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Effect of Indole Acetic Acid on Growth and Physiology of a Cyanobacterium Spirulina Platensis

Kawalpreet Kaur Bhatia*

Assistant Professor, Department of Botany, Sri Guru Gobind Singh College Sector 26, Chandigarh, India

Abstract* – *Growth and developmental processes of cyanobacteria can be influenced by a variety of environmental factors including phytohormones. Present study investigated the role of Indole-3-acetic acid (IAA), one of key phytohormones, in promoting Spirulina growth and its photosynthetic pigments. The results showed that IAA supplementation resulted in immediate increase in growth just after 24 hours of inoculation which may be due to the fact that IAA causes fairly rapid increase in cell wall extensibility. Notably, there was significant increase in growth of Spirulina supplemented with IAA at 25-50 μg ml^{-1} by 20^{th} day (p value < 0.01). Optical microscopy showed that IAA supplementation resulted in rapid growth after 1^{st} day of inoculum. On 20^{th} day at 50 μg ml^{-1} of IAA application, there was approximately 30% increase in chlorophyll a content, 17% increase in carotenoid content and 14% increase in phycocyanin content. The protein content measured serially from 0-20 day showed significant difference when supplemented with different IAA regimes (F-statistics 77.39; p value < 0.001). Based on results, it is concluded that IAA, in appropriate quantity, can be used as growth promoter to increase the quantity of Spirulina produced with increased amount of photosynthetic pigments like chlorophylls, carotenes and phycocyanin thus enhancing antioxidant potential of Spirulina.

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INTRODUCTION

Spirulina platensis has been prominently catalogued as a rich source of proteins and micronutrients in the pyramid of the marketed nutraceuticals (Belay, 1997; Bhatia *et al.* 2016). Its original use as a dietary supplement is credited to natives of Central Africa around Lake Chad, and in East Africa along the Great Rift Valley. After the discovery of its immense nutritive potential, widespread commercial production had already jump started in different parts of the world. Nutraceutical importance of *Spirulina* has rapidly increased over last two decades with emerging role in healthcare (Belay 1997; Bhatia *et al.*, 2014). Vast amount of research is directed to optimize *Spirulina* growth and production for its emerging demand worldwide (Vonshak and Tomaselli, 2000; Danesi *et al*., 2001; Ogbonda 2007; Celekli *et al*., 2009; Sena *et al.,* 2011; Bhatia et al 2017). Availability of improved production techniques and better understanding of underlying biochemical properties are indeed vital for its commercial use. Growth and developmental processes of cyanobacteria can be influenced by a variety of environmental factors. Indole-3-acetic acid acts as growth promoter of various cyanobacteria has a wide range effect on growth and morphogenesis. Enhanced effect of IAA (1-6µg/ml) on antioxidant phenolic compounds has been indicated (Mohammed, 2011). Indole-3-acetic acid neither increased the nitrogenase activity nor enhanced the heterocyst frequency in cyanobacteria (Leganes *et al.,* 1987). However, little is known about the possible effect of Indole-3-acetic acid on growth and physiological performance *Spirulina*.

Various studies on algal systems (Czerpak *et al.,* 1994; Czerpak *et al.,* 1999; Ahmad and Winter (1970) reported increase in cell count and dry mass of *Chlorella* sp. due to exogenous application of auxins, auxin precursors and analogs. Single study on effect of IAA on carotenoids of *Spirulina* reported increased production of carotenoid content with exogenous IAA supplementation (Mohammed and Mohammed, 2011). In this context, role of phytohormones has recently attracted attention in optimizing growth of *Spirulina*. The present study, therefore, aimed to assess the efficacy of IAA in stimulating *Spirulina* growth.

CULTURE AND MEDIUM

The experimental cultures were propagated in Zarrouk medium (Zarrouk 1966) at 35 ± 2°C, pH of 9 – 9.5. The cultures were illuminated for 14 h light at 1800 lux on the surface of culture vessels. Wide range of Indole-3-acetic acid concentration was initially tested to identify the lowest and highest endpoints of concentrations showing no or inhibitory effect respectively. The samples were taken every 4th day in triplicate from each container to observe the effect of various concentrations of Indole-3-acetic acid on biomass, protein content, chlorophyll content, carotenoids and phycocyanin content.

