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EFFECT OF PLANT GROWTH REGULATORS ON GROWTH AND PIGMENT COMPOSITION OF MICROALGA, *Nannochloropsis salina* D.J. Hibberd

S. Shyam Kumar[1]* and A.V. Saramma[2]

Department of Botany, Maharaja's College, Ernakulam - 682 011, Kerala (India)
[2]Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Ernakulam - 682 016, Kerala (India)
*e mail: *gokulamshyam01@gmail.com*

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ABSTRACT

***Nannochloropsis salina* D.J. Hibberd, a marine microalga, has aquaculture and biotechnological potential. The present study was aimed at to assess the influence of common plant growth regulators on growth and pigment composition of microalga *N. salina*. The addition of indole-3-acetic acid (IAA) to growth medium at lower concentration (0.1 mg L^{-1}) slightly promoted algal growth while 2,4-dichlorophenoxy acetic acid (2,4-D) decreased the growth. Lower concentrations of auxins enhanced chlorophyll *a* production in *N. salina* while higher concentration inflicted inhibition. Addition of kinetin in medium negatively influenced the growth rate and pigment production while 6-benzylaminopurine (BAP) promoted algal growth at lower concentrations (0.1 mg L^{-1}) but positively influenced pigment production at 2 mg L^{-1}.**

Key words: Chlorophyll, growth regulators, microalgae, *Nannochloropsis salina*, pigments

INTRODUCTION

Phytohormones are chemical substances present in minute amounts in plant tissues which regulate plant growth and development. Auxins and cytokinins are the two main types of hormones used in *in vitro* plant culture. Auxins influence plant cell growth through cell expansion, division initiation, cell wall acidification, callus formation and rooting in *in vitro* culture. In developed tissues, auxins play key role in apical dominance and fruit ripening. Indole-3-acetic acid (IAA), the most commonly detected natural auxin, reportedly is the first chemically characterized plant growth substance which promotes growth elongation (Tarakhovskaya *et al*., 2007). IAA and several conjugates are used as growth regulators in tissue culture. 2,4-dichlorophenoxy acetic acid (2,4-D) is the most commonly used synthetic auxin in plant tissue culture for callus induction, somatic embryogenesis and cell suspension culture (Quainoo and Dwomo, 2012). 2,4-D is also used as a weedicide. Cytokinins stimulate cell division, release lateral bud dormancy and induce adventitious bud formation. Joint action of auxins and cytokinins is the main growth regulating mechanism in higher plants. Auxins affect DNA replication while cytokinins influence the events controlling mitosis. Cytokinins also retard leaf senescence, promote chlorophyll biosynthesis and enhance chloroplast development. Zeatin is a naturally occurring cytokinin, but its high production cost restricts its use in *in vitro* culture. Many cytokinins-like growth regulators are used in plant tissue culture and the most commonly used cytokinins are substituted purines; kinetin and 6-benzylaminopurine (BAP).

Unicellular plants like microalgae are capable of producing some low-molecular chemical compounds in their growth medium which have proved beneficial as protective and cell-cell attractive

in function (Tarakhovskaya *et al*., 2007). The knowledge on algal hormone system is fragmentary. Stirk and Van (1997) discovered the presence of zeatin in fucoid algae. Ordog *et al.* (2004) confirmed the presence of hormonal systems in microalgae. However, the impact of exogenous plant growth regulators on the growth of microalgae have not properly been studied. The present study was, therefore, aimed to assess the influence of common plant growth regulators on growth and pigment production of marine microalga *Nannochloropsis salina.*

MATERIALS AND METHODS

The culture of marine microalga *Nannochloropsis salina* D.J. Hibberd, maintained at the Department of Marine Biology, Microbiology and Biochemistry, CUST, Kerala (India), was used in the present study. *N. salina* culture was raised in 1000 mL Erlenmeyer flasks containing 600 mL f/2-Si medium (Guillard, 1975) with a salinity of 30 ppt (parts per thousand). Illumination was provided by cold white fluorescent light of 2000 lux for a light/dark period of 12:12 h. Cultures were maintained at a temperature of 30±2ºC.

