Proc. Indian natn. Sci. Acad. **B52** No. 2 pp 308–312 (1986)

Influence of the Herbicide 2,4-D on Nitrogen Fixation and Ammonia Excretion by the Cyanobacterium *Anabaena*

G SUBRAMANIAN
Department of Botany, Bharathidasan University Tiruchirapalli 620 023
and
S SHANMUGASUNDARAM
Department of Microbiology, School of Biological Sciences, Madurai Kamaraj University, Madurai 625 021

(Received 29 October 1985)

2,4-D (2,4-Dichlorophenoxyacetic acid) at concentrations of 1–100 ppm did not have any adverse effect on six strains of *Anabaena*. The growth was stimulated in some strains. While in some strains, nitrogenase activity was considerably stimulated, in others, the activity decreased with increasing concentrations. The excretion of ammonia under the influence of 2,4-D varied with the strains and showed no correlation to nitrogenase activity.

Key Words : Herbicide, 2,4-D, cyanobacteria, *Anabaena*, Growth, Nitrogenase activity, Ammonia

Introduction

The introduction of chemical pest control measures has brought into use many powerful pesticides including herbicides, some of which are even added to irrigation water (Venkataraman 1975). Pesticides, in general, may be inhibitory, stimulatory or neutral depending on the nature of the chemical, its concentration and the algal strain (Roger & Kulasooriya 1980). 2,4-D (2,4-Dichlorophenoxyacetic acid) is a common herbicide employed in rice cultivation. Since cyanobacteria are increasingly used as biofertilizers in rice-fields (Venkataraman 1981), it is imperative that the influence of various agrochemicals on these organisms be thoroughly understood. Attempts with different chemicals on different organisms have been made by many workers (Metting 1981). The influence of 2,4-D on some of the cyanobacteria with special reference to nitrogen fixation has been reported earlier (Singh 1974). The present study is on the effect of 2,4-D on growth, nitrogen fixation and also on ammonia excretion by different strains of *Anabaena*.

Materials and Methods

Six strains of *Anabaena* were obtained from

the Culture Collection of Microalgae, Division of Microbiology, IARI, New Delhi, India.

The cultures were maintained in Bothe's (1968) medium, substituting nitrates with chlorides and ammonium molybdate with sodium molybdate in equimolar concentrations. The experiments were performed in test tube still cultures containing 5 ml of the medium under continuous illumination with fluroscent tubes (1400 lux) at room temperature (27±2°C). The herbicide stock solution was added to the media to give a final concentration of 1, 10 and 100 ppm.

Growth was measured in terms of Chlorophyll *a*, following the method of McKinney (1941). Nitrogenase activity was measured by acetylene reduction assay (Stewart et al. 1967) using Poropak T column in a gas chromatograph (Nukon Engineers, India). Ammonia estimation was carried out in the culture filtrate following the method of Solorzano (1969).

Results

2,4-D, at all concentrations used (1, 10, 100 ppm) did not significantly affect the growth in most of the strains studied and with *Anabaena* ARM 308, it positively stimulated the growth (figure 1).

Nitrogenase activity was significantly stimulated in strains ARM 39, ARM 220 and ARM 287. However, in the latter two, there was a decrease at 100 ppm level, which was nevertheless higher than the control (table 1). In *Anabaena* ARM 299 the stimulation of nitrogenase activity at 1 ppm was three-fold, while at 10 ppm it came down considerably but yet higher than control and at 100 ppm it was reduced to less than half of control activity. In *Anabaena* ARM 308 and ARM 311 the activity was reduced with increasing concentrations well below control (table 1).

Table 1 *Effect of 2,4-D on nitrogenase activity and ammonia excretion by* Anabaena

Strain	Conc. of 2,4-D in ppm	n moles C_2H_4 mg chl^{-1} hr^{-1}	μg NH_3 mg chl^{-1}
Anabaena ARM 39	0	8	17.2
	1	15	13.2
	10	17	7.9
	100	38	5.5
Anabaena ARM 220	0	18	1.5
	1	38	3.1
	10	41	5.6
	100	26	4.7
Anabaena ARM 287	0	77	2.4
	1	135	19.2
	10	112	21.9
	100	105	8.9
Anabaena ARM 299	0	118	54.5
	1	380	23.6
	10	155	21.6
	100	75	15.2
Anabaena ARM 308	0	125	3.9
	1	70	15.1
	10	52	2.3
	100	12	1.2
Anabaena ARM 311	0	135	22.9
	1	108	22.6
	10	108	22.6
	100	82	9.3

