The Efficacy of Different Concentrations of Gibberellic Acid on Growth Rate, Dry Biomass, Endogenous Gibberellic Acid, Lipid Content, and Some Photosynthetic Pigments of *Nannochloropsis salina* Microalgae in Guillard (f/2) Medium

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Abstract

Nannochloropsis salina is a promising candidate for lipid and biomass production used in aquaculture and biofuel production. On the other hand, phytohormones have different effects on the metabolism of single-celled algae. One of the objectives of this study was to investigate the effect of gibberellic acid treatment on growth, photosynthetic pigments, dry biomass, endogenous gibberellic acid level, and total lipid content in *N. salina* cultivated in f/2 medium. Experimental treatments included concentrations of 0 (control), 0.5, 5, 10, and 20 mg/L of gibberellic acid. This experiment was conducted using a completely randomized design (CRD) with three replications in the year during 2021-2022 at the Persian Gulf Science and Technology Park in Bushehr, Iran. The measured traits encompassed growth rate, doubling time, chlorophyll a and b content, total chlorophyll and carotenoid, dry biomass, endogenous gibberellic acid levels, and total lipid content. The analysis of variance (ANOVA) showed that gibberellic acid treatment has a significant effect on examined traits at a significant level of ($p < 0.01$). Exogenous application of gibberellic acid treatment increased the growth rate, chlorophyll-b content, dry biomass, and total lipid content in *N. salina* microalgae, and the highest amount of total lipid and dry biomass with 24.69 % and 0.79 g/L were observed in 20 mg/L GA treatment, respectively. This was while the highest growth rate was obtained at the concentration of 0.5 mg/L gibberellic acid (GA_3). It suggested that a concentration of 20 mg/L GA be used in the exponential growth phase to increase biomass production and total lipid content in *N. salina* microalgae under an f/2 medium.

Keywords: Endogenous gibberellic acid, Growth, Biomass, Pigment, Lipid, *Nannochloropsis salina*

Introduction

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Nannochloropsis microalgae are less than 5 μm size ionic coccids. This microalgae is mainly known for the marine environment (Liu et al., 2013). various species of *Nannochloropsis* hold significant importance due to their substantial biomass and lipid production capabilities, as well as their potential for large-scale cultivation (Lichtenthaler, 1988). *N. salina* is a unicellular green microalgae, is classified within the class Eustigmatophyceae and the family Monodopsidaceae. Currently, it is regarded as a promising candidate for the sustainable production of high-value by-products, including long-chain polyunsaturated fatty acids (PUFAs), omega-3 fatty acids, omega-6 fatty acids, and eicosapentaenoic acid (EPA) (Pan et al., 2008). Nanochloropsis microalgae are utilized in aquaculture for fish farming, either directly or via rotifers, due to their highly stable characteristics during the cultivation process. (Falkowska et al., 2011). This species of microalgae predominantly contains chlorophyll-a, while the main accessory pigments, such as violaxanthin and vaucheriaxanthin esters, are crucial for light absorption (Du et al., 2017). Certain minor xanthophylls such as canthaxanthin, anthraxanthin, zeaxanthin, and carotenes like beta-carotene are also found in significantly lesser amounts (Chia et al., 2013). All species of *Isochyrsis*, *Nannochloropsis*, *Pavlova*, and *Phaeodactylum* have been investigated for biomass and lipid productivity, as well as omega fatty acid content (Yu et al., 2016). Numerous species of Nannochloropsis are found to be rich in lipids, comprising as much as 60% of their dry weight, and are also significant sources of eicosapentaenoic acid (EPA). (Osório et al., 2020). Additional studies have shown that lipid production and EPA in *Nannochloropsis* sp., *N. ditana, N. oceanica, N. oculata,* and *N. salina* can be increased through abiotic stresses. These stresses may include genetic manipulation, nitrogen starvation, changes in light intensity, and altering temperature conditions (Madani et al., 2021). A separate study focused on the metabolism and regeneration of lipids across various species of *Nannochloropsis*. These findings showed that environmental stressors, including variations in light intensity and nitrogen deprivation, lead to lipid regeneration and triacylglycerol (TAG) accumulation, respectively (Lin et al., 2018). The application of plant growth regulators in microalgae presents novel possibilities for the development of microalgae lipids aimed at biofuel production, as well as for the synthesis of carotenoids intended for use as dietary supplements. Plant growth regulators and their analogs stimulate the growth and production of metabolites such as carotenoids, lipids, carbohydrates, and proteins (Mansouri and Talebizadeh, 2016). Plant hormones play an essential regulatory role in multicellular higher plants. However, the specific features of hormone metabolism across various groups of microalgae remain largely unknown (Mansouri et al., 2011). Gibberellins represent a group of plant growth regulators that are essential for the growth and development of plants, as well as their response to stress. GA is a plant hormone, diterpene, which can regulate various physiological responses to stress (Ghasemi et al., 2016). GA_3 is recognized as