Estimation of Growth: The growth was enumerated on 1st day and then every 4th day till 20 days. Growth was quantified as an increase in spectrophotometric absorbance. The absorbance was recorded at 550 nm. The experiments were run in triplicate

Estimation of Biomass: The Homogenous cultures were centrifuged and washed in double distilled water and dried at 70℃. The growth was expressed in terms of dry weight (µg ml^{-1}).

Estimation of Total Protein Content: The total protein content of the cultures was estimated by the method of Herbert *et al*. (1971),

Estimation of Chlorophyll: Cold extraction method using acetone was employed for chlorophyll estimation. The amount of chlorophyll was determined using the specific absorbance coefficient of 13.9 for chlorophyll (Mackinney 1941).

Estimation of Caroteins Carotenoids were extracted in 90% acetone by cold extraction method. The concentration of carotenoids was determined using specific absorbance coefficient (α) of 12 (Weber and Wetton 1981).

Estimation of Phycocyanin: The Phycocyanin was estimated by the method given by Tandeau de Marsac (1977) and was measured by the equations developed by Bennet and Bogorad (1973)

Morphological and ultrastructural studies

Cell morphology was observed for microscopically detectable morphological alterations, in *Spirulina* culture. The cultures were viewed under light microscope at 400X till the development of hormogonia in control cultures.

The structural modifications at finer scale were studied using scanning electron microscopy. The specimen was fixed with 2.5% gluteraldehyde. Dehydration was done in increasing grades of ethanol. Critical point dried specimens were loaded on metallic specimen stubs and sputtering was done in JOEL JFC -1100 (Bozzola and Russell 1992). The specimens were examined under JOEL-JSM 6100.

Statistical analysis

All values were expressed as mean ± standard error mean (SEM) of the three replicates. Serial measurements of growth from baseline to day 20 were stratified by various ascorbic acid groups. They were further analyzed and compared by summary measures of area under concentration time curve (AUC_{0-t} max), time weighted average and maximum percentage difference from baseline using non-parametric Kruskal Wallis test. This was followed by Mann-Whitney- U test for post hoc test for the pair wise comparisons between the various treated groups. Statistical analysis was carried out using Stata 12 IC version and MedCal 3.1 version. Values having $p < 0.05$ were considered as statistically significant.

RESULTS

The morphological and biochemical effects of Indole acetic acid (IAA) on *Spirulina* were notable. Both immediate and long term effects of IAA on *Spirulina* were observed in present study

Effect on growth: *Spirulina* showed fairly rapid and significant increase in early response and overall positive correlation between IAA supplementation and *Spirulina* growth. During 1st hour of IAA treatment, 14% increase was recorded in *Spirulina* growth at 25 µg ml^{-1}. There was significant increase in growth after 1st day of culture indicating effective rapid response. The growth of *Spirulina* showed 26% increase after 24 hours, however, 21% increase was observed at the end of 20 days. The growth levels measured serially from day 0-20 showed overall statistically significant differences when stratified by different doses of IAA (F-statistics 6.38; p value < 0.01). On further posthoc pair-wise analysis, IAA showed statistically significant difference from control. Comparison of serial measurement of growth between 0 hour and 1 hour across different groups was highly significant (p value < 0.01) indicating that IAA induced immediate increase in the growth of *Spirulina.* There was significant increase in growth of *Spirulina* supplemented with IAA at 25-50 µg ml^{-1} by 20th day (p value < 0.01) thus indicating positive correlation between IAA supplementation and growth of *Spirulina* (F-statistics 351, p value < 0.01). At relatively higher concentrations, IAA showed inhibitory effect on *Spirulina* (Fig. 1)

