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Research Article
Impact of Salinity and Light Intensity Stress on B Vitamins Content in Marine Diatom *Skeletonema costatum*

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Abstract

Background and Objective: The impact of environmental factors on the B vitamins (B1, B2, B6 and B12) content of marine diatom *Skeletonema costatum* has not yet been evaluated so far. Here, we aim to optimize the B vitamins production (vitamin B1, B2, B6 and B12) through culture of *S. costatum* on different salinity levels as well as light intensity exposures. **Materials and Methods:** The marine diatom *Skeletonema costatum* was cultured in different salinity levels (24, 29 and 34 g L^{-1}) to optimize B vitamins production, prior to exposure to different light intensity levels (20, 34 and 47 µmol m^{-2} sec^{-1}) for 3 days in f/2 medium. **Results:** Twenty four grams per liter salinity exposure produced the highest vitamin B1, B6 and B12 content of 90.08±2.6, 410.03±12.97 and 61.22±27.67 µg g^{-1}, respectively. When cultivated either below or above light intensity of 34 µmol m^{-2} sec^{-1}, vitamin B1, B6 and B12 content decreased. The highest total B vitamins obtained at 34 µmol m^{-2} sec^{-1} was 563.46 mg L^{-1} (vitamin B1: 90.08±1.48 µg g^{-1}, vitamin B2: 2.87±0.79 µg g^{-1}, vitamin B6: 410.03±12.97 µg g^{-1} and vitamin B12: 61.22±27.67 µg g^{-1}). **Conclusion:** Optimum growth, biomass and total B vitamins produced was achieved by culturing at salinity of 24 g L^{-1} and light intensity 34 µmol m^{-2} sec^{-1}. So far, optimum growth, biomass and total B vitamins produced was achieved by culturing at salinity of 24 g L^{-1} and light intensity 34 µmol m^{-2} sec^{-1}. Note that when aiming at high vitamin productivities, it is better to culture *S. costatum* in a two step process: A nutrient sufficient phase in optimum environmental growth conditions to produce enough cells (e.g., salinity, illumination, pH, temperature, supply of CO_2 and nutrients etc.), followed by suitable stress to stimulate B vitamins synthesis in a controlled manner.

Key words: Environment factor, biomass production, light intensity, salinity level, supply of CO_2

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Indonesia is an archipelagic island country consist of 64.97% sea and ocean of its total area[1]. These water region which supports life of various natural resources, including marine microalgae diversity[2,3]. Marine microalgae play an important roles as primary producer for small marine zooplankton such as rotifer, copepod, daphnia, brine shrimp, etc., which they are fed further to larval and juvenile fish and crustaceans. For that reason, marine microalgae have been widely used in aquaculture practice, particularly in larval stage to provide many essential nutrients such as protein, lipid and other key nutrients, such as vitamins, pigment, sterols, etc.[4]. Some microalgae commonly used in aquaculture that have good nutritional properties, either as monoculture or mixed culture are *Chaetoceros calcitrans*, *Chaetoceros muelleri, Pavlova lutheri*, *Isochrysis* sp. (T.ISO), *Tetraselmis suecica*, *Skeletonema costatum* and *Thalassiosira pseudonana*[5].

As previously mentioned, vitamins are one of the key nutrient that should be sufficient in feed even though they are required only in small quantities. Deficiency of vitamins will result in abnormal biochemical function and organ dysfunction that leads to disruption of the animal growth. In addition, vitamin deficiency results in depressed immune function and slow or no recovery from disease. This is particularly for early developmental stages in which the developing immune system requires an optimum level of vitamins[6]. Among of all vitamins, B vitamins are more crucial because they are involved in many cellular metabolisms that act as enzyme activators and play a key role in carbohydrate, protein and lipid metabolism. Chinook salmon fed with diet that contains riboflavin (B2), panthotenic acid (B5), pyridoxine (B6) and folic acid (B9) based on NRC suggestion levels for optimum growth also enhanced optimum immune function[6].

