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Effect of the Pyrethroid Insecticide Cypermethrin on Photosynthetic Pigments of the Cyanobacterium *Anabaena doliolum* Bhar.

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Abstract

The degradation of chlorophyll *a* (Chl *a*), carotenoids and phycobiliproteins, and the fluorescence responses of the filamentous cyanobacterium *Anabaena doliolum* Bhar. were studied with short exposure (45 min-30 hours) to the pyrethroid insecticide cypermethrin by taking 20 μM and 50 μM of the chemical as treatment concentrations. There was significant reduction in Chl *a*, carotenoids and phycobiliprotein contents of cells in each of the selected concentrations of the insecticide. Pheophytin *a* and fluorescing and non-fluorescing chlorophyll catabolites (FCCs and NCCs) were produced as degradation products of Chl *a* during the exposure period. The amount of the degradation products gradually decreased with prolonged exposure of the cells to the insecticide. The pattern of degradation of carotenoids was similar to that of Chl *a* but the rate of degradation was less than for Chl *a*. The amount of lycopene also continuously decreased with increase of the insecticide concentration. There was gradual reduction of cellular phycocyanin and phycoerythrin contents of cells with rapid degradation during the first 6 hours of treatment. The fluorescence emission of phycobilisomes and photosystem II decreased with increased concentrations of insecticide and prolongation of treatment. The cyanobacterium did not show any recovery from insecticide stress during 30 hours of exposure.

Keywords: pyrethroids, *Anabaena*; chlorophyll *a*, pigment degradation, photosystem fluorescence

Introduction

The insecticidal properties of natural pyrethroids have been recognized since the middle of the 19th century, when commercial production of insecticidal powder from pyrethrum flower heads began. The low residual toxicity, lack of persistence in the environment and rapid knock down action of natural pyrethroids have prompted the manufacture and use of synthetic pyrethroids in agriculture. Pyrethroids are preferred over other insecticides because of their degradation into nontoxic or less toxic metabolites under natural conditions by biological organisms [1-3].

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It is known that bacteria and cyanobacteria efficiently degrade synthetic insecticides, including pyrethroids, to harmless products and the degradation processes are enzymatic [3-5]. However, despite degradation, non-target effects of pyrethroids have been reported and the intensity of effect is dependent on the wash away concentration of the insecticides and exposure time [6, 7]. Therefore, it is worthwhile to critically evaluate such effects of pyrethroids on soil and aquatic microorganisms to regulate their field application. Even though assessments are regularly being done by quite different agencies, reports are scanty on the short-term toxicity of these chemicals on soil cyanobacteria. There is also very little information on the response of photosynthetic pigments, the pattern of degradation of photosynthetic pigments and

the response of their biosynthetic pathways to the toxicity of pyrethroids. The rate of degradation of different pigments and their metabolites by pyrethroid toxicity is also not known. The present paper deals with the effects of a synthetic pyrethroid insecticide, cypermethrin, on the photosynthetic pigments and fluorescence responses of the filamentous cyanobacterium *Anabaena doliolum.*

Material and Methods

The axenic stock cultures of the filamentous cyanobacterium *Anabaena doliolum* was grown in modified nitrogen containing Chu No 10^+ medium [8] and were kept at 29 ± 2^0C with a continuous irradiance of 35 μE/m^2 s. The cultures were regularly hand shaken to maintain their homogeneity. Filaments at the mid-log growth phase were harvested and diluted with sterile medium to an initial absorbance of 0.30 at 678 nm (3.664 ± 0.113 μg Chl *a* / 10^7 cells). The experimental cultures were grown in 100 ml borosilicate conical flasks containing 30 ml of sample under conditions similar to that of stock cultures. The stock solution (10 mM) of cypermethrin 10 EC [(R, S)-α - Cyano – 3 – Phenoxybenzyl – 2, 2 – dimethyl (1R, 1S) cis, trans – (2, 2- dichlorovinyl) cyclopropane carboxylate] was prepared by dissolving the commercial formulation of the insecticide in acetone and then diluting with sterile nutrient medium. The required treatment concentrations (20 and 50 μM a. i. /l) of the insecticide were achieved in the experimental cultures by adding the required amount of the freshly prepared insecticide stock solutions. The acetone level at 50 μM concentration was 0.82 μl/l.