The influence of 2,4-D on ammonia release by the strains showed variations and had no strict correlation to nitrogenase activity (table 1). In three out of the six strains (*Anabaena* ARM 39, ARM 299 and ARM 311) where the original release of ammonia in control was high, there was a decrease in ammonia release with increasing concentrations of 2,4-D, whereas in two others (*Anabaena* ARM 220 and ARM 287) where the release of ammonia in control cultures was low, there was a significant increase with 2,4-D. In *Anabaena* ARM

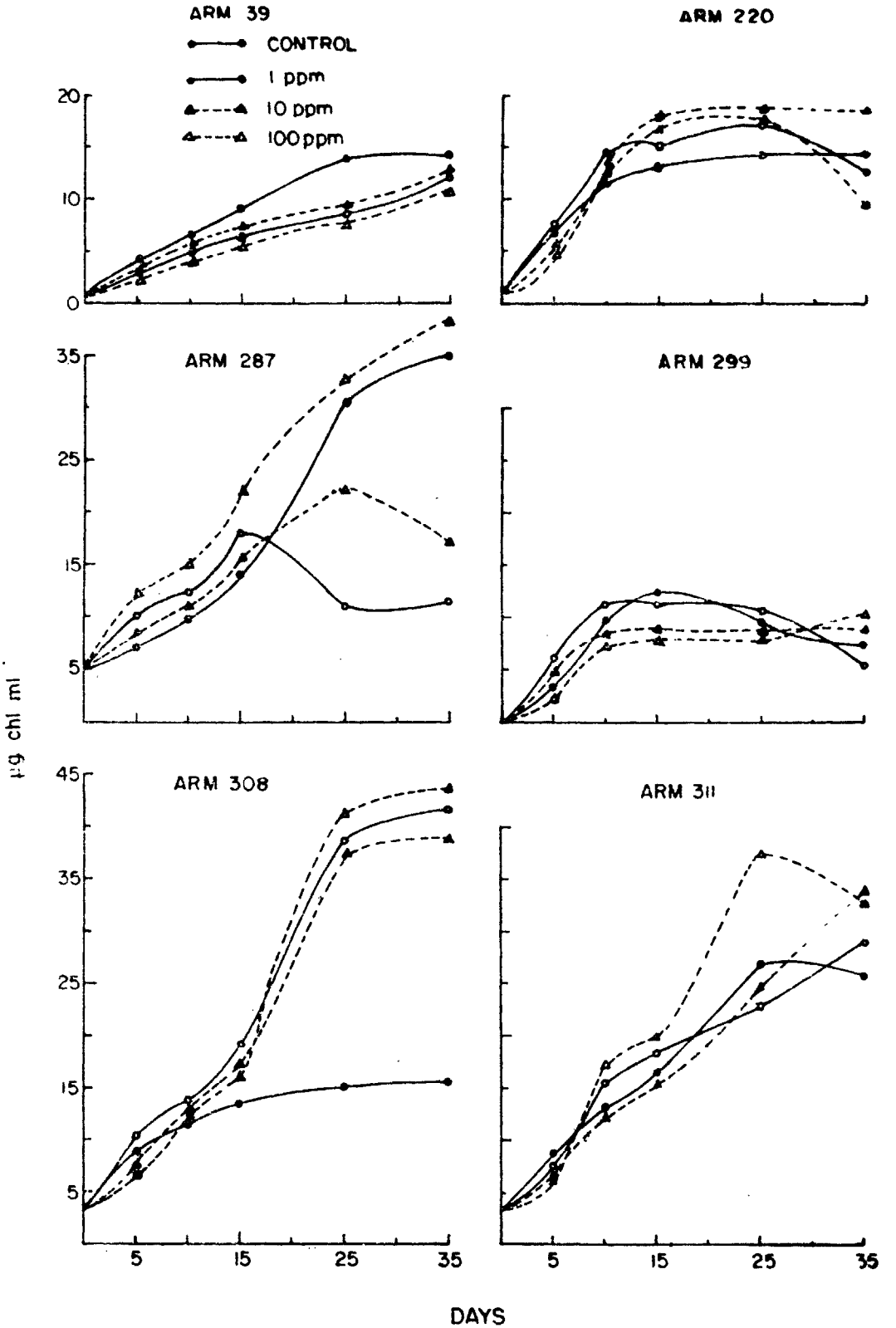

Figure 1 Time-course growth of six strains of *Anabaena* at different concentrations of 2-4,D

308 2,4-D at 100 ppm decreased ammonia release while at lower concentrations there was a stimulation.

