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ENVIRONMENTAL HAZARD OF THE HERBICIDE BENTHIOCARB ON THE NON TARGET CYANOBACTERIUM, *GLOEOCAPSA* SP.

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Abstract

A variety of responses were noticed when examining the influence of the rice field herbicide benthiocarb (saturn) on *Gloeocapsa* sp. The herbicide affected growth and cellular Chl *a* content of the Cyanobacterium. This suggested investigations on some other metabolic activities. Oxygen evolution was reduced by 20-55% in response to treatment with low herbicide concentrations (4-10 ppm, respectively). The process was time dependent. The rate of protein synthesis was highly sensitive to benthiocarb at low concentrations, whereas high concentrations caused an impairment of the protein synthesis process and a reduction in the nitrogen fixation ability of the Cyanobacterium. The results are of considerable significance, since the applied concentrations are less than normal field application.

Key Words: herbicides, *Gloeocapsa* sp., environmental hazard.

Introduction

Benthiocarp is a carbamate herbicide that has been widely used for weed control in rice crops. It was moderately toxic to aquatic invertebrates and fishes in acute toxicity tests (Sanders and Hunn, 1982, Kodama, *et al.* 1997).

The importance of monitoring the effect of pesticides on non-target organisms has been emphasized (Hill and Wright 1978, Abdullah, *et al.* 1997). Among those non-target organisms potentially susceptible to pesticides are the soil algae and Cyanobacteria. These organisms are important component of soil microflora and contribute to soil fertility (McCann and Cullimore 1979). They contribute photosynthesized organic material to the carbon cycle, increase the moisture holding capacity of the soil, and help to bind together soil particles. Many of the studies conducted with herbicides have focused on Cyanobacteria, in particular those of paddy field soils due to the growing awareness of the vital role of these organisms in the nitrogen economy of rice fields (Sharma, 1986). This reflects the justifiable concern for the fate of nitrogen fixing Cyanobacteria in pesticide treated paddy fields (Mishra *et al.* 1989), given that, according to Watanabe (1962), the average amount of nitrogen fixed in a flooded paddy field is 30 kg/ha/yr.

Extensive use of herbicides and pesticides for improving crop yields has given rise to their effect on soil and aquatic microorganisms. The presence or absence of certain species can be used for the evaluation of the water and soil quality, since the application of environmental pollutants can greatly affect the distribution of this genus. It is therefore imperative that regular monitoring be undertaken to evaluate the influence of pesticides on these organisms (Abdullah, *et al.* 1997). Evaluation of the possible impact of the rice field herbicide benthiocarb on the non-target soil isolate Cyanobacterium *Gloeocapsa*, as a component of the soil nitrogen fixing microflora, was assessed in the present investigation. This recognizes the environmental hazard of pesticides.

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Materials and methods

Organism and culture conditions: *Gloeocapsa* sp., a local isolate of soil Cyanobacteria, was grown photoautotrophically at 25°C in axenic batch cultures under continuous fluorescent illumination (25 Wm^{-2}) on the medium described by Allen (1968), for 5-6d, and monitored for reaching O.D. of about 0.28-0.33, to start the experiments. Growth was determined by measuring the culture density at 800 nm and Chl *a* was estimated after extraction with methanol using the extinction coefficient given by Mckinney (1941). Benthiocarb S[(chlorophenyl)methyl] diethylcarbamothioate (pesticide grade) was prepared in stock solution and added aseptically to the culture medium to the final concentrations indicated for each treatment. The applied herbicide concentrations ranged between 2 and 25 ppm.

Nitrogenase activity measurement: The cultures were grown without a nitrogen source for measuring the nitrogen fixation ability of the cyanobacterium. The activity of nitrogenase was measured according to Stewart *et al.* (1968) by the acetylene reduction technique by GLC gas chromatography.

Oxygen evolution: Photosynthetic oxygen evolution was measured polarigraphically by following the changes in O_2 concentrations in the medium with a calibrated Clark type oxygen electrode (Ono and Murata 1981). Three ml aliquots of cell suspensions, with a cell density of 0.1 mg ml^{-1}, were placed in temperature controlled cuvette and illuminated with a quantum flux density of 300 μEm^{-2} s^{-1}.

