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Effect of Herbicide Monuron on Nitrogen Fixing Blue Green Algae (BGA) *Anabaena doliolum and Nostoc Linckia*

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ABSTRACT

The herbicide monuron [3-(4 Chlorophenyl),1,1-dimethyl urea] has been found to be inhibitory to cyanobacterial growth and Heterocyst differentiation in the Nitrogen fixing blue green algae *Nostoc linckia* and *Anabaena doliolum.* In the present investigation, in vitro tolerance of different strains of blue green algae to herbicide monuron was studied.

Key words: Monuron, *Nostoc linckia* and *Anabaena doliolum*

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INTRODUCTION

Agro-chemicals such as herbicides, fungicides and insecticides are either stimulatory or inhibitory or neutral to cynobacterial growth and nitrogen fixation. Pesticides which usually kill their target organisms by being either an inhibitor of photosynthesis or respiration or growth.(Dodge 1975).

The cynobacteria possesses photosynthetic machinery identical to the chloroplasts of higher plants (Fogg *et al.,* 1973, Stewart 1973). Herbicides are, therefore, expected to interfere with the photosynthetic machinery of the naturally occurring cynobacteria. Herbicides which inhibit photosynthesis thus indirectly effect the nitrogen fixing machinery of Cynobacteria.

MATERIAL AND METHOD :

Organisms

Blue Green algae Noxtoc linckia and Anabaena doliolum have been selected for evaluating the toxicity of the herbicide monuron. These algae were selected because of their wide distribution, hetrocystus nature and their ability to fix atmospheric nitrogen, collected from rice fields and identified after Desikachary, 1959.

Medium and Culture

The organisms were grown routinely in Chu No.10 medium as modified by Gerloff et al (1950) with or without combined inorganic nitrogen source in form of KNO3. KNO2 and NH4Cl. Growth was measured in terms of optical density at 663nn. The cultures were maintained in a culture cabinet at 30 ± 2°C under 2500 LUX intensity provided by fluroscent tubes. The pH of all the test media was adjusted to 7.8. Heterocyst frequency was measured in terms of number of heterocysts per 100 vegetative cells. Each individual reading was based on a random sample of twelve different filaments.

Methods

Experiments were conducted on solid agar medium as well as in liquid medium. Experiments were conducted by inoculating actively growing organisms into 25 ml of culture medium in 100 ml conical flasks.

The biological effects of the pesticide monuron was seen on per cent survivality, the growth and heterocyst forming capacity of Noxtoc linckia and Anabaena doliolum at various concentration of the herbicide monuron was prepared in sterile combined nitrogen free medium.

Growth was recorded at the interval of 4 days of incubation whereas heterocyst frequency was recorded at alternate day from parallel set of experiments. A log phase of 5 mM KNO_3 grown, non heterocystus culture of Noxtoc linckia and Anabaena doliolum was harvested, washed repeatedly and were homogenized with glass – distilled water to remove traces of NO_3.

For survival percentage of the two strains, equal amount of treated culture was spread over agar plates containing culture media without nitrogen sources (N_2, KNO_3, KNO_2 or NH_4Cl). All samples were inoculated in growth chamber. After 15 days of incubation the colony forming units in control as well as Petri plates containing both herbicides treated cultures were counted & percent survival was calculated considering 100% colony forming units developed in control plates.

For the study of Relative tolerance of the algal culture of *Nostoc linckia & Anabaena doliolum* in 10 ml liquid N_2-free medium. Equal amount of culture was inoculated in 10 ml liquid medium containing herbicides at various concentrations.

RESULT AND DISCUSSION

On the basis of the experiments conducted on survivality and relative tolerance of both algae 3 doses of monuron (10, 20, 25, 50, 75, and 100 ppM) were selected for further study. The effect of herbicide monuron on the growth of the two algae summarized in Table 1 and Table 2.

It is evident from Table 1 and 2 that low doses of the herbicide monuron has little or no impact on the survivality of nitrogen fixing blue green algae *Anabenna doliolum* and *Nostoc linckia* whereas the stimulatory decreases with increase in the concentration.

