with or without artificial feed on growth performance and feed utilization of rabbitfish (*Siganus rivulatus*) fry," *Journal of Aquaculture Research & Development*, vol. 8, Article ID 482, 2017.

[5] N. E. El-Tawil, "Effects of green seaweeds (*Ulva sp.*) as feed supplements in red tilapia (*Oreochromis sp.*) diet on growth performance, feed utilization and body composition," *Journal of the Arabian Aquaculture Society*, vol. 5, no. 2, pp. 179–194, 2010.

[6] H. Pereira, J. Silva, T. Santos et al., "Nutritional potential and toxicological evaluation of *Tetraselmis sp.* CTP4 microalgal biomass produced in industrial photobioreactors," *Molecules*, vol. 24, no. 17, Article ID 3192, 2019.

[7] M. O. Becerra, L. M. Contreras, M. H. Lo, J. M. Díaz, and G. C. Herrera, "Lutein as a functional food ingredient: stability and bioavailability," *Journal of Functional Foods*, vol. 66, Article ID 103771, 2020.

[8] V. Andreotti, A. Chindris, G. Brundu, D. Vallainc, M. Francavilla, and J. García, "Bioremediation of aquaculture wastewater from *Mugil cephalus* (Linnaeus, 1758) with different microalgae species," *Chemistry and Ecology*, vol. 33, no. 8, pp. 750–761, 2017.

[9] M. H. A. Michels, M. Vaskoska, M. H. Vermuë, and R. H. Wijffels, "Growth of *Tetraselmis suecica* in a tubular photobioreactor on wastewater from a fish farm," *Water Research*, vol. 65, pp. 290–296, 2014.

[10] C. Darwin and F. Darwin, *The Power of Movement in Plants*, D. Appleton, 1896.

[11] V. Ördög, W. A. Stirk, J. Van Staden, O. Novák, and M. Strnad, "Endogenous cytokinins in three genera of microalgae from the Chlorophyta," *Journal of Phycology*, vol. 40, no. 1, pp. 88–95, 2004.

[12] S. Singh, A. Singh, G. Bashri, and S. M. Prasad, "Impact of Cd stress on cellular functioning and its amelioration by phytohormones: an overview on regulatory network," *Plant Growth Regulation*, vol. 80, no. 3, pp. 253–263, 2016.

[13] W. A. Stirk, J. van Staden, O. Novák et al., "Changes in endogenous cytokinin concentrations in *Chlorella* (chlorophyceae) in relation to light and the cell cycle," *Journal of Phycology*, vol. 47, no. 2, pp. 291–301, 2011.

[14] A. Piotrowska-Niczyporuk, A. Bajguz, E. Zambrzycka, and B. Godlewska-Żyłkiewicz, "Phytohormones as regulators of heavy metal biosorption and toxicity in green alga *Chlorella vulgaris* (Chlorophyceae)," *Plant Physiology and Biochemistry*, vol. 52, pp. 52–65, 2012.

[15] J. Ludwig-Müller, "Auxin conjugates: their role for plant development and in the evolution of land plants," *Journal of Experimental Botany*, vol. 62, no. 6, pp. 1757–1773, 2011.

[16] J. Zhao, Y. Zhou, Z. Li, W. Wang, and K.-W. Chang, "Learning gender-neutral word embeddings," in *Proceedings of the 2018 Conference on Empirical Methods in Natural Language Processing*, pp. 4847–4853, Association for Computational Linguistics, Brussels, Belgium, 2018.

[17] R. Salama, M. Sadaie, M. Hoare, and M. Narita, "Cellular senescence and its effector programs," *Genes & Development*, vol. 28, no. 2, pp. 99–114, 2014.

[18] X. Yu, L. Chen, and W. Zhang, "Chemicals to enhance microalgal growth and accumulation of high-value bioproducts," *Frontiers in Microbiology*, vol. 6, Article ID 56, 2015.

[19] D. W. S. Mok and M. C. Mok, "Cytokinin metabolism and action," *Annual Review of Plant Physiology and Plant Molecular Biology*, vol. 52, pp. 89–118, 2001.

[20] P. Mousavi, M. H. Morowvat, N. Montazeri-Najafabady et al., "Investigating the effects of phytohormones on growth and β-carotene production in a naturally isolates stain of *Dunaliella salina*," *Journal of Applied Pharmaceutical Science*, vol. 6, no. 8, pp. 164–171, 2016.

