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EFFECT OF BHC ON GROWTH, CHLOROPHYLL CONTENTS AND NITROGEN FIXATION OF *AZOLLA - ANABAENA* SYMBIONTS

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Benzene hexachloride (BHC) at lower concentrations stimulated the growth of *Azolla-Anabaena* symbionts upto 10 µg/ml exhibiting biomass yield as 4.57, 5.12 and 2.25 % over the control at 5 µg/ml after 7, 14 and 21 days of treatment respectively. Higher concentrations proved to be toxic and plants did not survive at such concentrations. Chlorophyll contents followed the similar pattern of stimulation and reduction like the growth of *Azolla*. But the stimulation of nitrogen fixation was evident upto 10 µg/ml with the progress of time during all the three intervals of treatment.

Keywords :*Azolla pinnata; Anabaena azollae;* Benzene hexachloride.

Introduction

Application of pesticides has become increasingly important in modern rice culture. Several high yielding strains of rice have been introduced necessitating the use of a variety of powerful pesticides. However, the frequent and indiscriminate use of pesticides may have undesirable secondary consequences.[1] BHC, an organochlorine insecticides is commonly used to control the major pest in cultivated rice. *Azolla pinnata* is a free floating fast growing N_2-fixing fern that is ubiquitous in the paddy fields of India. Each leaf of *A. pinnata* is bilobed, the upper lobe is chlorophyllous containing *Anabaena azollae* in its cavity and the lower thin lobe is non-chlorophyllous. It has tremendous importance as a green biofertilizer.

The present investigation was designed to study the influence of BHC on the growth (biomass yield), chlorophyll metabolism and nitrogen fixation in *Azolla pinnata.*

Materials and Methods

Azolla pinnata was collected from a rice field nearest to Guwahati, Assam and made a stock culture. BHC was obtained from Rallis India Ltd., Bombay. The active ingradient amounting to 70% is 1,2,3,4,5,6-hexachlorocyclohexane.

The experiment was conducted under out door condition in 18 cm deep earthen pots of 804.57 sq.cm surface area. A roof of transparent white polythene sheet was used to protect cultures from rain fall. Fishing net was also used to protect the culture from bird damage. The internal surface of each earthenware pot was covered with polythene sheet to check absorption of chemicals and filled with one kilogram of sterilized paddy field soil. One hundred mg superphosphate, 25mg potash and 5g wood ash were applied per pot as basal fertilizer dose. A series of dilutions viz. 1, 5, 10, 50, 100, 250, 500 and 1000 µg/ml was prepared by addition of appropriate amount of stock solution and tape water against the total volume of solution used 5 litres/pot. One treatment was considered as control where only tap water of the same volume was used. The level of

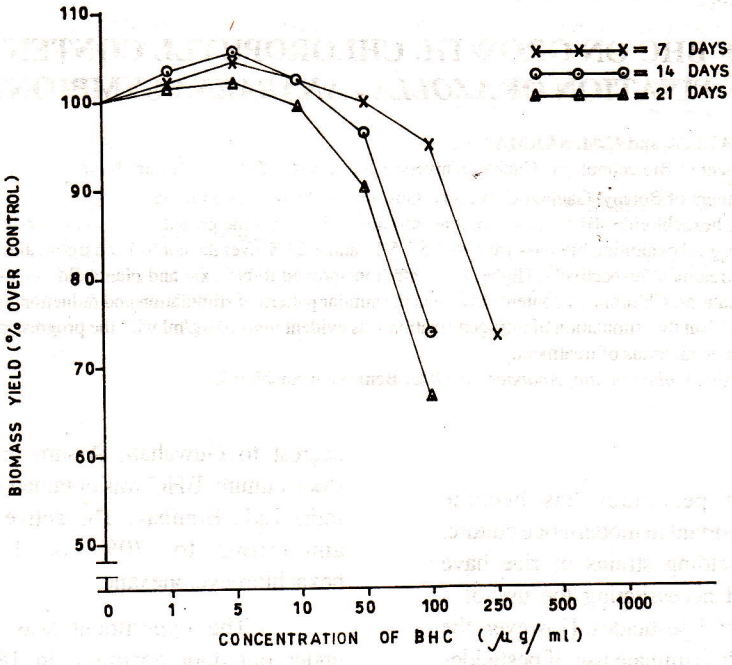

Fig. 1. Action-Curves of BHC on Biomass Yield.

solution in all the pots was maintained at 8 cm by addition of water throughout the period of study, to maintain proper dilutions. Each treatment was replicated three times and growth measurements were recorded after 7, 14 and 21 days treatment. Healthy *Azolla* inocula were screened out from stock culture, washed several times with tap water and blotted with blotting paper to remove surface water. One gm of *Azolla* was weighed and inoculated in each pot. The plants were removed from each treatment after specific time, washed carefully, blotted and fresh weights were taken for recording the growth (biomass yield). Chlorophyll content of fresh *Azolla* was determined following the method of Arnon[2]. Dried *Azolla* was used to estimate the nitrogen fixation in terms of total nitrogen content. Dry weights were taken after drying the samples in hot air oven at 60°C for 24 hours. The total nitrogen percentage of dried *Azolla* (100 mg of sample) was determined by micro-Kjeldahl method[3].