Isotope labeling conditions: Logarithmically growing *Gloeocapsa* sp. cultures (O. D. of approximately 0.3) were treated with the herbicide at the above designated concentrations. To determine the rate of protein synthesis, one ml portions of the culture was removed for each treatment concentration (2, 4, 6, 10, 15, 20 and 25 ppm), incubated with 0.5 Mbq of ^{14}C-uniformly labeled protein hydrolysate at 25°C for 3h. The total protein was immediately precipitated by adding 10% trichloroacetic acid. The precipitates were collected by centrifugation and washed with cold 5% trichloroacetic acid and ethanol and recentrifuged. The labeled newly synthesized proteins were estimated by measuring the radioactivity in a Delta Searle 300 liquid scintillation spectrometer.

Results and discussion

Growth of the Cyanobacterium *Gloeocapsa* sp., as monitored by measuring optical density at 800 nm and estimation of Chl *a* content, was only slightly reduced by the addition of benthiocarb to the cultures (Table 1). The herbicide induced reduction in growth at higher concentrations. The degree of inhibition increased by increasing benthiocarb concentration (4-25 ppm) and reached 85%. The recorded percentage inhibition values were slightly lower as estimated by Chl *a* content (Table1). Growth of *Gloeocapsa* sp. exhibited less sensitivity to the carbamate herbicides, as compared with the previously investigated Cyanobacteria. Thiocarbamate benthiocarb, was reported to inhibit growth in *Nostoc linckia* (Singh *et al.*. 1983). In addition, they recorded reduction in heterocyst formation. At 4 ppm growth was completely inhibited and heterocyst differentiation ceased.

Table 1. Effect of benthiocarb on growth, chlorophyll *a* (Chl *a*) content in *Gloeocapsa* sp., as monitored for 48h. Data are expressed as percentage of control cultures.

Benthiocarb (ppm)	% inhibition	
	Growth	Chl *a*
2	1.2	2.0
4	13.5	12.2
6	26	24.6
10	34.4	29.7
15	58	42
20	85	70
25	85	76

The interference of benthiocarb with growth and photosynthetic Chl *a* was further clarified by testing the herbicide's effect at different concentrations on the photosynthetic electron flow (Fig.1). The photosynthetic activity in *Gloeocapsa*, measured by O_2 evolution was markedly reduced when the cells were exposed to high concentrations of benthiocarb (10,15,20 and 22 ppm). The reduction in O_2 evolution rate reached approximately 55 to 77% within a narrow herbicide treatment range (10-15 ppm). Furthermore, time course correlation study was conducted. When *Gloeocapsa* cells were treated with 4, 10 and 15 ppm of benthiocarb for different time intervals, the photosynthetic O_2 evolution was affected by the treatment period (Fig. 2). Cultures treated with 4 ppm showed a maximum of 26% reduction in photosynthetic activity after 4h and were not affected by longer incubation time. Treatment at 10 and 15 ppm induced a rapid loss in activity of about 20 and 30%, respectively after one hour and approximately 58 and 73%, respectively after 4h. However, about 22-24% of the photosynthetic activity seemed to be persistent to longer treatment periods (up to 7h). The results showed a correlation pattern between photosynthetic activity and growth response to benthiocarb.

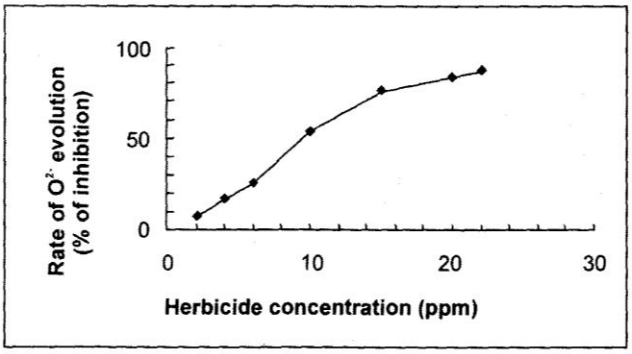

Fig. 1. The rate of photosynthetic O_2-evolution in *Gloeocapsa* sp. Cells as affected by the herbicide concentrations. The cells were treated at concentration for 3h. The values are averages of three independent measurements.