Six concentrations (0, 0.1, 0.5, 1.0, 1.5 and 2.0 mg L^{-1}) of 2,4-D and IAA (auxins), and kinetin and 6-benzylaminopurine (cytokinins) were assessed in the present study. The experiments were set up using 250 mL Erlenmeyer flasks containing 150 mL f/2 medium appended with above respective growth regulator as per treatment. Ten percent volume of logarithmically growing culture was used as inoculum. Illumination was provided by cold white fluorescent light of 2000 lux for a light/dark period of 12:12 h and flasks incubated at 29±1°C for 12 days. The observations were taken at 2 days intervals. The experiment was laid in a completely randomized design and each treatment replicated three times. The algal population was estimated by counting the cells with the help of a haemocytometer and growth rate calculated as per Guillard's equation (Guillard, 1973):

$$\text{Exponential growth rate (r)} = \frac{\ln N_t - \ln N_0}{\Delta t}$$

Wherein, $\ln N_t$ = population size at the end of time interval; $\ln N_0$ = population size at the beginning of experiment; Δt = length of time interval

The growth rate was expressed in terms of growth day^{-1}. For the estimation of pigments, 1 mL culture was filtered through 13 mm Whatman GF/C filter paper by using gentle vacuum filtration (Kumar and Saramma, 2013). The filter papers with algal cells were introduced into screw-capped test tubes containing 5 mL 90% acetone. The test tubes were covered with aluminum foil to prevent the entry of light. Algal samples were ground in a glass homogenizer and refrigerated for 1 h. The samples were then centrifuged at 3000 rpm for 5 min. The clear supernatant was taken, made up to 5 mL with 90% acetone and used for pigment quantification. The experiments were performed in dark in triplicate (Strickland and Parsons, 1972; Kumar and Saramma, 2013). The absorbance of algal samples was estimated by using 90% acetone as a blank, at 750, 665, 645, 630 and 480 nm in a spectrophotometer (Hitachi U-2001). The extinction at 750 nm was subtracted from the extinctions observed at 665, 645, 630 and 480 nm (Jeffrey and Humphrey, 1975). The amount of chlorophyll a (Chl *a*) in samples was estimated by using the equation:

$$C_a = 11.85(OD_{665}) - 1.54(OD_{645}) - 0.08(OD_{630})$$

Chlorophyll *a* (µg L^{-1}) = C_a × extract volume (mL)/ **v**olume of sample (L) × path length of cuvette (cm)

$$C_{Car} = 4.0(OD_{480}) \text{ …… (Strickland and Parsons, 1972)}$$

Total carotenoids (µg L^{-1}) = C_{Car} × extract volume (mL)/ **v**olume of sample (L) × path length of cuvette

Statistical analysis was performed using one-way analysis of variance using SPSS version 17. The values were compared using Tukeys test ($p < 0.05$) (Moreira *et al*., 2017).

RESULTS AND DISCUSSION

Plant growth regulators play vital role in algal growth and metabolism (Bradley and Cheney, 1990; Evans and Trewavas, 1991). The exogenous plant hormones modify the metabolism in algae (Piotrowska *et al.*, 2012) yet their effect on growth and metabolism in microalgae has not been studied much (Stirk *et al.*, 2013). The present study revealed that plant growth regulators can either promote or inhibit the growth of *N. salina*. The effect varied with change in the concentration of growth regulators in medium. In present study two auxins used were IAA (a natural auxin) and 2,4-D (a synthetic hormone). IAA supplementation to growth medium @ 0.1 mg L^{-1} improved growth rate (0.197 day^{-1}) as compared to the control (0.189 day^{-1}). However, IAA @ 0.5 mg L^{-1} in medium gave very low growth rate (Fig. 1). Our results are in agreement with Ahmad and Winter (1968) who reported that lower concentration of IAA promote growth whereas high concentration show inhibitory effect in *Anabaena cylindria*. Bajguz and Piotrowska (2013) also found enhanced growth in microalgae at low concentrations of IAA. In *Haematococcus pluvialis* and *Dunaliella salina*, 2,4-D showed an increase in biomass (de Jesus Raposo *et al.*, 2015). 2,4-D is an auxin that promotes cell enlargement (Schenck *et al.*, 2010). The auxins may be involved in cell division in microalgae. The application of IAA in growth medium increased the growth rate in *Chlorella pyrenoidosa* (Vance, 1987) and *Scenedesmus obliquus* (Prasad, 1982).

