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Methyl Parathion Induced Changes in Primary Photochemistry of A Natural Nitrogen Engineer *Nostoc Punctiforme*

KEYWORDS: Cyanobacteria. Pesticide Toxicity, Nitrogen Engineers, Photosynthetic efficiency, O2 evolution

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ABSTRACT *Cyanobacteria are an ancient life form which adapt themselves to a variety of extreme environments, including exposure to pesticides. The present study was under taken to investigate the influence of an organophosphate pesticide Methyl Parathion on the growth and photosynthetic properties of a rice field cyanobacterium Nostoc punctiforme. under controlled laboratory conditions. The test algae was isolated from the rice field soils near Sambalpur University,Jyoti Vihar, Western Odisha, India and grown in nitrogen free BG 11 culture medium. The growth rate of the organism declined with increasing incubation time as well as with increase in the concentration of pesticides. The pesticide exposure is clearly manifested with decrease in pigment and protein content. Increase in initial fluorescence (F_O) and loss in photosynthetic efficiency (Fv/Fm) suggested alteration in oxygen evolving complex. Further, significant decrease in peak height of the absorption spectra in treated condition indicates loss in the pigments during the experimental periods with higher concentration of pesticides.*

1. INTRODUCTION

Cyanobacteria are the mother of oxygenated photosynthesis in this planet. All present day plants have probably evolved from cyanobacteria which first colonized and created an oxygenated environment. Since their origin,3.5 billion years ago they have been surviving on the earth in almost all habitats. Today cyanobacteria are found to be abundant in rice fields (Peschek *et al.,* 1994) and aquatic biosystems (Deep *et al.,* 2013). Its unique structure and environmental adaptability have generated great interest among recent researchers in various fields like agriculture, medicine, textile, alternative energy etc. They are well known nitrogen engineer and maintain the nitrogen budget and homeostasis of the rice field (Choudhary,2011) by photo biological nitrogen fixation in heterocyst. The tropical rice field of Western Odisha, provide a favorable ecosystem for the growth of cyanobacterial flora due to swamped condition and crop canopy. However, continued dependence on different types of pesticides such as insecticides, fungicides, herbicides, etc in order to minimize the crop losses stands as a threat to resurgence of cyanobacterial flora.

Loss in agricultural yield due to biotic stress is a major factor. The loss due to pest comprise more than 15% yield loss in paddy crop (Herdt.,1991). Stem borer, plant hopper, pathogenic disease are some of the biotic factors which minimize rice production. Therefore to achieve the yield level, farmers of Western Odisha, India are largely dependent on several agrochemicals to control the pests and to achieve the target yield.

Organophosphate pesticides are among the top five pesticides used in India (Nayak *et al.,* 2012) Methyl parathion is frequently used by the farmers of Western Odisha particularly in the district of Sambalpur, Sundargarh and Bargarh. Paddy is mostly dependent on the soil for nitrogen which is fixed by the natural nitrogen engineers i.e. cyanobacteria. Heterocystous cyanobacteria can fix atmospheric nitrogen aerobically in heterocyst, whereas non heterocystous cyanobacteria fix nitrogen anaerobically. A symbiotic relationship of rice root and cyanobacteria has been reported by Nilson *et al.,* (2002). Uses of biofertilizer and chemical fertilizer have caused great concern after the green revolution. Use of cyanobacteria in the paddy field as biofertilizer is considered to be a good way as it is eco-friendly and sustainable. In 2007, Rajendran *et al.,* had studied the effect of fungicides, insecticides and biopesticides on *Tolypothrix scytonemides*, a common cyanobacterium grown in rice field. Literature on the influence of pesticides such as cypermethrin (Mohapatra et a l., 2003), bentazone (Bagchi *et al.,* 2003), lindane (Bueno *et al.,*2004), furadan(Islam *et al.,* 2007), monosulfuron (Shen and Luo, 2011), endosulfun (Kumar *et al.,*2008, Kumar *et al.,* 2012), chloropyrifos (Shoaib *et al.,*2012) on the growth, nitrogen fixation, and metabolic activities of several cyanobacteria are also available. The present study was aimed to investigate the growth and photosynthetic status of *N. punctiforme* exposed to organophosphate pesticide Methyl Parathion.

