Full Paper

Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium

*Plectonema boryanum* to endosulfan stress

Sheo Mohan Prasad,* Deelip Kumar, and Mohd. Zeeshan†

Ranjan Physiology and Biochemistry Laboratory, Department of Botany, University of Allahabad, Allahabad 211002, India

(Received May 17, 2004; Accepted January 12, 2005)

The present paper deals with the insecticide endosulfan (5, 10 and 20 μg/ml)-induced changes in physiological and biochemical parameters related to photosynthesis and defense systems in paddy field cyanobacterium *Plectonema boryanum* grown under laboratory conditions. Growth and photosynthetic pigments, i.e., chlorophyll *a*, carotenoids and phycocyanin, were adversely affected by endosulfan treatment and the inhibition was found to be dose dependent. The toxic effect of endosulfan was more pronounced on phycocyanin; however, a considerable reduction in chlorophyll *a* and carotenoids was also noticed. 14C-fixation appeared to be more sensitive to insecticide than whole cell oxygen evolution. Spheroplasts treated with endosulfan exhibited a severe effect on PSII activity which was mainly due to blocking of the electron flow at the water oxidation side. In contrast to this, similar doses of endosulfan caused the least effect on PSI activity (DCPIP/ASC→MV). Furthermore, endosulfan with increasing doses accelerated the formation of active oxygen species, i.e., O₂⁻ and H₂O₂, in cells progressively, whereby an enhanced peroxidation of lipid and leakage of cell membrane were noticed. As a consequence of active oxygen species (AOS) generation in endosulfan-treated cells, the activity of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) was enhanced considerably. Besides the accelerated action of enzymatic defense systems, chemical antioxidant ascorbate showed a decreasing trend with the rising concentration of endosulfan (5, 10 and 20 μg/ml).

**Key Words**——active oxygen species; antioxidants; 14C-fixation; endosulfan; lipid peroxidation; membrane leakage; photosynthetic electron transport chain; *Plectonema boryanum*

Introduction

The application of insecticides, a group of pesticides, in crop fields for selective control of pests in the modern age has led to serious environmental contamination resulting in greater loss of crop productivity and growth of many beneficial micro-organisms (Shetty et al., 2000). Though, the application of many insecticides are forbidden, the low cost, easy availability, lack of awareness and lax regulatory implementation have contributed to the continuous use of the insecticides in tropical and subtropical regions. The removal of these insecticides from soil and aquatic systems has become a difficult problem and as a result of this, they persist in these ecosystems for a long period of time (Das and Singh, 1977; Singh, 1973). Water-bodies such as paddy fields, shallow water, aquaculture, ponds and water reservoirs are highly eutrophic and maintain...
large standing crops of phytoplankton, particularly cyanobacteria (Millie et al., 1992). Cyanobacteria, a group of ubiquitous, photosynthetic prokaryotes which perform two key biological processes such as oxygenic photosynthesis and nitrogen fixation together in same the cells/filaments, and enrich the paddy soil particularly with nitrogen and humus contents (Watanabe and Kiyohara, 1960). During photosynthesis, cyanobacteria harvest solar energy and assimilate it into carbon compounds which provide cellular energy and a carbon skeleton for metabolic process such as nitrogen fixation in heterocystous cyanobacteria. In recent years, it has been explored whether non-heterocystous cyanobacteria which are predominantly found in paddy fields (Desikachary, 1959) may also fix atmospheric nitrogen under aerobic conditions as Ohki et al. (1992) demonstrated the potential of a non-heterocystous cyanobacterium Trichodesmium in nitrogen fixation. Insecticide endosulfan-induced adverse impact on photosynthesis may cause a severe effect on other related metabolic processes and over all growth performance of cyanobacteria. Because of dual characteristic features, the cyanobacteria occupy an important place in both aquatic and terrestrial ecosystems (Venkataraman, 1981). Since time immemorial cyanobacteria have been applied in rice fields as a biofertilizer for better yield of paddy (Relwani, 1963). A large number of pesticides are used in rice fields to protect the rice seedlings and crops, and selectively destroy the pests, but the indiscriminate use of pesticides causes great danger to the rice field cyanobacteria and other beneficial micro-organisms (Da Silva et al., 1975). Under water logged conditions as commonly observed in rice fields, pesticides may induce many cellular disorders in cyanobacteria (Greaves, 1982; Singh et al., 1986). It has been shown that insecticide, carbofuran reduced the growth of cyanobacteria Oscillatoria sp., and Westiellopsis prolifica considerably (Ravindran et al., 2000). Owing to extensive usage of endosulfan in various tropical and subtropical countries to control the insect population, it also declines the growth of microflora including cyanobacteria considerably (Satish and Tiwari, 2000; Shetty et al., 2000).