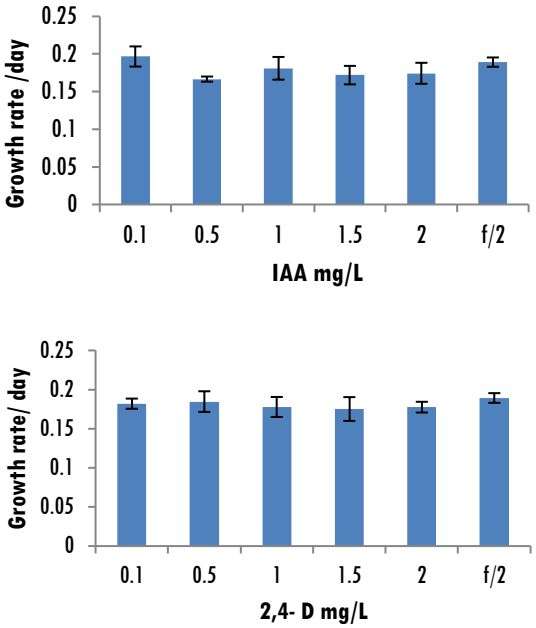

Fig. 1: Effect of IAA (upper) and 2,4-D (lower) on growth rate of *N. salina*

The addition of 2,4-D in medium decreased growth rate of *N. salina*. An inverse relationship was observed between the concentrations of auxins and growth rate (Fig. 1). Earlier reports also reveal that lower concentrations of auxins and their derivatives stimulated the growth of *Chlorella* sp. (Czerpak *et al.*, 1994; Czerpak *et al.*, 1999; Piotrowska *et al.*, 2012).

At lower concentrations, IAA promoted chlorophyll *a* production in *N. salina* (Fig. 2), whereas at higher concentration the chlorophyll content showed a decrease. Carotenoid production was more in the medium supplemented with low levels of IAA (0.5 mg L^{-1}). This may be attributed to the good growth

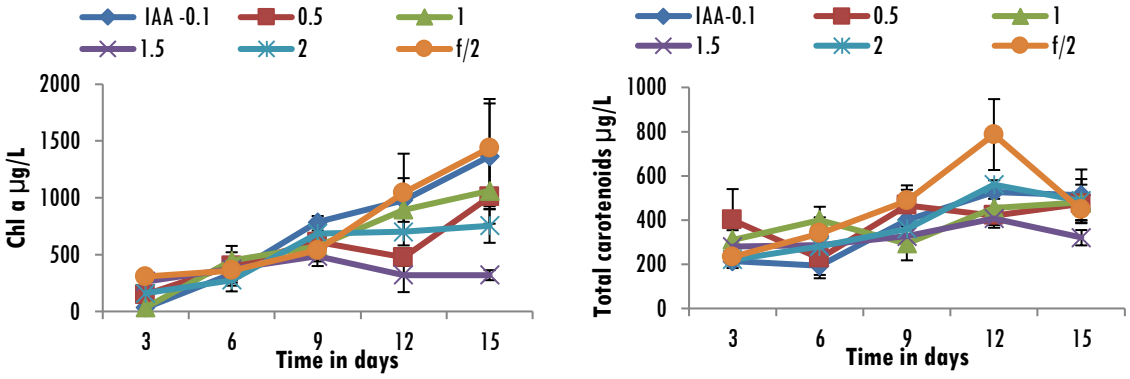

Fig. 2: Effect of IAA on chlorophyll a (Chl*a*)and total carotenoid production in *N. salina*

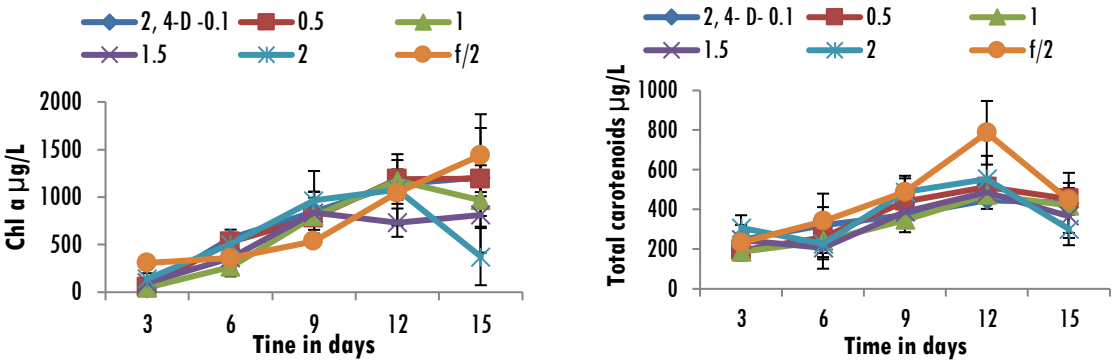

Fig. 3: Effect of 2, 4-D on chlorophyll *a* and total carotenoid production in *N. salina*