2. Methodology

2.1Culture Method and Treatment of Insecticide

Test organism (*N. punctiforme*) isolated from soils of rice field of Sambalpur, Odisha, was grown in nitrogen free BG 11 medium (Rippka *et al.,* 1979). The pH of all the media was adjusted to 7.8 prior to sterilization. The algal cultures were incubated at 26±2°C and 7.5Wm^{-2} light intensity in culture room up to 16 days.

2.2 Growth Analysis

The inoculated samples were taken and homogenized for 5 minutes and shaken thoroughly in order to obtain uniform-suspension. Growth was measured using light scattering technique (Guillard., 1973) by taking the absorbance at 760 nm at an interval of 2 days upto 16th day of inoculation of the control as well as the treated samples by using UV- visible spectrophotometer.

2.3 Pigment Estimation

Homogenate algal suspensions were centrifuged at 5000

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rpm for 10 minutes. Residues were extracted with 5 ml of 80% chilled acetone and kept in the dark for 2 hours. Then it was kept in 60 ^{O}C water bath for 1-2 minutes and finally centrifuged at 5000 rpm for 15 minutes to obtain a clear supernatant. Absorbance of the extract was recorded at 660 nm for chl-*a* and at 470 nm for Car in a spectrophotometer. The quantity of chl-*a* was determined using the formula of Mackinney (1941) and Car as per Jensen (1978).

2.4 Protein Estimation

Trichloroacetic acid (6%) was added to the residue left after extraction of chl-*a*, kept for 1 hr and centrifuged. The supernatant was discarded and protein content in the precipitate was estimated according to Lowry *et al.*,(1951).

2.5 Measurement of Initial Fluorescence and Photosynthetic Efficiency

Photosynthetic efficiency was measured in terms of Chl fluorescence using a Handy PEA(Hansatech Instruments, Norfolk, UK). The algal suspension was dark-adapted for 20 minutes before the measurements and then F_O, F_m and F_V/F_m were recorded and analyzed by the Handy PEA as describes by Prakash *et al.*,(2003).

2.6 Absorption Spectra

Absorption spectrum of whole cells of algal suspension was measured by a visible spectrophotometer (Cary 50 Bio, Varian, Australia) according to Thomas and Nagaraja (1973). The absorption spectra was recorded by double diffusion technique where the reference and the sample beam were scattered to the same extent by keeping the ground surface of the matched cuvettes in the light path. Algal suspension equivalent to 5µg of Chl per ml was taken in the final volume of 3 ml for recording the absorption spectra of cell sample.

2.7 Measurement of Oxygen Evolution

Photosynthetic oxygen evolution was measured directly from the algal suspensions as the function of incident photon flux density of 1000µmole m^{-2} s^{-1} with a liquid phase electrode unit (Chlorolab 2, Hansatech, UK). Before the measurement of oxygen evolution the instrument was calibrated in the liquid medium with a pinch of sodium dithionate. 1 ml of homogenized algal cells was poured into the sample cuvette with continuous stirring. The signals from the instrument were analyzed using Oxylab V1.15 and rate was determined from the graph.

2.8 Measurement of Electron Transport Activity

The 2, 6-dichlorophenol indophenol (DCPIP) photoreduction activity of whole algal cells was measured as the method described by Spiller (1980). Algal suspension equivalent to 10mg Chl was mixed with 3ml reaction mixture having 50mM Tris buffer (pH 7.8), 175mM NaCl and 0.5ml of DCPIP was added to the prepared mixture and placed under saturating white light for 60sec. Photoreduction of the dye was measured at 600nm.

