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Effect of blue light intensity and photoperiods on the growth of diatom *Thalassiosira pseudonana*

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ABSTRACT

Diatom *Thalassiosira pseudonana* photosynthetic properties and generation of fucoxanthin and lipid production under different blue light intensity with different photoperiods (dark/light cycle) at temperature 23 ± 1 °C were studied in this work. The growth (cell number) and the biomass concentration of the cells were found to be doubled at 120 μE m^{-2} s^{-1} blue light intensity as compared to 200 μE m^{-2} s^{-1} at 8:16 h dark light photoperiods. The rise in blue intensity from 40 to 120 μE m^{-2} s^{-1} has increased the synthesis of fucoxanthin and lipid in *Thalassiosira pseudonana* at dark-light cycle 8:16 h. It was found that at optimal amount of blue light and photoperiod ratio, evidently influence the growth of *Thalassiosira pseudonana* by yielding 35.6 % lipid and 1.18 mg/g fucoxanthin.

1. Introduction

Marine diatom found to have higher amount of fucoxanthin ($C_{42}H_{58}O_6$) content, one of the primary carotenoids, as compare to macroalgae (Wang et al., 2018; Yang and Wei, 2020). Fucoxanthin and fatty acids have numerous health properties in the aspects of nutraceutical and functional food (Woo et al., 2010; Lauritano et al., 2016; Bhattacharjya et al., 2020). It is an economically important carotenoid derived with a gramme costing around USD 30,000 (Su et al., 2019; Seth et al., 2021). The fucoxanthin largely extracted from marine seaweeds for commercial production. But they have certain drawbacks such as slower growth rate, low fucoxanthin concentration with lower quality and possess environmental negative effects (Lu et al., 2018). To avoid these circumstances, microalgae can be a potential alternative source especially the diatoms due to the presence of principal cellular pigments which contain 0.2 % to 2.6 % of dry cell weight; potentially 100 times higher than in brown seaweeds (Guo et al., 2016; Wang et al., 2021).

In order to survive, diatoms are adapted swiftly to a dramatic variation in light intensity, temperature and nutrition availability (Lockhart, 2013; Govindan et al., 2021). Among them, the rate of photosynthesis is highly influenced by the light intensity (Maltsev and Maltseva, 2021). Chlorophyll a and b, are highly sensitive to blue and red-light wavelengths, which are the central light harvesting pigments in algae (Lockhart, 2013).

Apart from regulating biological processes, light intensity affects the content of biomass as well as its production. Light is required for microalgae to make adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate ($NADPH_2$), as well as to synthesis necessary chemicals for the growth (Xie et al., 2021). Algal growth is hampered by a lack of light, but too much light can be harmful. For net growth, diatoms require sufficient light to exceed their light compensation point (Govindan et al., 2020; Nagarajan et al., 2022). Increases in light intensity beyond the compensation point result in faster development until the culture becomes light saturated, at which time greater light intensities might cause photoinhibition (Chen et al., 2011; Hu et al., 2018). On the other hand, microalgae growth will be reduced if intensity decreases below the saturation point (Metsoviti et al., 2019).

The majority of research on light effects has focused solely on irradiance. Despite the fact that spectrum has been found to alter algal metabolism and growth (Prins et al., 2020). Furthermore, the photosynthetic rate, pigment content, biochemical composition of most species was highly affected by the spectrum quality and the metabolic

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makeup, pigment concentration, and photosynthetic rate of numerous species have all been shown to be influenced by spectrum quality. Blue light cause higher production of biochemical composition of pigments and lipid when compare with red or white light (Runkle, 2017).

Diatoms contain mainly two pigments, chlorophylls and carotenoids. Both are involving in photosynthesis and photo defence mechanisms. Blue and red wavelengths are captured by chlorophylls and utilised for photosynthesis (Kuczynska et al., 2015). Carotenoids are highly contributing in photoprotection whereas fucoxanthin involves in light harvesting. Diatoms respond far more favourable to blue light than the white light (Wagner et al., 2006). It is producing more cells with higher photosynthetic activity. In addition, planktonic diatoms that float in the water column are subject to variations in the proportion of blue to red light (Kuczynska et al., 2015).