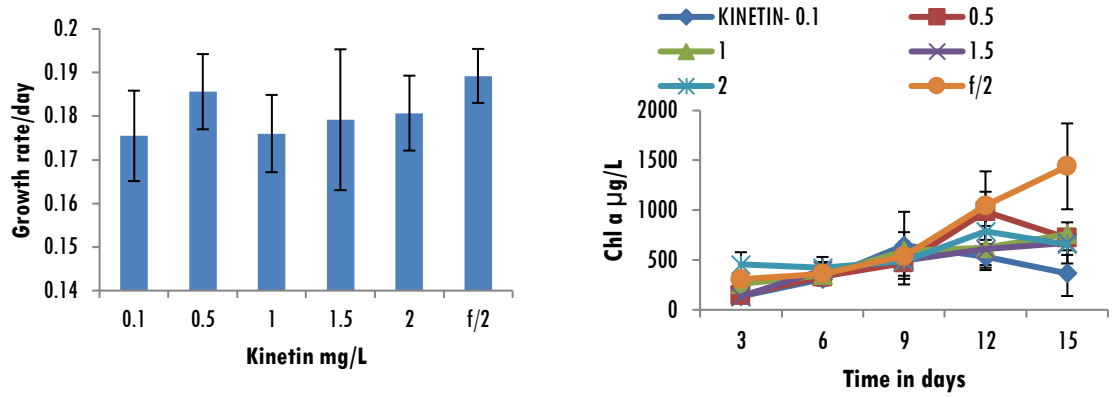

Fig. 4: Effect of kinetin on growth rate and chlorophyll a production of *N. salina*

rate observed at this concentration as compared to their higher concentrations or control. In 2,4-D supplemented growth medium, the chlorophyll *a* production by microalgae was high upto 9th day of incubation and decreased thereafter (Fig. 3). There was no notable influence of 2-4 D on carotenoid production in *N. salina*. In a similar study, Czerpak *et al.* (1999) and Piotrowska *et al.* (2012) observed increase in carotenoid concentration in *C. vulgaris* when exogenous auxins were added to the medium. The use of kinetin @ 0.5 mg L^{-1} in growth medium enhanced algal growth (Fig. 4). Vance (1987) noted that the addition of kinetin in culture medium increased cell division rates of *C. pyrenoidosa* by 12-16%. In present study, the addition of kinetin adversely affected chlorophyll *a* and carotenoid production (Fig. 4 & 5). Contrary to our observations, Bajguz and Czerpak (1996) found stimulatory effects of kinetin on carotenoid production in alga *C. pyrenoidosa.* Cytokinins activate cell division (Dobrev *et al.*, 2002) and protein synthesis in algae.

All the test concentrations of BAP showed growth promoting effect, except the concentration of 0.5 mg L^{-1} which depicted no effect (Fig. 6). The results are in agreement with Burkiewicz (1987)

