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Effect of pH, irradiance and population size on the toxicity of *Furadan* to two species of *Anabaena*

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Abstract

In two selected nitrogen-fixing cyanobacterial species *Anabaena fertilissima* and *Anabaena variabilis* pH, irradiance and different inocula sizes considerably modified the toxic effect of 50 % effective concentration (EC 50) dose of the pesticide *Furadan* (carbofuran 75 DB). Maximum growth and chlorophyll (Chl) *a* content of *A. fertilissima* was observed in the pH range of 8 - 9 and that of *A. variabilis* at pH 7 - 8, while at acidic pH (5 - 6) and at pH above 9 these parameters were considerably retarded. Toxicity of the EC 50 dose of *Furadan* was increased further at pH 5 - 6, whereas reduction in the toxicity to the test cyanobacteria was observed at pH 7.8 - 9.0. The experimental organisms grew comparatively better and synthesized higher amount of Chl *a* at an irradiance of 12.5 than at 7.5 or 2.5 W m^{-2}. The toxicity of EC 50 dose of the pesticide gradually decreased with the increasing irradiance. The toxic effect of *Furadan* was larger when the initial cyanobacterial population concentration was low and *vice versa*.

Additional key words: chlorophyll *a*, cyanobacteria, growth, pesticide.

Introduction

The environment (pH, temperature, irradiance, *etc.*) and nutrient balance of soil and/or water fluctuations affect the toxicity of pesticides to microorganisms, *e.g.* nitrogen-fixing cyanobacteria of rice fields. About 28 % of soil samples from different agroclimatic zones of Orissa state, India, have a pH from neutral to slight alkaline. However, nearly 70 % of the soils of the state are slightly acidic (pH 6.0 - 6.8). Therefore the effect of different doses of the pesticide *Furadan* on two important soil cyanobacteria (*Anabaena fertilissima* and *A. variabilis*) at various pH of the culture was studied. In the rice fields, seedling growth during few weeks after transplantation leads to canopy coverage due to which radiant energy seldom

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Abbreviations: Chl - chlorophyll; DB - dustless base; EC 50 - 50 % effective concentration.

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penetrates to the bottom soil. This is why the effect of *Furadan* was tested also under different irradiance. Experiments were carried out also at various initial cyanobacteria culture density in order to find out whether high population of cyanobacteria modified the toxicity of the pesticide.

Materials and methods

Pure culture of *A. fertilissima* and *A. variabilis* differing in their tolerance to *Furadan* were grown in hardglass test tubes (15 × 150 mm) containing 10 cm^3 of nitrogen free BG 11 medium (Rippka *et al.* 1979) and 1 cm^3 of inoculum. 10 cm^3 of the sterilized medium in different culture tubes was adjusted to different pH (5.0, 6.0, 7.0, 7.8, 8.0, 9.0 and 10.0) by aseptical adding 0.1 M HCl or 0.1 M NaOH. Homogenised culture suspension of equal size (absorbance of the culture suspension at 760 nm after inoculation was 0.02) were inoculated to each of the experimental tube. Sterile solution of the analytical grade *Furadan* was added to the culture medium of various pH so as to get the EC 50 dose of the pesticide (at which approximately 50 % growth of the respective organism was decreased in comparison to control) in each experimental tube and then incubated at 26 ± 1 °C under an irradiance of 7.5 W m^{-2} upto 8 or 16 d. In experiments studying the irradiance effect, the irradiance 2.5, 7.5 or 12.5 W m^{-2} were provided by day-light fluorescent tubes. In experiments testing the effect of different population size the absorbances of the inoculum at 760 nm were 0.1, 0.2, 0.3 and 0.4 (1 cm^3 to 10 cm^3 medium). Incubation conditions were 26 ± 1 °C, 7.5 W m^{-2}, 8 or 16 d. Growth of the homogenised culture suspension was recorded at 760 nm using a *Systronics* model *105* spectrophotometer. The amount of Chl *a* was estimated by using the extinction coefficients given by Mackinney (1941).

Results

Maximum growth and Chl *a* content of *A. fertilissima* were found in the pH range of 8 - 9 and that of *A. variabilis* at the pH of 7 - 8, though both the cyanobacteria tolerated a wide pH range (Table 1). Toxicity of EC 50 dose of the *Furadan* (100 and 250 gm^{-3} for *A. fertilissima* and *A. variabilis*, respectively) increased further in the acidic medium of the culture at pH 5 to 6, and there was a gradual reduction in the relative toxicity of the pesticide in the alkaline range of pH 7.8 (control) to 9.0. Larger reduction of growth at suitable culture pH by the pesticide observed at prolonged incubation suggesting that at pH from 7 to 10, both the organisms grew maximally within few days of incubation, and during their exponential growth the toxic effect of *Furadan* decreased.