For the study of toxicity of herbicide (Mode of Action and biological effects) experiments were restricted to three different concentration of herbicide monuron such as 10, 50 and 100 for 30 MTS. The effect of herbicide monuron was also studied on the frequency of heterocyst Nostoc linckia and Anabenna doliolum form 6.5 ± 0.115 and 5.4 ± 0.115 heterosysts in combined inorganic nitrogen free medium (N_2 Medium). The heterocyst frequency of two cynobacterial strain is adversely affected by treating with monuron. The effect of monuron on heterocyst frequency is reversed by glucose as organic carbon source as shown in Table 3.

Both the algae tolerated the lower dose of herbicide but the cultures failed to grow at higher concentration. The toxicity increases with increase in the concentration of the herbicide monuron and also it is more in ammonical nitrogen (NH^{4+}) medium followed by nitrite, nitrate and elemental nitrogen medium.

These experiments were conducted in the medium with or without supplementing them with exogenous source of organic carbon (500 ppm glucose) as shown in Table 4 and Table 5. Microscopic examination of the treated cultures growing in different culture medium revealed that the cells underwent lysis after growing for sometime.

The response of selected BGA Nostoc linckia and Anabenna doliolum towards the herbicide monuron, it is found that Nostoc linckia appears to tolerate monuron more than Anabenna doliolum. Similar pattern of the toxicity has been observed when the growth of cultures of Nostoc linckia and Anabenna doliolum were studies in different media containing various sources of inorganic nitrogen (NH_4Cl, KNO_2, KNO_3 and elemental Nitrogen). The effect of monuron on the growth was found more in NH^{4+} medium since the herbicide monuron is more toxic in ammonical nitrogen (NH^{4+}) medium at higher doses.

The effect of monuron appears to be similar to that of DCMU [3-(3,4-dichlorophenyl)-1,1 dimethyl urea] a well known photosynthetic inhibitor (mainly by preventing chloroplast electron flow photosystem II in higher plant systems). The inhibitory effect of

monuron on heterocyst differentiation in both algae has also been reversed by glucose supplementation which is similar to the reversal of heterocyst differentiation effect by DCMU. So far as the inhibitory effect of monuron is concerned both the inhibitory effects (growth and heterocyst formation) are reversed by supplementing media with 500 ppm glucose.

It is strongly believed that monuron inhibits the photo system II generated reducing power and thereby affects the N_2 fixing mechanism either indirectly by affecting heterocyst formation or directly by inhibiting nitrogenase activity due to a poor supply of $NADPH_2$ and hence the marked growth inhibition.

It is not advisable to use herbicide monuron in combination with N2 fertilizers like ammonia and urea. Such laboratory results would be helpful in monitoring the use of herbicide as well as the cynobacterial strains as bio fertilizers in paddy fields.

Table -1. Effect of Monuron on Survival of *Anabaena doliolum* and *Nostoc linckia* No. of colony forming units [CFU] in control – 224 ± 3.055

Pesticide	Concentration	% Survival	
		Nostoc linckia	*Anabaena doliolum*
Monuron	10	95.60 ± 0.346	97.35 ± 0.375
	20	78.50 ± 0.317	85.50 ± 1.675
	25	68.75 ± 0.433	73.65 ± 0.404
	50	48.25 ± 0.288	58.50 ± 0.317
	75	12.50 ± 0.404	14.50 ± 0.375
	100	00	04. 50 ± 0.348

Table-2. Relative Tolerance of the algal culture in combined inorganic nitrogen free medium

Pesticide	Dose	Growth (OD on 24th Day)	
		Nostoc linckia	*Anabaena doliolum*
Monuron	10	0.48 ± 0.295	0.52 ± 0.312
	20	0.45 ± 0.274	0.48 ± 0.323
	25	0.35 ± 0.274	0.39 ± 0.293
	50	0.24 ± 0.306	0.26 ± 0.301
	75	0.12 ± 0.342	0.18 ± 0.323
	100	00	00

