AJES
ASIAN JOURNAL OF ENVIRONMENTAL SCIENCE ■ VOLUME 7 | ISSUE 2 | DECEMBER, 2012 | 200-203

RESEARCH ARTICLE

Effect of solar ultraviolet-B radiation on growth and enzymes of nitrogen assimilation in *Cylindrospermum* sp.

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Article Chronicle :
Received **:**
30.07.2012;
Revised **:**
21.09.2012;
Accepted **:**
12.11.2012

SUMMARY : A study was undertaken, under controlled laboratory conditions to investigate the influence of ultraviolet-B (UV-B) radiation on the growth, nitrate uptake, nitrate reductase (NR) ammonium uptake, glutamine synthetase (GS) and nitrogenase enzyme of cyanobacterium, *Cylindrospermum* sp. Test alga was isolated from rice field soils of Allahabad, India and grown in BG-11 culture medium. Culture of log phase was treated with UV-B (0-120 min exposure) which showed inhibitory effect on growth (chlorophyll-a) and found to be dose dependent. Ammonium uptake and GS were also inhibited by UV-B treatment but there was no total loss of these activities. Among the various parameters, nitrogenase enzyme was most sensitive for all the doses of UV-B and was not detected beyond 90 min exposure. In contrast, a significant increase in nitrate uptake and nitrate reductase following exposure of *Cylindrospermum* sp. to UV-B was also observed.

HOW TO CITE THIS ARTICLE : Chris, Abhishek (2012). Effect of solar ultraviolet-B radiation on growth and enzymes of nitrogen assimilation in *Cylindrospermum* sp. *Asian J. Environ. Sci.*, **7** (2): 200-203.

Key Words :
Cylindrospermum sp. UV-B, Growth, Nitrate uptake, Ammonium uptake, Nitrate reductase, Glutamine synthetase, Nitrogenase

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There is mounting evidence that the solar flux of UV-B radiation (280-320 nm) has increased at the earth's surface due to the depletion of the stratospheric ozone layer by anthropogenically released atmospheric pollutants such as chlorofluorocarbons (Callaghan *et al.*, 2004; Cen and Bornman, 1990). As a result, UV-B reaches the earth's surface directly which is harmful to living organisms. This created interest on the study of UV-B radiation induced effects and its recovery on the living system (Dohler,1985; Gao *et al.*, 2007).Although only a small fraction of the electromagnetic spectrum, UV-B light is sufficiently actinic to evoke a wide range of damaging photobiological effects because of its absorption by biomolecules *i.e.* nucleic acids and proteins (Sinha and Hader, 2008). The fluence rates of UV-B radiation reaching to the green plants is of major concerns specially cyanobacteria. Since cyanobacteria were among the early photosynthetic prokaryotes, thus it seems probable that during their evolutionary history, they might have faced more intense ambient solar UV-B radiation than others and may have acquired the ability to accumulate UVA/UV-B screening pigments to attenuate UV-B induced damage (Sinha and Hader, 2008). Cyanobacteria with a cosmopolitan distribution are the most common photosynthetic prokaryotes on earth playing an important role as a atmospheric nitrogen fixers in both aquatic as well as terrestrial ecosystems. The role of cyanobacteria as natural biofertilizers in rice paddy fields is well documented (Sinha and Hader, 1996). According to an assumption cyanobacteria fix 15-18 kg N ha^{-1} yr^{-1} through the process of biological nitrogen fixation with the help of enzyme nitrogenase. Cyanobacteria also posses nitrate reductase and nitite reductase enzyme which convert nitrate to nitrite and nitrite to ammonia (Sinha and Hader, 1996). In cyanobacteria, after transport by specific permeases, ammonium is incorporated into carbon skeletons by the sequential action of glutamine synthetase (GS) and glutamate synthetase (GOGAT) enzyme. Enhanced level of UV-B radiation has been reported to cause severe inhibition of growth as well as nitrogen fixation in legumes (Cen and Bornman, 1990) and inhibition

of NO_3^- and NH_4^+ uptake has also been reported in diatoms and higher plants (Dohler, 1985; Appenroth *et al.*,1993).

However little, if any, study has been made on some other important enzymes of nitrogen metabolism especially in blue-green algae (cyanobacteria) so, in the present study, the impacts of UV-B on growth and enzymes of nitrogen assimilation in a N_2- fixing cyanobacterium, *Cylindrospermum* sp. has been assessed, which is the dominant strain of the locality.