The marine diatom *Skeletonema costatum* has been recognized a potential species as source of some essential B vitamins for maricultured animals such as thiamine (B1)[7], riboflavin (B2)[8], pyridoxine (B6)[4] and cobalamin (B12)[4]. In addition, *S. costatum* has been cultivated as feed for bivalve, shrimp larvae and zooplanktons since 1980's[2,9]. Although, its biomass culture has gone a long way, so far little progress was achieved regarding the optimization of B vitamins content of *S. costatum*[10].

As antioxidant compounds, B vitamins regulates defense mechanism in microalgae cell towards external stresses[11]. Several environmental factors associated with cell metabolism, such as light intensity and salinity have been reported to influence the chemical composition of microalgae, as well as vitamins contents[2,12,13]. Light intensity affects the growth of photoautotrophic organisms in general including diatoms[9], while salinity accounts as one important factor which influences growth and vitamin production of most Bacillariophyceae species and some *S. costatum* strains present in temperate climate[13,14].

In this study, it is expected that B vitamins content (vitamin B1, B2, B6 and B12) can be optimized through culture of *S. costatum* with environmental stress by changing the salinity levels and exposure to a different light intensity levels. For the future, this study can support to improve the quality of live feed in aquaculture practice.

MATERIALS AND METHODS

Measuring growth rate of *Skeletonema costatum*: The microalgae strain used for the experiments *Skeletonema costatum* (wild-type) was obtained from Balai Besar Pengembangan Budidaya Air Payau (BBPBAP Jepara, Central Java). The diatom were cultured in a 16 L breeding reactor with f/2 medium (Guillard©) for measuring growth of *S. costatum*[15]. To prevent reaction vessel overflow, only 8 L of the microalgae suspension filled to the reactors. The aeration rate was maintain at 100 mL min^{-1} using a light-dark cycle[16] of 24:0 at 34 μmol m^{-2} sec^{-1}. Salinity was adjusted at 29 g L^{-1} and incubated at room temperature 27±1°C. Cell density was counted daily using a hemocytometer and specific growth rate (μ) was calculated using equation below:

$$\mu = \frac{\ln X_{t2} - \ln X_{t1}}{t_2 - t_2}$$

where, μ is the specific growth rate and $\ln X_t$ is the natural log of the cell density at t, time of count (cell mL^{-1}). Integration over times t1 and t2 yields the log-linear growth curve[17,18].

Optimizing salinity and light intensity factor: To explore the influence of different salinity and light intensity levels on B vitamins content, a two-stage culturing were conducted. The *S. costatum* was cultured at 8 L in f/2 medium with 3 salinity levels (24, 29 and 34 g L^{-1}) and light intensity of 34 μmol m^{-2} sec^{-1} from initial cell density of 5×10^4 cell mL^{-1}, following harvesting and B vitamins content analysis (methods described below). Further, optimum light intensity level (20, 34 and 47 μmol m^{-2} sec^{-1}) in culture was analyzed using optimum salinity condition. All treatments were carried out in triplicates for 3 days.

Harvesting of *Skeletonema costatum*: Biomass of *S. costatum* was harvested by filtration yielding wet

biomass[19]. Ash-free dry weight was obtained by air drying the biomass for 24 h at room temperature, following vacuum drying at 62°C for 4 h[20].

Vitamin B1, B2, B6 and B12 analysis: About 0.5 g ash-free dry biomass was mixed in 15 mL, 0.01 N HCl and boiled in a water bath for 15 min, centrifuged at 1400 rpm, 4°C and the supernatant was filtered through a 0.45 µm membrane filter before HPLC analysis[20]. The C_{18} column with size 250×4.6 mm was used for vitamins separation, with mobile phase 30:70 of methanol:dH_2O. The flow rate was maintained at 1 mL min^{-1}. Wavelength of detection was 254 nm. Twenty mictoliters sample was injected and chromatographed, performed at ambient temperature[20].

Statistical analysis: Vitamin B1, B2, B6 and B12 content were evaluated using one-way variance analysis (ANOVA) using SPSS 20.0 (IBM, USA) with significance level of $\alpha = 0.05$ and Duncan's multiple range test at 5% level[21].

RESULTS

Growth of *Skeletonema costatum*: Optimum time for transferring inoculants as well as harvesting was determined from the growth curve in Fig. 1 and growth rate of *S. costatum* on each day. Referring to this data, the highest biomass (1.64×10^6 cell mL^{-1}) as well as specific growth rate (1.175 day^{-1}) occurred at the third day of culture. Therefore, *S. costatum* innoculum transfer to initiate a fresh culture batch was conducted after the culture reached three days old.