one of the most potent forms of GA, which can be industrially synthesized through the fermentation of fungi, particularly from the species *Gibberella fujikuroi* (Wen et al., 2018). This compound has a variety of different commercial applications in the agricultural sector, specifically in plant cultivation, where it is employed to stimulate growth rate, prevent chlorophyll decomposition, increase fruit size, and reduce reactive oxygen levels (Verma et al., 2019). Several studies have shown that GA can affect the growth and metabolic processes of microalgae by enhancing the absorption and utilization of nutrients from the culture medium (Gao et al., 2012; Park et al., 2013). For example, the addition of an optimal concentration of GA has been shown to elevate the levels of carotenoids and monosaccharides in *Chlamydomonas reinhardtii* (Zarandi Miandoab et al., 2015), increase lipid content in *Chlorella pyrenoidosa* (Wen et al., 2018), boost unsaturated lipids in *Aurantiochytrium* sp. (Sivaramakrishnan and Incharoensakdi, 2020), chlorophyll a, phycocyanin, and protein in *Microcystis aeruginosa* (Park et al., 2013.) Research indicates that various plant growth regulators, including ABA, EBR, ETH, and GA, can significantly enhance the synthesis of astaxanthin and carotenoids in *Haematococcus pluvialis*. These regulators also promote the mRNA expression of eight carotenogenic genes, each exhibiting distinct expression patterns. Additionally, it has been observed that salicylic acid (SA) positively affected the expression of carotenoid and astaxanthin-related genes (Wen et al., 2018). Despite the investigation of various plant growth regulators in microalgae, the impact of varying concentrations of GA across different culture media and pH levels on specific morpho-physiological characteristics such as growth traits, accumulation of photosynthetic pigments, endogenous GA levels, lipid content, and biomass production—has not been thoroughly examined in this particular species and strain. This study aims to investigate the effect of different treatments of GA on the growth rate, accumulation of chlorophyll a and b, total carotenoid content, dry biomass, endogenous GA, and total lipid content of *N. salina* in Gillard (f/2) culture medium.

Materials and methods

Species and cultivation conditions

N. salina (SAG 40.85) was acquired from the Algae collection at the University of Gottingen, Germany (SAG). This study utilized a slightly modified (f/2) Gillard medium with a salinity of 25 ds/m and seawater as the base water for microalgae cultivation. For this purpose, the seawater of the Persian Gulf was collected from the shore of Bushehr Student's Park, at a distance of one kilometer from the shore and at a depth of about one meter, and subsequently transported to the algae and artemia cultivation greenhouse of the Persian Gulf Research Institute, located at Persian Gulf University. During the filtration process, a 20-liter gallon was prepared and transferred to the laboratory of the Persian Gulf Microalgae Technology Development Center affiliated with the Persian Gulf Science and Technology Park in Bushehr.

The formulation of the modified Gillard (f/2) culture medium utilized in this experiment was created in accordance with the preparation protocol established by Moradi et al. (2015), and the details regarding this formulation are found in Table 1. Solutions of vitamins, trace micronutrients, sodium nitrate, and monosodium phosphate of Gillard (f /2) medium were prepared in four separate containers. Subsequently, 1 mL of each solution was mixed with 1 L of seawater. The containers containing the culture medium, inoculated with microalgae, were then transferred to 500 ml Erlenmeyer flasks, maintained at a temperature of 25 ± 2 ºC. These flasks were illuminated with white fluorescent lamps providing a light intensity of 4500 lux, following a photoperiod of 16 hours of light and 8 hours of darkness The cultures were incubated for a duration of 28 days, with aeration facilitated by a shaker, all under controlled laboratory conditions.

Hormonal treatments

Experimental treatments comprised a control group and concentrations of GA at 0.5, 5, 10, and 20 mg/L. This experiment was conducted in a completely randomized design (CRD) with three replications during the period of 2021-2022. The GA hormone utilized in this experiment dissolved readily in double-distilled water without the need of a magnetic stirrer. To prepare GA stock, 30 mg of substance, sourced, sourced from Acros Ornics, New Jersey, USA was accurately measured using a digital scale with a precision of 0.0001g. Subsequently, the GA was dissolved in 30 ml of distilled water. Finally, the resulting hormonal stock was then stored in the refrigerator at 4 °C until it was used for the treatment of microalgae. For the treatments, 150 µl of the hormonal stock was allocated for a 0.5 mg GA treatment, 1500 µl for a 5 mg GA treatment, 3000 µl for a GA treatment 10 mg, and 6000 µl for a 20 mg GA treatment all of which were added to Erlenmeyer flasks. The growth phase was characterized as exponential, and each Erlenmeyer flask was stirred uniformly.