Table-3. Effect of Monuron Heterocyst Frequency of *Nostoc linckia* & *Anabenna doliolum*

Pesticide	Dose	Heterocyst Frequency			
		Nostoc linckia		*Anabaena doliolum*	
Monuron	10	2.8 ± 0.118	5.2 ± 0.233	1.9 ± 0.481	4.8 ± 0.175
	50	0.6 ± 0.120	4.5 ± 0.118	0.8 ± 0.175	4.5 ± 0.156
	100	00	4.5 ± 0.118	00	4.5 ± 0.172
No Pesticide		5.5 ± 0.115	5.6 ± 0.120	5.0 ± 0.115	5.5 ± 0.118

Table- 4. Effect of Monuron in different media un supplemented with 500 ppm Glucose

On *Nostoc linckia*					
Dose	**Days**	**N_2**	**KNO_3**	**KNO_2**	**NH_4Cl**
10	0	0.19	0.19	0.19	0.19
	4	0.21±0.014	0.21±0.014	0.20±0.009	0.19±0.006
	8	0.23±0.011	0.22±0.009	0.24±0.009	0.22±0.009
	12	0.36±0.011	0.34±0.010	0.33±0.008	0.28±0.012
	16	0.38±0.012	0.34±0.010	0.35±0.009	0.31±0.008
	20	0.38±0.013	0.36±0.011	0.36±0.010	0.34±0.012
	24	0.39±0.009	0.36±0.012	0.36±0.010	0.34±0.013
50	4	0.19±0.009	0.19±0.009	0.20±0.010	0.18±0.008
	8	0.22±0.012	0.23±0.009	0.22±0.012	0.20±0.010
	12	0.28±0.014	0.27±0.006	0.24±0.012	0.22±0.012
	16	0.29±0.009	0.28±0.011	0.25±0.012	0.24±0.009
	20	0.30±0.009	0.30±0.010	0.28±0.014	0.25±0.011
	24	0.33±0.008	0.30±0.010	0.28±0.013	0.25±0.004
100	4	0.19±0.012	0.19±0.012	0.16±0.008	0.13±0.009
	8	0.18±0.009	0.19±0.011	0.16±0.008	0.13±0.009
	12	0.18±0.009	0.16±0.007	0.12±0.007	0.06±0.008
	16	0.12±0.008	0.09±0.011	0.08±0.010	00
	20	0.09±0.011	0.03±0.010	0.05±0.006	00
	24	0.06±0.008	00	00	00
On *Anabaena doliolum*					
Dose	**Days**	**N_2**	**KNO_3**	**KNO_2**	**NH_4Cl**
10	0	0.2	0.2	0.2	0.2
	4	0.22±0.012	0.22±0.009	0.20±0.008	0.20±0.008
	8	0.25±0.004	0.28±0.014	0.26±0.009	0.24±0.012
	12	0.30±0.010	0.32±0.009	0.29±0.010	0.26±0.013
	16	0.34±0.012	0.34±0.012	0.32±0.013	0.30±0.010
	20	0.37±0.008	0.38±0.006	0.34±0.012	0.30±0.010
	24	0.37±0.008	0.38±0.006	0.34±0.012	0.30±0.011
50	4	0.20±0.009	0.20±0.009	0.20±0.010	0.18±0.010
	8	0.20±0.009	0.19±0.006	0.18±0.008	0.15±0.008
	12	0.18±0.012	0.15±0.008	0.13±0.007	0.12±0.013
	16	0.18±0.009	0.14±0.011	0.13±0.009	0.12±0.008
	20	0.16±0.008	0.12±0.007	0.09±0.010	0.06±0.016
	24	0.09±0.010	0.12±0.011	00	00

On *Anabaena doliolum*					
Dose	**Days**	**N_2**	**KNO_3**	**KNO_2**	**NH_4Cl**
100	4	0.20±0.013	0.16±0.008	0.16±.008	0.12±0.007
	8	0.16±0.006	0.14±0.010	0.13±0.009	0.09±0.011
	12	0.12±0.007	0.10±0.013	0.06±0.008	0.05±0.006
	16	0.12±0.013	0.09±0.011	0.04±0.008	0.02±0.006
	20	0.08±0.012	0.06±0.008	0.04±0.008	00
	24	0.06±0.008	0.06±0.007	00	00