3. RESULTS:

The kinetic of growth and total protein content of N. *punctiforme* in BG 11 medium with and without different concentration of pesticides has been depicted in Fig1a. The growth curve indicated that the organism exhibited lag phase up to 4 days followed by gradual increase and reached to a steady state on the 16th day in control whereas in pesticide treated samples the growth was maximum on the 12th day. The growth rate declined up to 7.19 % in 10ppm and 30.65% in 80ppm of MP treated N. *punctiforme* compared to the control. The percentage of decline on 16th day of inoculation was 19.7%, 23.8%, 23.95%, 35% and 42.7% respectively for 10, 20, 45, 50 and 80ppm of pesticide treatment. The total protein content also followed the same kinetics as that of the growth curve, however the percentage of loss in total protein content was much more than that of the growth and it was 46.82, 51.4, 63.6, 69.2 and 77.3 percent respectively for 10,20,40,50,and 80ppm of MP treatment.

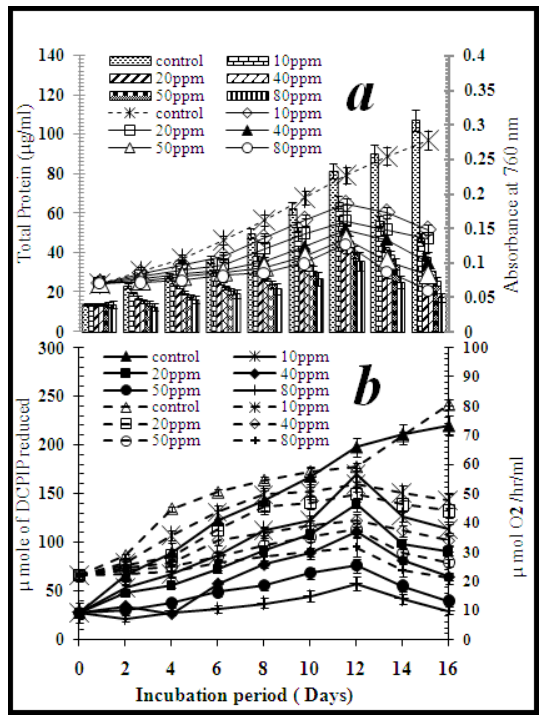

Figure.1. Effect on growth, total protein content, DCPIP photoreduction and rate of oxygen evolution of *Nostoc* sp treated with different concentration of Methyl Parathion. **a.**, histogram shows changes in growth and line shows Changes in total protein contents. **b.,** Solid line shows Changes in electron transport activity of PS II in terms of DCPIP photoreduction (µmole DCPIP reduced/mg of chloroplast/h) Dotted line Shows MP induces changes in the rate of oxygen evolution (µ mole O_2/hr/ml) in *Nostoc* sp. Data means ± SD (n= 5).

Fig. 1b showed the changes in the rate of oxygen evolution (dotted line) and electron transport activity of PS II (solid line). The rate of decline in the oxygen evolution at 12th day was 28, 33.83, 46.6, 57.66 and 65.89 percent respectively with 10, 20, 40, 50 and 80ppm of pesticide treated condition compared to the control, whereas loss in DCPIP reduction was 39.92, 53.17, 61.2, 73.98 and 80.13 % respectively with the increasing concentration of pesticide at 12th day of inoculation as compared to the control.

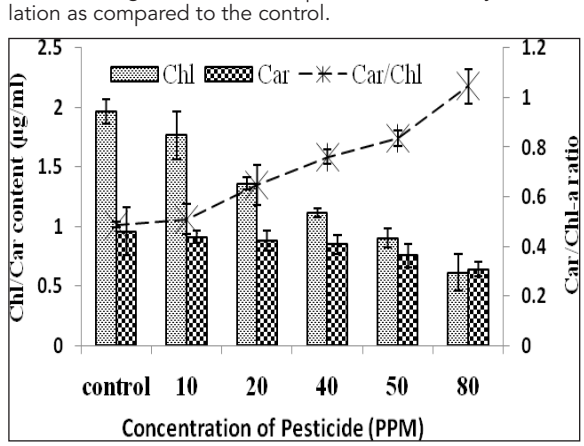

Figure.2. Effect of Methyl Parathion on Chlorophyll a content, Carotenoid content and Car/chl-*a* ratio of *Nostoc* sp at 12th day of incubation. Histogram shows total Chl-a and Car content, Line Shows changes in Car/ Chl ratio. Data means ± SD (n= 5).