The cyanobacteria grew better and synthesized more Chl *a* at high irradiance (12.5 W m^{-2}) than at 7.5 or 2.5 W m^{-2} (Table 2). The toxic effect of EC 50 dose of *Furadan* on the experimental organisms was lower when the cultures were incubated under higher irradiance, especially in the phase of exponential growth. Both the cyanobacteria did not grow in the dark.

Toxicity of EC 50 dose of *Furadan* to the experimental organisms was more pronounced when the initial inoculum was low (absorbance at 760 nm = 0.01) (Table 3). When the population size of the inoculum was increased by 2, 3 or 4, the toxicity of the EC 50 dose decreased.

Discussion

The deleterious effect of EC 50 dose of *Furadan* on growth and Chl *a* content of *Anabaena* was higher in acidic medium at the pH range of 5 to 6 than at the alkaline pH. Mishra and Pandey (1989) have reported that toxicity of herbicides to *Nostoc linckia* was alleviated at the alkaline pH of the culture (up to pH 9). Inhibitory effect of different concentrations of $CuSO_4$ and *Karmex* on *Microcystis aeruginosa* was reduced at the alkaline pH but not at the acidic pH (Swain 1994). Kar and Singh (1977) found that carbofuran was most toxic at pH 5.0 - 6.0 and the least at pH 7.5 - 10.0 in *Nostoc muscorum*. They suggested that better growth of cyanobacterium in the alkaline medium reduced the toxicity of the pesticide. However, carbofuran is hydrolysed in alkaline media (Getzein 1973) and therefore, the reduction in toxicity might also be due to hydrolysis of the pesticide, although metabolic products of some pesticides are as inhibitory to growth of microorganisms as the parent compound (Batterton *et al.* 1971).

The uptake of toxic chemicals by photosynthetic microorganisms is higher in light than in the dark. The uptake and assimilation of these substances are linked with the energy metabolism which requires radiant energy (Kar 1978). In this study variation in culture irradiance considerably affected the toxicity of *Furadan* on the growth and Chl *a* content of the cyanobacteria. Decrease in the toxicity of the pesticide at high irradiances indicated that carbofuran might accelerate photosynthesis with the increase in irradiance that leads to better growth of the organisms. The reduction in toxicity at EC 50 dose of the pesticide at high irradiance might also be due to the formation of some photosynthates antagonistic to the pesticide.

Also the population size of cyanobacteria altered the toxicity of pesticides on them. The inhibitory effect of EC 50 dose of *Furadan* gradually decreased with the increase of population size in the inoculum of both *A. fertilissima* and *A. variabilis*. Existence of the dose-inoculum relationship of various algicides, herbicides and other toxic chemicals to cyanobacterial species have been reported by Fitzgerald (1973), Das and Singh (1978), and Singh and Tiwari (1988). Our results showed that toxicity of *Furadan* was greatly altered due to various pH of the culture medium irradiance of the culture and by the population size. Thus various factors of the rice field environment such as soil/water pH, irradiance reaching the cyanobacterial population, field application rates of the pesticide, and the sensitivity of a particular population size of cyanobacteria towards a particular pesticide dose should be taken into consideration for evaluating the pesticide toxicity to rice field cyanobacteria.

Table 1. Effect of *Furadan* on the growth and chlorophyll (Chl) *a* content of *Anabaena fertillisma* and *A. variabilis* at different pH of the culture. The organisms were inoculated into the culture vessels containing BG 11 medium and an EC 50 dose of the pesticide (*A. fertilissima* 100 g m^{-3}, *A. variabilis* 250 g m^{-3}) and incubated at 26 ± 1 °C under an irradiance of 7.5 W m^{-2} up to 8 or 16 d. Absorbance of the culture after inoculation on the initial day of the experiment at 760 nm = 0.02; Chl *a* content of the initial inoculum was 1.34 g m^{-3} for *A. fertilissima* and 2.01 g m^{-3} for *A. variabilis*. Values in parentheses indicate per cent decrease (-) over control; means of three independent determinations ± S.D.