Though a considerable amount of work relating to the pesticide induced inhibitory effects on growth, photosynthetic pigment contents and nitrogen fixation in cyanobacteria has been done, insecticides particularly endosulfan induced effects on photosynthesis, AOS generation, antioxidants viz. enzymatic, non-enzymatic and lipid peroxidation in cyanobacteria in general and Plectonema boryanum in particular are yet to be investigated. Furthermore, another group of pesticides such as herbicides are shown to generate singlet oxygen and other active oxygen species at various sites of photosynthetic electron transport chain (Halliwell, 1987) and create oxidative stress in cells. Cellular systems scavenge these active oxygen species by invoking an increased antioxidative machinery such as enzymes superoxide dismutase, catalase and peroxidase etc., and organic chemicals like proline, ascorbate and carotenoids etc. Herbicide induced oxidative stress accelerates lipid peroxidation, thereby affecting structural integrity and permeability of cellular membranes (Halliwell, 1987). Considering the importance of cyanobacteria in rice fields, and frequent use of pesticides against pests, the authors set forth the objective of investigating the impact of insecticide endosulfan on growth, photosynthetic pigments and photosynthetic electron transport activity, 14C-fixation, active oxygen species formation, antioxidant systems, lipid peroxidation and cell membrane leakage in a cyanobacterium Plectonema boryanum.

Materials and Methods

Organism and culture conditions. The filamentous, non-heterocystous, cyanobacterium Plectonema boryanum AUCC 143 was isolated from rice fields near Allahabad and raised to axenic culture. The culture was grown in BG-11 medium (pH 7.5) containing sodium nitrate (1.5 g/L) as source of nitrogen and maintained in the culture room at 27±2°C under 75μmol m⁻² s⁻¹ photon flux density (PFD) with a photoperiod of 14:10 h. The exponentially grown cyanobacterial cells were used throughout the experiment. Each experiment was conducted in the replicate of three.

Endosulfan treatment. Endosulfan used in the present study is a quickly penetrating organochlorine insecticide. Various concentrations of endosulfan were prepared from stock solution by dissolving it into the sterilized nutrient medium. The stock solution was prepared in 70% ethanol. On the basis of a series of experiments, the effective doses of 5, 10 and 20 μg/ml of endosulfan were selected for present study. For each experiment, the solution of endosulfan was freshly prepared and sterilization was done by passage through a Millipore membrane filter (0.22 μm). The control and endosulfan-treated samples contained the same
amount of ethanol.

**Growth and photosynthetic pigments measurement.**

Growth was determined by estimating protein content at regular intervals for 12 days. Protein content was determined with Folin phenol reagent using lysozyme as the standard (Lowry et al., 1951). Chlorophyll a and carotenoids from each sample were extracted in 80% acetone and the content of both the pigments was determined from absorbance at 663 nm and 450 nm, respectively using the method of Myers and Kratz (1955). Phycocyanin from treated and untreated cells was extracted in 2.5 mM potassium phosphate buffer (pH 7.0) after repeated freezing and thawing and absorbance of supernatant was recorded at 620 nm. The amount of phycocyanin was determined by the method of Blumwald and Tel-Or (1982).

**Measurement of photosynthetic oxygen evolution and 14C-fixation.** Cyanobacterial cells, pretreated with endosulfan for 24 h, were used for photosynthetic oxygen evolution and 14C-fixation studies. Photosynthetic oxygen evolution was measured at 28°C for 5 min under an illumination of 360 μmol m−2 s−1 PAR light using a Clark type O2 electrode (Rank Brothers, UK). Carbon fixation in cyanobacterial cells was measured by recording the incorporation of 14CO2 from NaH14CO3 (specific activity 9.25 mCi/ml) into acid stable products. In each assay spheroplasts equivalent to 6 μg Chl a ml−1 were used.