Salinity effect on growth, biomass and vitamin B1, B2, B6 and B12 content: Figure 2 presents *S. costatum* grown in batch culture under 3 levels of salinity. During 4 days of culture period, *S. costatum* reached the highest cell density and biomass at the 24 g L^{-1} salinity. The cell density was similar compared to growth at 29 g L^{-1} salinity ($p<0.05$) but higher than growth at 34 g L^{-1} salinity ($p>0.05$). Total biomass was not significantly different among salinity treatments. Table 1 revealed that B vitamins content obtained from culture at 24, 29 and 34 g L^{-1} salinity were 563.46, 401.10 and 352.45 µg g^{-1}, respectively.

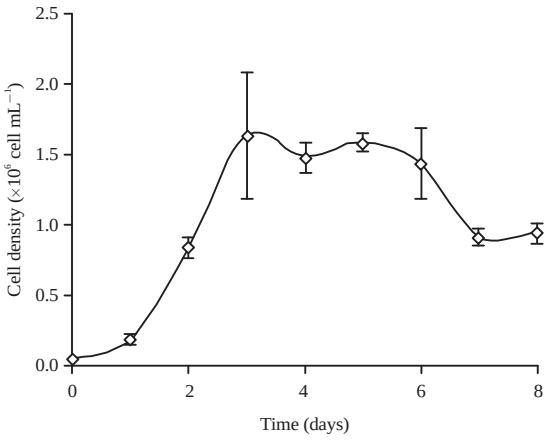

Fig. 1: Growth curve of *Skeletonema costatum*, at room temperature 27±1°C, salinity 29 g L^{-1}, light intensity 34 µmol m^{-2} sec^{-1}

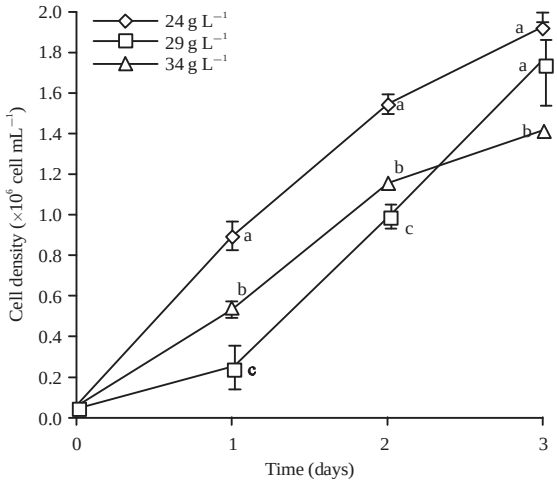

Fig. 2: Growth of *Skeletonema costatum* under different salinities. Different letters denote significant statistic results ($p<0.05$, $n = 3$)

Table 1: Growth, biomass production and vitamin B1, B2, B6 and B12 content at 3 levels of salinity

	Treatments		
Parameters	24 g L^{-1}	29 g L^{-1}	34 g L^{-1}
Vitamin (µg g^{-1})*			
B1	90.08±2.60[a]	73.75±1.96[b]	58.34±2.79[c]
B2	2.30±0.79[a]	29.71±8.94[b]	11.68±1.33[a]
B6	410.03±12.97[a]	245.40±71.74[b]	241.91±28.50[b]
B12	61.05±27.61[a]	52.24±2.24[a]	40.51±1.26[a]
Total	563.46	401.10	352.45
Cell density (×10^6 cell mL^{-1})*	1.93±0.06[a]	1.75±0.21[a]	1.42±0.01[b]
Biomass (g L^{-1})*	0.19±0.03[a]	0.146±0.05[a]	0.172±0.00[a]

*Mean results of three determinants. Different letters within a row denote significant statistic result ($p<0.05$)

Table 2: Data of growth, biomass production and vitamin B1, B2, B6 and B12 content at 3 levels of light intensity

