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RESEARCH ARTICLE

TOXIC EFFECT OF AN HERBICIDE ON GROWTH AND HETEROCYST FORMATION OF TWO N_2-FIXING CYANOBACTERIA

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ABSTRACT

The physiological effect of a widely used herbicide Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride) was studied at different concentrations on two filamentous N_2-fixing Cyanobacteria *Anabaena oryzae* and *Nostoc ellipsosporum* in laboratory conditions for 10 days. The results demonstrated that the increasing concentration of the applied pesticide proved to be toxic for the organism in terms of growth kinetics and heterocyst formation.

INTRODUCTION

Use of agrochemicals (i.e., pesticides/insecticides/herbicides) is a widespread practice in modern agronomical processes, but at the same time massive use of such agrochemicals has been evaluated to be a potential danger to naturally occurring biofertilizer, i.e., N_2-fixing Cyanobacteria. (Hawxby *et al*., 1977; Ma, 2005; Vaishampayan *et al*., 2001; Vaishampayan, 1984; Venkataraman and Rajyalaxmi, 1972). As a toxic or mutagenic chemical, a pesticide affects the Cyanobacteria by being either an inhibitor of photosynthesis, biological-oxidation or growth. (Dodge, 1975). The toxicity of various pesticides on few N_2-fixing Cyanobacteria. (Gangwane 1980; Mishra *et al.,* 1989; Suseela, 2001;Vaishampayan, 1981, 83; Venkataraman and Rajyalaxmi, 1971) has been reported in India. In the present investigation, an attempt has been made to study the effect of an herbicide Paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride), commonly used by the farmers, on the growth kinetics and heterocyst formation of two filamentous, N_2-fixing Cyanobacteria *Anabaena oryzae* and *Nostoc ellipsosporum.*

MATERIALS AND METHODS

The filamentous, heterocystous and N_2-fixing Cyanobacteria *Anabaena oryzae* and *Nostoc ellipsosporum,* isolated from local paddy fields, were cultured in modified Chu-10 medium. (Gerloff *et al*., 1950). Combined nitrogen medium were supplemented with 5mM KNO_3^-, 5mM NO_2^- & 1mM NH_4^+ inorganic respectively. The pH of the medium was adjusted to 7.5 after sterilization, and for the preparation of solid medium the liquid growth medium was gelled with about 1% (w/v) agar-agar. All the experimental examples were inoculated in a growth cabinet at a continuous light intensity of 2800±200 Lux and a temperature of 28 ± 2^0C in aseptic condition, after Vaishampayan, 1981. Physiology effects of the graded concentrations (15, 20 & 25 µg/ml) of Paraquat were examined on these organisms in N_2, NO_3^-, NO_2^- & NH_4^+ media, un-supplemented or supplemented with 3mM glucose after Prasad *et al.,* 1986 in both the liquid & solid media. Growth was measured every alternate day till 10th day by optical density determination at 663nm. Heterocyst frequency of the N_2-fixing samples was assessed daily microscopically as the number of heterocysts per hundred vegetative cells, after Vaishampayan 1982a. Final assessment was done on ten day's old culture. All the analytical chemicals and medium constituents were of Qualigens & Loba grade and the glassware's used were of Borosil make. The results were statistically analysed for assessing the biological significance and reproducibility of findings.

RESULTS AND DISCUSSION

All the employed concentrations of Paraquat reduced the growth and heterocyst formation, which was completely inhibited at its 25 µg/ml concentration in N_2, NO_3^-, NO_2^- & NH_4^+ media (Table 1 & 2) on both the isolates. The pesticide proved to be toxic on agar medium as well. However, effect of employed pesticide on growth indicated that the growth inhibition was resisted to some extent on supplementation of exogenous carbon (3mM glucose) (Table 1). The effect on

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Table 1: Growth of 10 Days Old Culture of *Anabaena oryzae & Nostoc ellipsosporum* in N_2, NO_3^-, NO_2^- & NH_4^+ Media with or without different concentration of Paraquat ±3 mM Glucose.