EXPERIMENTAL METHODOLOGY

Organism and growth conditions:

The filamentous heterocystous cyanobacterium, C*ylindrospermum* sp. was isolated from rice fields near Allahabad and was raised to axenic culture. The culture was axenically grown in nitrogen free BG-11 medium (Rippka *et al.,* 1979) at 27 ± 2^0 C pH under 75µ mol $m^{-2}s^{-1}$ photon flux density (PFD) with a photoperiod of 14:10 h. Mid logarithmic phase cultures were used for experimentation.

UV-B Treatment and estimation of growth:

For UV-B treatment the culture suspension (dry weight 0.1 mg ml^{-1}) occupying a depth of 0.25 cm sterilized 7.5 cm Petri dishes were exposed to artificial UV-B produced from single UV-B lamp with its main output 312 nm alongwith cool fluorescent light of 20 µ mol $m^{-2}s^{-1}$ PAR intensity. Radiation dose of 15,30,60,90 and 120 min exposure at the surface of culture were 0.18, 0.36, 0.72, 1.08 and 1.44 KJ^{-2}, respectively. Growth was measured by estimating chlorophyll content of cyanobacterium after four days of treatment following the method of Myres and Kratz (1955).

Estimation of nitrate uptake, ammonia uptake, nitrate reductase, glutamine synthetase and nitrogenase:

The uptake of NH^+_4 and NO^-_3 was estimated colorimetrically by Nessler's reagent (Herbert *et al.,* 1974), brucine sulfuric acid (Nicholas and Nasen, 1957) methods respectively by measuring the depletion of these nutrients due to consumption by alga. *In vivo* nitrate reductase activity was estimated by the method of Camm and Stein (1974). Nitrogenase activity was measured by estimating the reduction of acetylene to ethylene (Stewart *et al.,* 1968) with a gas liquid chromatograph (CIC Baroda, India). Glutamine synthetase activity was estimated by the method of Shampiro and Stadtman (1979).

Statistical analysis:

The different parameters were statistically analyzed using one way analysis of variance (ANOVA).

EXPERIMENTAL FINDINGS AND DISCUSSION

Cyanobacterium, *Cylindrospermum* sp. showed inhibitory growth response against UV-B treatment. Fig. 1 shows the effect of various doses (15, 30, 60, 90 and 120 min) of UV-B on the chlorophyll content of *Cylindrospermum* sp. A reduction of 10, 13, 20, 38 and 50 per cent in the chlorophyll content was recorded at 15, 30, 60, 90 and 120 min of UV-B as compared to control. Growth of any autotrophic organism is closely linked to the photosynthesis. Chl-a is the major photosynthetic pigment in the photochemical reaction centre of PSI and PSII. Pigments, proteins and membranes of the photosystems are sensitive to UV radiation (Chris *et al.*, 2006). Evidence showed that the detrimental effects of UV-B radiation of solar light on cyanobacteria are mediated by ROS (He and Hader, 2002; Chris *et al.*, 2006). As a result, UV stress leads to the damage of photosynthetic membrane, DNA and D1 protein of PSII and leads to inhibition of photosynthesis and finally growth (Nultsch and Agel, 1986).

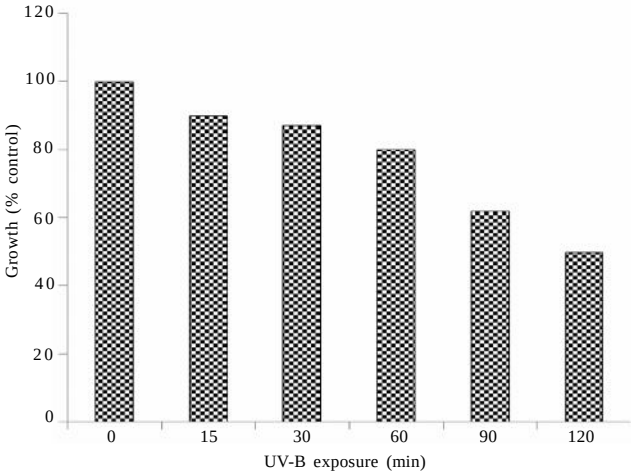

Fig. 1: Growth (chlorophyll-a) characteristic of *Cylindrospermum* sp. under different doses of UV-B. Chlorophyll-a in untreated control was 4.68 µg ml^{-1}. Values are mean ±SE of three replicates. Values are significant at 0.05 (5%) level of significance (ANOVA).