	Treatments		
Parameters	20 μmol m^{-2} sec^{-1}	34 μmol m^{-2} sec^{-1}	47 μmol m^{-2} sec^{-1}
Vitamin (μg g^{-1})*			
B1	74.39±5.60[a]	90.08±1.48[b]	86.89±1.57[b]
B2	2.87±1.48[a]	2.87±0.79[a]	1.46±0.07[a]
B6	356.27±41.03[a]	410.03±12.97[a]	345.76±16.13[a]
B12	45.92±5.07[a]	61.05±27.61[a]	49.36±4.09[a]
Total	476.25	563.46	483.48
Cell density (×10^6 cell mL^{-1})*	0.97±0.19[b]	1.93±0.06[a]	1.53±0.46[ab]
Biomass (g L^{-1})*	0.09±0.02[a]	0.19±0.03[b]	0.13±0.04[ab]

*Mean results of three determinants. Different letters within a row denote significant statistic result ($p<0.05$)

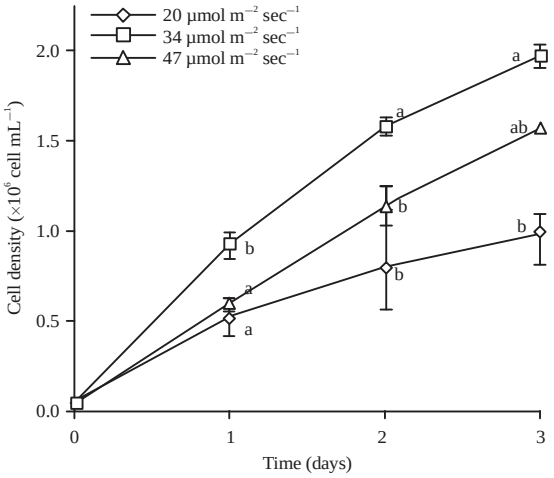

Fig. 3: Growth of *Skeletonema costatum* under different light intensities. Different letters denote significant statistic results ($p<0.05$, $n = 3$)

Light intensity effect on growth, biomass and vitamin B1, B2, B6 and B12 content: The growth of *S. costatum* in batch culture under 3 levels of light intensity is represented in Fig. 3. After four culture days, the highest cell density and biomass was achieved at culture with light intensity of 34 μmol m^{-2} sec^{-1}, followed by 47 and 20 μmol m^{-2} sec^{-1} (Table 2) with total B vitamins contentof each batch were 563.46, 483.48 and 476.25 μg g^{-1}, respectively.

DISCUSSION

Skeletonema costatum underwent a lag phase for a day, following an exponential growth for 2 days at specific growth rate of 1.175 day^{-1}, reaching cell density of 1.64±0.45×10^6 cell mL^{-1} (Fig. 1). From day 4th-6th growth rate decreased and cell density remained constant. This result was similar to Rajeswari and Balasubramanian[22] that showed the same trend in growth curve of *S. costatum* which was cultured in various culture media at 28±1°C, salinity 28 PSU and light intensity 50 μmol m^{-2} sec^{-1}.

Refer to the growth curve, optimum time to transfer and harvest *S. costatum* was on the 3rd day. This period of time applies when *S. costatum* was activated prior to culture in a larger batch to minimize growth in lag phase[23]. Harvesting biomass after the culture age exceeds 4 days were not suitable as growth rate has decreased, affected by numerous factors (e.g., high cell density reduces the amount of light available to individual cells, decreased nutrients as well as increasing metabolic waste excreted to the medium and reduced buffering capacity[24,25].

During cultivation, manipulation of the biochemical composition of the microalgae by changing the environmental growth conditions (e.g., salinity, illumination, pH, temperature, supply of CO_2 and nutrients, etc.) has various effect between species[26,27]. In this study, the best growth rate and the highest total biomass were obtained at the lowest salinity treatment (24 g L^{-1}). Based on Khan *et al.*[28] *S. costatum* could tolerate a wide range of salinity 3-55 ppt and the best growth rate occurred at salinity of 20 g L^{-1}. Salinity greater than 20 g L^{-1} in a range of 20-55 g L^{-1}, showed that the cell density was more decreased when the salinity increased. This indicates that the salinity was inversely related to growth rate. This is consistent with this study, among three salinity (24, 29 and 34 g L^{-1}), the highest salinity showed the lowest cell density. According to Moradi and Ismail[29], the photosynthetic rate decreased due to inefficient water intake in hyperosmotic environment. Based on Sylvander *et al.*[14] optimum salinity is 25-30 g L^{-1} for *S. costatum*[15], therefore it suggests that ionic stress occurred at 34 g L^{-1} salinity. Previous studies reported that culture at high salinity lead to a low chlorophyll-a content, as it is the main photosynthetic pigment present in photoautotrophic organisms[30,31]. This further resulted in a reduced photosynthesis and growth as well[32].