Diatoms are subjected to various light intensities during day time. To sense blue light, diatoms employ photoreceptors called aureochromes (Lockhart, 2013). Beyond the light intensity, dark: light cycle makes a considerable contribution to the phytoplankton metabolic process by influencing the oscillation pattern, spectral composition and photoperiod. The optimum irradiance, oscillation pattern, night length is affecting metabolic activity of phytoplankton (Oostlander et al., 2020). In diatoms, where photosynthesis requires deep connection between intercellular components, photoperiod length correlates with light intensity and oscillation pattern to influence photosynthesis and metabolism (R. Li et al., 2017; G. Li et al., 2017).

Therefore, to investigate the effects of blue light intensity on diatom *Thalassiosira pseudonana* under different blue intensities and photoperiods. As a consequence, the productivity of biomass especially the productivity of fucoxanthin and fatty acid content after the extract and purification have been studied. The changes in accumulation of fucoxanthin and lipid in the dry biomass have monitored closely to determine the influences of blue light intensity.

2. Material and methods

2.1. Diatom strain

Algae Culture Collection Centre and Laboratory, Faculty of Industrial Sciences and Technology (FIST), Universiti Malaysia Pahang provided the stock culture of *Thalassiosira pseudonana*. The culture was inoculated an artificial sea water F/2 + Si medium at 18 ± 1 °C maintained in 2 L Erlenmeyer flask under continuous illumination with low conditions 80 μE m^{-2} s^{-1} (photosynthetically active radiation) for 24 h. The culture was aerated with filtered air (1–2 %, v/v) to avoid settlement of culture at bottom of Erlenmeyer flask (Govindan et al., 2020; Govindan et al., 2021).

2.2. Experiment set up

Diatom *Thalassiosira pseudonana* were grown with an inoculum density of 4 × 10^5 cells mL^{-1} pH at 7.6 ± 0.1. The diatom suspension was centrifuged at 2500 ×*g* for 5 min and resuspended into the fresh sterilised media for light induction experiment. In aim of optimise the growth and accumulation of fucoxanthin in the cells, different blue light intensity such as 40, 80, 120, 160 and 200 μE m^{-2} s^{-1} were used to maximise the growth. The quantity of light intensity was measured using US-SQS/WB Micro Quantum Sensor WALZ which connected with LI-190R quantum sensor. In experiment, all the flasks culture were exposed at light intensity 40 μE m^{-2} s^{-1} in first day. The culture flasks were placed 10 cm away from the blue light emitting diode (LED) source. After optimised, the culture under various light intensity in first phase. In second phase the culture was cultivated in different photoperiods (16:8; 12:12, 8:16, 0:24 h dark:light cycle) to optimise the fucoxanthin production. The inoculum was made by transferring cells from a stock culture and incubating them in a 2 L Erlenmeyer flask with 1.5 L of F/2 media in an aseptic manner and aerated with filtered air containing 1.5 % (w/w) CO_2 at a rate of 0.5 vvm using air bump. Spectrophotometer (Thermo Scientific TM GENESYSTM 10S UV–Vis) was used to quantity cell growth by measuring the optical density value at 780 nm. The diatoms cell number was counted microscopically using bright field microscopy with a hemacytometer. Each experiment was carried out three times for both light intensities to minimize the errors.

2.3. Fucoxanthin analysis

The harvested biomass from the lyophilized cells (25 mg) were crushed and extracted with methanol by heating at 60 °C for 15 min. The supernatant was separated after centrifuged at 5100 ×*g* for 4 min. The extract was separated over 10 % petroleum ether, and the carotenoids were recovered in the upper phase. Water (5 %) was mixed with methanol extract to partition against petroleum ether. The different phases were separated and partitioned against 50 % petroleum ether. The phases separation done by mixing saturated sodium chloride (NaCl) solution and gently transferred the upper phases. The upper phases were evaporated in a nitrogen stream. Then it evaporated in a stream of nitrogen and redissolved prior to use. For phase separation and pigment transfer into the upper phase, a saturated NaCl solution was added. This was collected, evaporated in a nitrogen stream, then redissolved before use. HPLC was performed on a Nucleosil C18 25 cm × 3 mm column with (85:10:5) ratio of acetonitrile/methanol/2-propanol as the mobile phase at 10 °C. Individual peak was captured online using a Kontron diode array detector 440 and analysed.