As the maximum growth and protein content of the species

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was on the 12th day from the date of inoculation, the major photosynthetic pigment chl-*a* and Car were analyzed on the 12th day of inoculaion (Fig.2). chl-*a* content significantly declined after 12th day of pesticide treatment and the loss increased with the increase in concentration of MP. The rate of decline was 65.4($p<0.05$), 72.8($p<0.05$), 75.8($p<0.01$), 78.6 ($p<0.01$) and 86.3($p<0.01$) percent respectively. But for car/chl-a ratio significantly increased along with the increasing concentration of pesticides. The percentage of increase in 10, 20, 40, 50 and 80ppm of pesticide was 4.3%, 32.68% ($p<0.05$), 56% ($p<0.01$), 71.2% ($p<0.01$) and 114.21% respectively.

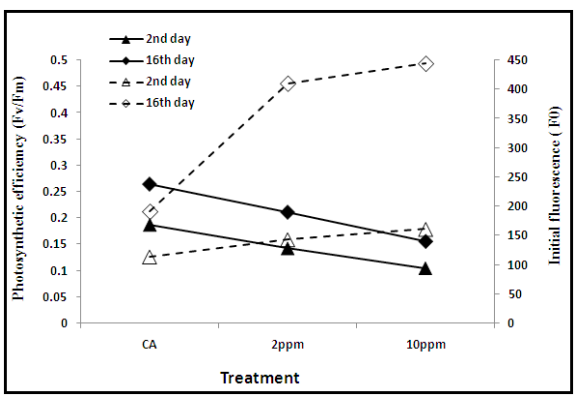

Figure.3. Changes in Initial fluorescence F_0(dotted line) and Photosynthetic efficiency of PS II in terms of Fv/Fm (solid line) of *Nostoc* sp to MP stress on 12th days of inoculation.

Fluorescence property of PS II was plotted in Fig3 on the 12th day of inoculation for both control and pesticide treated sample. Initial fluorescence (F_0) is shown to have significantly increased but the photosynthetic efficiency (F_v/F_m) ratio has declined significantly.

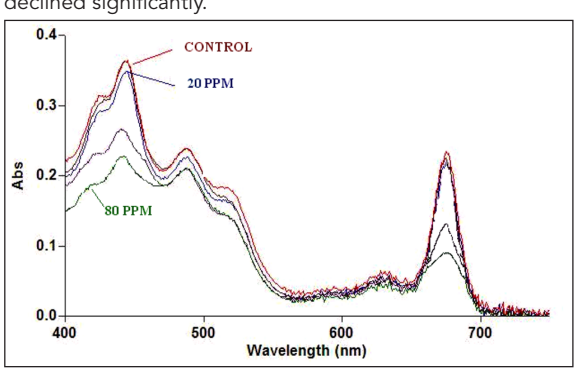

Figure.4. Methyl parathion induces changes in absorption spectra of *Nostoc sp* on 12th days of inoculation.

Fig 4 depicts the absorption spectra of *N. punctiforme* on the 12th day. Pesticide treated samples showed significant reduction in the peak height as compared to the control. As the concentration of pesticide increased the peak height decreased.

4. DISCUSSION

The Western region of Odisha, India is completely dependent on agricultural activity, which receives massive irrigation from Hirakud dam on Mahanadi river. To control pest during rice cultivation farmers use indiscriminately various pesticide. The eco-toxicological effects generated by several pesticides on cyanobacteria are of great concern to the biologist. In the present study the effect of MP pesticide was studied on growth pattern and photosynthetic parameters of N. *punctiforme* under laboratory condition in concentration and time dependent manner. A number of researchers have studied the effect of several insecticides on various strains of cyanobacteria from time to time (He *et al.*, 2013, Nayak *et al.*, 2012, Sheeba *et al.*, 2011, Chen *et al.*, 2007, Xia *et al.*, 2005.). The inhibition in growth of the alga might have been due to the inhibition in photosynthetic activities (Manikar *et al.*, 2013, Bhattacharyya *et al.*, 2011.) or impairment of macromolecule synthesis. The inhibitory effect of pesticides on growth rate of cyanobacterium is also dose dependent as suggested by Tiwari *et al.*, (2001) and Yamamoto and Tsukada (2009).