Extraction of chlorophyll *a* and carotenoids was done with DMF as per the method described by Porra *et al.* [9]. The corrected absorbance values at 664 nm and 463 nm of the extract were used to quantify chlorophyll *a* and carotenoids using the equations of Moran [10] and Chamovitz *et al.* [11], respectively. For measuring lycopene, the pigment was extracted with absolute acetone following the method described by Geider and Osborne [12]. After extraction, the absorbance was measured at 505 nm using absolute acetone as blank. Pheophytin *a* was quantified following the procedure of Geider and Osborne [12] and the absorbance of the extract was measured at 662 nm before and after acidification with 0.1 N HCl to pH 2.0. The amount of pheophytin *a* present in the sample before acidification was determined by subtracting the net pheophytin *a* produced by the acidification of chlorophyll *a* from the gross pheophytin *a* content of the sample.

Production of fluorescing chlorophyll catabolites (FCCs) was observed by taking the treated and control cultures. The filaments were harvested (5000 rpm, 10 min) and washed with normal growth medium. The washed filaments were diluted to the initial cell density with insecticide free medium and were homogenised (2 min) to get a homogenous suspension. The fluorescence emissions of the suspensions were observed at 450 nm by a Hitachi 4010 spectrofluorometer (Hitachi, Kyoto, Japan) after exciting the sample with 320 nm monochromatic light. The band pass for both excitation and emission monochromators was 10 nm. For measuring non-fluorescing chlorophyll catabolites (NCCs), reaction was made with acid ninhydrin following the method of Bates *et al.*[13]. After treatment the cells were harvested, washed and homogenized in 5 ml of 3% aqueous sulfosalicylic acid. The homogenates were centrifuged and 2 ml of the supernatant was added to 2 ml of acid ninhydrin and 2 ml of glacial acetic acid. The reaction was conducted for 1 hr at 100^0C followed by immediate cooling in ice bath. Extraction from the mixture was done with toluene, mixed vigorously and the absorbance was measured at 520 nm.

Extraction of phycobiliproteins was done with phosphate buffer (200 mM) following the method of Beer and Eshel [14]. The cells were homogenised with glass beads (0.5 mm dia) to optimise extraction. The absorbance peaks at 455 nm, 564 nm, 592 nm, 618 nm, and 645 nm of the extract were used to quantify the phycocyanin (PC) and phycoerythrin (PE) contents of cells. In order to measure the fluorescence emissions of phycobilisomes (PB) and photosystem II (PS II), treatments were done at a single time and the samples were collected at required intervals. After treatment the cells were harvested, washed with growth medium and diluted to the initial cell density with insecticide-free medium. Fluorescence emission spectra were determined at 580 nm excitation using 10 nm and 5 nm as band passes for excitation and emission monochromators, respectively. DCMU-induced fluorescence was measured by adding 10 μl DCMU (from 5 mM stock) to 2 ml sample in the cuvette followed by a dark incubation for 30 sec. Fluorescence values at 685 nm and 660 nm were taken as the emissions coming from PS II and PB, respectively.