C*onditions and methods of hormonal treatments*

GA treatments were administered into the culture medium using a micropipette in the exponential growth phase of microalgae, specifically over a period of 8 hours of darkness. Following a four-week (28-day) culture duration, the biomass of both control and treated samples was collected. The samples were subsequently subjected to centrifugation (Model K241R, Scientific Centrifuge, UK) at 6000 rpm for 5 minutes at a temperature of 4 °C. The excess culture medium was removed, and the precipitate at the bottom of 50 ml plastic falcon tubes was washed and centrifuged twice with distilled water to remove elements and salts from the culture medium completely. The samples were then stored in the freezer at -20 °C for 24 hours, after which they were dried using a freeze dryer (Model FD-8505/ FD-5005-BT, Dena Vacuum, SanatPardaz Dena, Iran) for a full 24 hours at a temperature -44°C. The lyophilized microalgae samples were subsequently kept in a -20 °C freezer until they were needed for further analysis.

Calculating growth rate and doubling time To measure daily light density in a certain period (10 am to 12 am), 2 ml of

samples were extracted from each 500 ml Erlenmeyer flask containing the microalgae culture medium using a micropipette. These samples were then placed in to the cuvette of the spectrophotometer, where cell density was measured at 750 using the PD-303UV model, Apple, Japan.

The growth rate indices (1) and doubling time (2) were calculated using optical density (OD) measurements at 750 nm according to the following equations (Madani et al., 2021):

$$\text{Growth rate } (d^{-1}) = \frac{\ln OD_{14\,d} - \ln OD_{0\,d}}{14\,d} \quad (1)$$

$$\text{Doubling time } (d) = \frac{\ln 2}{\text{Growth rate}} \quad (2)$$

Dry biomass measurement

Microalgae samples were initially collected during their dormant phase. These samples were then centrifuged at 6000 rpm for 5 minutes. In the next step, an excess of culture medium was added to the sediment, and to remove nutrients and excess salts, the remaining sediment, was washed with distilled before being stored in a freezer at -20 °C for 24 hours. The precipitates were then subjected to drying using a freeze dryer for 24 hours at -44 °C (Trinh et al., 2017). Finally, the dried samples were weighed using a precise digital scale, and the dry weight of the samples was recorded.

Extraction and measurement of total lipid content

Total lipid extraction was performed according to Bligh & Dyer method (Stirk et al., 2019). Initially, one gram of lyophilized microalgae was weighed, and then 10 ml of chloroform: methanol solution (2:1 ratio) was added. The samples were placed in a shaker for 2 minutes (model R-430, Pole Ideal Pars Company, Tehran, Iran) and for 8 minutes in a sonicator (Misonix Sonicator 3000, made in the USA) to break the thick wall of microalgae completely. They were then centrifuged at room temperature at 3000 rpm for 10 minutes to form two phases. The lipid-containing organic phase was isolated and added to the remaining 5 ml of pure chloroform. As in the previous step, the lipid extraction steps were repeated. The isolated organic phase was poured into a pre-weighed microtube (W1) and placed in an oven (Memmert, Germany) at 50 °C to evaporate the solvent. The microtubes were weighed again (W2), and then the total lipid content was calculated from the difference between the primary and secondary weights (W1-W2) in terms of weight-weight percentage (Liu et al., 2013).

Extraction and quantification of endogenous GA concentration by HPLC

Endogenous GA was extracted from one gram of lyophilized microalgae, according to Liu et al. (2013). The concentration of endogenous GA was determined using high-performance liquid chromatography (HPLC) (Model 1220 Infinity LC, Agilent, USA) with an ion trap mass spectrometer. HPLC-grade solvents (methanol, formic acid) were obtained from J.T. Baker (Phillipsburg, NJ, USA), and analytical-grade chemicals (ammonium hydroxide) were purchased from the Chemical Reagent Company of Beijing (China). Oasis MCX 3-cc (60 mg) and Oasis MAX 3-cc (60 mg) SPE cartridges were products of Waters (Milford, MA, USA). A high-performance liquid chromatography (HPLC) system, featuring

a Surveyor Autosampler and a Surveyor LC pump (Thermo Finnin, Waltham, MA, USA), along with a reversed-phase column (ZORBAX 300SBC18, 2.1×150 mm, 3.5 µm; Agilent, Santa Clara, CA, USA), was employed for the separation of mixed compounds. An ion trap mass spectrometer (LCQ DECA XP MAX) coupled to an ESI source (Thermo Finnin) was used to identify the target compounds. Data processing was performed in Xcalibur 2.1 (Thermo Finnin).

Assessment of chlorophyll a, b, and carotenoid pigments

The Lichtenthaler method was employed to measure the concentration of chlorophyll-a, b, and total carotenoids (Aftab et al., 2011). Initially, 10 mg of lyophilized microalgae powder was accurately weighed using a digital scale and then transferred into a glass test tube to extract these photosynthetic pigments. Following this, 5 ml of 90 % acetone was added to the test tubes, and the samples were stored in the dark at 4 °C for 48 hours. In the next step, the test tubes containing the samples were agitated well using a shaker for 2 hours, after which all samples were centrifuged at 5000 rpm for 5 minutes. The clear green supernatant was then carefully extracted with a micropipette, and 3 ml of this solution was placed into a quartz cuvette compatible with the spectrophotometer model (APEL PD-303UV, Kawaguchi, and Saitama, Japan). The 90% acetone served as a blank solution to calibrate the spectrophotometer. The absorbance readings for chlorophyll-a, chlorophyll-b, and carotenoids were taken at wavelengths of 662, 645, and 470 nm, respectively. The following formulas were utilized to calculate the concentrations of chlorophyll a, b, and carotenoids, with results expressed in micrograms per gram of dry weight.