Carlucci and Bowes[7] stated that vitamin uptake was greatest during the first few days of incubation and on continued incubation the rate of uptake decreased. *Skeletonema costatum* was reported can produced both thiamine and biotin when growing either 12 or 2 ng vitamin

B12 L^{-1}. Biosynthesis of thiamine has positive correlation between cell growth. Thiamine is vital to all cells due to its role as a cofactor for enzyme critical production in carbon and amino acid metabolism[33]. However, it is still unclear how the mechanism of thiamine biosynthesis pathway performed by the *S. costatum*, recent study has showed that *Thalassiosira pseudonana* has riboswitches that act as metabolite-sensing mRNA-based gene regulators for thiamine biosynthesis genes[34]. In this study, the highest content of thiamine observed at salinity 24 g L^{-1} which has the highest growth rate and biomass production. Sylvander *et al.*[14] also reported that reducing salinity below 25 g L^{-1} yields the higher vitamin B1 content.

On the other hand, vitamin B6 play a crucial role in protecting cells from oxidative stress as an antioxidant. Previous studies shown that several genes involved in the vitamin B6 synthesis pathways were induced by low salinity environment and Reactive Oxygen Species (ROS) presence[35]. Low salinity results an imbalance condition between nutrients and trace metal concentration in the medium and high metal is toxic and often influence *S. costatum* metabolism negatively[19]. Therefore, low salinity mostly induced the production of vitamin B6 among other B vitamins, as in line with result of the experiment. Based on Kumar and Prabu[4], vitamin B6 was observed 0.528 mg g^{-1} in *S. costatum* biomass. The *S. costatum* is vitamin B12 auxotrophy (unable to produce vitamin B12) and requires vitamin B12 for amino acid and one-carbon metabolism. Vitamin B12 contains cobalamin that is only produced by selected bacteria and archaea. Biosynthesis of vitamin B12 involves over 30 enzymatic steps and significant consumption of cellular energy, carbon, nitrogen, cobalt, zinc and in some cases iron[36]. Vitamin B12 was initially added 3.69×10^{-10} M from f/2 media[15] and was taken up by *S. costatum* most rapidly during the initial incubation on log phase and decreased with longer incubation period[7]. Vitamin B12 content was not significantly higher at 24 g L^{-1} salinity than other treatments. Based on present study, vitamin B12 might also involve in protecting microalgae cells from salt stress, but its specific role still needs further studies.

In contrast, the highest vitamin B2 content was reached at 29 g L^{-1} salinity. Either way, the positive correlation between cell density and vitamin B1, B6 and B12 content was observed in all 3 salinity levels but no correlation was observed between cell density and vitamin B2 content in all treatments. Previous studies observed a negative correlation between cell density and the total vitamin B1 in marine diatom culture[14,37-39]. The positive correlation indicates an early stress response cells to maintain its growth. It has been observed that vitamins accelerate cell division and cell enlargement to simprove membrane integrity towards either hypo osmotic or hyper osmotic environment in an effort to maintain osmotic balance in cell's cytoplasm[40]. Therefore, cell growth can continue and its rate was less affected.

Optimum light intensity for culturing *S. costatum* ranges between 34-68 μmol m^{-2} s^{-1}, in line with this study's result as reflected in Fig. 3, a slower growth rate with lower total biomass at 20 and 47 μmol m^{-2} sec^{-1} light intensity cultures than the 1 cultured at 34 μmol m^{-2} sec^{-1} light. Low light intensity reduces photosynthesis rate and thus decreasing biomass growth rate[41]. Moreover, either under or over optimum light intensity, metabolic disruption on photosynthesis would likely increase photo respiration process, which can subsequently increase production of reactive oxygen species (e.g., ROS, O^{-}_{2}, H_2O_2 and OH)[42]. These radicals can cause oxidative damage to microalgae cells. Recent studies have shown that ROS play disturbs signal transduction molecules involved in mediating responses to stress stimuli in autotropic organisms, including the growth of *S. costatum*[43,44] at 20 and 47 μmol m^{-2} sec^{-1}.