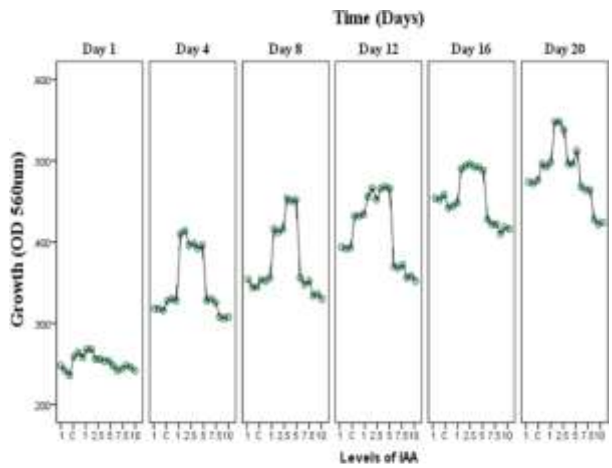

Fig 1: Analysis of Spirulina growth under different concentrations of IAA on Spirulina measured in terms of absorbance at 560nm. Spirulina showed significant increase in growth

with IAA supplementation. F-statistics 451; p-value < 0.01.

Effect on dry weight: Dry weight increased by 7% after 1 hour of IAA supplementation indicating its enhancing effect however these values were statistically not significant implying that IAA showed early and faster effect on growth of *Spirulina* however the increase in dry weight was recorded on 4th day of experimentation. There was about 22% increase at 25 and 50 μg ml^{-1} on 20th day of growth indicating its overall beneficial effect on the growth of this cyanobacterium. *Spirulina* showed significant increase in dry weight (F-statistics 451; p value < 0.01). Maximum cell productivity of about 0.57 μg ml^{-1}/day was observed with IAA supplemented group in comparison with control at 45 μg ml^{-1}/day (Fig.2).

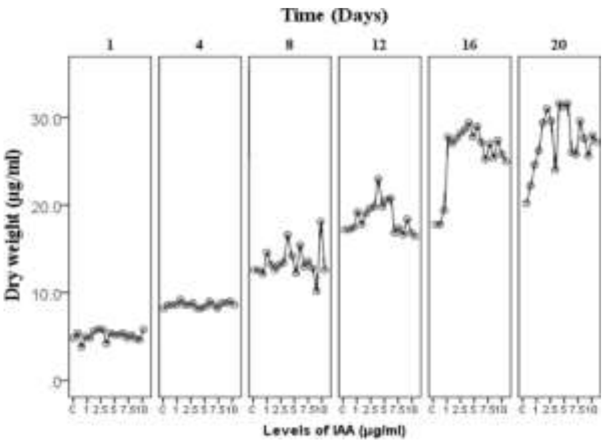

Fig 2: Analysis of Spirulina growth in in μg ml^{-1} under different IAA concentrations measured by dry weight. F-statistics 313; p value < 0.01.

Effect on chlorophyll *a*:

Chlorophyll *a* content also showed similar trend as an increase of 19% was observed in chlorophyll *a* content after 1 day of growth (Fig.3). On 20th day, there was approximately 30% increase in chlorophyll *a* content at 25 and 50 μg ml^{-1} of IAA application. The mechanism of IAA action on chlorophyll *a* has not been elucidated so far but some protective role of IAA on chlorophyll *a* content of *Spirulina* seems plausible. The chlorophyll *a* level measured serially from 0-20th day showed statistically significant difference when stratified with different IAA concentrations (F-statistics 313). Further within groups contrast analysis the chlorophyll shows significant difference between the groups (p value < 0.01).

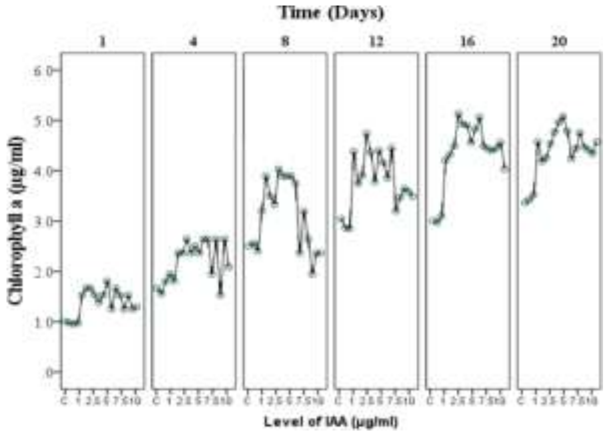

Fig 3: Chlorophyll a content of Spirulina expressed in μg ml^{-1} at different concentrations of IAA on every 4th day of growth. Values are expressed as Mean ± S.E.; n = 3. F-statistics 313; p value < 0.01.