2.4. Lipid analysis

Hexane (40 ml) solvent was used to sock the 5.0 g of dry biomass in 50 mL of closed falcon tube, then placed at ultrasonication water bath at 60 °C with 1 kW sonication power for 60 min. The solvent turns from colourless to light yellow/brown. Centrifugation was used to separate the supernatant and biomass at 2300 ×*g* for 5 min. Once separated the solvent from biomass, steps were repeated until the solvent turns colourless. The extracted lipids were transesterified to collect fatty acid methyl ester and analysed via gas chromatography mass spectroscopy according procedures stated elsewhere (Palanisamy et al., 2021).

2.5. Statistical analysis

Each experiment was performed in triplicate. Each sample was quantified in triplicate for each experimental repeat. The statistics data are provided in mean values and standard deviation. The significant difference was measured at 95 % using through Tukey's test.

3. Results and discussions

Photosynthetically active radiation between 400 and 700 nm wavelength used for photosynthesis. LED light is highly effective way to change electrical energy to light energy. At the same time, it does not waste much energy in heat form. The anabolic demands for the formation of cellular carbohydrate obtained from CO_2 simplify the computation of photoautotrophic microalgae's light photon demand. Chlorophyll accessory antenna converting chemical energy from photosynthesis in the form of $NADPH_2$ to synthesis lipid, protein and nucleic acids. The impact of varied wavelengths exposure on microalgae cellular chemical composition was determined.

LEDs produce light with narrow wavelength ranges and the ability to readily change the colour of the light. According to research, the red light spectrum has a significant impact on algal growth, promoting rapid multiplication of cells. Blue light spectrum enhances cell proliferation. Single wavelength at different light intensities or light frequencies can be used to change the biochemical content of diatom cells. Besides that, this light is long last up to 50,000 h, quick response which emitting monochromatic light at different wavelength and free of mercury

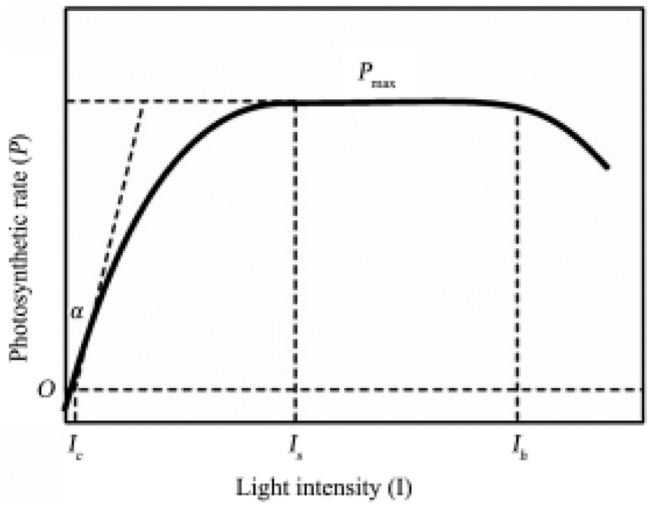

Fig. 1. The photosynthetic rate versus light intensity curve of microalgae (Chowdury et al., 2020).

(Schulze et al., 2014). The photosynthetic rate of cell rises when intensity of light increase until it reaches the photosynthetic maximum rate point (P_{max}) as shown in Fig. 1.

Many plants, including diatoms need minimum intensity of blue in addition to red wavelength to meet their metabolic process. Blue wavelengths are used for enzyme activation and gene transcription regulation in cells. It been claimed that cell damage could be take place by pure red light. The diatom *Thalassiosira pseudonana* cells were cultured with high nutritional regime supported by supplementation of macro and micronutrient (Palanisamy et al., 2022). The growth curves of cells were monitored. *Thalassiosira pseudonana* strain was cultured under optimum light intensity and photoperiod to enhance fucoxanthin production.