Total vitamin B1, B2, B6 and B12 content at 47 μmol m^{-2} sec^{-1} light intensity was not significantly lower than the result in 34 μmol m^{-2} sec^{-1} light intensity culture ($p<0.05$). It is possible that ROS molecule production was embraced by the defence mechanisms of *S. costatum*. Successive counteracts towards radical accumulation during external environmental stress greatly influenced by the production rate of both radical molecules and antioxidant molecules, in this case: B vitamins[45]. Thus, higher radical production stimulates higher B vitamins production, but it will cause an inhibition on growth and total biomass produced. This study proved that salinity and light intensity affects B vitamins content of *S. costatum.* So far, the optimum growth, biomass and total B vitamins produced was achieved by culturing at salinity of 24 g L^{-1} and light intensity 34 μmol m^{-2} sec^{-1}.

Even more intensive research and field trials are necessary to study the mechanisms of salinity and light intensity stresses on B vitamins production in *S. costatum*. In general, stress conditions lead to activation of related biomolecules in the particular stress-related defense mechanisms, which affects the growth rate negatively.

CONCLUSION

To conclude, when aiming at high vitamin productivities, it seems better to culture *S. costatum* in a two-step process: A nutrient sufficient phase in optimum environmental growth

conditions to produce enough cells (e.g., salinity, illumination, pH, temperature, supply of CO_2 and nutrients, etc.) followed by suitable stress to stimulate B vitamins synthesis in a controlled manner.

SIGNIFICANCE STATEMENTS

- Lower than 34 g L^{-1} salinity mostly induced the production of vitamin B6 among other B vitamins
- Cultivation of *S. costatum* on either below or above light intensity of 34 µmol m^{-2} sec^{-1} decreases vitamin B1, B6 and B12 content
- In general, stress conditions lead to activation of related biomolecules in the particular stress-related defense mechanisms, which affects the growth rate negatively
- More intensive research and field trials are necessary to study the mechanisms of salinity and light intensity stresses on B vitamins production in *S. costatum*

