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Effect of an amide herbicide on nucleic acid and protein metabolism in a diazotrophic cyanobacterium *Anabaena doliolum*

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ABSTRACT

Effect of an amide herbicide alachlor was studied on the cyanobacterium *Anabaena doliolum* for nucleic acids (DNA and RNA) and protein metabolism. Total DNA content in the cells, treated with 10 μgml^{-1} of alachlor after 72 hrs of treatment,was reduced upto 63.4% against control culture. In contrast to DNA total RNA values under similar conditions showed a marked inhibition with the increasing concentration of alachlor. The decreased in RNA was nearly 57.12%. The increasing ratio between RNA/ DNA is also suggesting for the sensitivity of DNA synthesis. The reduction in total protein was 39% at 10 μgml^{-1} and RNA/protein ratio showed a decreasing order with increasing concentration suggesting for sensitivity of nucleic acids (DNA and RNA) under alachlor stress which ultimetly affects protein metabolism.

Key words: Amide herbicide, protein metabolism, *Anabaena doliulum.*

INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes with their light capturing machinery being of higher plants type in that it consists of two photosystem, using water as electron doner and evolving oxygen as byproduct of photosynthesis. The role of nitrogen-fixing cyanobacteria in the nitrogen economy of paddy fields is well recognized (Singh 1961; Watnabe et al 1951). The nitrogen fixation in axenic culture of *Anabaena* and *Nostoc* was first demonstrated by Drews (1928) From then, almost all physiological, biochemical and genetical studies of nitrogen fixation have been carried out in these two filamentous and heterocystous cyanobacteria.

A large number of agrochemicals including herbicides are used in rice agriculture(Rudd,1971; Dodge, 1975). Most of them evidently affect weed by inhibiting their structural and functional aspects of photosynthesis. There are, howere many reports of direct effects which herbicide can exert upon nitrogen-fixing cyanobacteria (DaSilva et al 1975; Kashyap and Pandey 1982; Vaishampayan, 1984; Suseela, 2001, Pandey et al 1984; Pandey, 1985; Pandey *et.al.* 2007). Effect of herbicides on nucleic acids and metabolism has been reviewed by Asthon and Bayer (1976) and Moreland(1980). Cherry (1976) concluded that majority of herbicides limit the synthesis of macromolecules by reducing availability of ATP. Correlation between inhibition of RNA, protein synthesis and reduced ATP concentrations were established by Gruenhagen and Moreland (1971). Vartually little is known about biological interaction of herbicide alachlor on N2-fixing cyanobacteria. It is, therefore, intended to study the effect of alachlor on nucleic acid and protein metabolism in N2-fixing cyanobacterium *Anabaena doliolum.*

MATERIAL AND METHODS

Anabaena doliolum, a heterocystous N2-fixing cyanobacterium, was isolated from local paddy field and raised to axenic culture by

conventional microbiological techniques. The cyanobacterium was grown in Allen and Arnon's nitrogen free medium (Allen and Arnon, 1955) at 24± 1ºC in a culture room under illuminance of fluorescent light (intensity approx. 2200 lux) for 14hrs photoperiod. The exponentially grown cultures were invariably used as inocula and added to the graded concentrations of alachlor. Volume of the medium including herbicide was mantained 50 ml each. Medium without herbicide always served as control. Nucleic acid (DNA and RNA) were estimated with diphenylamine and orcinol reagent method respectively and total protein content was determined by using Folin-phenol reagent as described by Herbert *et al* (1971) , after 72 hrs of alachlor treatment.

Alachlor is a trade name given to 2 chloro-2' 6'-diethyl-N(methoxymethyl) acetanilide and contains 50% (0.5 g.ml^{-1}w/w)active ingradient. The required concentrations were prepared by diluting filter sterilized (0.22µm pore size) stock solution.

RESULTS AND DISCUSSION

The content of DNA ,RNA, and protein during active growth (after 72 hrs), in presence and absence of graded concentrations of the herbicide alachlor are given in table1. Total DNA cntent in the cells treated with 10µg ml^{-1} was reduced upto 63.4% against control and DNA content was found to be reduced from 4.05± 0.15µg ml^{-1} protein to 1.65± 0.19 µg ml^{-1} protein. In contrast to DNA, total RNA content under the similar condition showed a marked inhibition with the increasing concentrations of alachlor (Table 1). Total RNA content in the cells of *A . doliolum* treated with 10µg ml^{-1} was 22.15± 0.75µg ml^{-1} protein against control (51.65± 2.56µg ml^{-1} protein) , a culture grown without alachlor. Total decrease in RNA was nearly 57.12% to that of control value (Table 1). The ratio of RNAand DNA (RNA/DNA)was grater than control ratio at 10µg ml^{-1} also suggesting for DNA synthesis sensitivity under alachlor stress. It is also possible that the herbicide might act only on the replicating DNA as

Table 1: Effect of different concentration of Alachlor on Nucleic acids (DNA and RNA) and protein synthesis in the cyanobacteria *A. doliolum*

Alachlor conc.	DNA µgmg^{-1} Protein	RNA µgmg^{-1} Protein	Protein µgmg^{-1}	RNA / Protien	RNA / DNA
Control	4.5±0.15	51.65±2.56	180±1.15	0.28±6.072	11.47±1.050
0.5	4.20±0.51	45.25±1.52	184±1.22	0.24±5.015	10.77±1.10
1.0	4.05±0.25	41.38±1.75	176±1.35	0.23±4.022	10.20±0.95
5.0	2.50±0.28	30.05±1.32	140±1.05	0.21±4.019	12.0±20.52
10.0	1.65±0.19	22.15±0.75	110±0.95	0.20±10.09	13.42±a0.85
20.0	0	0	0	0	0

reported by Drake Blatz(1967) for N-methyl-N-nitro-N-nitrosoguanidine (MNNG).

Protein content in the cells of *A. doliolum* in presence of different concentrations of alachlor decreased with the increasing concentrations except 0.5µg ml^{-1} (184± 1.22µg ml^{-1}) against control (180± 1.5µg ml^{-1}) . The reduction in total protein was 39% at 10µg ml^{-1} and RNA/protein ratio showed a decreasing order with the increasing concentration of alachlor. It is suggesting for more sensitivity of protein to herbicide. The control of protein synthesis might be possible at different level: (a) at transcription level (i.e. specific regulation of DNA directed RNA synthesis). (b) at the level of translation (i.e. to read out of m-RNA to protein). Similar observations are reported by Cherry (1976) for phenoxy herbicide. Many of the herbicides block nucleic acidand protein synthesis by reducing ATP production(Gruenhagen and Moreland,1971). Inhibition of DNA synthesis is similar to observation reported with panacide in *Scenedesmus obliques* (Amla and Saxena, 1980).

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