pH of the medium	Growth (absorbance of the culture at 760 nm)				Chl *a* content [g m^{-3}(culture)]			
	8 d		16 d		8 d		16 d	
	Control	EC 50	Control	EC 50	Control	EC 50	Control	EC 50
A. fertilissima								
5.0	0.11 ± 0.02	0.02 ± 0.01 (-81.8)	0.16 ± 0.02	0.03 ± 0.01 (-81.2)	0.52 ± 0.08	0.06 ± 0 (-88.4)	0.49 ± 0	0
6.0	0.17 ± 0.03	0.04 ± 0.02 (-76.5)	0.18 ± 0.01	0.04 ± 0.02 (-77.8)	0.57 ± 0.08	0.12 ± 0 (-78.9)	0.55 ± 0.10	0.06 ± 0 (-89.1)
7.0	0.24 ± 0.02	0.11 ± 0.01 (-54.2)	0.27 ± 0.01	0.07 ± 0.01 (-74.1)	0.60 ± 0.12	0.18 ± 0.06 (-70.0)	0.65 ± 0.07	0.12 ± 0 (-81.5)
7.8	0.25 ± 0.03	0.13 ± 0.02 (-48.0)	0.52 ± 0.01	0.23 ± 0.02 (-55.8)	0.85 ± 0.07	0.53 ± 0.07 (-37.6)	1.95 ± 0.12	0.89 ± 0.07 (-54.3)
8.0	0.32 ± 0.01	0.20 ± 0.01 (-37.5)	0.54 ± 0.01	0.28 ± 0.02 (-48.1)	1.14 ± 0.07	0.67 ± 0 (-41.2)	2.14 ± 0.07	1.26 ± 0.07 (-41.1)
9.0	0.56 ± 0.02	0.34 ± 0.03 (-39.3)	0.76 ± 0.03	0.33 ± 0.02 (-56.6)	1.68 ± 0.13	0.97 ± 0.12 (-42.3)	2.23 ± 0.07	1.26 ± 0.12 (-43.5)
10.0	0.22 ± 0.01	0.08 ± 0.02 (-63.6)	0.20 ± 0.02	0.06 ± 0.01 (-70.0)	0.57 ± 0.07	0.18 ± 0.07 (-68.4)	0.56 ± 0.07	0.16 ± 0.07 (-71.4)

Table 1 (continued)

A. variabilis								
5.0	0.08 ± 0.01	0.03 ± 0.01 (-62.5)	0.13 ± 0.02	0.04 ± 0.01 (-69.2)	0.26 ± 0.03	0.11 ± 0.03 (-57.7)	0.32 ± 0.07	0.11 ± 0.04 (-65.6)
6.0	0.21 ± 0.02	0.08 ± 0.01 (-61.9)	0.25 ± 0.03	0.09 ± 0.01 (-64.0)	0.53 ± 0.07	0.23 ± 0.07 (-56.6)	0.51 ± 0.04	0.21 ± 0.04 (-58.8)
7.0	0.35 ± 0.04	0.15 ± 0.01 (-57.1)	0.61 ± 0.05	0.25 ± 0.02 (-59.0)	1.23 ± 0.09	0.59 ± 0.08 (-52.0)	1.9 ± 0.05	0.84 ± 0.10 (-55.8)
7.8	0.35 ± 0.03	0.15 ± 0.03 (-57.1)	0.65 ± 0.03	0.28 ± 0.02 (-56.9)	1.22 ± 0.04	0.67 ± 0.14 (-45.1)	2.19 ± 0.07	1.13 ± 0.07 (-48.4)
8.0	0.39 ± 0.04	0.22 ± 0.02 (-43.6)	0.65 ± 0.04	0.38 ± 0.02 (-37.0)	1.65 ± 0.07	0.97 ± 0 (-41.2)	2.19 ± 0	1.29 ± 0.07 (-41.0)
9.0	0.39 ± 0.05	0.23 ± 0.03 (-41.0)	0.54 ± 0.03	0.34 ± 0.01 (-37.0)	1.62 ± 0.07	0.97 ± 0.12 (-40.1)	1.73 ± 0.04	1.01 ± 0.07 (-41.6)
10.0	0.21 ± 0.03	0.08 ± 0.02 (-61.9)	0.25 ± 0.02	0.07 ± 0.01 (-72.0)	0.53 ± 0.07	0.26 ± 0.04 (-50.9)	0.53 ± 0.07	0.14 ± 0.03 (-73.5)

Table 2. Effect of *Furadan* on the growth and chlorophyll (Chl) *a* content of *Anabaena fertillisma* and *A. variabilis* at different irradiances. (See Table 1 for details.)