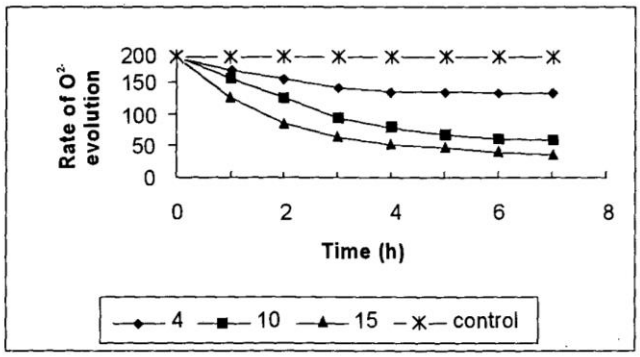

Fig. 2. The rate of photosynthetic O_2-evolution in *Gloeocapsa* sp. Cells as affected by the herbicide treatment time in cells treated with 4 (¦), 10 (¦), and 15 ppm (^) of benthiocarb.

Tolerance of the unicellular Cyanobacterium to low concentrations was obvious from the above data of the photosynthetic activity. Investigations by Singh *et al.*. (1983) showed that low concentrations of benthiocarb (4 ppm) induced a reduction in oxygen evolution by the Cyanobacterium (*N. Linckia*) to nearly 25% of the control level. This level was induced by applying 6 ppm for 3h, whereas, treatment with 4 ppm for a period of 4h produced comparable degree of inhibition (approximately 26%). On the other hand, studies by Mishra and pandy (1989), on some paddy field isolates of filamentous Cyanobacteria showed that similar concentrations of benthiocarb (Saturn) (6-8 ppm) were recorded to be leathal to *N. linckia*, *Anabaena doliolum*, *N. calciola* and *Nostoc* sp. This revealed that O_2 evolution sensitivity to benthiocarb in *Gloeocapsa* sp. was demonstrated at higher levels of the reported concentrations.

Aliquots of cultures were labeled with ^{14}C-labeled protein hydrolysate at different herbicide concentrations. The results (Fig. 3) indicated that treatment was not leathal. However, the rate of protein synthesis was drastically reduced upon treatment with 6-25 ppm, where minimum incorporation of radioactivity was obtained. An obvious gradual decreased rate of protein synthesis was measured by increasing herbicide concentration. It has been reported that eukaryotes and prokaryotes respond to environmental changing by altering their pattern of growth and protein synthesis (Carr, 1973). Thiocarbamate benthiocarb herbicides were classified mainly as protein synthesis inhibitors (Singh *et al.*. 1983, Pipe, 1992).

Nitrogen fixation by Cyanobacteria, is an important source of nitrogen input in the nitrogen cycle of rice fields and could limit pollution problems by lowering the demand for chemical fertilizers (Quesada *et al.*. 1997). The nitrogen fixing ability of *Gloeocapsa* was affected by applying the herbicide and approximately 53% maximum reduction in nitrogenase activity was produced (Table 2). The unicellular nitrogen fixing Cyanobacterium, under investigation, carry out N_2 -fixation and provide the microenvironment for the O_2 - sensitive nitrogenase (as measured according to Stewart *et al.* 1968). N_2 -fixation requires both ATP and a source of reducing power. Reductants for N_2 -fixation are dependent upon a supply of carbon compounds from photosynthesis. The

above results revealed that the photosynthetic activity of the Cyanobacterium was reduced by the treatment. In addition, the reduction in nitrogenase activity could be attributed to inhibition in protein synthesis by benthiocarb, as previously reported in *Nostoc linckia* (Singh *et al.*. 1983). Much higher concentrations of benthiocarb were applied by Zargar and Dar (1990). They reported that growth, nitrogen fixation and heterocyst formation in rice field isolates (*Anabaena* sp., *Nostoc* sp. and *Oscillatoria* sp.) were unaffected by 35 ppm of the herbicide. However, treatment of the cultures with 45 ppm and higher caused drastic inhibition of all the tested processes.