**Assay of antioxidant enzymes.** Cyanobacterial cells were harvested by centrifugation after 24 h of endosulfan treatment and then homogenized at 4°C in 100 mM EDTA-phosphate buffer (pH 7.8) for superoxide dismutase (SOD) activity and in 100 mM phosphate buffer (pH 7.8) for peroxidase activity. Supernatant obtained after centrifugation of the homogenate at 20,000×g for 30 min was used as a crude extract for the enzyme assay. SOD (EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium chloride (NBT) according to the method of Giannopolitis and Ries (1977) using a reaction mixture (3 ml) consisting of 1.3 μM riboflavin, 13 mM methionine, 63 μM NBT, 0.05 μM sodium carbonate (pH 10.2) and crude extract (600 μg protein ml−1). Peroxidase (EC 1.11.1.7) activity in a reaction mixture (3 ml) containing 16 mM H2O2, 10 mM pyrogallol and crude extract (650 μg protein ml−1) was determined spectrophotometrically according to the method of Gahagen et al. (1968) and the activity was measured as rise in optical density at 430 nm. Catalase (EC 1.11.1.6) activity was determined by recording O2 release from dissociation of H2O2 in darkness for 1 min after the addition of 5 ml of 50 mM phosphate buffer (pH 7.0) containing 50 mM H2O2 directly to 1 ml of cell suspension in a reaction vessel (Egashira et al., 1989). Oxygen release due to enzymatic dissociation of H2O2 was measured by a Clark type O2 electrode (Rank Brothers) and the oxygen produced by enzymatic reaction was calculated after correction for autoproduction of oxygen from H2O2. One unit of catalase is the amount of enzyme producing 1 μmol O2 min−1 as described by Sgherri et al. (2001).

**Determination of ascorbate.** Ascorbate was extracted from the pellets of 10 ml test samples equivalent to 3 mg dryweight with 5% w/v sulfosalicylic acid and the amount of ascorbate was determined in the supernatant obtained after centrifugation at 14,000×g for 10 min, using the method given by Oser (1979) and expressed as μmol ascorbate (g dry wt)−1.

**Determination of total peroxide and superoxide radi-
cal. For total peroxide, test samples were homogenized in 3.5 ml of 5% TCA and after centrifugation at 10,000×g for 15 min, the total peroxide in the supernatant was analyzed by following the ferrithiocyanate method as described by Sagisaka (1976). The superoxide radical was measured according to the method of Elstner and Heupel (1976) by monitoring the nitrite formation from hydroxylamine in the presence of O2 in supernatant obtained from homogenates of 24 h treated and untreated samples with some modification as described by Jiang and Zhang (2001).

**Measurement of malondialdehyde.** Thiobarbituric acid reactive MDA production as a result of lipid peroxidation in test samples was measured by the method of Heath and Packer (1968).

**Membrane leakage.** Intactness of plasma membrane in treated and untreated cells of *P. boryanum* was measured as the leakage percentage of electrolytes, as described by Gong et al. (1998). Test samples were washed with EDTA buffer (pH 7.6) followed by subsequent thorough washing with deionized water and thereafter pellets were placed in test tubes containing 30 ml deionized distilled water at 30°C for 24 h. The samples were centrifuged and the initial electrolyte conductivity of the supernatant (*EC*1) was measured by digital conductivity meter CC-607, Century, India. One set of control samples was boiled at 100°C for 15 min to release all electrolytes, cooled, centrifuged and the final electrical conductivity (*EC*2) of the supernatant was measured. The leakage percentage of electrolytes was calculated by using the formula: (EC1/EC2) × 100.

**Results**

The cyanobacterium *Plectonema boryanum* AUCC 143 showed inhibitory growth response against insecticide endosulfan. Figure 1 shows the effect of various doses (5, 10 and 20 µg/ml) of endosulfan on the growth pattern of *P. boryanum*. Untreated control and endosulfan (5 and 10 µg/ml)-treated samples exhibited a lag phase of 3 days, while the culture treated with 20 µg/ml insecticide did not show significant growth even up to 6 days. Upon raising the concentration of endosulfan from 5 to 20 µg/ml, at the sixth day of treatment the growth declined from 6–62% that of control, depicting a concentration-dependent inhibition of growth. After 12 days of treatment, 5, 10 and 20 µg/ml endosulfan diminished the growth of cyanobacterium by 2, 14 and 42%, respectively.