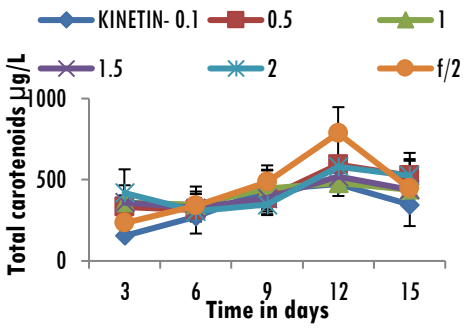

Fig. 5: Effect of kinetin on total carotenoid production in *N. salina*

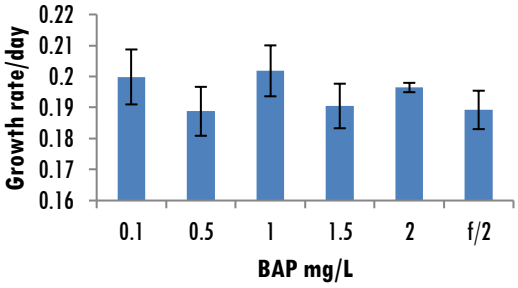

Fig. 6: Effect of BAP on the growth rate of *N. salina*

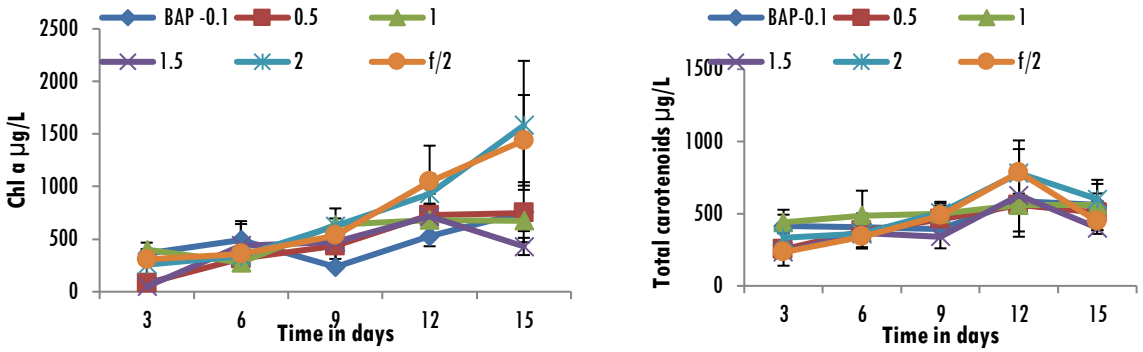

Fig. 7: Effect of BAP on chlorophyll *a*(left) and total carotenoid (right) production in *N. salina*

and Piotrowska and Czerpak (2009) who reported that exogenous cytokinins increase cell number in *Scenedesmus quadricauda* and *Chlorella vulgaris*. In present study, 2 mg L^{-1} BAP produced more chlorophyll *a* in *N. salina* (Fig. 7). Similarly, the carotenoid production was high in medium containing all test concentrations of BAP, except 1.5 mg L BAP. Our findings are supported by Piotrowska and Czerpak (2009) who also found that exogenous cytokinins increased carotenoids in *Chlorella vulgaris*. Satpati *et al.* (2014) observed good growth of algae in presence of cytokinins. Auxins and cytokinins are involved in cell division and regulate the activity of cyclin-dependent kinases (Hutchison and Joseph, 2002). If auxins and cytokinins are to be successfully used to improve microalgal growth and yield in *in vitro* culture, a better understanding of their role in microalgae metabolism is essential.

REFERENCES

Ahmad, M.R. and Winter, A. 1968. Studies on the hormonal relationships of algae in pure culture. *Planta*, **81:** 16-27.

Bajguz, A. and Czerpak, R. 1996. Effect of brassinosteroids on growth and proton extrusion in the alga *Chlorella vulgaris* Beijerinck (Chlorophyceae). *Journal of Plant Growth Regulation*, **15**(3): 153-156.

Bajguz, A. and Piotrowska-Niczyporuk, A. 2013. Synergistic effect of auxins and brassinosteroids on the growth and regulation of metabolite content in the green alga *Chlorella vulgaris* (Trebouxiophyceae). *Plant Physiology and Biochemistry*, **71:** 290-297.

Bradley, P.M. and Cheney, D.P. 1990. Some effects of plant growth regulators on tissue cultures of the marine red alga *Agardhiella subulata* (Gigartinales, Rhodophyta). pp. 353-360. **In**: *Thirteenth International Seaweed Symposium*. Springer, Netherlands.

Bradley, P.M. 1991. Plant hormones do have a role in controlling growth and development of algae. *Journal of Phycology*, **27:** 317-321.

Burkiewicz, K. 1987. The influence of gibberellins and cytokinins on the growth of some unicellular Baltic algae. *Botanica Marina*, **30:** 63-70.

Czerpak, R., Bajguz, A., Białecka, B., Wierzchołowska, L.E. and Wolańska, M.M. 1994. Effect of auxin precursors and chemical analogues on the growth and chemical composition in *Chlorella pyrenoidosa* Chick. *Acta Societatis Botanicorum Poloniae*, **63:** 279-286.

Czerpak, R., Krotke, A. and Mical, A .1999. Comparison of stimulatory effect of auxins and cytokinins on protein, saccharides and chlorophylls content in *Chlorella pyrenoidosa* Chick. Pol. Arch. *Hydrobiology*, **46**: 71-82.

de Jesus Raposo, M.F., de Morais, A.M.B. and de Morais, R.M.S.C. 2015. Marine polysaccharides from algae with potential biomedical applications. *Marine Drugs*, **13**: 2967-3028.