3.1. Effect of blue light intensity on Thalassiosira pseudonana

Photosynthetic active radiation helps to absorb various wavelength of light based on the types of pigments presence in the cells. *Thalassiosira pseudonana* culture were grown under blue light emitting diode (LED) which can enhance the photosynthesis of species without wasting much heat energy. Radiation with wavelengths between 400 and 500 nm is commonly referred to as a blue light. This waveband is part of the visible spectrum, has a lot of energy, and has a big impact on plant growth. When it comes to driving photosynthesis, blue light is just as effective as green or red light. It has a lot of energy that accelerates plant growth. The photosynthetic reaction is driven by blue photons, which are less efficient than green or red photons in terms of energy because their high energy is not fully utilised; some of the energy is basically lost when compared to photosynthetic photons with a longer (less energetic) wavelength (Runkle, 2017).

The growth, biomass productivity of *Thalassiosira pseudonana* under various blue light intensities shown in Fig. 2. The cells undergo lag phase in first 2 days where all flasks placed under 40 μE m^{-2} s^{-1} low intensity. So, to prevent the over exposure of light which causes shock to cell. It can adapt itself to the new physiological environment as temperature, pH and radiation. The cells were growing at a faster rate as the light intensity rises up to 120 μE m^{-2} s^{-1}. When the light intensity optimal range is exceeded, the growth rates turn to maximum. From day three, the exponential phase begins where the growth rate and biomass density rapidly increase under all light intensities. The highest cell number (482 × 10^4) and biomass (1245 ± 105 mg/l) was found at 120 μE m^{-2} s^{-1}. It is hypothesised that at these light settings, the light intensity has no deleterious impact on cell division or other light-sensitive processes in the cell (Oostlander et al., 2020). Optimum intensity highly supports the maximum multiplication of cells and steady growth under optimum condition. However, it does not produce sufficient biomass as expected. On other hand, number of cells begins to drop under 160 and 200 μE m^{-2} s^{-1} from day 6 after it reached the stationary phase and reached to decline phase. The lowest cell number (293 × 10^4) and biomass (321 ± 41 mg/l) was found at 200 μE m^{-2} s^{-1}. In order to prevent PSII damage when exposed to high irradiances, phytoplankton cells must dissipate surplus energy as reported by Gao et al. (2018). Following the upward light shift, non-photochemical satisfying in the cells rise with exposure time (Jayakumar et al., 2021). Furthermore, diatoms have evolved an elaborate antioxidant network to scavenge reactive oxygen species generated by excessive light (Yuan et al., 2018). Brindhadevi et al. (2021) reported that, *Scenedesmus* sp. cultivated between 250 and 400 μE m^{-2} s^{-1} light conditions, revealed a significant change in storing lipid content. In addition, overall lipid of *Isochrysis galbana* LB987, *Nanochloropsis oculata* CCAP849/1, and *Dunaliella salina* rise up when the