The effect of different concentrations of endosulfan on photosynthetic pigments after 6 and 12 days of treatment is represented in Table 1. After 6 days of treatment a low concentration (5 µg/ml) of insecticide reduced Chl a, carotenoid and phycocyanin contents by 11, 7 and 17%, respectively. The declining trend in the pigment contents continued with the rising concentration of insecticide as 20 µg/ml of endosulfan sharply lowered chlorophyll a, carotenoid and phycocyanin contents by 60, 57 and 70%, respectively. However, 20 µg/ml of insecticide after 12 days of exposure exhibited less reduction in pigment contents, showing a significant recovery in the levels of chlorophyll a, carotenoid and phycocyanin contents. The growth of autotrophic organisms reflects the status of a key physiological process such as photosynthesis, which regulates the biomass production in autotrophs. Therefore, to understand the impact of endosulfan on biomass production of cyanobacterium, photosynthesis and protective mechanisms were investigated in detail. Table 2 shows the photosynthetic activity, i.e., oxygen evolution and 14C-fixation, in whole cell and photosynthetic electron transport of *P. boryanum* under the insecticide stress. After 24 h of treatment of cells, 4–17% reduction in oxygen evolution and 6–30% in 14CO2 fixation rates were recorded when endosulfan concentration was raised from 5 to 20 µg/ml. The photosynthetic electron transport activity, i.e., PSII, PSI
and whole chain, in spheroplasts of *P. boryanum* was investigated. Lower dose (5 μg/ml) of endosulfan treatment caused insignificant decrease in PSI activity. However, higher dose (20 μg/ml) of insecticide reduced the PSI activity marginally (7%). Contrary to this, PSII activity and whole chain mediated photosynthetic electron transport rates decreased rapidly following the exposure of endosulfan. The decline in the activity of PSII by 21% and whole chain by 34% was observed in the spheroplasts treated with the higher dose (20 μg/ml) of endosulfan for 10 min. However, the degree of inhibition of whole chain activity increased with the time of incubation (Fig. 2). In order to understand the mode of action of endosulfan on PSII activity, spheroplasts were incubated with various concentrations (10 and 20 μg/ml) of endosulfan for 10 min in darkness and the rate of DCPIP photoreduction in the presence and absence of electron donors (DPC, MnCl₂ and NH₂OH) was measured. The results presented in Table 3 indicate that an addition of saturating concentration of these electron donors to insecticide treated spheroplasts restored PSII activity significantly. On comparing electron donors, DPC and NH₂OH were found to restore the PSII activity maximally (36%). However, the extent of restoration of PSII activity with all donors tested, decreased with increasing concentrations of endosulfan.

The generation of superoxide radical and singlet

### Table 1. Photosynthetic pigments of *P. boryanum* after 6 and 12 days of endosulfan treatment.

<table>
<thead>
<tr>
<th>Photosynthetic pigments (μg/ml)</th>
<th>Insecticide treatment (μg/ml)</th>
<th>After 6 days/endosulfan (μg/ml)</th>
<th>After 12 days/endosulfan (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td></td>
<td>3.8±0.1</td>
<td>3.4±0.1*</td>
</tr>
<tr>
<td>Carotenoids</td>
<td></td>
<td>1.4±0.2</td>
<td>1.3±0.1ns</td>
</tr>
<tr>
<td>Phycocyanin</td>
<td></td>
<td>22.8±0.8</td>
<td>19.0±0.5</td>
</tr>
</tbody>
</table>

The values are means±SE. Values in parentheses indicate % decrease. All treatments are significantly different (*p*<0.01) and (*p*<0.05) from control (Student’s *t*-test). ns = not significant.

### Table 2. Effect of endosulfan on whole cell O₂ evolution, ¹⁴C fixation and photosynthetic electron transport activity of *P. boryanum*.

<table>
<thead>
<tr>
<th>Endosulfan (μg/ml)</th>
<th>Whole cell O₂ evolution [μmol O₂ (mg Chl a)⁻¹ h⁻¹]</th>
<th>¹⁴C fixation [cpm (mg Chl a)⁻¹ h⁻¹ × 10³]</th>
<th>Electron transport rate O₂ evolution/consumption rate [μmol (mg Chl a)⁻¹ h⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ASC/DCPIP → MV</td>
<td>H₂O → p-BQ (PSII)</td>
<td>H₂O → MV (whole chain)</td>
</tr>
<tr>
<td>Control</td>
<td>478 ± 5</td>
<td>856 ± 8</td>
<td>642 ± 6</td>
</tr>
<tr>
<td>5</td>
<td>458*±5</td>
<td>804 ± 7</td>
<td>629*±4</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(6)</td>
<td>(2)</td>
</tr>
<tr>
<td>10</td>
<td>445 ± 3</td>
<td>770 ± 7</td>
<td>622* ± 4</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(10)</td>
<td>(3)</td>
</tr>
<tr>
<td>20</td>
<td>398 ± 4</td>
<td>599 ± 9</td>
<td>598 ± 5</td>
</tr>
<tr>
<td></td>
<td>(17)</td>
<td>(30)</td>
<td>(7)</td>
</tr>
</tbody>
</table>