Irradiance [W m^{-2}]	Growth (absorbance of the culture at 760 nm)				Chl *a* content [g m^{-3}(culture)]			
	8 d		16 d		8 d		16 d	
	Control	EC 50	Control	EC 50	Control	EC 50	Control	EC 50
A. fertilissima								
2.5	0.10 ± 0.02	0.05 ± 0.01 (-50.0)	0.18 ± 0.01	0.08 ± 0.01 (-55.6)	0.36 ± 0	0.16 ± 0.04 (-55.5)	0.69 ± 0.14	0.30 ± 0.06 (-56.5)
7.5	0.25 ± 0.03	0.13 ± 0.01 (-48.0)	0.52 ± 0.03	0.23 ± 0.02 (-55.8)	0.85 ± 0.07	0.53 ± 0.07 (-37.6)	1.95 ± 0.07	0.89 ± 0.07 (-54.3)
12.5	0.49 ± 0.01	0.27 ± 0.02 (-44.9)	0.91 ± 0.03	0.44 ± 0.02 (-51.6)	1.5 ± 0.07	0.83 ± 0.07 (-44.7)	3.57 ± 0.07	1.49 ± 0.14 (-58.3)
A. variabilis								
2.5	0.12 ± 0.01	0.05 ± 0.01 (-58.3)	0.22 ± 0.02	0.09 ± 0.01 (-59.1)	0.43 ± 0.1	0.19 ± 0 (-55.8)	0.77 ± 0.07	0.33 ± 0.07 (-57.1)
7.5	0.35 ± 0.03	0.15 ± 0.01 (-57.1)	0.65 ± 0.03	0.28 ± 0.02 (-56.9)	1.22 ± 0	0.67 ± 0.06 (-45.1)	2.19 ± 0.12	1.14 ± 0.07 (-47.9)
12.5	0.74 ± 0.03	0.36 ± 0.03 (-51.3)	1.33 ± 0.03	0.65 ± 0.02 (-51.1)	2.11 ± 0.07	1.18 ± 0.07 (-44.1)	5.15 ± 0.07	2.65 ± 0.12 (-48.5)

Table 3. Effect of population density of the initial inoculum (absorbance of culture after inoculation at 760 nm) on the toxicity of analytical grade *Furadan* on the growth and chlorophyll (Chl) *a* content of *Anabaena fertillisma* and *A. variabilis*. (See Table 1 for details.) Values in the parentheses indicate per cent decrease (-) over control; means of three independent determination ± S.D.

Population size	Growth (absorbance of the culture at 760 nm) 8 d		16 d		Chl *a* content [g m^{-3}(culture)] 8 d		16 d	
	Control	EC 50	Control	EC 50	Control	EC 50	Control	EC 50
A. fertilissima								
0.01	0.11 ± 0.02	0.04 ± 0.01 (-63.6)	0.18 ± 0.03	0.07 ± 0.01 (-61.1)	0.45 ± 0.14	0.12 ± 0 (-73.3)	0.40 ± 0.07	0.12 ± 0 (-70.0)
0.02	0.25 ± 0.01	0.13 ± 0.02 (-48.0)	0.52 ± 0.03	0.23 ± 0.02 (-55.8)	0.85 ± 0.07	0.53 ± 0.07 (-37.6)	1.95 ± 0.07	0.89 ± 0.07 (-54.3)
0.03	0.34 ± 0.02	0.19 ± 0.01 (-44.1)	0.68 ± 0.02	0.3 ± 0.03 (-55.9)	1.34 ± 0.12	0.80 ± 0.09 (-40.3)	2.44 ± 0.12	0.97 ± 0.07 (-60.2)
0.04	0.52 ± 0.01	0.30 ± 0.02 (-42.3)	0.75 ± 0.02	0.33 ± 0.02 (-56.0)	1.99 ± 0.07	1.17 ± 0.12 (-41.2)	2.48 ± 0.07	0.90 ± 0.07 (-63.7)
A. variabilis								
0.01	0.21 ± 0.03	0.08 ± 0.01 (-61.9)	0.25 ± 0.01	0.1 ± 0.02 (-60.0)	0.93 ± 0.14	0.44 ± 0.07 (-50.7)	1.42 ± 0.07	0.65 ± 0.07 (-54.2)
0.02	0.35 ± 0.02	0.15 ± 0.01 (-57.1)	0.65 ± 0.03	0.28 ± 0.01 (-56.9)	1.22 ± 0	0.67 ± 0.10 (-45.1)	2.19 ± 0	1.14 ± 0.06 (-47.9)
0.03	0.52 ± 0.03	0.26 ± 0.02 (-50.0)	0.93 ± 0.03	0.56 ± 0.01 (-39.8)	2.27 ± 0.14	1.27 ± 0.14 (-44.0)	4.67 ± 0.07	2.58 ± 0.07 (-44.7)
0.04	0.65 ± 0.01	0.37 ± 0.02 (-43.1)	1.10 ± 0.02	0.77 ± 0.02 (-30.0)	2.96 ± 0.07	1.72 ± 0.07 (-41.9)	5.52 ± 0.07	3.29 ± 0.12 (-40.4)

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