Discussion

Among the pesticides, the herbicides have been more extensively studied and have been found to have varied effects in various organisms (Metting 1981). The growth of *Anabaena* strains is little affected by 2,4-D. On the other hand, there was a stimulation with a few strains. Other cyanobacteria such as *Cylindrospermum* have been reported to tolerate 2,4-D even at a concentration of 800 ppm (Singh 1974) while in *Nostoc linckia* not only growth was stimulated upto 100 μg ml^{-1} of 2,4-D but even the heterocyst frequency was enhanced and the organism could counteract the effect of Rifampicin (Tiwari et al. 1981). Though much is not known about the biochemical interaction of herbicides and cyanobacteria, there have been reports of both detoxification as well as uptake and metabolism of herbicides like 2,4-D by various algae (Tiwari & Pandey 1981, Meeting 1981).

The effect of herbicides on the process of nitrogen fixation has been shown to vary considerably depending upon the organism and the herbicide. Herbicides like 2,4-D have been reported to be inhibitory (Inger 1970), neutral (Singh 1974) and stimulatory (Meeting & Rayburn 1979) to nitrogen fixation in different cyanobacteria. This study has revealed that even in the single genus *Anabaena*, the effect of 2,4-D could vary depending upon the strain. It is possible that the herbicides or their products influence the enzyme systems concerned with nitrogen fixation and assimilation either directly or indirectly and that the differential response could be the result of differences in the ability of strains either to detoxify or take up and metabolize the herbicide.

The response of the different strains to 2,4-D in releasing ammonia into the medium showed variation and had no correlation to nitrogenase activity. A very similar situation was reported on the influence of insecticides and fungicides on *Anabaena* (Subramanian 1982). This suggests that ammonia excretion is not simply a function of nitrogen fixation but may be more complicated involving ammonia assimilatory process and membrane permeability.

Acknowledgements

The authors express their deep sense of gratitude to Dr G S Venkataraman, Division of Microbiology, IARI, New Delhi for making available the cyanobacterial cultures and crtically going through the manuscript.

References

Bothe, H 1968 Ferredoxin and phytosynthetischen reaktionen einer preparation aus der Blanalgne *Anacystis nidulans*. Dissertation, Univ. Gottingen pp 125

Inger L 1970 Effect of two herbicides on N_2 fixation by BGA; *Svensk. Bot. Tidskr.* **64** 460-461

McKinney G 1941 Absorption of light by chlorophyll solutions; *J. Biol. Chem.* **140** 314–322

Metting B 1981 The systematics and ecology of soil algae; *Bot. Rev.* **47** 195–312

—— and Rayburn 1979 The effects of the pre-emergence herbicide di-allate and the post-emergence herbicide MCPA on the growth of some soil algae; *Phycologia* **18** 269–272

Roger P A and Kulasooriya S A 1980 *Blue-green Algae and Rice* (Phillippines: Int. Rice Res. Inst.) 112 pp

Singh P K 1974 Algicidal effect of 2,4-D on the BGA *Cylindrospermum* sp.; *Arch. Mikrobiol.* **97** 69–72

Solorzano L 1969 Determination of ammonia in natural waters by the phenol hypochlorite method; *Limnol. Oceanogr.* **14** 799–801

Stewart W D P, Fitzgerald G P and Burris R H 1967 *In situ* studies on N_2 fixation using the acetylene reduction technique; *Proc. Natn. Acad. Sci. USA.* **58** 2071–2078

Subramanian G 1982 The effect of pesticides on nitrogen fixation and ammonia excretion by *Anabaena*; *Proc. National Symp. on Biological N_2-fixation* IARI New Delhi

Tiwari D N and Pandey A K 1981 Bio-detoxification of different groups of herbicides by BGA *Nostoc calcicola*; *Indian J. Microbiol.* **21** 163–164

——, —— and Mishra A K 1981 Action of 2,4-D and Rifampicin on heterocyst differentiation in the BGA *Nostoc linckia*; *J. Biosci.* **3** 33–39

Venkataraman G S 1975 BGA in tropical rice cultivation; in *Nitrogen Fixation by Free-diving Microorganisms.* ed. W D P Stewart pp 207–218 (Cambridge Univ. Press)

—— 1981 BGA for Rice Production FAO Soils Bull. M. 46, Rome, 102 pp