Results and Discussion

It has been observed in senescent tissues that degradation of Chl *a* produces a number of degradation products like chlorophyllide *a*, pheophytin *a*, pheophorbide *a*, epimers, allomers and pyroderivatives [15]. In addition, numerous other catabolites including FCCs and NCCs are also produced [16]. In the present experiment the insecticide at the two selected concentrations caused reduction in Chl *a* content of cells, which was found significant after 4 hr and 45 minutes of treatment with 20 μM and 50 μM, respectively (Fig. 1 A). At both treatment concentrations, the cellular Chl *a* content decreased with prolongation of exposure. This indicated that the insecticide is a potent inhibitor of photosynthesis. The metabolic cost hypothesis of Calow and Sibly [17] suggests that all types of toxic stresses induce metabolic change in the organism, leading to depletion of its energy reserve that results in an adverse effect on its growth and biochemical composition. The inhibition of photosynthesis of *Anabaena* by cypermethrin enhanced the degradation of pigments mainly for producing carbon skeleton to satisfy the energy demand of cells. It has also been proposed that when photosynthesis is

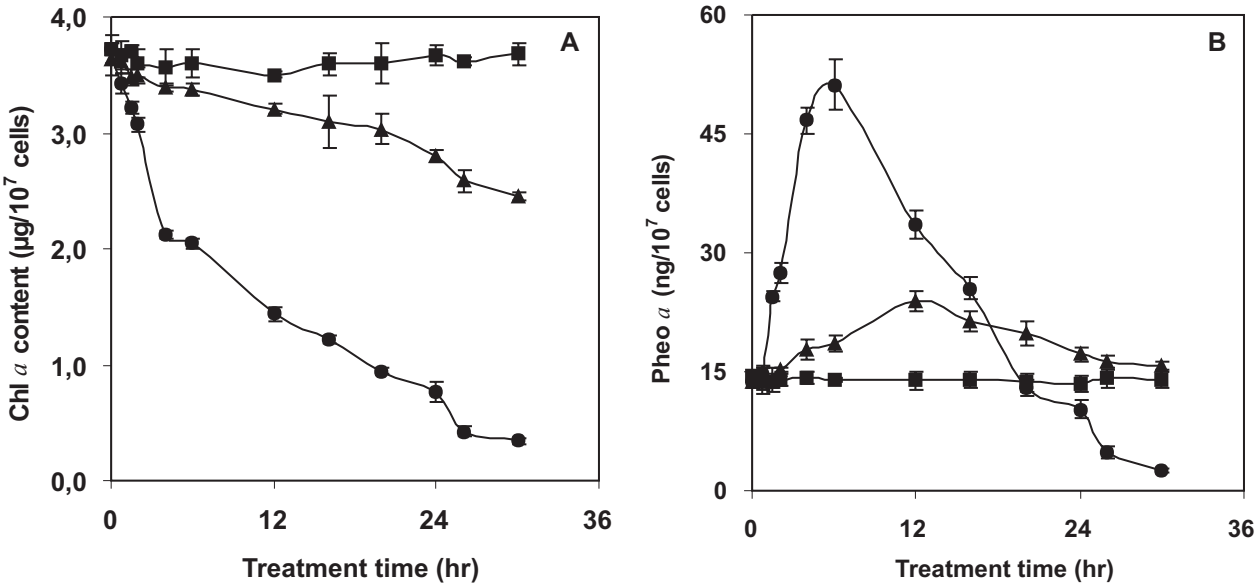

Fig. 1. Effect of cypermethrin treatment on (A) chlorophyll *a* and (B) pheophytin *a* content of *Anabaena doliolum* at different times of exposure. Insecticide concentrations: ■ - control, ▲ - 20 µM, ● - 50 µM.

Table 1. The least significant difference (LSD) values of comparison among means of the selected parameters at p = 0.05.

Parameter	Insecticide concentrations (µM)		
	0	20	50
Chl *a* (µg/10^7 cells)	0.179	0.173	0.106
Pheophytin *a* (ng/10^7 cells)	1.766	1.946	2.406
Carotenoids (µg/10^7 cells)	0.072	0.058	0.046
Lycopene (rel. units)	0.024	0.019	0.019
FCCs (rel. units)	0.21	0.29	0.16
NCCs (rel. units)	0.004	0.008	0.007
PC (ng/10^7 cells)	4.187	3.855	2.578
PE (ng/10^7 cells)	0.601	0.318	0.255

inhibited cells accelerate degradation of light harvesting pigments to maintain the balance between energy use and absorption.