Effect on carotenoid content:

Spirulina showed 17% increase in carotenoid content at the end of 20th day at 50µg/l IAA concentration (Fig. 4). The carotene content measured serially from 0-20th day showed significant difference when stratified with different IAA regimes (F-statistics 4.52; P value < 0.01). On further posthoc pairwise analysis, IAA supplementation showed statistically significant differences in carotenoid concentrations at 25 μg ml^{-1} (p value < 0.05) and 50 μg ml^{-1} (p value < 0.01) from the control. Carotenoid content showed significant increase on 4th day of growth with *Spirulina* supplementation indicating effective immediate response of IAA on carotenoid production of *Spirulina*.

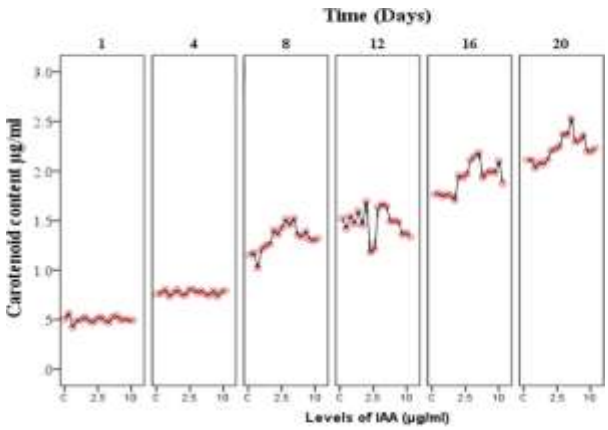

Fig 4: Carotenoid content of Spirulina expressed in μg ml–1 at different concentrations of IAA on every 4th day of growth. Carotenoid content of Spirulina expressed in μg ml^{-1} on different ways of growth. Values are expressed as Mean ± SE, n = 3. F-statistics 4.52; p value < 0.01.

Effect on phycocyanin content:

The phycocyanin content of *Spirulina* did not show any significant increase on first day of growth, However, there was 14% increase in phycocyanin content after 20th day of growth at 50 μg ml^{-1} concentration (Fig. 5). Analysis of phycocyanin content measured serially from initial day to 20th day when stratified by different IAA doses groups, overall differences showed significant differences (F-statistics 3.37; p value < 0.01) on further posthoc pairwise comparison 25 and 50 μg ml^{-1} (p value < 0.01). IAA supplemented groups showed significant difference over control.

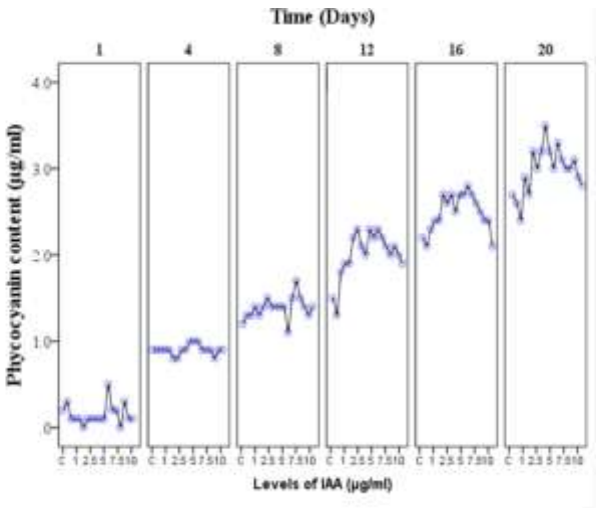

Fig 5: Phycocyanin content of Spirulina expressed in μg ml^{-1} at different days of growth. IAA supplementation increase phycocyanin till 20th day. F-statistics 3.37; p value < 0.01.