Analysis of Protein content of cyanobacteria during laboratory culture has been used by several authors as an index of the developmental status of the organisms. The low level of protein content in higher concentrations of pesticide treated sample (Fig 1a) may be due to the inhibition of protein synthesis (Aldehni *et al.*, 2003) or stimulation of nonspecific proteases activity (Anand and Subramanian, 1997) which may cause the degradation of protein in stress condition. At lower concentration, i.e. 10ppm of pesticide, protein content comparatively increases which indicates stimulation of some stress induced protein synthesis in low dose of pesticide (Karthikeyan and Gopalaswamy, 2009).

Photosynthetic pigments of cyanobacteria are very much susceptible to stress such as oxidative stress,(Latifi *et al.*,2009), osmotic stress (Sharma *et al.*, 2012) or under any biocides (Tiwari *et al.*, 2001). In this study significant decline in Chl content in pesticide treated condition (Fig. 2) supports the work of Kumar (2008) on *Nostoc, Anabaena and Aulosira* treated with endosulfun and Kumar *et al.*, (2010) on *A. fertilissimia* treated with 2,4 D. Latifi *et al.*, (2009) suggested that carotenoid content in cyanobacterial spheroplast is a vital component for scavenging free radical. The loss in the carotenoid contents on 12th day of inoculation (Fig.2) could be due to metabolic degradation of pigments under MP stress (Galhano *et al.*, 2011). However, interestingly carotenoid to Chl ratio is increased significantly ($p<0.01$) while treated with 80ppm of pesticides. This signifies that Chl is at a greater risk than Car to this pesticide stress. Similar type of inference was made by Galhano *et al.*, (2009) for bentazon and molinate on the diazotrop *Anabaena cylindrica*.

In photosynthetic organism electron transport activity is the major target site of inhibition during stress. In the present study the rates of DCPIP photoreduction in control and pesticide treated samples (Fig. 1b) are almost similar with their kinetics of growth and protein content (Fig1a). Photoreduction in treated samples is lower than in the control (Fig. 1b). This could be due to loss of protein content and pigment synthesis of the alga under the stress (pesticide treated) condition. This could also have been caused by possible changes in the thylakoid microenvironment. The degradation of D1 protein under stress condition could be another reason for low photoreduction (Long and Humphries, 1994). Because of the harmful effect of the treatment, with increase in the concentration of pesticide the dye reduction gradually gets reduced. These observations support the findings of Shikha and Singh (2004). The loss of PS II electron transport ability is supported by loss of oxygen evolution. Pesticide induced loss in oxygen evolution is ascribed to distortion in the RC II core complex and the OEC of PS II (Prasad *et al.*, 2005). Status of the absorption spectra in control and treated sample shows (Fig.4) a significant decline in peak height which may be due to the loss in pigment content.

Chlorophyll *a* fluorescence is a responsive tool to measure photosynthesis (Bjorn *et al.*, 2009). It signifies the efficiency of primary photochemical reactions. The calculation of the rate for competing energy dissipation pathways in the sample of light saturated (F_m) and dark adapted (F_O) conditions had shown that maximal fluorescence (F_V/F_m) is directly proportional to the quantum efficiency of PS-II. Increasing trend of initial fluorescence (F_o) during MP (Fig. 4) stress may be due to either uncoupling of antenna molecules or slower reoxidation of Q_A by the PQ pool or both. The increase in F_o has also been regarded as an apparent sign of damage to

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the reaction centre and D1 protein degradation (Komenda *et al.*, 2007). The possible reasons may be detachment of LHC II from RC II, dark reduction of Q_A, inactivation of RC and decrease in rate of energy transfer in the antenna as reported by Nayak *et al.*, (2003) in *Triticum aestivum*. The result exhibits a significant decrease in F_v/F_m along with increasing concentration of MP.