$Chl_a = 11.75A662 - 2.35A64$

$Chl_b = 18.61A645 - 3.96A662$

$$\text{Carotenoid} = \frac{1000A470 - 2.70Chla - 81.4Chlb}{227}$$

In the above formula, Chla chlorophyll-a, Chlb chlorophyll-b, and the amount of carotenoid were calculated according to equations.

Statistical analysis of data

Statistical analysis of data was performed using one-way analysis of variance (ANOVA-one way) with the help of statistical software MSTAT-C and DSAASTAT, version 022.0 Perugia, Italy 2010. All tests were performed in three replications in a completely randomized design. Data were calculated and plotted with Excel software, 2007 version. The means of the treatments were compared using Duncan's multiple range test at a 5 % probability level ($P < 0.05$).

Results

The analysis of variance (ANOVA) showed that the effect of GA treatment on all studied characteristics of *N. salina* was statistically significant at the 1% level (Table 2). The mean comparison was performed for the measured traits under different treatments of GA, and it was found that there were significant differences between different concentrations of GA for all the studied parameters.

Effect of different concentrations of GA on growth rate

Figure 1 shows the effect of different treatments of the GA on the growth of *N. salina* based on the amount of light density during the culture days. According to Figure 1, after induction of GA, the growth curve of microalgae for different GA treatments, except 0.5 mg/L, did not significantly affect the growth trend of microalgae compared to the control treatment. The results showed that the control and 0.5 mg/L GA treatments showed the highest growth rate in *N. salina*. According to these results, it can be said that the GA can stimulate the growth of *N. salina* in low concentrations. However, the growth response of all microalgae species to hormonal treatments will be varied in different conditions and various culture media.

The effect of GA treatments on growth rate and doubling time

Based on the optical density (OD) data at 750 nm, the highest growth rate and doubling time with values of 0.085 per day and 8.2 days has belonged to the treatment of 0.5 mg/L GA. Also, the lowest growth rate and doubling time with values of 0.073 per day and 9.5 days were related to the treatment of 10 mg/L GA (Table 3).

Effect of GA treatment on chlorophyll-a content

The analysis of variance showed that the chlorophyll-a content in the *N. salina* was significantly affected by GA treatments at 1% level (Table 2). Based on the results, the exogenous application of GA resulted in a significant decrease in chlorophyll-a in the *N. salina* compared to the control. The highest amount of chlorophyll-a with an average of 3.28 µg/g dry weight was related to the control treatment. The lowest amount of chlorophyll-a with an average of 1.74 µg/g dry weight belonged to the treatment of 20 mg/L GA (Figure 2).

The impact of GA treatment on total carotenoid accumulation

Table 1. Components of the Gillard (f/2) medium used in this experiment

Component	Primary stock solution	Quantity	Molar concentration in the final medium
$NaNO_3$	75 g/L	1 mL	8.82×10^{-4} M
NaH_2PO_4	5 g/L	1 mL	3.62×10^{-5} M
Micronutrients solution			
$6H_2O.FeCl_3$	-	3.15 g	1.17×10^{-5} M
$Na_2EDTA.2H_2O$	-	4.36 g	1.17×10^{-5} M
$CuSO_4.5H_2O$	9.8 g/L	1 mL	3.93×10^{-8} M
$2H_2O.Na_2MoO_4$	6.3 g/L	1 mL	2.60×10^{-8} M
$2H_2O.ZnSO_4$	22 g/L	1 mL	7.65×10^{-8} M
$6H_2O.CoCl_2$	10 g/L	1 mL	4.20×10^{-8} M
$4H_2O.MnCl_2$	180 g/L	1 mL	9.10×10^{-7} M
Vitamin solution			
Thiamine HCl (Vit. B_1)	-	200 mg	2.96×10^{-7} M
Biotin (Vit. B_7)	1 g/L	1 mL	2.05×10^{-9} M

The results showed that GA did not enhance the carotenoid accumulation in whole microalgae when compared to the control treatment. Consequently, the control treatment exhibited the highest total carotenoids content, averaging of 1.99 µg/g dry weight. In contrast, the treatment with 20 mg/L GA recorded the lowest total carotenoid level, averaging of 0.45 µg/g dry weight for this characteristics. (Figure 4).

