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RESEARCH ARTICLE

MODIFYING EFFECT OF INDOLE-3-ACETIC ACID ON FURADAN TOXICITY ON TWO SPECIES OF RICE FIELD CYANOBACTERIA

D. Sahu, I. Priyadarshani and B. Rath

Department of Biotechnology, North Orissa University, Baripada-757003

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ABSTRACT

It has been reported earlier that hormones are secreted by cyanobacteria under water logged rice fields, and thus when cyanobacterial populations are abundant in such environment, might be secreting such substance in considerable amounts which possibly ameliorate the toxic effect of the pesticide on these non-target organisms present in the same environment. The present study infers that the exogenous hormone IAA supported growth and chlorophyll-a content of *Nostoc sp* and *Anabaena sp* when supplemented at a concentration of 0.01mM /10ml culture to the nitrogen free BG 11 medium though their effect to stimulate growth differ among the two experimental organisms. However,exogenous hormone induced growth increase was only about 12 and 13 per cent more in the test species than the control. The concentration of IAA which supported growth increase of experimental organisms could decrease the toxic effect of mostly the lower concentration of Furadan on the growth, chlorophyll-a content and carotenoid content whereas at higher concentration no such promising result was observed in both the organisms.

INTRODUCTION

Cyanobacteria, a group of ubiquitous, photosynthetic prokaryotes which perform two key biological processes such as oxygenic photosynthesis and nitrogen fixation together in the same cells/filaments, and enrich the paddy soil particularly with nitrogen and humus contents (Watanabe and Kiyohara, 1960). The beneficial effect of nitrogen fixation by cyanobacteria for rice cultivation has been recognized for many years (Roger and Kulasooriya, 1980; Roger and Ladha, 1992). The N_2-fixing cyanobacteria form a prominent component of microbial populations in wetland soils, especially in rice paddy fields, as they significantly contribute to fertility as a natural biofertilizer (Kumar and Kumar, 1988; Sinha and Kumar, 1992). The agronomic potential of free-living and symbiotic cyanobacteria has been recognized as one of the most promising biofertilizer systems for wetland soils (Mian and Stewart, 1985). Moreover it has been documented that Cyanobacteria present in rice fields also liberate some growth promoting substances (hormones) which add to crop productivity (Venkoteraman, 1979). On the otherhand agriculture is heavily dependent on chemical pesticides (Gadkari, 1988; Greaves *et al*., 1988) and these pesticides are observed to exert detrimental effect on non target microbial processes, which play an important role in plant growth, crop productivity, and soil fertility. Though, the application of many insecticides are forbidden, the low cost, easy availability, lack of awareness and lack of regulatory implementation have contributed to the continuous use of the insecticides in tropical and subtropical regions. Thus the importance of monitoring the effect of pesticides on non target organisms has been recognized (Hill and Wright, 1978). Among those non target organisms potentially susceptible to pesticides are the cyanobacteria. These are known to contribute to the maintenance of soil fertility and are consequently considered to be an important component of soil microflora (McCann and Cullimore, 1979). It is therefore imperative that regular monitoring be undertaken to evaluate the influence of pesticides on these organisms. In the rice fields cyanobacterial species occur abundantly resulting in liberation of a considerable amount of these extracellular metabolites which might be resulting in the amelioration of toxicity of pesticides applied during the paddy cultivation cycle. In the present study the synthetic hormone IAA , mostly reported to support cell division and elongation have been tried whether it can alter the toxicity of commercial grade Furadan(carbofuran 3%G) to the non target nitrogen fixing rice field cyanobacterial isolate *Nostoc sp.* and *Anabaena sp.*, which occurs abundantly in the paddy fields and contributes to the maintenance of soil fertility.

***Corresponding author:** brath_2000@yahoo.com

MATERIALS AND METHODS

Insecticide preparation

Furadan-Carbofuran3% G (2, 3- dihydro- 2, 2- dimethyl benzofuran-7-yl- methyl carbamate) a commercial grade pesticide was prepared in stock solution and added aseptically

to the culture medium to the final concentrations indicated for each treatment.