Corresponding to degradation of Chl *a* there was also a concentration plus time-dependent increase/decrease of pheophytin *a* (pheo *a*) of the cultures (Fig. 1B). At 20 µM concentration, significant enhancement of pheo *a* level was also observed after 4 hr of treatment (Table 1) while at 50 µM concentration pheo *a* significantly increased after 1.5 hr of treatment. However, compared to the amount of Chl *a* degraded by the insecticide during the period of exposure, the amount of pheo *a* detected was less than 1% of the expected production. In both cases prolongation of treatment beyond 12 hr caused a gradual reduction of pheo *a* level of cultures but no such trend was observed in the control. It is due to the fact that removal of phytol chain and the central magnesium atom from Chl *a* is followed by the processes that cause the loss of green colour.

The decrease of Chl *a* content of cultures was followed by an increase in the fluorescence of homogenized cell suspension at 450 nm, considered to be coming from FCCs (Fig. 2 A). The intensity of fluorescence continuously increased with increase of treatment concentrations and incubation period and showed positive correlation with pheo *a* degradation. No such trend was, however, reported in the control, indicating that such response was due to the effect of cypermethrin on the cyanobacterium. A similar trend of fluorescence enhancement was also found after acidification of samples, which was considered to be the result of fluorescence emitted by lipofuscin--like fluorescing compounds produced by lipid peroxidation (Fig. 2B). The difference in the fluorescence values before and after acidification showed that FCCs were produced as a degradation product of Chl *a*. Unlike fluorescence, the absorbance of ninhydrin extract of the cultures, treated with 20 µM of cypermethrin, increased gradually up to 16 hr and remained almost stable thereafter while at the other test concentration the increase was followed by a gradual decrease of absorbance after 12 hr of incubation (Fig. 3). However, in both cases the increase/decrease was found significant and a considerable part of absorbance remained after 30 hr of treatment, indicating the stability of NCCs in the treated cultures. Meir *et al.* [18] have observed that like some chlorophyll catabolites, the products of lipid peroxidation also give blue fluorescence. Kale *et al.* [19] have observed that cypermethrin and fenvalerate induce lipid peroxidation and increase the activity of antioxidant enzymes such as super oxide dismutase and catalase. Deltamethrin like pyrethroids penetrate into the