The Effects of GA treatment on dry biomass production

The application of GA at the specified concentrations resulted in a notable

Table 2. One-way analysis of variance (ANOVA) for measured traits in *N. salina* under different GA treatments

		MS					
S.O.V	DF	Chlorophyll-a (µg/g DW)	Chlorophyll-b (µg/g DW)	Total carotenoids (µg/g DW)	Dry biomass (g/L)	Total lipid content (%W/W)	Endogenous GA (ng/ml)
Treatment	4	1.44**	0.22**	1.27**	0.154**	32.047**	182.865**
Error	10	0,066	0.045	0.012	0.001	1.78	1.31
Total	14	0.46	0.095	0.372	0.045	1.0435	53.183
C.V	-	11.45	11.24	12.81	7.93	7.01	1.46

Symbol (*) and (**): indicates statistically significant differences between treatments at ($\rho \leq 0.05$) and ($\rho \leq 0.01$) levels respectively and the letters (ns): show statistically non-significant differences between treatments. S.O.V: Source of variation, DF: Degree of freedom, MS: Mean squares, GA: gibberellic acid

Table 3. Growth parameters for *N.salina* under various GA treatments during 28 days

	GA concentration				
Parameter	0 Control	0.5 mg/L	5 mg/L	10 mg/L	20 mg/L
Growth rate (d^{-1})	0.08^{3b}	0.085^{a}	0.078^{ab}	0.073^{ab}	0.084^{b}
Doubling time (d)	8.3^{ab}	8.2^{ab}	8.9^{b}	9.5^{a}	8.3^{ab}

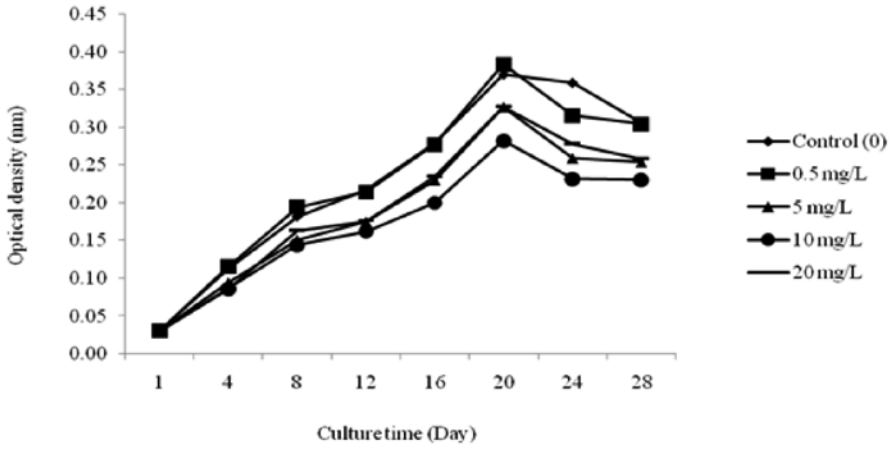

Fig. 1. Growth curve of *N. salina* at different GA concentrations

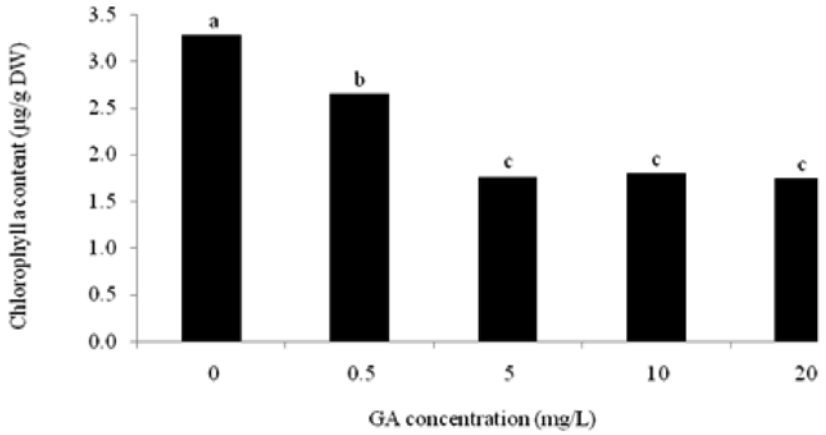

Fig. 2. Effects of different GA treatments on chlorophyll-a content in *N. salina*

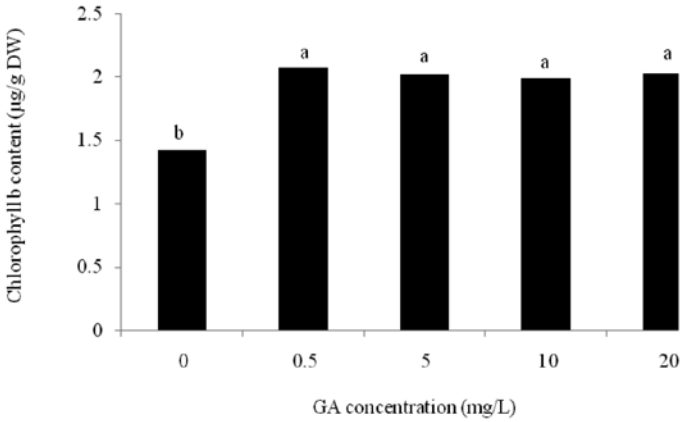

Fig. 3. Effects of different GA treatments on chlorophyll b content in *N. salina*