Organism	Paraquat Conc. in µg/ml	Growth (O.D. at 663nm)							
		N_2		NO_3^-		NO_2^-		NH_4^+	
		Gl-	Gl+	Gl-	Gl+	Gl-	Gl+	Gl-	Gl+
Anabaena oryzae	0	0.44±0.02	0.48±0.04	0.49±0.03	0.52±0.02	0.54±0.05	0.58±0.045	0.60±0.01	0.62±0.011
	15	0.42±0.02	0.44±0.05	0.43±0.01	0.45±0.05	0.45±0.06	0.49±0.04	0.46±0.01	0.48±0.012
	20	0.23±0.03	0.25±0.02	0.25±0.05	0.28±0.01	0.29±0.00	0.32±0.01	0.29±0.06	0.33±0.02
	25	0.00	0.00	0.00	0.021	0.00	0.051	0.00	0.062
Nostoc ellipsosporum	0	0.46±0.02	0.49±0.04	0.51±0.03	0.54±0.02	0.56±0.06	0.59±0.05	0.62±0.02	0.64±0.04
	15	0.42±0.02	0.45±0.02	0.44±0.04	0.46±0.03	0.48±0.02	0.54±0.03	0.56±0.00	0.59±0.01
	20	0.24±0.06	0.25±0.03	0.26±0.02	0.28±0.01	0.29±0.02	0.32±0.03	0.31±0.01	0.36±0.04
	25	0.00	0.00	0.00	0.022	0.00	0.052	0.00	0.062

Table 2: Heterocyst frequency of *Anabaena oryzae & Nostoc ellipsosporum* in N_2, NO_3^-, NO_2^- & NH_4^+ Media with or without various concentration of Paraquat ± 3 mM Glucose

Organism	Paraquat Conc. in µg/ml	Heterocyt Frequency							
		N_2		NO_3^-		NO_2^-		NH_4^+	
		Gl-	Gl+	Gl-	Gl+	Gl-	Gl+	Gl-	Gl+
Anabaena oryzae	0	5.62±0.62	6.74±0.56	0.00	0.00	0.00	0.00	0.00	0.00
	15	4.17±0.34	7.52±0.42	0.00	0.00	0.00	0.00	0.00	0.00
	20	3.85±0.24	6.79±0.64	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	2.18	0.00	0.00	0.00	0.00	0.00	0.00
Nostoc ellipsosporum	0	5.80±0.21	6.74±0.31	0.00	0.00	0.00	0.00	0.00	0.00
	15	5.21±0.61	7.29±0.25	0.00	0.00	0.00	0.00	0.00	0.00
	20	4.55±0.01	6.66±0.51	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

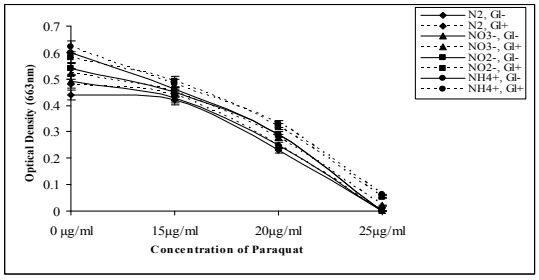

Fig 1: Growth pattern of 10 days old culture of *Anabaena oryzae* in different concentrations of herbicide Paraquat in ♦ N_2; ▲ 5mM NO_3^-; ■ 5mM NO_2^- & ● 1mM NH_4^+ medium, supplemented (---------) or unsupplemented (———) with 3mM glucose

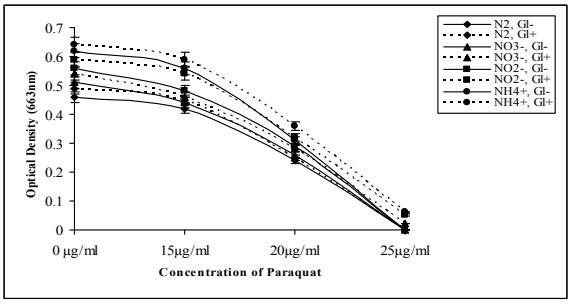

Fig 2: Growth pattern of 10 days old culture of *Nostoc ellipsosporum* in different concentrations of herbicide Paraquat in ♦ N_2; ▲ 5mM NO_3^-; ■ 5mM NO_2^- & ● 1mM NH_4^+ medium, supplemented (---------) or unsupplemented (———) with 3mM glucose