Hormone preparation

For studying the modifying effect of pesticide toxicity due to exogenous hormones, stock solutions of Indole-3-Acetic acid was prepared in doubled distilled water, filter sterilized by passing through millipore membrane filter (0.45 μm) and then added aseptically to the culture medium containing the pesticide to obtained the desired concentration in the experimental tubes.

without various concentration of commercial grade Furadan (Carbofuran, 3%G) and 1ml of homogenized suspension of the organisms. Experimental cultures were incubated at 26± 1^0C under 3000 lux light intensity. The cyanobacterial cultures were maintained in a culture room at a temperature with a photoperiod of 16h light and 8h dark. Each treatment was of four replicates. The liquid cultures in the flask and tubes were hand shaken daily 2- 3 times to provide uniform light, aeration and nutrient to the suspension culture and to avoid sticking of cyanobacteria cell to the walls of the glass vessel which may result in uneven growth and subsequent experimental error.

Table 1. Effect of different concentration of IAA on the Growth and Chlorophyll-a content of *Nostoc sp* and *Anabaena sp*.The cultures were incubated at 25 ± 1^0C under 3000 lux light intensity and were harvested after 10 days of incubation

Hormone (IAA) Concentration (mM)	Growth (Absorbance at 760nm)		Chlorophyll-a content (μg/10ml)	
	Nostoc sp	*Anabaena sp*	*Nostoc sp*	*Anabaena sp*
Control	0.25 ± 0.03	0.30 ± 0.02	10.24 ± 1.14	14.22 ± 1.02
0.005	0.25 ± 0.02	0.31 ± 0.03	11.28 ± 1.04	16.39 ± 1.11
0.01	0.28 ± 0.01	0.34 ± 0.02	15.09 ± 0.89	18.34 ± 1.01
0.02	0.24 ± 0.02	0.28 ± 0.02	10.19 ± 1.11	13.01 ± 0.89
0.04	0.22 ± 0.01	0.24 ± 0.01	9.25 ± 0.89	11.12 ± 0.77
0.06	0.19 ± 0.02	0.23 ± 0.02	9.45 ± 0.76	10.09 ± 0.98
0.08	0.18 ± 0.02	0.23 ± 0.02	8.89 ± 0.45	10.07 ± 0.70
0.1	0.16 ± 0.02	0.23 ± 0.01	8.69 ± 0.38	6.59 ± 0.79
0.15	0.11 ± 0.03	0.19 ± 0.01	7.85 ± 0.77	5.01 ± 0.77
0.2	0.10 ± 0.02	0.14 ± 0.03	3.36 ± 0.89	3.88 ± 0.78

Initial inoculum concentration = 0.06 (Absorbance at 760nm). Initial chlorophyll-a content = 1.34 μg/10ml and 2.01μg/10ml of *Nostoc sp* and *Anabaena sp* respectively. Values represent mean of three independent determinants ± S.D.

Table.2.Modifying effect of Furadan toxicity on growth and pigment content of *Nostoc sp* and *Anabaena sp.* by supplementing Indole 3-acetic acid (IAA) hormone to the culture media

Conce. of Furadan (μg/10ml)	± IAA (0.01 mM)	Growth (Absorbance at 760nm)		Chlorophyll-a content (μg/10ml)		Carotenoid content (μg/10ml)	
		Nostoc Sp	*Anabaena sp*	*Nostoc Sp*	*Anabaena sp*	*Nostoc Sp*	*Anabaena sp*
_	(Control)	0.25±0.01	0.30±0.05	12.052±1.57	12.286±1.19	0.013±0.002	0.013±0.002
_	+	0.28±0.02 (+12%)	0.34±0.02 (+13.3%)	13.758±0.05 (+14.1%)	13.991±0.65 (+13.8%)	0.014±0.002 (+7.7%)	0.015±0.001 (+15.4%)
0.5	_	0.19±0.03 (-24%)	0.22±0.02 (-26.6%)	8.917±0.69 (-26.1%)	8.914±0.60 (-27.4%)	0.010±0.002 (-23.01%)	0.010±0.001 (-23.01%)
1	_	0.13±0.04 (-48%)	0.14±0.01 (-53.3%)	6.032±0.40 (-49.9%)	5.662±1.35 (-53.9%)	0.007±0.001 (-46.1%)	0.006±0.002 (-53.846%)
2	_	0.07±0.01 (-72%)	0.07±0.04 (-76.6%)	3.343±0.28 (-72.2%)	2.126±0.63 (-82.7%)	0.003±0.001 (-76.9%)	0.003±0.001 (-76.9%)
0.5	+	0.24±0.02 (- 4%)	0.29±0.03 (-3.3%)	10.034±1.35 5 (-16.7%)	11.733±1.26 (-4.5%)	0.012±0.002 (-7.6%)	0.012±0.001 (-7.6%)
1	+	0.20±0.02 (-20%)	0.19±0.01 (-36.6%)	8.677±1.044 (-28.03%)	10.115±0.11 (-17.6%)	0.009±0.002 (-30.7%)	0.010±0.002 (-23.01%)
2	+	0.08±0.01 (-68%)	0.09±0.05 (-70%)	6.631±0.348 (-44.9%)	3.919±0.01 (-68.1%)	0.004±0.001 (-69.2%)	0.003±0.001 (-76.9%)