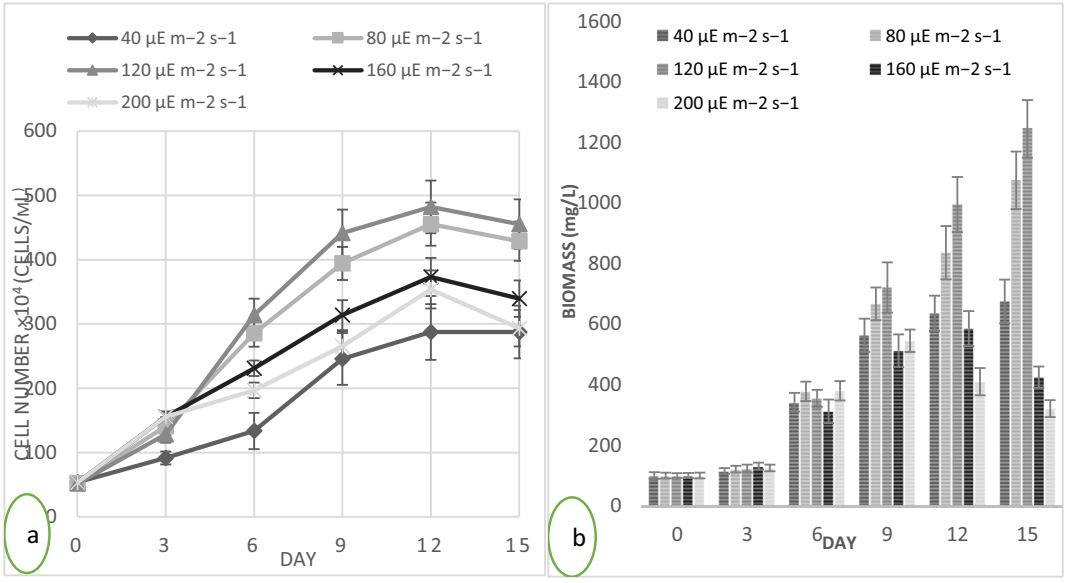

Fig. 2. Effect of blue light intensities on (a) cell number, (b) dry biomass. (The growth of cells and biomass concentration monitored daily and measure three days once in order to avoid contamination while handling.)

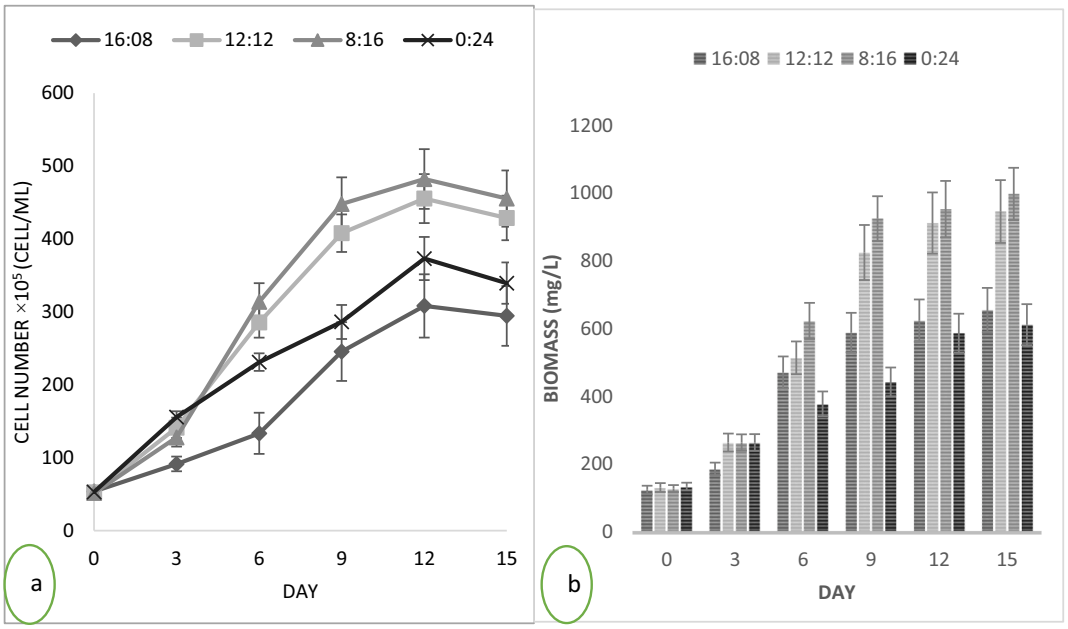

Fig. 3. Effect of photoperiod (16:08, 12:12, 8:16, 0:24 h) on (a) cell number ($\times 10^4$) and (b) biomass productivity of *Thalassiosira pseudonana*.

photon flux is increased until with 150 μE m^{-2} s^{-1} of intensity.