Decline in growth rate, pigments and protein content and loss of photochemical efficiency is supported by increase in initial fluorescence and decrease in photosynthetic efficiency, results stress induced loss in oxygen evolution. As the concentration of pesticide increases primary photochemistry wane up. Long run use of pesticide like MP may be a threat to the cyanobacterial population which are the pioneer and keystone species of the present oxygenic atmosphere. Business oriented temptation of modern agronomy by the use of pesticide and inorganic fertilizer is the major risk factor to the natural photosynthetic engineer.

REFERENCE

[1]. Aldehni, M. F., Sauer, J., Spielhaupter, C., Schmid, R. & Forchhammer, K. 2003, Signal transduction protein PII is required for NtcA-regulated gene expression during nitrogen deprivation in the cyanobacterium Synechococcus elongatus strain PCC 7942. J Bacteriol 185,2582–2591. | [2]. Anand, N., Subramanian, T.D., 1997, Effect of certain pesticides on the physiology of Nostoc calcicola. Phykos 36, 15–20. | [3]. Bagchi, SN., Pistorius, EK., Michel, KP., 2003, A Synechococcus sp PCC7942 mutant with high tolerance towards bentazone, Photosynthetica.75,171-182. | [4]. Bhattacharyya, S., Nayak, B., Choudhury, NK., 2011, Response of diazotrophic cyanobacterium Nostoc carneum under pesticide and UV-B stress. Chemosphere 84,131–135. Doi: 10.1016/j.Chemosphere. 2011.02.031. | [5]. Bjorn, LO., Papageorgiou, C., Blankenship, RE., Govindjee., 2009, A viewpoint: why chlorophyll a, Photosynth Res,99,85-98. DOI: 10.1007/s11120-008-9395-x. | [6]. Bueno, M., Fillat, F., Strasser, RJ., Rodriguez, RM., Marina, N., Smienk, H., Moreno, G., Barja, F., 2004, Effect of lindane on photosynthetic appararus on Cyanobacterium Anabaena, Fluorescence induction studies and immunolocalization of ferredoxin- NADP+reductase, ESPR-Environ Sci & Pollut Res11(2),98-106. | [7]. Chen, Z., Juneau, P., Qiu, B., 2007, Effect of three pesticides on the growth photosynthesis and photoinhibition of the edible cyanobacterium Ge-Xian-Mi (Nostoc). Aquat. Toxicol 81,149-157. | [8]. Choudhary, KK.,2011, Occurance of nitrogen fixing cyanobacteria during different stages of paddy cultivation, Bangladesh J.Plant Taxon. 18(1),73-16. | [9]. Deep, PR., Bhattacharyya, S., Nayak B., 2013, Cyanobacteria in wetlands of the industrialized Sambalpur District of India. Aquatic Biosystem, Biomed 9,14.Doi: 10.1186/2046-9063-9-14. | [10]. Galhano, V., Figueiredo, D., Alves, A., Correia, A., Pereira, M., Gomes-Laranjo, J., Peixoto, F.2011, Morphological, biochemical and molecular characterization of Anabaena, Aphanizomenon and Nostoc strains (Cyanobacteria, Nostocales) isolated from Portuguese freshwater habitats. Hydrobiologia. 663 (1),187-203. DOI 10.1007/s10750-010-0572-5. | [11]. Galhano, V. Peixoto, F. Gomes-Laranjo, J. & Fernández-Valiente, E. 2009, Differential effects of bentazon and molinate on Anabaena cylindrica, an autochthonous cyanobacterium of Portuguese rice field agro-ecosystems. Water Air and Soil Pollution, 197, 211-222, ISSN: 0049-6979. | [12]. Guillard, R.L. 1973, Division rates. In: J.R. Stein (Ed.), Handbook of Physiological Methods, Culture Methods and Growth Measurement, Cambridge University Press, New York, USA, pp.290-311. | [13]. He, H., Li Y., Chen, T., Huang, X., Guo, Q., Li S., Yu