Initial inoculum concentration = 0.06 (Absorbance at 760nm). Initial chlorophyll-a content = 1.34 μg/10ml and 2.01μg/10ml of *Nostoc sp* and *Anabaena sp* respectively. Values represent mean of three independent determinants ± S.D.

Test Organisms

Pure culture of two species of heterocystous rice field cyanobacteria, *Nostoc sp.* and *Anabaena sp.* were used as the experimental organism. The test organisms were isolated from local rice fields and kept in unialgal condition at department of Biotechnology, North Orissa University.

Culture media and culture conditions

Experiments were conducted in 15 × 150mm hard glass test tubes containing 10ml of nitrogen free BG_{11} medium with or

Test methods

Growth of the organism was determined by measuring the absorbance of the homogenized suspension of the cultures in a UV-VIS (Systronics model.103) spectrophotometer at 760nm with reference blank as culture medium (Adhikary, 1983).The experimental cultures were homogenized in an electric homogenizer with Teflon pestle to get an uniform culture suspension. The suspensions were centrifuged at 3,500 rpm for 15 minutes in a tabletop centrifuge. The supernatant was discarded and to the pellet 80% (v/v) acetone was added and

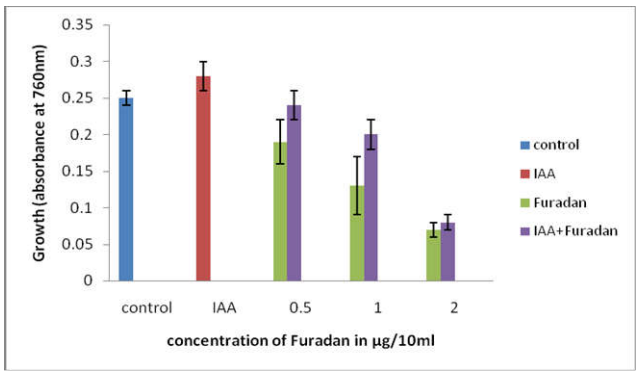

Fig.1(a): Effect on Growth of *Nostoc sp*. at different doses of Furadan supplimented with IAA in the culture media

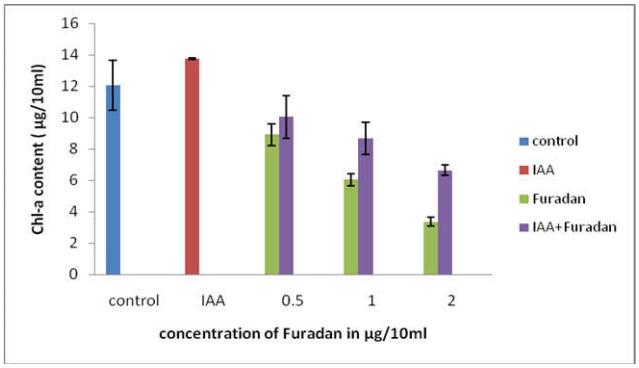

Fig.1(b):Effect on Chlorophyll-a content of *Nostoc sp.* at different doses of Furadan supplimented with IAA in the culture media

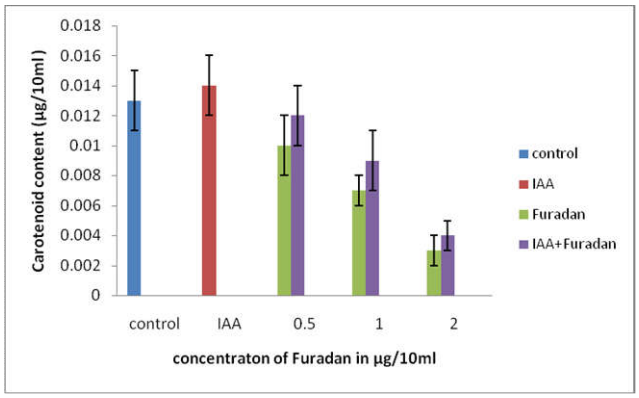

Fig.1(c):Effect on Carotenoid content of *Nostoc sp.* at different doses of Furadan supplimented with IAA in the culture media