Effect on proteins content:

The protein content observed on 20th day with different concentrations of IAA was found to be more than the control (Fig. 6). Maximum increase was recorded with supplementation of 25 μg ml^{-1} and closely followed by 50 μg ml^{-1} and 10 μg ml^{-1}, concentrations above 75 μg ml^{-1} resulted in protein content equal to or less than control. The protein content measured serially from 0-20 day showed significant difference when stratified with different IAA regimes (F-statistics 77.39; p value < 0.01). On further posthoc pairwise analysis 25 and 50 μg ml^{-1} dose groups of IAA showed statistically significant difference from the control. IAA resulted in significant increase in protein content in general.

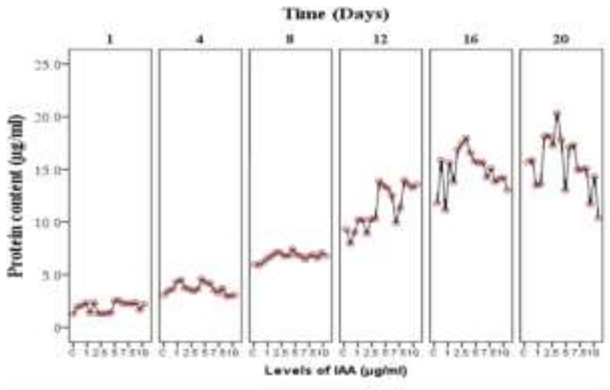

Fig 6: Effect of IAA on protein content of Spirulina expressed in μg ml^{-1} at different days of growth. Values are expressed as Mean ± SD, n = 3. F-statistics 77.39; p value < 0.001.

Morphological study: Optical microscopy showed that IAA supplementation resulted in rapid growth after 1st day of inoculum. The helical trichomes showing different degree of coiling and straight uncoiled filaments were observed after repeated subculturing (Fig. 7 a,b). The development of hormogonia was observed on 24th day in control as well as IAA supplemented group. Further scanning electron microscopy (SEM) illustrates some structural alterations and enlargement of trichomes when supplemented with IAA (Fig. 7 c,d).

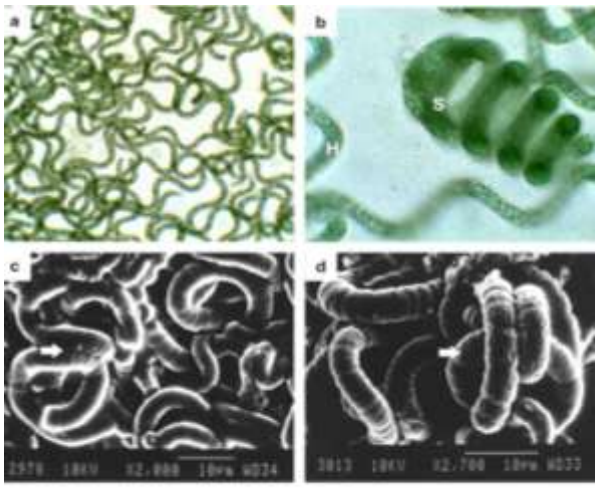

Fig. 7 Effect of Indole Acetic acid (IAA) on morphology of Spirulina platensis.

a- optical microscopy showing increased growth with IAA supplementation at 100X; b-Spirulina filaments at 1000X showing helical and spiral morphology;c,d scanning electron micrograph of Spirulina filaments showing altered morphology(white arrow) with IAA supplementation

Abbreviation

H-helically coiled filaments; S- spirally coiled filaments.

DISCUSSION

One of the known auxins identified as a plant growth hormone, indole acetic acid (IAA) is involved in nearly every aspect of the plant development including cell division, expansion and differentiation (Tsavkelova *et al*., 2006; Zhao, 2010). The present study indicated that IAA supplementation resulted in immediate increase in growth after just 24 hours of inoculation, which may be due to the fact that IAA causes fairly rapid increase in cell wall extensibility. Importantly, IAA causes responsive cell to extrude protons actively into the cell wall resulting in decreased pH which is responsible for activation of wall loosening enzymes that promote the breakage of cell wall bonds thus resulting in increased wall extensibility (Kutschera and Schopfer, 1986; Hussain *et al.*, 2015). Further, it could also increase osmotic solutes of the cells thus reducing the wall pressure thereby, promoting the permeability of the cells to water. Ahmad (1971) also reported similar results where they observed 77% increase in growth of *Nostoc muscorum* (cyanobacterium) in dry weight, which continued even after 24 days. Our results indicated that addition of appropriate quantities of IAA, resulted in significant positive effect on growth and pigments of *Spirulina* which could be due to widening of anion channels present in various cell membranes and may have central role in cell signaling, osmoregulation, plant nutrition and metabolism (Barbier-Brygoo *et al.,* 2000).