[21] R. H. Wijffels, M. J. Barbosa, and M. H. M. Eppink, "Microalgae for the production of bulk chemicals and biofuels," *Biofuels, Bioproducts and Biorefining*, vol. 4, no. 3, pp. 287–295, 2010.

[22] J. Barciszewski, M. Mielcarek, M. Stobiecki, G. Siboska, and B. F. Clark, "Identification of 6-furfuryladenine (Kinetin) in human urine," *Biochemical and Biophysical Research Communications*, vol. 279, no. 1, pp. 69–73, 2000.

[23] R. Czerpak, A. Bajguz, B. Białecka, L. E. Wierzchołowska, and M. M. Wolańska, "Effect of auxin precursors and chemical analogues on the growth and chemical composition in *Chlorella pyrenoidosa* Chick," *Acta Societatis Botanicorum Poloniae*, vol. 63, no. 3-4, pp. 279–286, 1994.

[24] W. A. Stirk, V. Ördög, O. Novák et al., "Auxin and cytokinin relationships in 24 microalgal strains[1]," *Journal of Phycology*, vol. 49, no. 3, pp. 459–467, 2013.

[25] C. Wang, M. Qi, J. Guo et al., "The active phytohormone in microalgae: the characteristics, efficient detection, and their adversity resistance applications," *Molecules*, vol. 27, no. 1, Article ID 46, 2022.

[26] P. R. Walne, "Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*," *Fishery Investigations*, vol. 26, no. 5, pp. 1–62, 1970.

[27] R. A. Andersen, *Algal Culturing Techniques*, p. 578, Elsevier Academic Press, New York, 2005.

[28] H. K. Lichtenthaler, "[34] Chlorophylls and carotenoids: pigments of photosynthetic biomembranes," *Methods in Enzymology*, vol. 148, pp. 350–382, 1987.

[29] Y. Li-Beisson, B. Shorrosh, F. Beisson et al., "Acyl-lipid metabolism," *The Arabidopsis Book*, vol. 11, Article ID e0161, 2013.

[30] A. Piotrowska-Niczyporuk and A. Bajguz, "The effect of natural and synthetic auxins on the growth, metabolite content and antioxidant response of green alga *Chlorella vulgaris* (Trebouxiophyceae)," *Plant Growth Regulation*, vol. 73, pp. 57–66, 2014.

[31] P. Mousavi, Z. Abolhassanzadeh, A. Mohagheghzadeh et al., "The effect of plant growth regulators on growth and production of β-carotene in *Dunaliella salina*," *Research in Pharmaceutical Sciences*, vol. 7, no. 5, Article ID S498, 2012.

[32] G. Towne and C. Owensby, "Cytokinins effect on protein and chlorophyll content of big bluestem leaves," *Journal of Range Management*, vol. 36, no. 1, pp. 75–77, 1983.

[33] L. Zarandi-Miandoab, M. A. Hejazi, and M. Nasiri, "The effect of cytokinin on growth and physiology of *Dunalilla salina*," *Journal of Applied Biology*, vol. 31, no. 1, pp. 121–132, 2018.

[34] N. Renuka, A. Guldhe, P. Singh, F. A. Ansari, I. Rawat, and F. Bux, "Evaluating the potential of cytokinins for biomass and

lipid enhancement in microalga *Acutodesmus obliquus* under nitrogen stress," *Energy Conversion and Management*, vol. 140, pp. 14–23, 2017.

[35] A. Piotrowska-Niczyporuk, A. Bajguz, U. Kotowska, M. Bralska, and M. Talarek-Karwel, "Growth, metabolite profile, oxidative status, and phytohormone levels in the green alga *Acutodesmus obliquus* exposed to exogenous auxins and cytokinins," *Journal of Plant Growth Regulation*, vol. 37, pp. 1159–1174, 2018.

[36] N. Renuka, A. Guldhe, P. Singh, and F. Bux, "Combined effect of exogenous phytohormones on biomass and lipid production in *Acutodesmus obliquus* under nitrogen limitation," *Energy Conversion and Management*, vol. 168, pp. 522–528, 2018.

[37] R. Sivaramakrishnan and A. Incharoensakdi, "Plant hormone induced enrichment of *Chlorella sp.* omega-3 fatty acids," *Biotechnology for Biofuels and Bioproducts*, vol. 13, Article ID 7, 2020.

[38] A. Udayan, S. Kathiresan, and M. Arumugam, "Kinetin and Gibberellic acid (GA3) act synergistically to produce high value polyunsaturated fatty acids in *Nannochloropsis oceanica CASA CC201*," *Algal Research*, vol. 32, pp. 182–192, 2018.