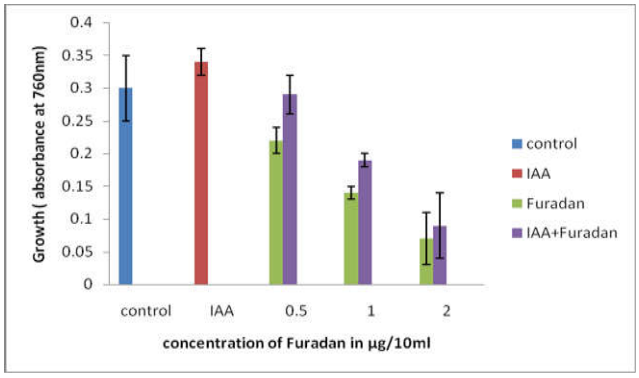

Fig.2(a): Effect on Growth of *Anabaena sp.* at different doses of Furadan supplimented with IAA in the culture media

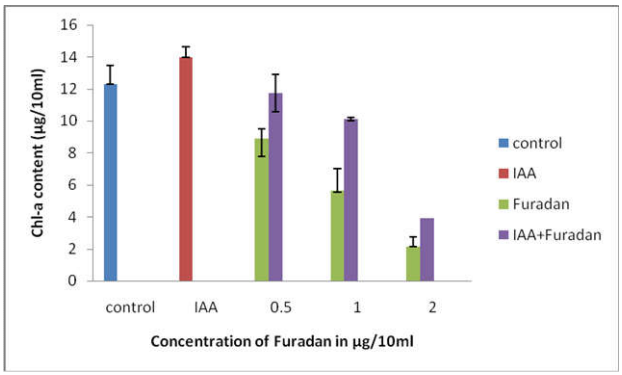

Fig.2(b): Effect on Chlorophyll-a content of *Anabaena sp.* at different doses of Furadan supplimented with IAA in the culture media

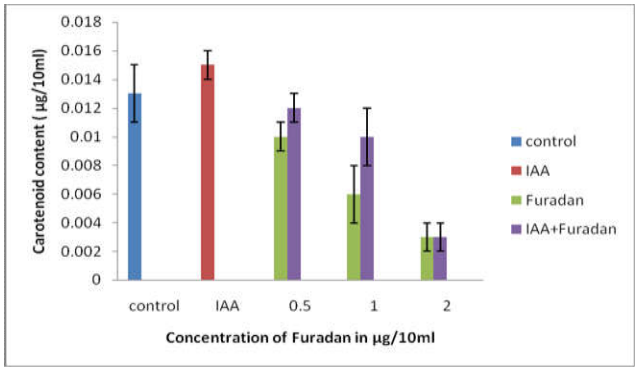

Fig.2(c): Effect on Carotenoid content of *Anabaena sp.* at different doses of Furadan supplimented with IAA in the culture media

kept for 2 hours at dark for extraction of chlorophyll. The pigment extracted was measured at 660 nm in a UV-VIS spectrophotometer and the amount of chlorophyll-a was estimated by using the extinction co-efficient given by Mackinney (1941).The carotenoid content of the same extract was measured at 470nm and the amount of carotenoid was estimated by using the extinction co-efficient given by Davis (1976).

Statistical evaluation

The experiments were set up in triplicates for each treatment and mean of such data was presented with the standard deviation.

RESULTS

Various concentration of the hormone Indole-3-acetic acid (IAA) was added to nitrogen free BG_{11} medium along with the inoculums. The results showed that IAA at 0.01mM concentration in 10ml culture supported increase in growth upto 12 and 13 per cent in *Nostoc sp* and *Anabaena sp* respectively. The chlorophyll-a content also increased to 47.4 and 28.9 per cent over control on the same concentration of IAA followed by decrease in subsequent concentrations. It was observed that the concentration of IAA, upto 0.01mM supported increase of growth as well as chlorophyll-a content of *Nostoc sp*. and *Anabaena sp*. over the control value followed by a decline with increase in the hormone concentration in the culture medium (Table.1).The concentration of IAA (0.01mM) which supported maximum