Results and Discussion

The increasing biomass yield of *Azolla pinnata*, upto 10 μg/ml of BHC after 7 and 14 days of treatment, indicate the stimulatory effect of BHC at lower concentrations for shorter durations. The rate of stimulation decreased after 21 days of treatment and recorded an increase upto 5 μg/ml after 7, 14 and 21 days of treatment (Fig. 1). The findings are also in conformity with that of Salazar and Paulsen[4], who reported stimulatory effect in seedlings of *Sorghum bicolor* with low

Table 1. Chlorophyll content of BHC treated *Azolla—Anabaena* symbionts.

(Mean of 3 replications ± SE)

Duration of Treatment	Concentration µg/ml	Chlorophyll-a mg/g	Chlorophyll-b mg/g	Total chlorophyll mg/g	% over control (Total Chlorophyll)	Chlorophyll a/b ratios
7 days	0	0.381±0.003	0.287±0.001	0.668±0.001	100.00	1.328
	1	0.397±0.002	0.302±0.002	0.699±0.001	104.64	1.315
	5	0.412±0.001	0.311±0.002	0.723±0.001	108.23	1.325
	10	0.387±0.002	0.293±0.001	0.680±0.001	101.80	1.320
	50	0.366±0.002	0.278±0.002	0.644±0.002	96.41	1.317
	100	0.310±0.002	0.237±0.003	0.547±0.001	81.89	1.308
	250	0.236±0.003	0.184±0.002	0.420±0.002	62.87	1.283
14 days	0	0.390±0.001	0.304±0.004	0.694±0.001	100.00	1.283
	1	0.403±0.001	0.316±0.001	0.719±0.004	103.60	1.275
	5	0.427±0.001	0.334±0.001	0.761±0.002	109.65	1.278
	10	0.390±0.002	0.305±0.001	0.695±0.002	100.14	1.279
	50	0.317±0.001	0.254±0.002	0.571±0.002	82.28	1.248
	100	0.234±0.001	0.191±0.001	0.425±0.002	61.24	1.225
21 days	0	0.378±0.002	0.293±0.001	0.671±0.003	100.00	1.290
	1	0.386±0.001	0.305±0.001	0.691±0.001	102.98	1.266
	5	0.394±0.001	0.309±0.003	0.703±0.001	104.77	1.275
	10	0.360±0.002	0.284±0.002	0.644±0.001	95.98	1.268
	50	0.274±0.004	0.222±0.002	0.496±0.001	71.22	1.234
	100	0.189±0.001	0.158±0.002	0.347±0.002	51.71	1.196

concentrations of BHC. The growth of cyanobacteria may be favourably[5] or adversely[6] affected depending upon the nature and concentrations of pesticides. The declining growth rate of *Azolla-Anabaena* symbionts at higher concentrations of organochlorine insecticides might be due to inhibition in DNA synthesis as has been reported by Anderegg *et al.*[7]

In the present study chlorophyll contents followed the similar pattern of stimulation and inhibition as that of growth exhibiting a linear correlation with growth of *Azolla pinnata* (Table 1). The poor growth and less accumulation of chlorophyll at higher concentrations might be due to the interference of excess chloride ion of BHC in the soil solution on the uptake of or possibly precipitating the iron and thereby reducing the iron availability to *Azolla*. The increased chlorophyll synthesis at lower concentrations is in conformity with that of Subramaniam *et al.*[8], who reported a marked increase of pigments of *Anabaena* with the application of BHC. The increased pigmentation in *Azolla-Anabaena* symbionts may be the result of mutagenesis or detoxification[9].

In the present study the trend of accumulation of total nitrogen was similar with biomass yield and chlorophyll content exhibiting highest nitrogen fixing ability at the concentration of 5 µg/ml (Table 2). The inhibition of nitrogenase activity of cyanobacteria by the application of pesticides at higher concentrations was also reported[10]. There are reports that ATP and reducing agent required for nitrogenase activity are derived mainly from photosythetic reactions[11].

Table 2. Total N-content of BHC treated *Azolla-Anabaena* symbionts (mean of 3 replications) as per cent of control. (Figures within parentheses are the mean of actual nitrogen content in percentage)

Concentration µg/ml	% total N-content			Total for Concn.	Mean
	7 days	14 days	21 days		
0	100.00	100.00	100.00	300.00	100.00
	(3.76)	(3.78)	(3.78)		
1	100.98	101.68	101.41	304.07	101.00
	(3.79)	(3.84)	(3.83)		
5	101.60	102.38	102.47	306.45	102.15
	(3.82)	(3.87)	(3.87)		
10	100.53	100.97	101.06	302.56	100.85
	(3.78)	(3.81)	(3.82)		
50	99.47	99.03	98.41	296.91	98.97
	(3.78)	(3.81)	(3.82)		
100	96.10	93.56	92.41	282.07	94.02
	(3.61)	(3.53)	(3.49)		
250	91.57			91.57	30.52
	(3.44)				
Total for time	690.25	597.62	595.76		
Mean	98.61	85.37	85.11		

CD for BHC (n = 9); At 5% probability level = 0.28; At 1% probability level = 0.36. CD for time (n = 21); At 5% probability level = 0.18; At 1% probability level = 0.23.

The reducing rate of total nitrogen accumulation at higher concentrations of BHC in *Azolla-Anabaena* symbionts might be due to the primary effect at the photosynthetic level.

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