Table 1 shows the impact of different doses of UV-B on nitrate uptake and nitrate reductase enzyme of *Cylindrospermum* sp. Nitrate uptake exhibited an increase of 18, 15 and 12 per cent after the treatment of 15, 30 and 60 min doses of UV-B as compared to control. At highest dose *i.e.* 120 min of UV-B treatment, a slight decrease of 5 per cent was also noticed. Generally the nutrient uptake is reduced under stress condition and it was earlier reported by some workers also (Fuggi *et al.*, 1984; Dohler, 1986;Tyagi *et al.*, 1992) but interstingly in the present findings NO_3^- uptake was stimulated after UV-B exposure.The stimulation of nitrate uptake by UV-B for short periods and thereafter slow depression is supported well with the findings of Prasad *et al.* (1998) and Rai *et al.* (1998) who observed stimulation in NO_3^-

uptake in cyanobacterium, *Anabaena doliolum* and in green alga, *Chlorella vulgaris*. A possible explanation for the initial stimulation of NO_3^- uptake might be due to the consumption of previously stored ATP and reductant inside the cell. Likewise the inhibition in nitrate uptake at higher doses may be due to depletion in ATP content and carbon assimilation, which are essential for nitrogen metabolism (Oelmueller *et al.*, 1988).

A similar trend of nitrate uptake was noticed when UV-B (15 – 120 min) exposed cells were analyzed for nitrate reductase. The levels of enzyme in cells of *Cylindrospermum* sp. increased with the increase in UV-B dose. Rising concentration of UV-B from 15 - 90 min stimulated NR enzyme by 24 –7 per cent. Beyond 90 min exposure NR was slightly reduced. Like nitrate uptake, we also observed stimulation in nitrate reductase activity following exposure of *Cylindrospermum* sp. to UV-B. This could be due to an accelerated uptake of NO_3^- and/ or presence of UV-B photoreceptors in the cells. The inhibition of nitrate reductase in the test alga at higher doses of UV-B may be attributed due to reduced carbon fixation as already reported by Dohler (1985) and Tyagi *et al.* (1992).The reduction in chlorophyll content was also noticed in this study due to UV-B that might be responsible for inhibition of nitrate reductase activity since nitrate reductase is bound to the chlorophyll- containing membrane fractions.

Data related to the effect of UV-B stress on the ammonium uptake and glutamine synthetase are compiled in Table 1. The ammonium content decreased by10, 18, 25, 40 and 58 per cent at 15, 30, 60, 90 and 120 min UV-B treatment whereas the GS was decreased by 16,21,29,35 and 63 per cent on the above same treatments .The inhibition in NH_4^+ uptake by UV-B suggests an alteration in the structure of enzyme responsible for its assimilation. The process of ammonium uptake is linked through the GS-GOGAT pathway and the enzymes of this pathway are highly sensitive to different stress. UV-B induced decrease in ammonium uptake could be due to a decreased ATP pool as a consequence of damaged photosynthetic electron transport chain (Thaper *et al.*, 2008). The enzyme glutamine synthetase which incorporates nitrogen into the carbon skeleton and produces amino acids was also affected by UV-B. Inhibition of GS activity by UV-B is in agreement with the findings of Dohler (1986) in marine diatom, *Dictylum brightweilli* and Rai *et al.* (1998) in cyanobacterium, *Anabaena doliolum*.

The exposure of the cyanobacterium to 15 and 60 min of UV-B decreased the nitrogenase activity from 4.1± 0.005 µmol C_2H_4 released mg^{-1} protein^{-1} h^{-1} to 2.7and 2.25 µmol C_2H_4 released mg^{-1} protein^{-1} h^{-1}, respectively. The intensified decrease in nitrogenase activity was noticed on the further doses of UV-B (Table 1). Nitrogenase the key enzyme for atmospheric nitrogen fixation in diazotrophic cyanobacteria, was severely affected by UV-B stress. An appreciable reduction in nitrogenase activity by UV-B might have been due to interruption in supply of ATP and reductants to the enzyme for its activity (Kumar *et al.*, 2003; Pandey and Rai, 2002). Decrease in nitrogenase activity may also be due to impact of UV-B in their morphology and heterocyst formation (Gao *et al.*, 2007).