The cell numbers begin to reduce not due to lacking of nutrients, however, over exposure of high blue intensity cause resistance to proliferation and disruption of diatom cells. Cahill et al. (2019) reported that, produce soluble microbial product produced during algae cultivation where it supports the growth of co-existing of heterotrophic bacteria especially under high light intensity. This condition causes predation of bacteria, release of lysogenic component, lacking of nutrients and ingesting desire product. As a result, the growing culture undergoing deleterious in a short period of time. Therefore, over exposure of light can cause photo-oxidative harm to the photosynthetic apparatus. As consequence it decreases in photosynthesis efficiency and rate due to the photoinhibition in cells.

3.2. Effect of photoperiod on Thalassiosira pseudonana

The growth of diatom cells was highly influenced by the duration of photoperiod (dark:light cycle). The optimum light intensity as 120 μE m^{-2} s^{-1} was selected from the result of Section 3.1 to conduct the photoperiod parameter and cultivate the *Thalassiosira pseudonana*. From Fig. 3, highest (475 × 10^4) cell number and biomass (953 ± 12 mg/l) production found under 8:16 h of dark light cycle on day 12. On other hand, continuous light 0:24 h causes lowest cell multiplication number (373 × 10^4) and biomass productivity (371 ± 21 mg/l) on day 15 of cultivation due to lower photosynthesis. Rapid exponential growth begins from third day of cultivation and reached stationary phase on day 12 and end up to growth decline. The growth begins to drop due to longer period light exposure causes the photoinhibition process in the cell and lack of nutrients in the culture. Therefore, cells could not perform photosynthesis process in order to cells multiplication. Under optimum light exposure cells were taken in inorganic carbon and convert it to organic stuff. Light is the source of energy that drives this reaction, and intensity, spectrum quality, and photoperiod must all be taken into account (Park and Craggs, 2011). Chen et al. (2021) mentioned that, *Thalassiosira pseudonana* cellular pigment concentration was lowered as the exposure period increased. Similar to earlier research, a light intensity beyond 150 μE m^{-2} s^{-1} was predictable to saturate the cell growth and may have oversaturated the cell growth under a longer photoperiod, evidenced by a small difference in the between the 8:16 and 16:8 dark: light cycles. It is self-evident that in oversaturated light, phytoplankton cells reduce Chl a synthesis and accumulation in order to reduce excessive energy harvesting and so ameliorate excessive light energy-caused photodamage or photoinhibition (R. Li et al., 2017; G. Li et al., 2017).

Krzemińska et al. (2014) mentioned that light intensity together with photoperiod highly impacting growth rate and intercellular composition. Changes in photoperiod cause impacts in generation of total protein, pigment, and fatty acid content in *Chlorella vulgaris*, growth and lipid formation in diatom *Porphyridium cruentum* (Oh et al., 2009) biomass production and nutrient utilisation by *Chaetoceros muelleri* (Minggat et al., 2021).

Table 1
Lipid and fucoxanthin obtained from *Thalassiosira pseudonana* under optimised conditions (120 μE m^{-2} s^{-1} and 8:16 photoperiod hour).

Parameter	Light intensity (μE m^{-2} s^{-1})	Photoperiod (h)	Average value
Total lipid (%)	36.7 ± 0.30	34.5 ± 0.20	35.6 ± 0.25
Fucoxanthin yield (mg/g)	1.13 ± 0.09	1.24 ± 0.04	1.18 ± 0.06

3.3. Fucoxanthin production

The diatom *Thalassiosira pseudonana* grown under different light intensities and photoperiod showed greater content in carotenoid. The extraction of pigments depends on the cell wall resistance, type of solvent and methods of extraction. Methanol was used for chlorophyll and fucoxanthin extraction. The maximum yield obtained at light intensity (120 μE m^{-2} s^{-1}) and photoperiod (8:16 h) was 1.13 ± 0.09 and 1.28 ± 0.04 mg/g. Compare with other light intensities and photoperiod, the maximum fucoxanthin was extracted. The biomass productivity determines the accumulation of lipid and fucoxanthin in their cellular composition before extracted. Study reported by Bhattacharjya et al. (2020), maximum fucoxanthin was obtained 0.241 ± 0.016 mg L^{-1}. Fucoxanthin is a secondary carotenoid that has been shown to play an important role in light harvesting, antioxidation. According to several studies, pigment content is highly influenced by the organic solvent, photoperiod, wavelength, nitrogen concentration and further physicochemical characteristics (Bhattacharjya et al., 2020).