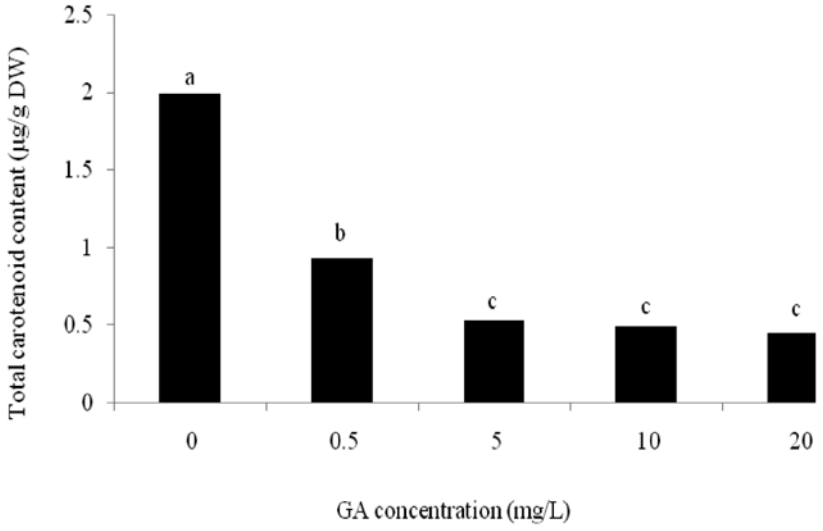

Fig. 4. The impact of various GA treatments on the overall carotenoid concentration in *N. salina*

enhancement in the dry biomass production of the microalgae *N. salina* when compared to the control group. The treatment with 20 mg/L GA yielded the highest average dry biomass, measuring 0.79 g/L. Conversely, the control treatment exhibited the lowest average dry biomass, recorded at 0.22 mg/L for this parameter (Figure 5).

GA treatment effect on total lipid content

The results of this experiment indicated that the application of GA treatment had a significant impact on the total lipid content of the microalgae *N. salina* at the concentrations tested, achieving a significance level of 1% (Table 2). The treatment with 20 mg/L GA exhibited the highest average total lipid content, measuring 24.69%. In contrast, the control treatment recorded the lowest average total lipid content at 16.65%. (Figure 6).

The effect of GA treatment on the endogenous GA content

The results showed that GA treatments did not elevate endogenous GA content in

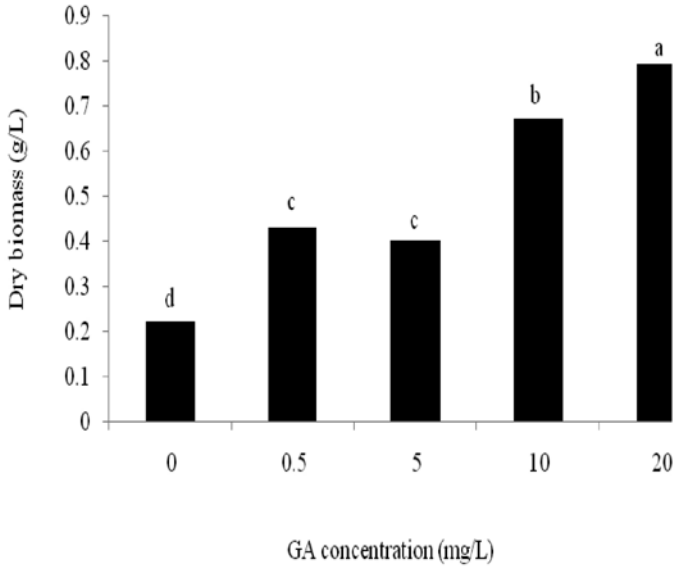

Fig. 5. The influence of various GA treatments on dry biomass in *N. salina*

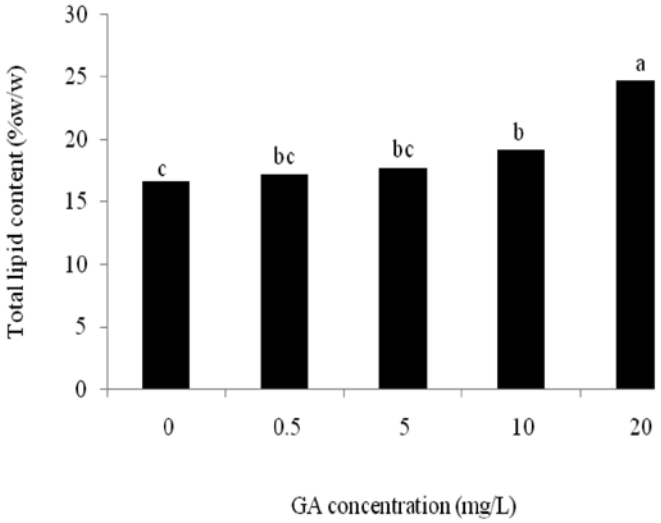

Fig. 6. Various GA treatments impact on total lipid content in *N. salina*

microalgae when compared to the control group. Consequently, the control group exhibited the highest concentration of endogenous GA, averaging of 88.98 ng/ml. In contrast, the treatment of 10 mg/L GA recorded the lowest endogenous GA concentration, averaging of 69.70 ng/ml (Figure 7).