T., Li, H.,2013, Butachlor induces some physiological and biochemical changes in a rice field biofertilizer cyanobacterium. Pesticide Biochemistry and Physiology 105,224-230. | [14]. Herdt, R.W. 1991, Research priorities for Rice Biotechnology. In Rice Biotechnology (ed. Khush G.S and Toenniessen G H). International Rice Research Institute. Los Banos, Phillipines. | [15]. Islam, MZ., Begum, S., Ara, H., Waliullah, TM., 2007 Effect of furadan on the growth and nitrogen fixation by blue green algae, J.bio-science 15,23-35. | [16]. Jensen, A.,1978, Chlorophyll and carotenoids: In: J.A. Hellebust and J.S. Craiyie (Eds.), Handbook of Physiological, Phycological and Biochemical Methods, Cambridge University Press, London, 59-70. | [17]. Karthikeyan, CV., Gopalaswamy, G., 2009, Studies on acid stress tolerant proteins of cyanobacterium, Int. . Biol Chem., 3.1-10. DOI: 10.3923/ijbc.2009.1.10. | [18]. Komenda, J., Tichý, M., Praśil, O., Knoppová, J.,Kuvikova´, S., Vries, RD., Nixon, PJ., 2007, The Exposed N-Terminal Tail of the D1 Subunit Is Required for Rapid D1 Degradation during Photosystem II Repair in Synechocystis sp PCC 6803,The Plant Cell, 19, 2839–2854, DOI: 10.1105/tpc.107.053868. | [19]. Kumar, J.I. N., Amb, M.K.., Bora, A., 2010, Chronic response of Anabaena fertilissima Rao, C. B. on Growth, Metabolites and Enzymatic Activities by Chlorophenoxy Herbicide, Pesticide Biochemistry and Physiology 98 (2), 168-174. | [20]. Kumar, N.,Bora, A., Kumar, R., Amb, MK., 2012, Differential effects of Agricultural pesticides endosulfan and tebuconazole on photosynthetic pigments, metabolism and assimilating enzymes of three heterotrophic, filamentous cyanobacteria, J Biol Environ. Sci.6(16),67-75. | [21]. Kumar, S., Habib, K., Fatma, T., 2008, Endosulfun induced biochemical change in nitrogen fixing cyanobacteria. Sci. Tot. Environ. (ELSEVIER) 403, 130–138. | [22]. Latifi, A., Ruiz, M., Zhang, CC., 2009, Oxidative stress in cyanobacteria, FEMS Microbiol. Rev.33,258-278. | [23]. Long, S.P. and Humphries, S., 1994, Photoinhibition of photosynthesis in nature. Annu. Rev. Plant Physiol. Plant Mol. Biol., 45: 633-662. | [24]. Lowry, O.H., Rosenbrough, N.J., Farr, A.I., Randall, R.J., 1951, Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-276. | [25]. Mackinney, G., 1941, Absorption of light by chlorophyll solution. J. Biol. Chem., 140: 315-322. | [26]. Manikar, N., Kumar, S., Habib, K., Fatma, T., 2013, Biochemical analysis of Anabaena variabilis exposed to malthion pesticide with special reference to oxidative stress and osmolytes, IJIRSET 2, 5403-5420. | [27]. Mohapatra , PH., Patra, S., Samantray, PK., Mohanty, RC., 2003, Effect of pyrithroid insecticide cypermethrin on photosynthetic pigments of cyanobacterium Anabaena doliolum bhar, Pol j. Environ Stud. 12, 207-2012. | [28]. Nayak, B., Bhttacharyya, S., Sahu, JK., 2012, Photosynthetic response of two rice field cyanobacteria to pesticides, In Pesticides- Advances in Chemical and Botanical Pesticides, Intech 151-168. | [29]. Nayak, L., Biswal B., Ramaswamy NK., Iyer RK, Nair JS., Biswal UC., 2003, Ultraviolet-A induced changes in photosystem II of thylakoids: effects of senescence and high growth temperature. J. Photochem. Photobiol. 