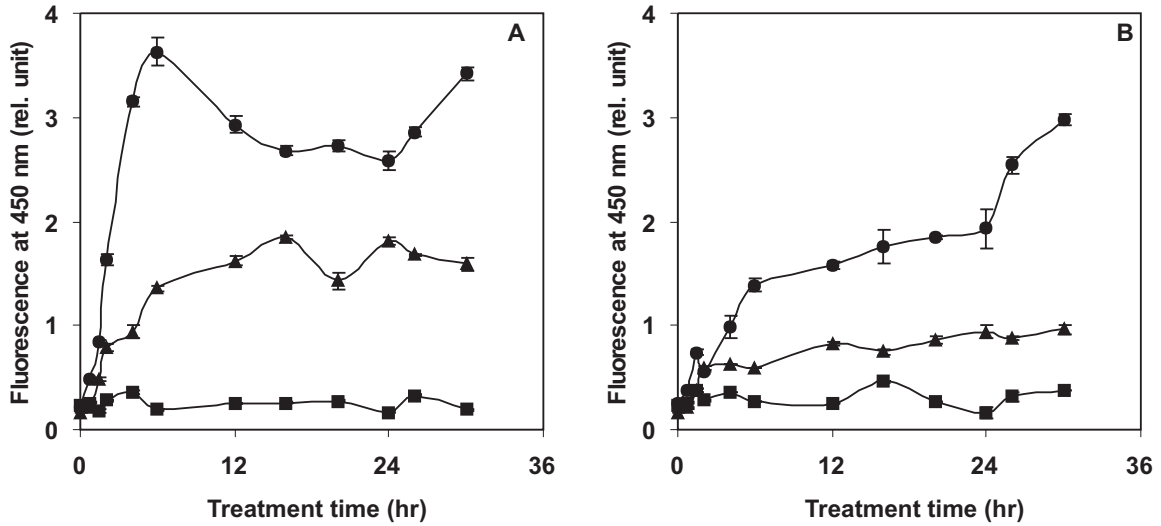

Fig. 2. Fluorescence emission by control and treated cultures of *Anabaena doliolum* at 450 nm (A) without and (B) with acidification to pH 2.0. Insecticide concentrations: ■ - control, ▲ - 20 μM, ● - 50 μM.

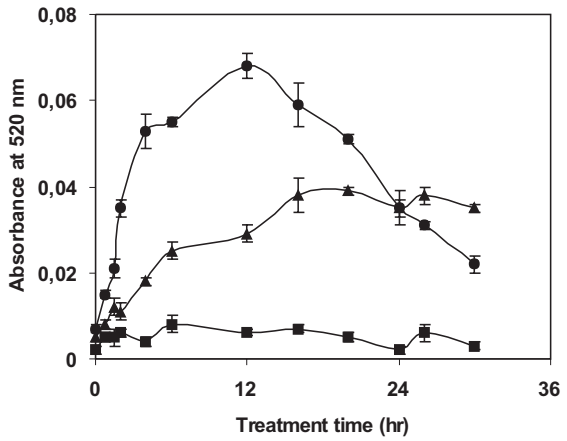

Fig. 3. Absorbance of ninhydrin extract of control and treated cultures of *Anabaena doliolum* at different treatment times. Insecticide concentrations: ■ - control, ▲ - 20 μM, ● - 50 μM.

hydrophobic interior of the phospholipid bilayer enhancing the lipid peroxidation and changing the membrane properties [20].

The carotenoids and lycopene contents of *Anabaena* cells decreased with cypermethrin treratment but the rate of reduction of carotenoids was slower than of Chl *a,* causing a time as well as concentration dependent increase of carotenoids: Chl *a* ratio of cultures (Fig. 4). At 20 μM concentration, significant reduction was observed after 16 hr of treatment while at the other concentration, it was noticed after 4 hr of treatment (Table 1). Reduction of carotenoids content of *Anabaena* showed that the insecticide not only accelerated the degradation but also blocked their biosynthesis. As most of the carotenoids synthesizing enzymes are membrane-bound, reduction of carotenoid biosynthesis might be due to the interaction of the insecticide with these enzymes. The interaction of