In the present study it was found that rice field cyanobacteria like *Cylindrospermum* sp. are at severe risk when exposed to unfiltered tropical solar radiation, particularly during summer season. It has been reported by several research groups that naturally occurring doses of UV-B are highly effective in causing damage to living organisms in their respective ecosystems. Thus, continued ozone layer depletion resulting in increased solar UV-B radiation might adversely affect the ecologically important cyanobacterial populations which in turn may affect the productivity of crops.

Acknowledgement:

Authors are thankful to Head, Department of Biological Sciences, Allahabad Agricultural Institute-Deemed University, Allahabad, India for providing lab facility. We are grateful to Prof (Dr.) R.B. Lal,Vice Chancellor AAI-DU for encouragement. A.C. is also thankful to Prof. S.B. Agrawal, Department of Botany, Banaras Hindu University for kindly providing the UV-B lamps.

Table 1: Effect of UV-B on nitrate uptake (µmol mg^{-1} protein h^{-1}),nitrate reductase (µmol $NO^-_3 mg^{-1}$ protein h^{-1}), ammonium uptake (µmol NH^+_4 mg^{-1} protein h^{-1}), glutamine synthetase (n-mol γ- glutamyl hydroxamate mg^{-1} protein min^{-1}) and nitrogenase (µmol C_2H_4 released mg^{-1} protein h^-) of *Cylindrospermum* sp.

Treatment	NO_3^- uptake	Nitrate reductase	NH_4^+ uptake	Glutamine synthetase	Nitrogenase
Control	12.1±0.3	24.5±0.6	0.32±0.003	3.86±0.04	4.1±0.05
UV-B_{15}	14.27±0.4 (+18)	30.38±0.8 (+24)	0.28±0.003 (-10)	3.24 ±0.04 (-16)	2.74± 0.03 (-33)
UV-B_{30}	13.91±0.4 (+15)	29.15±0.8 (+19)	0.26±0.002 (-18)	3.04±0.03 (-21)	2.54±0.03 (-38)
UV-B_{60}	13.55±0.4 (+12)	27.71 ±0.7 (+15)	0.24±0.002 (-25)	2.74 ±0.02 (-29)	2.25±0.02 (-45)
UV-B_{90}	12.58±0.3 (+4)	26.21 ±0.6 (+7)	0.19±0.001 (-40)	2.50±0.02 (-35)	1.02±0.01 (-75)
UV-B_{120}	10.89±0.2 (-10)	23.27 ±0.5 (-5)	0.13±0.001 (-58)	1.42±0.01 (-63)	ND

Mean±SE, values in parenthesis are per cent decrease or increase with reference to respective control. All treatments are significantly different (0.05) from control (ANOVA). ND= Not detected

REFERENCES

Appenroth, K.J., Augsten, H., Mattner, A., Teller, S. and Dohler, G. (1993). Effect of UVB radiation on enzymes of nitrogen metabolism in turious of *Spirudela polyrhiza* (L.) Schleiden. *Photochem. Photobiol. B: Biol.,* **18** (2-3) : 215-220.

Callaghan, T.V., Bjorn, L.O., Chemov, Y., Chapin. T., Christensen, T.R., Huntley, B., Ims, R.A., Johansson, M., Jolly, D., Jonnason, S., Matveyena, N., Pnikov, N., Oechel, W.,Shaver, G., Elster, J., Jonnsdottir, I.S., Laine, K., Taulavuori, K., Taulavuori, E. and Zockler, C. (2004). Responses to projected changes in climate and UV-B at the species level. *Ambio.*, **33** (7) : 418-435.

Camm, E.L. and Stein, J.R. (1974). Some aspects of nitrogen metabolism of *Nodularia spumigena* (Cyanophyceae). *Can. J. Bot.*, **52** (4) : 119-126.

Cen, Y.N. and Bornman, J.N. (1990). The response of bean plants to UV-B radiation under different irradiances of background visible light. *J. Exp. Bot.*, **41**:1489-1495.

Chris, A., Zeeshan, M. and Abraham, G. (2006). Effect of exogenous supply of antioxidants on UV-B induced oxidative stress in a cyanobacterium *Cylindrospermum* sp. *Algo. Stud.*, **121**:107-118.

Dohler, G. (1985). Effect of UV-B radiation (290-320 nm) on the nitrogen metabolism of several marine diatoms. *J. Plant Physiol.*, **118**: 391-400.

Dohler, G. (1986). Impact of UV-B radiation on [^{15}N] ammonia and [^{15}N] nitrate uptake of *Dictylum brightweilli*. *Photochem. Photobiophy*, **11**:115-121.