The values are means±SE. Values in parentheses indicate % inhibition. All treatments are significantly different (*p*<0.01) and (*p*<0.05) from control (Student’s *t*-test). Whole cell oxygen evolution was measured in suspension supplemented with 0.5 mM NaHCO₃.
oxygen may occur at various sites of PSI and PSII in photoautotrophs, and therefore, the status of superoxide radical and hydrogen peroxide in cells treated with insecticide for 24 h was investigated. These active oxygen species undergo deleterious reactions and cause oxidative stress in the cellular systems. Thus, the deleterious effects of endosulfan on \emph{P. boryanum} were correlated by estimating the rate of lipid peroxidation, superoxide radical and H$_2$O$_2$ production, and status of antioxidants. Results depicted in Table 4 and Figs. (3b, c and 4) demonstrate the increased antioxidant enzymes activity, superoxide radical and total peroxide content, lipid peroxidation and electrolyte leakage in the cells. Rising concentration of endosulfan from 5–20 $\mu$g/ml stimulated lipid peroxidation by 10–23%, electrolyte leakage by 3–14%, superoxide radical formation by 15–75% and the levels of H$_2$O$_2$ by 22–125%. At all the concentrations tested, endosulfan accelerated the activity of defense enzymes in \emph{P. boryanum}. Endosulfan, at 5, 10 and 20 $\mu$g/ml, caused a significant increase of 23, 41 and 58% in the activity of SOD; 9, 16 and 40% in catalase activity and 20, 30 and 50% in peroxidase activity, respectively. In contrast to the activity of these defense enzymes, antioxidant low molecular compound ascorbate declined progressively from 10–28% with increasing concentration (5–20 $\mu$g/ml) of endosulfan (Fig. 3a).

**Discussion**

The results of this study show endosulfan-induced changes in growth, photosynthetic pigment contents, photosynthesis, active oxygen species generation, lipid peroxidation, leakage of cell membrane and antioxidants in a paddy field cyanobacterium \emph{Plectonema boryanum} AUCC 143. Decreased growth of cyanobacterium with increasing doses of endosulfan could be explained on the basis of the damaging effect of the insecticide on light harvesting (PSII, PSI) and carbon assimilating systems, photosynthetic pigments synthesis
and protein synthesis. Similar inhibitory effect on growth of cyanobacteria with other insecticides—BHC, carbofuran, phorate and malathion—was correlated with the inhibition in chlorophyll a synthesis, photosynthetic activity and nitrogen fixation in Oscillatoria, Haplosiphon sp. and Calothrix braunii ARM 367 (Kaushik and Venkataraman, 1983; Torres and O'Flaery, 1976). An isolate of paddy field cyanobacterium Nostoc linkia exhibited 6–75% reduction in growth when exposed to 1 μg/ml to 25 μg/ml of endosulfan (Satish and Tiwari, 2000).