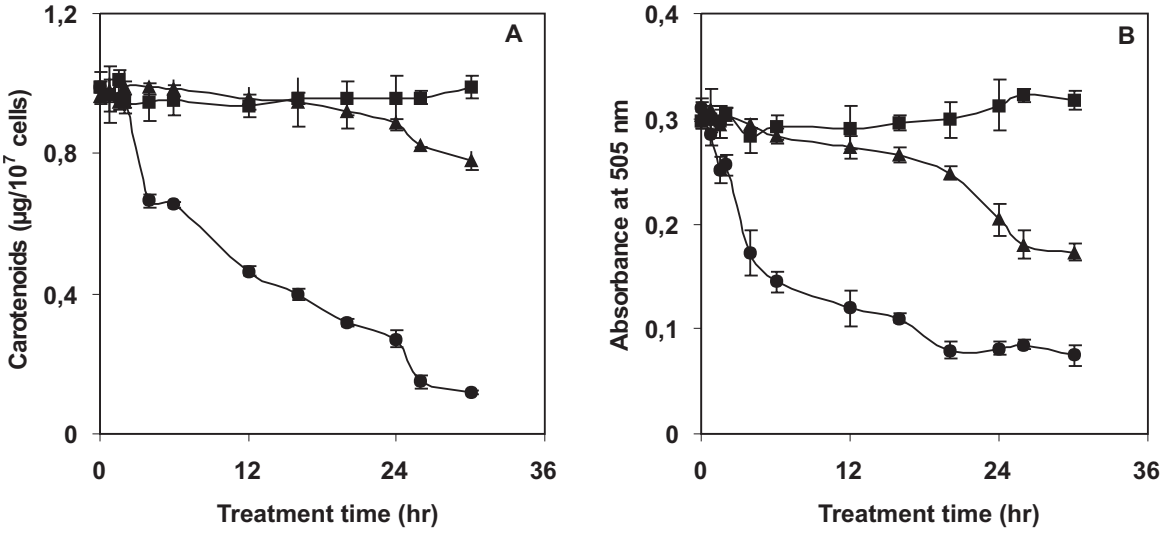

Fig. 4. Effect of cypermethrin treatment on (A) carotenoids content and (B) lycopene level of *Anabaena doliolum*. Insecticide concentrations: ■ - control, ▲ - 20 μM, ● - 50 μM.

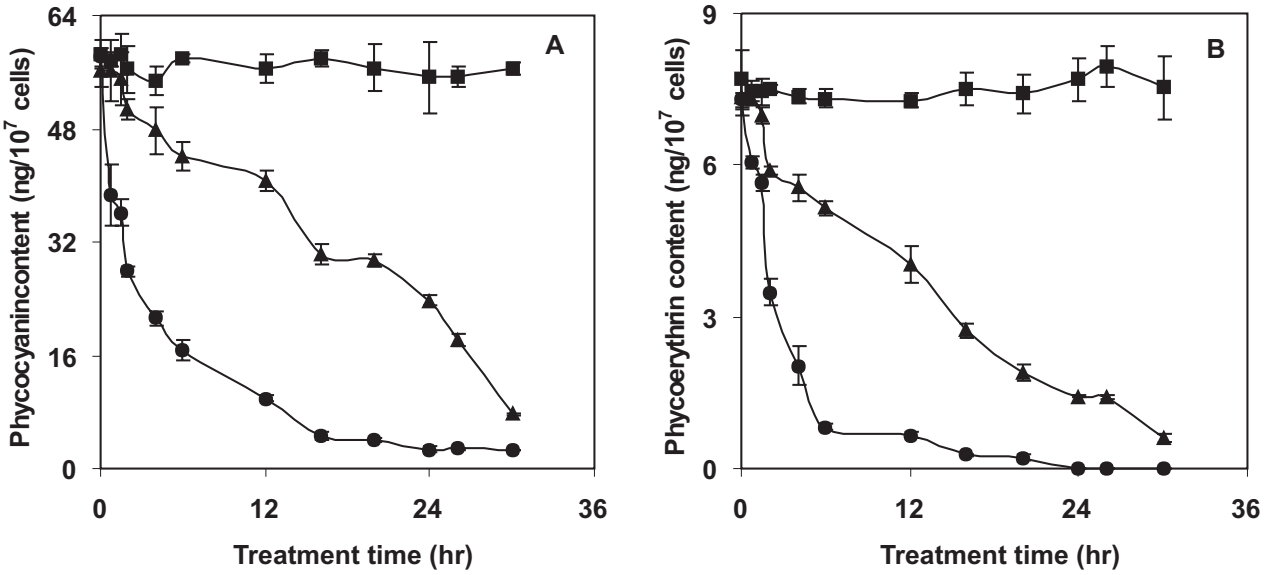

Fig. 5. Phycocyanin and phycoerythrin contents of *Anabaena doliolum* on treatment with cypermethrin. Insecticide concentrations: ■ - control, ▲ - 20 μM, ● - 50 μM.

Table 2. Effect of cypermethrin on fluorescence responses of treated and control *Anabaena* cells. Observations were made with excitation of cultures at 580 nm. The excitation and emission band passes during the measurement were 10 nm and 5 nm, respectively.