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REFERENCES

1. Hutomo, M. and M.K. Moosa, 2005. Indonesian marine and coastal biodiversity: Present status. Indian J. Mar. Sci., 34: 88-97.
2. Barsanti, L. and P. Gualtieri, 2006. Algae: Anatomy, Biochemistry and Biotechnology. 1st Edn., CRC Press, Florida, pp: 213.
3. Iba, W., 2016. The potential of Indonesian microalgal strains to support eastern white shrimp (*Litopenaeus vannamei*) aquaculture. Ph.D. Thesis, University of Rhode Island, Kingston.
4. Kumar, C.S. and V.A. Prabu, 2015. Nutritional value of *Skeletonema costatum* (Cleve, 1873) from parangipettai, southeast coast of India. Int. J. Pharmaceut. Sci. Res., 6: 3463-3466.
5. Brown, M.R., 2002. Nutritional Value of Microalgae for Aquculture. In: Avances en Nutricion Acuicola VI: Memorias del VI Simposium Internacional de Nutricion Acuicola, Cruz-Suarez, L.E., D. Ricque-Marie, M. Tapia-Salazar, M.G. Gaxiola-Cortes and N. Simoes (Eds.). Centro de Investigaciones de Quintana Roo, Mexico.
6. Lall, S.P., 2000. Nutrition and Health of Fish. In: Avances en Nutricion Acuicola V. Memorias del V Simposium Internacional de Nutricion Acuicola, Cruz-Suarez, L.E., D. Ricque-Marie, M. Tapia-Salazar, M.A.Y. Olvera-Novoa and R. Civera-Cerecedo (Eds.). Centro de Investigaciones de Quintana Roo, Mexico.
7. Carlucci, A.F. and P.M. Bowes, 1970. Production of vitamin B_{12}, thiamine and biotin by phytoplankton. J. Phycol., 6: 351-357.
8. Brown, R.M. and L.C. Farmer, 1994. Riboflavin content of six species of microalgae used in mariculture. J. Applied Phycol., 6: 61-65.
9. Becker, E.W., 2007. Micro-algae as a source of protein. Biotechnol. Adv., 25: 207-210.
10. De Roeck-Holtzhauer, Y., I. Quere and C. Claire, 1991. Vitamin analysis of five planktonic microalgae and one macroalga. J. Applied Phycol., 3: 259-264.
11. Berglund, T. and A.B. Ohlsson, 1995. Defensive and secondary metabolism in plant tissue cultures, with special reference to nicotinamide, glutathione and oxidative stress. Plant Cell Tissue Org. Cult., 43: 137-145.
12. Sarkiyayi, S. and H. Ikioda, 2010. Estimation of thiamin and ascorbic acid contents infresh and dried *Hibiscus sabdarriffa* (Roselle) and *Lactuca sativa* (Tettuce). Adv. J. Food Sci. Technol., 2: 47-49.
13. Parida, A.K. and A.B. Das, 2005. Salt tolerance and salinity effects on plants: A review. Ecotoxicol. Environ. Saf., 60: 324-349.
14. Sylvander, P., N. Haubner and P. Snoeijs, 2013. The thiamine content of phytoplankton cells is affected by abiotic stress and growth rate. Microb. Ecol., 65: 566-577.
15. Guillard, R.R. and J.H. Ryther, 1962. Studies of marine planktonic diatoms. I. Cyclotella nana Hustedt, and Detonula confervacea (cleve) Gran. Can. J. Microbiol., 8: 229-239.
16. Rudiyanti, S., 2011. [The growth of *Skeletonema costatum* on various salinity level's media]. Jurnal Saintek Perikanan, 6: 69-76, (In Indonesian).
17. Cappuccino, J.G. and N. Sherman, 2010. Microbiology: Laboratory Manual. 1st Edn., Pearson Benjamin Cummings, San Fransisco, pp: 75.
18. Andersen, R.A., 2005. Algal Culturing Techniques. Elsevier Academic Press, USA., ISBN-13: 9780120884261, pp: 272.
19. Grima, E.M., E.H. Belarbi, F.G.A. Fernandez, A.R. Medina and Y. Chisti, 2003. Recovery of microalgal biomass and metabolites: Process options and economics. Biotechnol. Adv., 20: 491-515.
20. Perveen, S., A. Yasmin and K.M. Khan, 2009. Quantitative simultaneous estimation of water soluble vitamins, riboflavin, pyridoxine, cyanocobalamin and folic acid in neutraceutical products by HPLC. Open Anal. Chem. J., 3: 1-5.
21. Zar, J.H., 1996. Biostatistical Analysis. 3rd Edn., Prentice Hall, USA., ISBN: 0130845426, Pages: 662.