The inhibitory effect of endosulfan on photosynthetic pigments of P. boryanum was found to be dose dependent and the deleterious effect was more pronounced on phycocyanin followed by chlorophyll a and carotenoids. These results are in consonance with the injurious effect of other insecticides on chlorophyll a, carotenoids and phycocyanin (Agrawal et al., 1993; Anand and Subramaniam, 1997; Kaushik and Venkataraman, 1983; Marco et al., 1990). Such decrease in chlorophyll a, carotenoid and phycocyanin contents may be ascribed to the inhibition of pigment synthesis directly by insecticide or accelerated degradation of pigments due to increased AOS formation at the various sites of the photosynthetic electron transport chain during stress. The decreased inhibitory effect of endosulfan on photosynthetic pigment contents after 12 days of treatment could be explained on the basis of the cellular degradation of endosulfan or due to the adaptability of cyanobacterium to the insecticide. Such a biodegradation of endosulfan by a microorganism has also been demonstrated in earlier study (Shetty et al., 2000). The greater sensitivity of phycocyanin to endosulfan may be due to direct interaction of the insecticide with phycocyanin. The proteinaceous nature and the exterior localization of phycocyanin on the thylakoid membrane could be one of the
Whole cell photosynthetic oxygen evolution, $^{14}$C-fixation rates and photosynthetic electron transport activity of *P. boryanum* were adversely affected by 5, 10 and 20 $\mu$g/ml endosulfan and a similar reduction in photosynthetic oxygen evolution in *Anabaena* PCC 7119 with a higher concentration (300 $\mu$g/ml) of trichlorfon has also been noticed (Marco et al., 1990). Endosulfan-induced inhibition in $^{14}$C-fixation in *P. boryanum* could be explained on the basis of a reduced supply of assimilatory power ATP and NADPH from the insecticide-inhibited photosynthetic transport system (Table 2). These results are in agreement with earlier findings where the insecticide diazinon was found to decrease ATP synthesis in green algae *Chlorella, Chlamydomonas* and *Euglena*; and fenithrothion and clorpyrifos were reported to inhibit $^{14}$C-fixation considerably in cyanobacteria *Anabaena* and *Aulosira*, respectively (Clegg and Koevenig, 1974; Lal et al., 1987). More reduction in $^{14}$C-fixation rate as compared to the photosynthetic oxygen evolution revealed that endosulfan might have also interfered with carboxylating and other enzymes involved in the Calvin cycle of this cyanobacterium.

Strong inhibitory effects on whole chain and PSII activity clearly demonstrate that endosulfan-induced effects might have resulted due to direct interaction with the water oxidation side of PSII, D$_1$ and D$_2$ proteins of the PSII reaction center and main antenna pigments phycobilisomes of *P. boryanum*. The results pertaining to the partial restoration of PSII activity by artificial electron donors MnCl$_2$, DPC and NH$_2$OH revealed that endosulfan arrested the electron flow on the oxidizing side of the PSII reaction center. One of the possible reasons for greater sensitivity of PSII to endosulfan could be correlated with reduced energy transfer from antenna pigment phycobilisomes to the PSII reaction center (P 680) as reported in the case of *Anabaena dolio* in the presence of the herbicide glyphosate (Shikha and Singh, 2004). The least effect on PSI activity (DCPIP $\rightarrow$ MV) by endosulfan even at 20 $\mu$g/ml confirms its stable nature against stress as reported earlier (Almog et al., 1991).

Endosulfan-induced acceleration in the active oxygen species formation in *P. boryanum* could be due to the strong inhibition of PSII and whole chain activities (Tables 2 and 3) as observed in case of other biotic stresses (Gaba et al., 1987). The stimulated generation of active oxygen species caused increased peroxidation of lipid in *P. boryanum* (Fig. 4b), and thus, resulted in greater membrane leakage (Fig. 4a). The enhanced level of O$_2$ and H$_2$O$_2$ following 20 $\mu$g/ml endosulfan treatment might have also caused greater damage to photosynthetic pigments (Table 1) and proteins (Fig. 1). Recent evidence has shown that AOS, especially H$_2$O$_2$ and O$_2^-$, are involved in cellular signaling processes as secondary messengers to induce a number of genes and enzymes such as CAT, POD and SOD (Mahalingam and Fedoroff, 2003), which invoke active oxygen species in stressed organisms. Thus, the increased level of O$_2^-$ and H$_2$O$_2$ triggered the activity of several antioxidant enzymes such as superoxide dismutase, catalase and peroxidase in *P. boryanum* at all the concentrations of endosulfan tested.

The present study demonstrates that the strong inhibitory effect on the growth of cyanobacterium *P. boryanum* could be correlated with the endosulfan-induced inhibition in PSII and whole chain activities. This inhibition might have not only increased the production of active oxygen species but also indirectly inhibited the removal of active oxygen species (H$_2$O$_2$) by reductive enzymatic reactions (glutathione reductase and dehydroascorbate reductase) due to less availability of NADPH and as a consequence, the level of ascorbate decreased rapidly in 20 $\mu$g/ml endosulfan-treated *P. boryanum* cells.

**Acknowledgments**

The authors thank the head, Department of Botany, University of Allahabad, Allahabad for providing necessary facilities.

**References**


Clegg, T. J. and Koevenig, J. L. (1974) The effect of four chlorinated hydrocarbon pesticides and one organophosphate pesticide on ATP levels in three species of photosynthesiz-


Marco, E., Martinez, F., and Orus, M. I. (1990) Physiological al-

2005 Response of *Plectonema boryanum* to endosulfan 123