Table 2
Fatty acid profile of transesterified lipid extracted from *Thalassiosira pseudonana*.

Fatty acids	(w/w, %)
Myristic acid (14:0)	7.73 ± 0.03
Tetradecanoic acid (14:1)	4.15 ± 0.21
Palmitic acid (16:0)	34.16 ± 0.02
Palmitoleic acid (16:1)	18.68 ± 0.04
Heptadecanoic acid (17:0)	18.23 ± 0.13
Stearic acid (18:0)	1.47 ± 0.06
Oleic acid (18:1)	3.21 ± 0.22
Elaidic acid (18:1)	2.95 ± 0.15
Linoleic acid (18:2)	1.24 ± 0.09
Linolenic acid (18:3)	4.32 ± 0.03
Eicosenoic acid (20:1)	0.33 ± 0.01
Arachidonic acid (20:4)	3.11 ± 0.06
Erucic acid (22:1)	0.42 ± 0.01
Saturated	61.59
Monounsaturated	29.74
Polyunsaturated	8.67

3.4. Fatty acid profile

The total lipid content was extracted from biomass of different light intensities (36.7 %) and different photoperiod (34.5 %) as mentioned in Table 1. In average, the strain produced 35.6 % of total lipid. Marella and Tiwari (2020) reported that, total lipid (% dry cell weight) of *Thalassiosira weissflogii* were 30.1 % and 19.5 % under modified and standard medium cultures. Bhattacharjya et al. (2020) reported that, extracted total lipid (52 %) as maximum from *Thalassiosira* sp. as compared to other species *Skeletonema* sp. (44 %) and *Chaetoceros* sp. (21 %). Palanisamy et al. (2022) reported that, under the optimal light dark cycle have produced (46.2 %) of lipid content from *Thalassiosira pseudonana* using palm oil mill effluent nutrient media for cultivation. Many factors influence the lipid yield such as strain, biomass composition, solvent extraction and harvesting period. In this study, light intensity and photoperiod highly have influenced the lipid accumulation even though obtained limited lipid as compared to other previous studies.

The transesterified lipid fatty acid methyl ester was analysed through gas chromatography mass spectrophotometry and the FAME profile given below in Table 2. *Thalassiosira pseudonana* lipid compositions contained most of saturated fatty acids, SFA (61.59 %) as compared to monosaturated fatty acids, SFA (29.74 %) and polyunsaturated fatty acids, PUFA (8.67 %). palmitic acid (16:0, 34.16 %) palmitoleic acid (16:1, 18.68 %) and linolenic acid (18:3, 4.32 %) are the most dominating fatty acids. The biochemical makeup of diatom cells, notably their lipid composition, is known to be phase dependent. The ratio of lipid classes, as well as their fatty acid content, changed in connection to the physiological condition especially light intensity of the culture and the life forms of the diatom. The fatty acid content determines the application of biomass and the strain for further biotechnological application.

4. Conclusion

This study was undertaken to determine the influence of blue light on *Thalassiosira pseudonana*. Experiments were conducted under laboratory condition which can control and manipulate the environmental parameters as temperature, blue light intensity and photoperiod. The exposure of blue wavelength on diatom cells enhances the growth and biomass compared to white light. Therefore, it confirms that, there is a significant influence in *Thalassiosira pseudonana* cell under blue wavelength condition.

CRediT authorship contribution statement

Karthick Murugan Palanisamy: Investigation, Validation, Writing – original draft. **Mohd Hasbi Ab. Rahim:** Resources, Methodology, Supervision. **Natanamurugaraj Govindan:** Resources, Methodology, Supervision, Writing – original draft. **Rameshprabu Ramaraj:** Formal analysis. **Palaniselvam Kuppusamy:** Resources, Methodology, Supervision. **Gaanty Pragas Maniam:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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