Treatment time (hr)	Without addition of DCMU						With addition of DCMU					
	660 nm			685 nm			660 nm			685 nm		
	Cypermethrin Conc. (μM)			Cypermethrin Conc. (μM)			Cypermethrin Conc. (μM)			Cypermethrin Conc. (μM)		
	0	20	50	0	20	50	0	20	50	0	20	50
0	13.98 ±0.63	13.68 ±0.59	13.95 ±0.86	8.92 ±0.42	8.19 ±0.36	8.28 ±0.18	15.26 ±0.83	15.17 ±1.01	15.39 ±0.46	15.86 ±0.78	14.91 ±0.92	14.95 ±0.48
6	13.87 ±0.78	10.62 ±0.42	6.26 ±0.21	9.21 ±0.29	7.59 ±0.58	4.17 ±0.18	15.11 ±0.76	12.05 ±0.43	7.95 ±0.43	17.59 ±0.86	11.57 ±0.48	7.23 ±0.42
12	13.97 ±0.43	9.86 ±0.35	5.24 ±0.28	8.64 ±0.49	7.21 ±0.13	3.01 ±0.09	15.29 ±1.04	11.56 ±0.27	5.93 ±0.19	16.86 ±0.49	11.27 ±0.73	4.59 ±0.11
24	14.11 ±0.57	7.52 ±0.18	1.28 ±0.11	9.35 ±0.24	7.56 ±0.46	1.79 ±0.11	16.62 ±0.43	8.57 ±0.46	1.30 ±0.11	18.05 ±1.18	10.16 ±0.42	2.01 ±0.16

membrane proteins and lipids with various pyrethroids has already been reported [1, 20]. As carotenoids provide photo protection to Chl *a*, pigment degradation may render the cyanobacterium more sensitive to photoinhibition under natural conditions.

PC and PE content of cells significantly decreased by the insecticide treatment at selected concentrations (Fig. 5). These two water-soluble protein pigments were degraded at a faster rate than those of Chl *a* and carotenoids. The degradation of other photosynthetic pigments, PC and PE, can also be attributed to the insecticide-thylakoid membrane interaction. Mohapatra and Schiewer [21] have demonstrated with organophosphorus insecticides that toxicant-membrane interaction is responsible for changes in fluorescence behaviour and pigment content of *Synechocystis* PCC 6803. The rate of reduction of cellular PC and PE levels of *Anabaena* was found to be significant with the treatment time at both the selected concentrations. Compared to PC, the cyanobacterium has a low amount of PE and thus has a high PC/PE ratio. By comparing the rates of their degradation it was observed that PE was degraded faster than PC, causing a time related increase of PE/PC ratio.

Fluorescence measurement of phytoplankton and cyanobacteria is the simplest and most economic method to investigate the pigment responses to light under stress conditions. It represents the part of energy effectively transferred to Chl *a* and PS II which is potentially usable in photosynthesis [22]. In our study there was a decrease of fluorescence with an increase of insecticide concentration. Such a trend was reported both with and without DCMU addition (Table 2). However, like in

control, DCMU addition caused significant enhancement of fluorescence of PS II and PB of the treated cultures. As expected, the fluorescence intensity of PS II showed a direct correlation with the cellular Chl *a* content of treated cultures (r=0.91 and 0.93 at 20 µM and 50 µM concentrations, respectively; n=12). A similar correlation could also be observed between PB fluorescence and PC content of treated cultures (r=0.89 and 0.92 at 20 µM and 50 µM concentrations, respectively; n=12). Due to a faster rate of reduction of PB fluorescence, the PB/PS II fluorescence ratio decreased with the prolongation of incubation time and treatment concentration of the insecticide. This agrees with our findings that cypermethrin caused faster degradation of phycobiliproteins than of other photosynthetic pigments. Similar observations have been reported with different OP insecticides and have been attributed to the accumulation of chemicals in the thylakoid membrane, resulting in change of membrane fluidity [21, 23]. However, this is the first report that cypermethrin causes enhancement of photosynthetic fluorescence of the cyanobacterium with short exposure. Michelangeli *et al.* [20] have observed that the accumulation of pyrethroids does not cause fluidity change of the lipid bilayer and inhibition of membrane bound enzymes are due to the interaction between membrane proteins and the insecticide molecules. Thus, the fluorescence enhancement of *Anabaena* by cypermethrin may be attributed principally to the enzymatic inhibition of photosynthesis.