In present study, IAA supplemented *Spirulina* indicated rapid increase in its growth in short span extending even up to 20 days of IAA supplementation. Subsequently, growth of *Spirulina* showed decline possibly due to overutilization of resources which caused scarcity and disequilibrium in demand and supply of the required nutrient resulting in death of filaments after 20 days. Latter observation, however, was not in consonance with study by Ahmad (1971) where the growth continued even after 24 days of growth.

The enhancement in pigments of *Spirulina* with IAA supplementation was in corroboration with Yin (1937) who reported that IAA distinctly affects the rate of production of photosynthetic pigments. This effect is indirect through changes in chlorophyll content and size of cells. In young cultures, the enhancement in directly affected by chlorophyll content while in older cultures it is affected by chlorophyll content and the cell surface. The present study observation mirrors the findings by Mohammaed and Mohammaed (2011) who studied the effect of IAA on carotenoid content of *Spirulina*. This may be attributed to the fact that maximum amount of nitrogen enters into the cells of *Spirulina* due to elevation of osmotic solutes uptake. Luxuriant uptake of nitrogen and its utilization in the synthesis of aromatic acids, result in the increase of pigments.

The altered morphology of *Spirulina* may be due to the cell wall extensibility on IAA supplementation as well as increased permeability of the cells to water. This study demonstrated positive correlation of IAA supplementation and chlorophyll *a*, carotenoids and phycocyanin production. Based on results, it is concluded that IAA, in appropriate quantity, can be used as growth promoter to increase the quantity of *Spirulina* produced with higher amount of photosynthetic pigments like chlorophylls, carotenes and phycocyanin thus enhancing antioxidant potential of *Spirulina*.

REFERENCES

Ahmad, M.R. (1971). Short and long term effects of indole-3-acetic acid on the growth of *Nostoc muscorum*. *Z. Pflanzen. physiol*. 64: pp. 175-177.

Ahmad, M.R. and Winter, A. (1970). The effect of weak auxin on the growth of blue-green algae. *Hydrobiologia* 36: pp. 305-316.

Barbier-Brygoo, H., Vinauger, M., Colcombet, J., Ephritikhine, G., Frachisse, J. and Maurel, C. (2000). Anion channels in higher plants: functional characterization, molecular structure and physiological role. *Biochim. Biophys. Acta.*1465: pp. 199-218.

Belay, A.(1997). Mass culture of *Spirulina* outdoors – the Earthrise experience. In: *Vonshak A (ed) platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London, pp. 131–158.

Bennett,A. and Bogard, L. (1973). Complimentary chromatic adaptation in a filamentous blue-green Alga. J. Cell Biol.58: pp. 419-435.

Bhatia, K.K., Puri, S. and Ahluwalia, A. S. (2014). Efficacy and safety of *Spirulina* in biomedical field : Evidence based critical appraisal. *Vegetos*. 27: pp. 104-116.

Bhatia, K.K., Puri, S. and Ahluwalia, A. S. (2017). Morphological and physiological performance of a cyanobacterium *Spirulina platensis* in presence of Ascorbic acid: a growth facilitator. *JABU*. 8: pp. 35-44.

Bhatia, K.K. , Puri, S., Kaur, R. and Ahluwalia K.K., Ahluwalia, A. S. (2016). Efficacy of *Spirulina* as Hepatoprotectant : A Review. *Vegetos*. 29: pp. 129-136.

Bozzola, J.J. and Russell, L.D. (1992) Electron microscopy – Principles and techniques for biologists. Jones and Barlett, Boston.

Celekli, A., Yavuzatmaca, M. and Bozkurt, H. (2009). Modeling of biomass production by *Spirulina platensis* as function of phosphate concentrations and pH regimes. *Bioresour. Technol.*100: pp. 3625-3629.