Fuggi, A., Vona, V., Rigano, D.I., Martino, C. Martella, A. and Rigano C. (1984). Evidence for two transport systems for nitrate in the acidophilic thermophilic alga, *Cyanidium caldarium*. *Arch. Microbiol.*, **137**: 281-285.

Gao, K., Yu, H. and Brown, M.T. (2007). Solar PAR and UV radiation affects the physiology and morohology of cyanobacterium *Anabaena* sp. PCC 7120. *J. Photochem. Photobiol. B: Biol.*, **89** (2-3): 117-124.

He, Y.Y. and Hader, D.P. (2002). Reactive oxygen species and UV-B: effect on cyanobacteria. *Photochem. Photobiol. Sci*., **1**: 729-736.

Herbert, D., Philips, P. J. and Strange, R. E. (1974).Chemical analysis of microbial cells. In: *Methods in microbiology*. Norris, J R, Ribbon, D.W. (Eds). Academic Press, London. pp. 209-344.

Kumar, A., Tyagi, M.B., Jha, P.N., Srinivas, G. and Singh A. (2003). Inactivation of cyanobacterial nitrogenase after exposure to ultraviolet-B radiation. *Curr. Micro.*, **46**: 380-384.

Myres, J. and Kratz, W.A.(1955). Relationship between pigment content and photosynthetic characteristics in blue-green algae. *J. Gen. Physiol.*, **39**:11-21.

Nicholas, D.J.D. and Nasen, A. (1957). Determination of nitrate and nitrite In: *Methods Enzymol.* III. Colowick , S.P., Kaplan, N.O. (Eds). Academic Press New York pp. 981-984.

Nultsch, W. and Agel, G. (1986). Fluence rate and wavelength dependence of photobleaching in the cyanobacterium, *Anabaena variabilis*. *Arch. Microbiol.*, **144**: 268-271.

Oelmueller, R., Schuster, C. and Mohr, H. (1988). Physiological characterization of a plastidic signal required for nitrate induced appearance of nitrate and nitrate reductase. *Planta*, **174**:75-83.

Pandey, V. and Rai, L.C. (2002). Interactive effects of UV-B and pesticides on photosynthesis and nitrogen fixation of *Anabaena doliolum*. *J. Micro. Biotech*., **12**:423-430.

Prasad, V., Kumar, A. and Kumar, H.D. (1998). Effect of UV-B on certain metabolic processes of the green alga, *Chlorella vulgaris*. *Internat. J. Env. Sci.*, **55**:129-140.

Rai, L.C., Tyagi, B., Rai, P.K. and Mallick, N. (1998). Interactive effects of UV-B and heavy metals (Cu and Pb) on nitrogen and phosphorus metabolism of a N_2- fixing cyanobacterium, *Anabaena doliolum*. *Env. Exp. Bot.*, **39**:221-231.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M. and Stanier R. Y. (1979). Generic assignments, strain histories and properties of purecultures of cyanobacteria.. *J. Gen. Microbiol.*, **111**:1–61.

Shampiro, B. M. and Stadtman, E. R. (1979). Glutamine synthetase (*E.coli*) In: *Methods Enzymol III.* Tabor, H. and Tabor, C.W. (Eds). Academic Press London and New York. pp. 910-922.

Sinha, R.P. and Hader, D.P. (1996). Photobiology and ecophysiology of rice field cyanobacteria. *Photochem Photobiol.*, **64**: 887-896.

Sinha, R.P. and Hader, D.P. (2008). UV-B protectants in cyanobacteria. *Plant Sci.*, **174**:278-289.

Stewart, W.D.P., Fitzgerald, G.P. and Burries, R.H. (1968). Acetylene reduction by nitrogen fixing blue-green algae. *Arch. Microbiol.*, **62**:336-348.

Thaper, R., Srivastava, A.K., Bhargava, P., Mishra, P., Mishra, Y. and Rai, L.C. (2008). Impact of different abiotic stresses growth, photosynthesis, electron transport chain, nutrient uptake and enzyme activities of Cu-acclimated, *Anabaena doliolum*. *J. Plant Physiol.*, **165**: 306-316.

Tyagi, R., Srinivas, G., Vyas, D., Kumar, A. and Kumar, H.D. (1992).Differential effect of ultraviolet-B radiation on certain metabolic processes in a chromatically adapting *Nostoc*. *Photochem. Photobiol.*, **55**:401-407.

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