growth of the experimental organisms over their respective control value was selected for further experiments in order to find out whether the hormone can alter the toxicity of commercial grade Furadan (carbofuran,3%G) on the growth and pigment content of the cyanobacterial species in the experimental cultures. The concentration of IAA--- (0.01Mm) which supported maximum growth of the experimental organisms over the control was selected for further experiments in order to find out whether the hormone can alter the toxicity of commercial grade Furadan on the growth, chlorophyll-a and carotenoid content of the cyanobacterial species in the experimental cultures. The results showed that 0.01mM IAA which supported about 12 per cent increase in growth of *Nostoc sp.* over the control could decrease the toxicity of 0.5,1 and 2 µg /10ml dose of Furadan from 24, 48 and 72 per cent to 4, 20 and 68 per cent respectively (Fig.1a). Similarly concentration of IAA (0.01mM) which supported 13.3 per cent growth increase over control in *Anabaena sp* could decrease the toxicity from 26.6, 53.3 and 76.6 per cent to 3.3, 36.6 and 70 per cent at 0.5, 1 and 2 µg /10ml dose of Furadan respectively (Table 2, Fig. 1a and 2a). In IAA (0.01mM) supplemented culture media the chlorophyll-a content increased to 14.1% and 13.8% in *Nostoc sp* and *Anabaena sp*. respectively. IAA induced amelioration at Furadan toxicity was found to decrease the chlorophyll-a content from 26.1, 49.9 and 72.2 per cent to 16.7, 28.03 and 44.9 per cent in *Nostoc sp* (Fig.1b). Similarly in *Anabaena sp.* IAA supplemented culture media along with Furadan at 0.5, 1 and 2 µg/10ml, the chlorophyll-a content decreased from 27.4, 53.9 and 82.7 per cent to 4.5, 17.6 and 68.1 per cent (Fig.2b). Similar trend was also marked in the carotenoid content in IAA supplemented culture media on selected concentration of Furadan (Fig.1c and 2c). It was observed that in both the species, IAA could able to nullify the toxic effect of Furadan (at the selective concentration) though little variation was observed among the two species. Among the two species IAA induced amelioration of Furadan toxicity was more prominent in *Nostoc sp* as compared to *Anabaena sp*. Further it was found that IAA could able to nullify toxicity of lower doses of Furadan (0.5 and 1 µg /10ml)in the culture media more prominently whereas at the higher dose of Furadan (2 µg/10ml) the nullifying effect of IAA was not much significant.

DISCUSSION

Cyanobacteria have been said to benefit rice plants by producing growth promoting substances. Most of the documentation is based on indirect evidence from field experiments (Venkataraman,1979). Most direct evidence for hormonal effects has come primarily from treatment of rice seedlings with cyanobacterial cultures on their extracts. Presoaking of seeds in cultures of cyanobacteria attributed to the enhancement of germination and faster seedling growth due to cyanobacteria (Jacq and Roger, 1977). These reports together with field trials have established the growth promoting effects of extracellular products on rice plant and such substances might be the growth promoting phytohormones. In the present study it was found that IAA though supported increase of growth and pigment content up to 0.01mM concentration, but the mode of action differed among the two test species. Cyanobacteria were used worldwide for a long time in Agricultural practice in paddy fields (Watanbe, 1962 and El Nawawy 1972). The increase in crop yields as a result of algal inoculation can not only be attributed to the nitrogen-fixing property of cyanobacteria, but may be largely due to the growth regulating substances endogenously produced by these algae. This suggestion is greatly supported by the fact that non-nitrogen fixing species as *Phormidium sp.* and *Oscillatoria sp*. stimulated the growth of rice (Gupta and Shukla 1967, Gupta and Gupta 1970). Moreover, it was found that cyanobacteria in addition to their stimulatory effect on plant growth, it enhanced the production of secondary metabolites and these mechanisms may be controlled with or mediated by hormones (Saker *et al.,* 2000 and Shanab 2001). In the results of the present study it was found that the concentration of the hormone (IAA) which supported growth increase of the test organisms could decrease the toxic effect of mostly the lower concentration of the pesticide on their growth and pigment content which was in accordance with the previously published data that pointed to the presence of auxin-like substances in algae. This study infers that toxicity of Furadan in rice plants field cyanobacteria can be ameliorated by exogenous hormones. Further study on biochemical, physiological and molecular aspect at the cellular level will reveal the cause, effect and remedy on pesticide toxicity amelioration or its elevation on cyanobacteria.

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