61, 221–230. http://dx.doi.org/10.1016/S1011-1344(03)00052-6. | [30]. Nilsson, M., Bhattacharya, J., Rai, AN., Bergman, B., 2002, Colonization of roots of rice (Oryza sativa) by symbiotic Nostoc strains, New Phytologist159,517-525. | [31]. Padhy, RN., 1985 Cyanobacteria and pesticide. Residue Rev.,95,1-44. | [32]. Peschek, G.A., Obinger, C., Sherman, D.M., Sherman, L.A.,1994, Immuno cytochemical localization of the cytochrome-c oxidase in a cyanobacterium, Synechococcus PCC7942 (Anacystis nidulans) . Biochem. Biophys. Acta 1187,369-372. | [33]. Prakash, JS., Srivastava,A., Strasser, RJ., Mohanty, P.,2003,Senescence-induced alterations in the photosystem-II functions of Cucumis sativus cotyledond: probing of senescence driven alterations of photosystem II by chlorophyll a induction transients, Indian Journal of Biochemistry Biophysics 40:160-168. | [34]. Prasad, SM, Kumar, D., Zeeshan, M.,2005, Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium Plectonema boryanum to endosulfan stress. J.Gen .Appl.Microbiol 51,115-123. | [35]. Rajendran, U.M., Kathirvel, E.,Narayanaswamy, A., 2007, Effects of a fungicide, an insecticide, and a biopesticide on Tolypothrix scytonemoides, Pesticide Biochemistry and Physiology,87,164-171. http://dx.doi.org/10.1016/j.pestbp.2006.07.006. | [36]. Rippka, R., Deruelles, J., Waterbuy, J.B., Herdman, M., Stanier, R.Y., 1979, Genetic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol., 111: 1-61. | [37]. Sharma,R.,Chahar,O.P.,Bhatnagar,M.,Bhatnagar., A.,2012, Impact of osmotic stress and temperature on pigments and proteins of Anabaena strains, Journal of Environmental Biology, 34, 941-943. | [38]. Sheeba, Sing V. P., Srivastava PK., Prasad, SM., 2011, Differential physiological and biochemical response of two cyanobacteria Nostoc muscorum and Phormidium foveolarum against oxyfluorfen and UV-B radiation. Ecotoxicology and Environmental Safety 74:1981-1993. Doi: 10.1016/j.ecoenv.2011.07.006. | [39]. Shen, J.,Luo,W., 2011, Effect of Monosulfuron on growth, Photosynthesis, and nitrogenase activity of three Nitrogen fixing cyanobacteria, Arch Environ Contam Toxicol (Springer)60,34-43. | [40]. Shikha., Singh, D.P., 2004, Influence of glyphosphate on photosynthetic properties of wild type and mutant strains of cyanobacterium Anabaena doliolum. Current Science, 86, 571-576. | [41]. Shoaib, N., Siddiqui, PJA.,Khalid, H., 2012, Toxicity of chloropyrifos on some marine cyanobacteria species, Pak.J.Bot.,44,1131-1133. | [42]. Spiler, H., 1980, Photophosphorilation capacity of stable spheroplasts preparation of Anabaena Plant Physiol, 66,446-450. | [43]. Thomas, JB., Nagaraja, R., 1973, Absorption spectra of algal chloroplast suspension: A simple method using conventional spectrophotometers, Indian journal of biochemistry and biophysics,19,266-268. | [44]. Tiwari O., Prasanna R., Yadav A., Dhar DW., Singh P., 2001, Growth potential and biocide tolerance of non-heterocystus filamentous cyanobacterial isolates from rice fields of Uttar Pradesh, Indian.Biol. Fertil Soils, 34,291-295.DOI 10.1007/s003740100402 . | [45]. Xia, J., 2005, Response of growth, photosynthesis and photoinhibition of the edible cyanobacterium N. sphaeroids colonies to thiobencarb herbicide. Chemosphere, 59, 561-566. | [46]. Yamamoto, Y., Tsukuda, H., 2009, Measurement of in situ specific growth rates of microcystis (cyanobacteria) from frequency of dividing cells, J. Phy. 45,1003, DOI: 10.1111/j.1529-8817.2009.00723.x. |