22. Rajeswari, M.V. and T. Balasubramanian, 2014. Comparative study on growth of *Skeletonema costatum*: Amicroalga as live feed for aquaculture importance. Int. J. Res. Fish. Aquacult., 4: 117-121.
23. Lincoln, R.E., 1960. Control of stock culture preservation and inoculum build-up in bacterial fermentation. J. Biochem. Microbiol. Technol. Eng., 2: 481-500.
24. Creswell, L., 2010. Phytoplankton culture for aquaculture feed. SRAC Publication No. 5004, Southern Regional Aquaculture Center, Stoneville, MS., USA., September 2010, pp: 1-13.
25. Hoff, F.H. and T.W. Snell, 2008. Phytoplankton Culture Manual. Florida Aquafarms Inc., Florida, pp: 186.
26. Xu, Y. and J. Lin, 2008. Effect of temperature, salinity and light intensity on the growth of the green macroalga, *Chaetomorpha linum*. J. World Aquacult. Soc., 39: 847-851.
27. Adenan, N.S., F.M. Yusoff and M. Shariff, 2013. Effect of salinity and temperature on the growth of diatoms and green algae. J. Fish. Aquatic Sci., 8: 397-404.
28. Khan, S., M.M. Haque, O. Arakawa and Y. Onoue, 1998. Physiological observations on a diatom *Skeletonema costatum* (Greville) cleve. Bangladesh J. Fish. Res., 2: 109-118.
29. Moradi, F. and A.M. Ismail, 2007. Responses of photosynthesis, chlorophyll fluorescence and ROS-scavenging systems to salt stress during seedling and reproductive stages in rice. Ann. Bot., 99: 1161-1173.
30. Munns, R., H. Greenway and G.O. Krist, 1983. Halotolerant Eukaryotes. In: Encyclopedia of Plant Physiology, Lange, O.L., P.S Noble, C.B. Osmond and H. Ziegler (Eds.). 12th Edn., Springer, New York, pp: 59-136.
31. Vonshak, A., N. Kancharaksa, B. Bunang and M. Tanticharoen, 1996. Role of light and photosynthesis on the acclimation process of the cyanobacterium *Spirulina platensis* to salinity stress. J. Applied Phycol., 8: 119-124.
32. Rai, A.K. and G. Abraham, 1993. Salinity tolerance and growth analysis of the cyanobacterium *Anabaena doliolum*. Bull. Environ. Contam. Toxicol., 51: 724-731.
33. Paerl, R.W., E.M. Bertrand, A.E. Allen, B. Palenik and F. Azam, 2015. Vitamin B1 ecophysiology of marine picoeukaryotic algae: Strain-specific differences and a new role for bacteria in vitamin cycling. Limnol. Oceanogr., 60: 215-228.
34. McRose, D., J. Guo, A. Monier, S. Sudek and S. Wilken *et al*., 2014. Alternatives to vitamin B_1 uptake revealed with discovery of riboswitches in multiple marine eukaryotic lineages. ISME J., 8: 2517-2529.
35. Ehrenshaft, M., A.E. Jenns, K.R. Chung and M.E. Daub, 1998. *SOR1*, a gene required for photosensitizer and singlet oxygen resistance in *Cercospora* fungi, is highly conserved in divergent organisms. Mol. Cell, 1: 603-609.
36. Bertrand, E.M. and A.E. Allen, 2012. Influence of vitamin B auxotrophy on nitrogen metabolism in eukaryotic phytoplankton. J. Front. Microbiol. 10.3389/fmicb. 2012.00375.
37. Abdel-Rahman, M.H.M., R.M. Ali and H.A. Said, 2005. Alleviation of NaCl-induced effects on *Chlorella vulgaris* and *Chlorococcum humicola* by riboflavin application. Int. J. Agric. Biol., 7: 58-62.
38. Brown, M.R., M. Mular, I. Miller, C. Farmer and C. Trenerry, 1999. The vitamin content of microalgae used in aquaculture. J. Applied Phycol., 11: 247-255.
39. Montagnes, D.J.S. and S. Brown, 1997. Early Mortality Syndrome in Salmonid Fishes from the Great Lakes. In: Chemically Induces Alterations in Functional Development and Reproduction of Fishes, Roland, R.M., M. Gilbertson and R.E. Peterson (Eds.). SETAC, Pensacola, pp: 135-152.
40. Ekmekci, B.A. and M. Karaman, 2012. Exogenous ascorbic acid increases resistance to salt of *Silybum marianum* (L.). Afr. J. Biotechnol., 11: 9932-9940.
41. Stanbury, P.F., A. Whitaker and S.J. Hall, 1995. Principles of Fermentation Technology. 2nd Edn., Butterworth-Heinemann Ltd., Oxford, UK., ISBN-13: 9780750645010, Pages: 357.
42. Niyogi, K.K., 1999. Photoprotection revisited: Genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol., 50: 333-359.
43. Kawano, I., T. Oda, A. Ishimatsu and T. Muramatsu, 1996. Inhibitory effect of the iron chelator Desferrioxamine (Desferal) on the generation of activated oxygen species by *Chattonella marina*. Mar. Biol., 126: 765-771.
44. Apel, K. and H. Hirt, 2004. Reactive oxygen species: Metabolism, oxidative stress and signal transduction. Annu. Rev. Plant Biol., 55: 373-399.
45. Mittler, R., S. Vanderauwera, M. Gollery and F. van Breusegem, 2004. Reactive oxygen gene network of plants. Trends Plant Sci., 9: 490-498.