Czerpak, R., Bajguz, A., Białecka, B., Wierzchołowska, L.E. and Wolan´ska, M.M. (1994). Effect of auxin precursors and chemical analogues on the growth and chemical composition in *Chlorella pyrenoidosa* Chick. Acta. Soc. Bot. Pol.63: pp. 279–286.

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

Danesi, E.D.G., Rangel, C.O., Pelizer, L.H., Carvalho, J.C.M., Sato, S. and Moraes, I.O. (2001). Production of *Spirulina platensis* under different temperatures and urea feeding regimes for chlorophyll attainment. In: Proceedings of the 8th International Congress on Engineering and Food. University of Waikato, New Zealand. pp. 1978-1982.

Herbert, D., Phipps, P. and Strange, R. (1971). Chemical analysis of microbial cells. Methods Microbiol.5: pp. 249-252.

Hussain A., Shah, S.T., Rahman, H., Irshad, M. and Iqbal.A. (2015). Effect of IAA on *in vitro* growth and colonization of *Nostoc* in plant roots. Front plant Sci.6: pp. 46.

Kutschera, U. and Schopfer, P. (1986). Effect of auxin and abscisic acid on cell wall extensibility in maize coleoptiles. Planta167: pp. 527-535.

Leganes, F., Sanchez-Maeso, E. and Fernandez-Valiente, E. (1987). Effect of indoleacetic acid on growth and dinitrogen fixation in cyanobacteria. Plant Cell Physiol.28: pp. 529-533.

Mackinney, G. (1941). Absorption of light by chlorophyll solutions. J Biol Chem 140: pp. 315-319

Mohammed, M.K. (2011). Production of carotenoids (antioxidants/ colourant) in *Spirulina platensis* in response to Indole acetic acid (IAA). Int. J. Eng. Sci. Technol. 3: pp. 4973-4979.

Mohammed, M.K. and Mohammed, M.K. (2011). Production of carotenoids (antioxidants /colourant) in *Spirulina platensis* in response to indole acetic acid (IAA). Int. J. Eng. Sci. Technol. 3: pp. 4973–4979.

Ogbonda, K.H., Aminigo, R.E. and Abu, G.O. (2007). Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina sp*. Bioresour. Technol. 98: pp. 2207-2211.

Sena, L., Rojas, D., Montiel, E., González, H., Moret, J. and Naranjo, L. (2011). A strategy to obtain axenic cultures of *Arthrospira sp*. cyanobacteria. World J. Microbiol. Biotechnol. 27: pp. 1045–1053.

Tandeau de Marsac, N. (1977). Occurrence and nature of chromatic adaptation in cyanobacteria J. Bacteriol.130: pp. 182-191

Tsavkelova, E.A., Klimova, S.Y., Cherdyntseva, T.A. and Netrusov, A.I. (2006). Microbial producers of plant growth stimulators and their practical use: A review. Appl. Biochem. Microbiol. 42: pp. 117-126.

Vonshak, A. and Tomaselli, L. (2000). *Arthrospira (Spirulina*) systematics and ecophysiology. *In*: B.A.Whitton , M. Potts (Eds) The Ecology of Cyanobacteria: Their Diversity in Time and Space. Kluwer academic, India. pp. 505-522.

Weber, A., Wetton, M. (1981). Some remarks on the usefulness of algal carotenoids as chemotoxic markers. *In:* F.C. Cygzan (Ed.) Academic Verlag, Pigments in Plants. Berlin, pp. 104-116.

Yin, H.C. (1937). Effect of auxin on *Chlorella vulgaris*. Proc. Nat. Acad. Sci. 23: pp. 174-176.

Zarrouk, C. (1966). Contribution à l'étude d'une cyanophycée. Influence de divers'facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*. Ph.D. Thesis, Université de Paris, Paris.

Zhao, Y. (2010). Auxin biosynthesis and its role in plant development. Annu. Rev. Plant Biol. 61: pp. 49-64.

Corresponding Author

Kawalpreet Kaur Bhatia*

Assistant Professor, Department of Botany, Sri Guru Gobind Singh College Sector 26, Chandigarh, India

kawalpreet38@gmail.com