References

1. STELZER K.J., GORDON M.A. Biochim. Biophys. Acta, **938**, 114, **1988**.
2. GOLOW A.A., GODZI T. A. Bull. Environ. Contam. Toxicol., **52**, 351, **1994**.
3. FARMER D., HILL I. R., MAUND S. J. Ecotoxicology, **4**, 219, **1995**.
4. HILL I.R., SHAW J.L., MAUND S.J. in. "Fresh Water Field Test for Hazard Assessment of Chemicals" (I.R Hill, F. Heimbach, P. Leeuwangh, P. Matthiessen; Eds), Lewis Publisher, Michigan, pp. 249-271, **1994a**.
5. HILL I.R., TRAVIS K.Z., EKONIAK P. in. "Aquatic Mesocosm Studies in Ecological Risk Assessment" (R.L. Graney, J.H. Kennedy, J.H. Rodgers; Eds) Lewis Publisher, Michigan, pp. 201-240, **1994b**.
6. VIJVERBERG H.P.M., VAN DEN BERCKEN J. CRC Crit. Rev. Toxicol., **21**, 105, **1990**.
7. PATRA S. Effect of the Pyrethroid Insecticide Cypermethrin on Photosynthetic Pigments of the Cyanobacterium *Anabaena doliolum* Bhar. M. Phil. Thesis, Utkal University, India, pp. 50, **2000**.
8. SAFFERMAN R.S., MORRIS M.E. J. Bact., **88**, 771, **1964**.
9. PORRA R.J., THOMSON W.A., KRIEDERMAN P.E. Biochim. Biophys. Acta, **975**, 384, **1989**.
10. MORAN R. Plant Physiol., **69**, 1376, **1982**.
11. CHAMOVITZ D., SANDMANN G., HIRCHBERG J. J. Biol. Chem., **268,** 17348, **1993**.
12. GEIDER R.J., OSBORNE B.A. Algal Photosynthesis. Chapman and Hall Inc., New York, pp. 256, **1992**.
13. BATES L.S., WALDRES R.P., TEARE L.D. Plant Soil, **39**, 205, **1973**.
14. BEER S., ESHEL A. Aust. J. Mar. Freshwater Res., **36**, 785, **1985**.
15. JEFFREY S.W., MANTOURA R.F.C., BJORNLAND T. in. "Phytoplankton pigments in oceanography: guidelines to modern methods" (S.W. Jeffrey, R.F.C. Mantoura, S.W. Wright; Eds) UNESCO Publication, pp. 449-559, **1997**.
16. MATILE P., HOERTENSTEINER S., THOMAS H. Annu. Rev. Plant Physiol. Plant Mol. Biol., **50**, 67, **1999.**
17. CALOW P., SIBLY R. Funct. Ecol., **4**, 283, **1990**.
18. MEIR S., PHILOSOPH-HADAS S., AHARONI N. J. Am. Soc. Hortic. Sci., **117**, 128, **1992.**
19. KALE M., RATHORE N., JOHN S., BHATNAGAR D. Toxicol. Lett., **105**, 197, **1999**.
20. MICHELANGELI F., ROBSON M.J., EAST J.M., LEE A.G. Biochim. Biophys. Acta, **1028**, 49, **1990**.
21. MOHAPATRA P.K., SCHIEWER U. Arch. Hydrobiol. Suppl., **134**, 79, **2000**.
22. LUTZ V. A., SATHYENDRANATH S., HEAD E.J.H., LI W.K.W. J. Phycol., **34**, 214, **1998**.
23. MOHAPATRA P.K., SCHUBERT H., SCHIEWER U. Ecotoxicol. Environ. Safety, **36**, 231, **1997**.