both the organism was observed to be almost same. (Figure 1 & 2). The resistance against growth inhibition on supplementation of exogenous glucose was similar to that of DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The effect of pesticide on heterocyst formation proved to be 100% lethal at all the employed concentrations in NO_3^-, NO_2^- & NH_4^+ media, however, survival in N_2 supplemented medium was observed to be decreasing with the increasing concentration of the pesticide (Table 2). But, on addition of exogenous carbon (3mM glucose) the reversion was observed in heterocyst formation. Reversion of the inhibitory action of heterocyst formation on addition of exogenous glucose suggested that 15 to 20 µg/ml concentration of Paraquat inhibits photosynthetically assimilation of CO_2. The results indicated the ensuring lethality in growth of organisms on exposure to higher concentrations of the treatment and tallies with the earlier findings. (Biondi *et al*., 2004; Greave, 1982; Ma *et al*., 2002; Ma, 2005;Mishra *et al*., 1989; Vaishampayan and Prasad, 1981, 83). The toxic effects of paraquat on both the test isolates were more or less simmilar to earlier report on *Nostoc muscorum* (Singh and Kshatriya 2002; Singh and Vaishampayan 1978). DCMU inhibits photosynthesis mainly by preventing chloroplast electron flow through PS II. (Rochaix and Erickson, 1988). *Nostoc muscorum* can photoassimilate organic substrates like glucose, amino acids as easily metabolizable carbon sources (Vaishampayan, 1981, 1982, 1984). It has, nevertheless, been, shown that DCMU-inhibition of both growth and heterocyst differentiation in photoheterotrophs, including *Nostoc* spp., occurs only when carbon is obtained by photosynthetic CO_2-fixation. Glucose in light, can serve as an alternative source of carbon for growth and differentiation. (Singh and Vaishampayan,1978). The finding that the same organic substrate (glucose) effectively reverses the inhibitory effects of Paraquat on growth and heterocyst differentiation in *Nostoc ellipsosporum* & *Anbaena oryzae* suggests that Paraquat is similar to DCMU in its mode of action. (Abou-Waly *et al*., 1991; Singh and Kshatriya, 2002; Singh and Vaishampayan, 1978). Paraquat is a specific inhibitor of PS II function and its application to oxygenic photosynthetic organisms is known to result in abolition of photochemically generated reducing power ($NADPH_2$) without causing any adverse effect on the generation of ATP through cyclic photophophorylation (De Lorenzo *et al*., 1999; Kotrikla *et al*., 1997). $NADPH_2$ is the major source of reductant for the nitrogenase reaction in the heterocystous

filamentous cyanobacteria and it has been found that paraquat inhibits nitrogenase activity by inhibiting the generation of $NADPH_2$. (Kotrikla *et al*., 1997). Moreover, photosynthetic assimilation of inorganic carbon (CO_2) is a reductive process occurring at the expense of photosynthetically generated reductant during oxygenic photosynthesis. Paraquat treatment, as expected blocks the CO_2 assimilation in such systems. Accordingly, while obligate photoautotrophs fail to recover from herbicide inhibition of growth in the presence of an organic carbon supplement like glucose, the photoheterotrophs show rapid recovery under similar conditions (Abou-Waly *et al*., 1991; Singh and Vaishampayan, 1978). Further, the reversal of heterocyst differentiation by exogenous supplementation of glucose suggests that, heterocyst differentiation requires a photosyntheticaly fixed carbon supply and that glucose (by feeding electrons during the light reaction) can effectively substitute for photosyntheticaly generated organic carbon in growth and differentiation (Singh and Kshatriya, 2002; Singh and Vaishampayan, 1978) favoring the fact that the same organic substrate (glucose) reversed the inhibitory effects of paraquat on growth. Similar results have also been reported in case of *Nostoc muscorum* in the presence of herbicides, fungicides and insecticides (Prasad *et al*., 1986; Vaishampayan and Prasad, 1981; Vaishampayan, 1982) and in *Anabaena doliolum* in presence of glyphosate (Shikha *et al*., 2004). Heterocyst differentiation, thus can to some extent, serve as a good index for assessing, whether a herbicide inhibits the cyanobacterial growth by inhibiting photosynthetic assimilation of inorganic carbon or by inhibiting protein synthesis (Singh and Vaishampayan, 1978).

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