Discussion

GA is a crucial plant growth regulator that affects several activities in microalgae, such as cell division (Pan et al., 2008; Falkowska et al., 2011). In the present study, six factors, including growth traits, accumulation of some photosynthetic pigments, total carotenoid content, dry biomass, endogenous GA, and total lipid content in *N. salina*, were studied under different treatments of GA. The results of this experiment indicated that GA treatment effectively enhanced the growth rate, chlorophyll-b accumulation, dry biomass, and total lipid levels in *N. salina*. The growth rate factor serves as a primary indicator of a species's adaptability and ecological success to varying conditions in both natural and laboratory environments (Salmaninejad, 2016). In the present study, GA treatments on *N. salina* microalgae showed that the growth rate is consistent with the exponential growth of microalgae during the 28-day growth period. The data obtained from this experiment indicated a trend of enhanced yield and efficiency in cultures that received treatment with GA. The experimental results demonstrated that the exogenous application of GA at low concentrations accelarates the growth rate and reduces the doubling time in *N. salina*. Research conducted by Du et al. (2017) found that the GA significantly increased the growth of *C. pyrenoidosa* ZF cells. Additionally, Chia et al. (2013) investigated the growth of *C. conceulris* across various culture media and reported a maximum growth rate of 0.14 per day. Recent findings by Yu et al. (2016) has showen that GA enhances both the growth rate and the overall rate of microalgae by facilitating nitrogen uptake and consumption of other nutrients, such as carbohydrates (Pan et al., 2008).

In marine algae, the primary photosynthetic pigments, namely chlorophyll-a, b, c, and d, play a crucial role in absorbing light energy at the photosynthetic reaction center (Osório et al., 2020). According to the results,

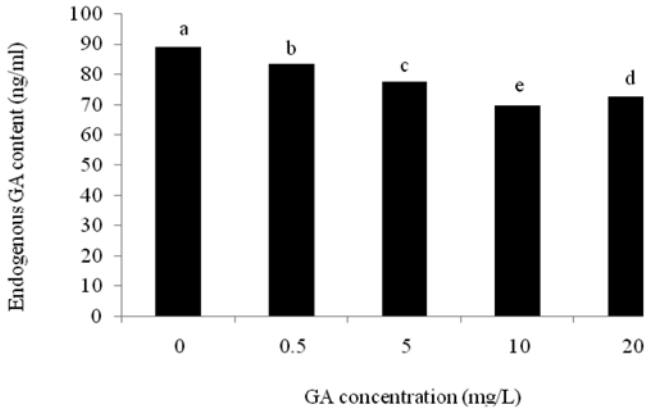

Fig. 7. The influence of different GA treatments on endogenous GA content in *N. salina*

exogenous application of GA did not stimulate and increase the chlorophyll-a content in the *N. salina* compared to the control treatment. Pan et al. (2008) reported that the application of appropriate concentrations of GA in a culture medium increased chlorophyll-a, phycocyanin, and protein in *Microcystis aeruginosa.* Furthermore et al. (2011) investigated the effect of GA on growth, photosynthetic pigments, and metabolism in *Chlorella vulgaris* revealing that GA promotes an increase in the number of photosynthetic pigments (Falkowska et al., 2011). Additionally, Lin et al. reported an increase in chlorophyll-a content in *Chlorella vulgaris* following GA treatment (Lin et al., 2018; Madani et al., 2021); however, the result do not correspond to the findings of the present study. In addition, Mansouri and Talebizadeh. (2016) reported that the levels of chlorophyll and carotenoids in *Nostoc linckia* decreased under GA treatment. Similarly, a decline in chlorophyll and carotenoid content was observed in cannabis plants when treated with GA, which indicates that the results of the present study are consistent with the results of the above research (Mansouri et al., 2011; Mansouri and Talebizadeh, 2016). Enhancing the overall chlorophyll content is a crucial and significant factor that affects the photosynthetic efficiency of plant organisms. Increasing the total chlorophyll content is among the most critical and impactfull elements affecting the photosynthetic capacity of these organisms. (Ghasemi et al., 2016). According to the results of this study, the GA also affects the accumulation of chlorophyll-b in the *N. salina*. Wen et al. (2018) reported that exogenous application of GA treatments significantly increased chlorophyll-b and a + b in the first and sixth leaves of *Camellia oleifera* Abel. The exogenous application of GA also affects the source-reservoir relationship, including the stability of the source and reservoir during carbohydrate uptake and distribution. In addition, GA increases resource strength by improving photosynthetic efficiency and improves reservoir strength by redistributing photosynthetic adsorbents (Verma et al., 2019). Chlorophyll plays a pivotal role in photosynthetic efficiency, as GA increases the chlorophyll concentration in leaves by augmenting both the number and size of chloroplasts, as well as enhancing the ultrastructural morphogenesis of plastids(Wen et al,. 2018).