Dobrev, P., Motyka, V., Gaudinová, A., Malbeck, J., Trávníčková, A., Kamínek, M. and Vaňková, R. 2002. Transient accumulation of cis-and trans-zeatin type cytokinins and its relation to cytokinin oxidase activity during cell cycle of synchronized tobacco BY-2 cells. *Plant Physiology and Biochemistry*, **40**: 333-337.

Evans, L.V. and Trewavas, A.J. 1991. Is algal development controlled by plant growth substances? *Journal of Phycology*, **27:** 322-326.

Guillard, R.R. 1973. Division rates. pp. 289-312. **In**: *Handbook of Physiological Methods.* Cambridge University, Cambridge, UK.

Guillard, R.R. 1975. Culture of phytoplankton for feeding marine invertebrates. pp. 29-60. **In**: *Culture of Marine Invertebrate Animals.* Springer, New York, USA.

Hutchison, Claire E. and Joseph J. Kieber. 2002. Cytokinin signaling in *Arabidopsis*. *The Plant Cell*, **14** (suppl) 1: S47-S59.

Jeffrey, S.T. and Humphrey, G.F. 1975. New spectrophotometric equations for determining chlorophylls *a, b, c*1 and *c*2 in higher plants, algae and natural phytoplankton. *Biochemie und Physiologie der Pflanzen,* **167:** 191-194.

Kumar, S.S. and Saramma, A.V. 2013. A revised method for pigment extraction from marine nannoplanktonic algal cultures. *Journal of Algal Biomass Utilization*, **4**: 47-52.

Moreira, J.B., Terra, A.L.M., Costa, J.A.V. and Morais M.G. 2017. Utilization of CO_2 in semi-continuous cultivation of *Spirulina* sp. and *Chlorella fusca* and evaluation of biomass composition. *Brazilian Journal of Chemical Engineering*, **33**: 691-698.

Ördög, V., Stirk, W.A., Lenobel, R., Bancířová, M., Strnad, M., Van Staden, J., Szigeti, J. and Németh, L. 2004. Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*, **16**: 309-314.

Piotrowska, A. and Czerpak, R. 2009. Cellular response of light/dark-grown green alga *Chlorella vulgaris* Beijerinck (Chlorophyceae) to exogenous adenine-and phenylurea-type cytokinins. *Acta Physiologiae Plantarum*, **31**: 573-585.

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

Prasad, P.V.D. 1982. Effect of some growth substances on three freshwater green algae. *Cryptogamie Algologie,* **4**: 315-321.

Pratt, R. 1938. Influence of auxins on the growth of *Chlorella vulgaris*. *American Journal of Botany*, **25**: 498-501.

Quainoo, A.K. and Dwomo, B.I. 2012. The effect of TDZ and 2,4-D concentrations on the induction of somatic embryo and embryogenesis in different cocoa genotypes. *Journal of Plant Studies*, **1**: 72-78.

Satpati, G.G., Nandi, C. and Pal, R. 2014. Influence of growth regulators (2,4-D and kinetin) on vegetative and reproductive behaviour of some green algae. *Phykos*, **44**(2): 54-64.

Schenck, D., Christian, M., Jones, A. and Lüthen, H. 2010. Rapid auxin-induced cell expansion and gene expression: A four-decade-old question revisited. *Plant Physiology*, **152**: 1183-1185.

Stirk, W.A. and Van Staden, J. 1997. Isolation and identification of cytokinins in a new commercial seaweed product made from *Fucus serratus* L. *Journal of Applied Phycology*, **9**: 327-330.

Stirk, W.A., Ördög, V., Novák, O., Rolčík, J., Strnad, M., Bálint, P. and Staden, J. 2013. Auxin and cytokinin relationships in 24 microalgal strains. *Journal of Phycology*, **49**: 459-467.

Strickland, J.D. and Parsons, T.R. 1972. *A Practical Handbook of Sea Water Analysis.* Fisheries Research Board of Canada, Ottawa, Canada.

Tarakhovskaya, E.R., Maslov, Y.I. and Shishova, M.F. 2007. Phytohormones in algae. *Russian Journal of Plant Physiology*, **54:** 163-170.

Vance, B.D. 1987. Phytohormone effects on cell division in *Chlorella pyrenoidosa* chick (TX-7-11-05) (chlorellaceae). *Journal of Plant Growth Regulation*, **5**(3): 169-173.