Table-5. Effect of Monuron in different media supplemented with 500 ppm Glucose

On *Nostoc linckia*					
Dose	**Days**	**N_2**	**KNO_3**	**KNO_2**	**NH_4Cl**
10	0	0.18	0.18	0.18	0.18
	4	0.19±0.010	0.20±0.009	0.22±0.012	0.19±0.010
	8	0.24±0.012	0.26±0.009	0.29±0.009	0.29±0.009
	12	0.34±0.010	0.32±0.009	0.33±0.008	0.36±0.010
	16	0.34±0.010	0.36±0.009	0.39±0.013	0.44±0.009
	20	0.36±0.011	0.41±0.012	0.41±0.012	0.52±0.015
	24	0.40±0.010	0.44±0.009	0.500.013±	0.54±0.011
50	4	0.19±0.009	0.20±0.009	0.22±0.010	0.21±0.010
	8	0.24±0.012	0.25±0.009	0.27±0.008	0.27±0.008
	12	0.31±0.013	0.31±0.012	0.33±0.008	0.34±0.008
	16	0.32±0.009	0.36±0.009	0.39±0.009	0.42±0.007
	20	0.35±0.011	0.40±0.010	0.41±0.012	0.49±0.009
	24	0.39±0.009	0.42±0.007	0.49±0.009	0.51±0.010
100	4	0.19±0.009	0.20±0.009	0.20±0.011	0.20±0.007
	8	0.21±0.010	0.23±0.011	0.28±0.011	0.26±0.011
	12	0.29±0.009	0.29±0.009	0.31±0.012	0.36±0.009
	16	0.32±0.005	0.34±0.010	0.37±0.008	0.42±0.009
	20	0.32±0.009	0.39±0.009	0.42±0.007	0.48±0.006
	24	0.36±0.011	0.41±0.012	0.47±0.008	0.48±0.006

On *Anabaena doliolum*					
Dose	**Days**	**N_2**	**KNO_3**	**KNO_2**	**NH_4Cl**
10	0	0.19±	0.19±	0.19±	0.19±
	4	0.21±0.014	0.21±0.014	0.22±0.012	0.24±0.014
	8	0.24±0.014	0.27±0.012	0.29±0.009	0.28±0.014
	12	0.30±0.010	0.29±0.009	0.31±0.008	0.35±0.009

On *Anabaena doliolum*					
Dose	**Days**	**N_2**	**KNO_3**	**KNO_2**	**NH_4Cl**
10	16	0.32±0.010	0.35±0.009	0.35±0.009	0.39±0.010
	20	0.36±0.009	0.39±0.014	0.43±0.012	0.46±0.007
	24	0.36±0.010	0.41±0.012	0.45±0.009	0.56±0.011
50	4	0.20±0.009	0.21±0.014	0.22±0.008	0.22±0.008
	8	0.21±0.010	0.25±0.009	0.25±0.009	0.26±0.009
	12	0.29±0.010	0.27±0.011	0.29±0.010	0.33±0.009
	16	0.30±0.010	0.32±0.009	0.33±0.009	0.37±0.010
	20	0.34±0.012	0.37±0.008	0.41±0.012	0.46±0.007
	24	0.34±0.012	0.39±0.010	0.44±0.009	0.54±0.011
100	4	0.20±0.006	0.19±0.009	0.20±0.008	0.20±0.008
	8	0.21±0.010	0.23±0.011	0.25±0.009	0.25±0.009
	12	0.27±0.010	0.28±0.012	0.29±0.010	0.31±0.008
	16	0.29±0.010	0.32±0.009	0.35±0.009	0.35±0.010
	20	0.32±0.009	0.35±0.009	0.39±0.011	0.42±0.009
	24	0.32±0.013	0.37±0.010	0.42±0.009	0.48±0.006

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