Carotenoids are non-polar pigments that play a crucial role in protecting against light by inactivating reactive oxygen species (ROS) that are formed when exposed to light (Osório et al., 2020). According to the results, the carotenoid content of *N. salina* did not increase under the influence of GA treatment. Park et al. (2013) reported that adding appropriate concentrations of GA to the culture medium increased the levels of carotenoids and monosaccharides in the *Chlamydomonas reinhardtii* . In another study, Lin et al. (2018) examined the effect of the GA on the *Chlorella vulgaris*and reported that carotenoid production and accumulation in the cells of this microalgae increased . Du et al. (2017) reported that the GA increased the total carotenoid content of *Chlorella pyrenoidosa* . Higher biosynthesis of carotenoids is usually associated with

inhibiting chloroplast damage in cells exposed to environmental stresses (Gao et al., 2012). In general, carotenoid accumulation could be enhanced by altering carbon and nitrogen balances under adverse conditions (Zarandi Miandoab et al., 2015).

Based on the results of this study, the dry biomass in *N. salina* was enhanced by GA treatment. Research conducted by Yu et al. (2016). demonstrated that GA increases the biomass in the *Aurantiochytrium* sp. Additionally, Sivaramakrishnan and Incharoensakdi. (2020) reported that both GA and IAA contributed to increased biomass production in *Chlorella sp*. However, the highest rate of biomass stimulation was observed in GA treatment (Sivaramakrishnan and Incharoensakdi, 2020). In a study, Aftab et al. (2011) showed that GA is a plant hormone that can stimulate growth, stomatal conduction, and photosynthesis in sweet wormwood *(Artemisia annua*). Gibberellic acid (GA) under conditions of nitrogen limitation enhances the activity of carbonic anhydrase, nitrate reeducates, and key enzymes involved in carbon and nitrogen metabolism (Aftab et al., 2011). Consequently, GA promotes an increase in biomass and bioproducts by regulating metabolic pathways (Madani et al., 2021).

Microalgae typically enhance lipid accumulation when subjected to adverse and stressful environments. Consequently, investigating the processes that induce lipid production to boost both the production and accumulation of lipid content is of significant importance (Moradi et al., 2015). The findings of this research indicate that the total lipid content of *N. salina* was elevated as a result of GA treatment. According to Yu et al. (2016), GA enhance lipid accumulation in the *Aurantiochytrium* sp. In another study, Sivaramakrishnan and Incharoensakdi. (2020) reported that GA treatment resulted in a 12 % increase in total lipid content in *Chlorella* sp. This study shows that the cellular response to GA varies across different species (Sivaramakrishnan and Incharoensakdi, 2020). GA promotes lipid accumulation by enhancing cell diameter as well (Trinh et al., 2017).

Phytohormones also play a significant role in the regulation of plant growth and development. Endogenous hormones in plants including gibberellins (GA), indole-3-acetic acid (IAA), and abscisic acid (ABA), interact with one another, influencing various physiological process. (Wen et al., 2018). According to the findings of this experiment, the exogenous application of GA did not lead to a significant increase in the levels of endogenous GA in *N. salina* compared to the control. Similarly, Stirk et al. (2019) reported that GA did not affect the endogenous GA concentration of *Chlorella minutissima*. Consequently, they inferred that the effects of the exogenous application of phytohormones might be mild due to the precise mechanism of homeostasis controlling hormone levels through biosynthesis, synthesis, and degradation. In another study, Do et al. (2020) reported that GA is an endogenous phytohormone produced by the *C. sorokiniana* TH01 under phototrophic and mixotrophic culture conditions that endogenous GA levels decrease under phototrophic growth under salinity stress (Do et al., 2020).

Conclusion

The current investigation demonstrated that a concentration of 20 mg/L GA at positively affect on both dry biomass and total lipid content in *N. salina,* while it doesnot significantly affect chlorophyll content. Additionally, the highest growth rate was obtained at a GA concentration of 0.5 mg/L. Mean comparisons indicated that the control treatment exhibited the highest values for chlorophyll-a, total carotenoids, and endogenous GA. However, *N. salina* achieved maximum highest efficiency in terms of both total lipid content and dry biomass at a 20 mg/L GA concentration. Consequently, the findings of this studysuggested that utilizinga concentration of 20 mg/L GA enhance the production of dry biomass and total lipids in *N. salina* within the Gillard culture medium. Furthermore, further research is warranted to explore varoius concentrations of other phytohormones across different culture media for this valuable species, particularly in relation to lipid production and biomass. In general, GA can increase the biomaterials, lipids, and biomass of the *N. salina* by regulating the metabolic pathways.

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