

## INFLUENCE OF SOME INSECTICIDES ON PHOTOSYNTHETIC OXYGEN EVOLUTION IN THE CYANOBACTERIUM ANABAENA

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In modern agriculture, several high-yielding strains of paddy have been introduced necessitating the use of a variety of powerful pesticides, some of which are even added to irrigation water<sup>1</sup>. BHC (benzene hexachloride), an organochlorine compound, and Ekalux (Quinolphos (0-odiethyl-o-quinoyalanyl-2-thiooxophosphate)), an organophosphorus compound, are commonly used in paddy cultivation in India. *Anabaena* is a nitrogen-fixing, heterocystous cyanobacterium that is ubiquitous in paddy fields forming a major constituent of the algal biotypes in nine out of fifteen states in India<sup>1</sup> and is also a component of the algal biofertilizer increasingly used in paddy cultivation. The effect of different pesticides including herbicides on growth, nitrogen fixation and ammonia excretion in *Anabaena* was reported earlier<sup>2,3</sup>. The present study pertains to the long term effect of BHC and Ekalux on photosynthesis and pigmentation in two paddy field strains of *Anabaena* which differ in their morphological and physiological characteristics<sup>4</sup>.

The two strains (*Anabaena* sp. ARM286 and ARM310) were maintained axenically in Bothe's medium<sup>5</sup> and for experimental purposes grown in a

medium free of combined nitrogen<sup>4</sup>, under continuous illumination with fluorescent tubes (1,400 lux) at room temperatures ( $27 \pm 2^\circ\text{C}$ ) in test tube still cultures. The insecticides were added to give a final concentration of 1 and 10 ppm in the concerned tubes. After 25 days of growth, oxygen evolution was measured polarographically, chlorophyll-*a* and phycobilins were estimated colorimetrically<sup>6,7</sup>.

Photosynthetic oxygen evolution was enhanced in both the strains of *Anabaena* after a long-term exposure (25 days) to the two insecticides at concentrations of 1 and 10 ppm (table 1). The increase with Ekalux was relatively higher than with BHC. In both cases the higher concentration (10 ppm) proved more stimulatory than the lower concentration (1 ppm). With 10 ppm of Ekalux the evolution of oxygen was nearly two-fold higher than that obtained with 10 ppm BHC. The basal level of oxygen evolution was higher in *Anabaena* sp. ARM310 than in *Anabaena* sp. ARM286 but the increase with insecticides was more significant in the latter (table 1). There is very little information available on the long term influence of insecticides on the process of photosynthesis by cyanobacteria. The present study indicated a favourable response of *Anabaena* to the application of insecticides similar to that reported for growth and nitrogen fixation<sup>2</sup>. To determine the reasons for stimulation of oxygen evolution, the pigment composition, especially the levels of chlorophyll-*a* and phycobilins, was examined. There was a marked increase in the levels of pigments with the two insecticides (table 2). Similar to oxygen evolution, the basal level of pigments in *Anabaena* sp. ARM310 was more than two-fold higher, compared to *Anabaena*

Table 1 Influence of BHC and Ekalux on photosynthetic O<sub>2</sub> evolution by *Anabaena* ( $\mu\text{l mgchl}^{-1} \text{hr}^{-1}$ )

Strain	Treatment	O <sub>2</sub> evolved	% increase over control
<i>Anabaena</i> sp. ARM286	Control	210.0	—
	BHC 1 ppm	252.8	20.0
	10 ppm	513.5	144.0
	Ekalux 1 ppm	591.3	181.0
	10 ppm	827.0	293.8
<i>Anabaena</i> sp. ARM 310	Control	365.0	—
	BHC 1 ppm	366.8	0.6
	10 ppm	410.0	13.0
	Ekalux 1 ppm	520.0	42.5
	10 ppm	840.0	130.0

Table 2 Influence of BHC and Ekalux on chlorophyll and phycobilins in *Anabaena* ( $\mu\text{g mg dry wt}^{-1}$ )

Strain	Treatment	Chlorophyll <i>a</i>	<i>c</i> -Phycocyanin	<i>allo</i> -phycocyanin	<i>c</i> -Phycocerythrin
<i>Anabaena</i> sp. ARM 286	Control	19.0	26.5	9.8	1.2
	BHC 1 ppm	28.0	53.0	13.1	3.1
	BHC 10 ppm	36.0	92.75	47.4	12.0
	Ekalux 1 ppm	29.0	99.4	52.3	6.9
	Ekalux 10 ppm	40.0	119.2	65.3	12.7
<i>Anabaena</i> sp. ARM 310	Control	45.0	52.0	20.0	27.0
	BHC 1 ppm	65.0	94.5	34.3	46.8
	BHC 10 ppm	89.0	125.3	45.7	68.4
	Ekalux 1 ppm	68.0	68.5	22.8	30.6
	Ekalux 10 ppm	160.0	115.8	59.9	55.8

sp. ARM286. Though both the insecticides enhanced pigmentation, Ekalux was more effective than BHC (table 2). BHC 1 ppm increased the levels of *c*-phycocyanin and *c*-phycocerythrin more than chlorophyll-*a* and *allo*-phycocyanin. At 10 ppm all the pigment levels were increased (table 2). The stimulation of oxygen evolution appeared to be the result of increased pigmentation. The increased pigmentation could not be easily explained since the mode of action of pesticides on Cyanobacteria has not been fully understood and it may range from mutagenesis<sup>8,9</sup> to detoxification<sup>10</sup> or even metabolizing the pesticide<sup>11</sup>. The earlier report<sup>2</sup> as well as this on *Anabaena* indicates the possibility of the organism metabolizing the pesticide since the initial inhibitory effect is overcome in about ten days<sup>2</sup>. The decrease in ammonia excretion coupled with increased nitrogen fixation in the presence of insecticides<sup>2</sup> may increase the nitrogen pool of cells, consequently increasing pigment synthesis, since pigments like *c*-phycocyanin have been regarded as nitrogen reserves<sup>12</sup>. However, further work is in progress to correctly assess the influence of pesticides on this process.

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*gical Nitrogen Fixation*, IARI, New Delhi 1982, p. 567

- Subramanian, G. and Shanmugasundaram, S., *Proc. Indian Natl. Sci. Acad.*, 1986, **B52**, 308.
- Subramanian, G., Ph.D. thesis, Madurai Kamaraj University, Madurai, 1982 (submitted).
- Bothe, H., Dissertation, Univ. Gottingen, Germany, 1968, p. 125.
- McKinney, G., *J. Biol. Chem.*, 1941, **140**, 315.
- Siegelman, H.W. and Kycia, J.H., In: *Handbook of phycological methods—Physiological and biochemical methods*, (eds) J.A. Hellebust and J.S. Craigie, 1978, p. 71.
- Singh, H.N. and Vaishampayan, A., *Environ. Exp. Bot.*, 1978, **18**, 87.
- Singh, H.N., Singh, H.R. and Vaishampayan, A., *Environ. Exp. Bot.*, 1979, **19**, 5.
- Das, B. and Singh, P.K., *Microbios. Lett.*, 1977, **4**, 99.
- Gregory, W.W., Reed, J.K. and Priester, L.E., *J. Protozool.*, 1969, **16a**, 69.
- Fogg, G.E., Stewart, W.D.P., Fay, P. and Walsby, A.E., *The blue-green algae*, Academic Press, New York, 1973.

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- Venkataraman, G.S., In: *Nitrogen fixation by free living microorganisms*, (ed.) W.D.P. Stewart, Cambridge University Press, 1975, p. 207.
- Subramanian, G., *Proc. Nat. Symp. on Biolo-*