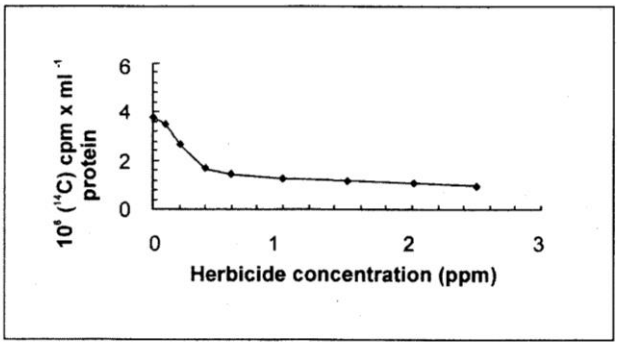

Fig.3. Effect of benthiocarb on the rate of protein synthesis. Aliquots of log-phase *Gloeocapsa* sp. culture were labled for 3h with ^{14}C-protein hydrolysate at the indicated herbicide concentrations.

Table 2. Effect of benthiocarb on the nitrogenase activity in logarithmically growing cultures of *Gloeocapsa* sp.

Herbicide concentration (ppm)	C_2H_4-reduction rate (nmol C_2H_4 x h^{-1} x mg $protein^{-1}$)
0	110 ± 2
4	86 ± 2
6	80 ± 0.8
10	74 ± 3
15	52 ± 1.2

Data in the present assessment established a variety of responses to the rice field herbicide benthiocarb (Saturn) on *Gloeocapsa* sp. High concentrations caused impairment of the tested processes. The sensitivity of the unicellular Cyanobacterium was apparent at the altered protein synthesis level, including nitrogenase activity. However, this reflected the limited photosynthetic activity and ATP (Van Hoogstraten, 1972). In addition, interspecific differences in benthiocarb sensitivity in unicellular and filamentous Cyanobacteria, though differential responses, has the potential to profoundly disrupt their balance.

Furthermore, the above responses have significantly increased the understanding of the potential threat of rice field herbicides to paddy field Cyanobacteria. However, the importance of these organisms to the nitrogen economy of other agricultural systems

should be considered. The harmful effect of the herbicide on Cyanobacteria, as important component of the soil microflora, is likely to occur at the onset of its field application. Therefore, better understanding of the environmental relevance of the present study requires further study of the effect of herbicides on natural populations of Cyanobacteria under field conditions. It is highly unlikely that *in vitro* data are completely transferable to *in vivo* situations, in the soil ecosystem, biological degradation and physical and chemical processes all govern the availability of a pesticide and its ability to interact with algae and Cyanobacteria (McCann and Cullimore, 1979).

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الخطر البيئى للمبيد العشبى (بنثيوكارب) على الطحلب الأخضر المزرق الغير مستهدف *Gloeocapsa* sp.

علا حمودة إبراهيم ومحمد سيد عبد الحميد
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عند دراسة تأثير المبيد العشبى (ساترن) المستخدم فى حقول الأرز، أظهرت التجربة عدة استجابات لطحلب *Gloeocapsa* sp.، حيث أثر المبيد العشبى على النمو والمحتوى اليخضورى أ (الكلوروفيل A) فى خلية الطحلب، مما أدى إلى التفكير فى البحث على بعض الأنشطة الحيوية الأخرى ذات الصلة، أوضحت الدراسات حدوث اختزال فى كمية الأكسوجين المنطلقة بنسبة من ٢٠-٥٥ ٪ كاستجابة للمعالجة بتركيز منخفض من المبيد العشبى (٤-١٠ جزء فى المليون على الترتيب) كما أكدت اعتماد العملية على الوقت. كان معدل بناء البروتين حساسا بالنسبة للمبيد العشبى بتركيزاته المنخفضة، بينما أدت التركيزات المرتفعة إلى توقف عملية بناء البروتين وإنقاص قدرة الطحلب على تثبيت النيتروجين الجوى. وتعتبر النتائج ذات مغزى كبير حيث أن التركيزات المستخدمة أقل من المستخدمة فى الحقول العادية.