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DIFFERENTIAL RESPONSE OF CYANOBACTERIA TO AN ORGANO-PHOSPHATE PESTICIDE, ROGOR (DIMETHOATE 30 EC)

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ABSTRACT

The differential response of cyanobacteria, collected from the rice field soils of Sambalpur district of Orissa to an organophosphate pesticide, Rogor (dimethoate 30 EC) was examined. Four different cyanobacterial strains *viz. Anabaena variabilis*, *Nostoc muscorum*, *Calothrix parietina* and *Westiellopsis prolifica* were selected for the study. They showed their relative tolerance to the test pesticide, Rogor in laboratory culture, so far their growth, chlorophyll-*a* content, carotenoid, protein and extra-cellular amino acids are concerned. Of the test organisms, *Calothrix parietina* was comparatively more tolerant, while *Anabaena variabilis* was more sensitive to Rogor. However, *Nostoc muscorum* that does not possess thick slime layer tolerated relatively higher pesticide doses. A pesticide concentration dependent reduction in chlorophyll-*a* content, carotenoid, protein and extra-cellular amino acids of the organisms was observed.

INTRODUCTION

Cyanobacteria form a living constituent of soil biotypes. They grow luxuriantly in submerged conditions of rice fields, which provide congenial habitat for their growth. They form most efficient system providing biologically fixed nitrogen to the crop. However, a variety of weedicides, fungicides and insecticides used for plant protection are known to affect the cyanobacterial population (Anand 1980) in the rice fields, thus influencing the total productivity (Singh 1973, Lal & Saxena 1980, Padhy 1985, Stratton 1987, Kolte & Goyal 1990, Subramanian et al. 1994, Venkataraman et al. 1994). Pesticides have been shown to have differential effect on various metabolic processes (Das & Singh 1977, Zargar & Dar 1990, Anand & Subramanian 1997).

In the present paper, the effect of Rogor (dimethoate 30 EC) on the growth and various macro-molecules of four indigenous cyanobacterial flora of Sambalpur district of Orissa have been studied.

MATERIALS AND METHODS

The filamentous cyanobacteria *viz. Anabaena variabilis*, *Nostoc muscorum*, *Calothrix parietina* and *Westiellopsis prolifica* were collected from the rice fields of Sambalpur district of Orissa (Das 2002). Unialgal cultures of these organisms were routinely grown in nitrogen free BG-11 medium (Rippka et al. 1979), maintained at 26±1°C under continuous illumination of 7.5 W/m^2. Commercial formulations of Rogor (dimethoate 30 EC), manufactured by Rallis India Ltd., Bombay was obtained from the local market. Stock solutions of this pesticide was prepared with double distilled water, sterilized at 15 lb pressure and added aseptically to the culture media to obtain the desired concentrations (0.01, 0.02, 0.05, 0.1, 0.2, 0.5. 1.0, 2.5, 10 and 20 ppm). Experiments were conducted in 15 × 150 mm Borosil test tubes. Pesticide was added aseptically after autoclaving and it was inoculated with 1 mL of homogenized 10-day old actively growing culture of each alga and incubated. Three replicates were used for each set. At the end of 15 days, cultures were harvested and growth was recorded as absorbance of the homogenized culture suspension at 760 nm by a spectrophotometer (Adhikary 1983). EC_{25}, EC_{50}, EC_{75} and sub-lethal doses (25% inhibition, 50% inhibition, 75% inhibition and 90-95% inhibition over control respectively) of the pesticide for each organism were calculated.

Chlorophyll-*a* and carotenoids were extracted in 80% acetone and absorbance was measured at 660 nm (Mackinney 1941) and 470 nm (Davis 1976) respectively. Cell protein content of cyanobacterial materials was determined by the methods developed by Lowry et al. (1951). The extra-cellular amino acids content was determined by Moore and Stein's ninhydrin method, modified by Spies (1957).

RESULTS AND DISCUSSION

Growth and Chlorophyll-*a* content of four different species of cyanobacteria in response to pesticide Rogor are given in Tables 1 and 2. Among the four species, *Nostoc muscorum* and *Calothrix parietina* tolerated up to 10 ppm, *Anabaena variabilis* up to 2 ppm and *Westiellopsis prolifica* up to 5 ppm; the lethal concentration of the respective cyanobacteria being 20, 20, 5 and 10 ppm in sequence. Lower concentration of the pesticide (0.01 ppm) had no effect on the growth of *Calothrix parietina*. Chlorophyll-*a* content of all the test organisms in presence and absence of Rogor followed a similar trend like that of growth (Table 2). The growth and chlorophyll-*a* content are maximum in *Anabaena variabilis* and minimum in *Calothrix parietina* in the absence and presence of pesticide. Hence, these two cyanobacteria were selected for further study.

Table 1: Effect of different concentrations of Rogor (dimethoate 30 EC) on the growth (absorbance of the culture at 760 nm) of four different nitrogen-fixing cyanobacteria.

Organism	Concentrations of Rogor (ppm) in BG-11_0 medium											
	0	0.01	0.02	0.05	0.1	0.2	0.5	1	2	5	10	20
Anabaena variabilis	0.66 ± 0.02	0.57 ± 0.03	0.52 ± 0.04	0.46 ± 0.04	0.4 ± 0.02	0.33 ± 0.02	0.16 ± 0.03	0.08 ± 0.01	0.03 ± 0	0	0	0
Nostoc muscorum	0.5 ± 0.02	0.48 ± 0.03	0.41 ± 0.02	0.36 ± 0.02	0.31 ± 0.03	0.28 ± 0.03	0.23 ± 0.03	0.16 ± 0.02	0.12 ± 0.02	0.06 ± 0.01	0.02 ± 0	0
Calothrix parietina	0.32 ± 0.03	0.32 ± 0.03	0.31 ± 0.03	0.28 ± 0.02	0.25 ± 0.01	0.22 ± 0.01	0.16 ± 0.02	0.12 ± 0.01	0.08 ± 0.01	0.04 ± 0	0.02 ± 0	0
Westiellopsis prolifica	0.58 ± 0.02	0.56 ± 0.02	0.54 ± 0.04	0.47 ± 0.02	0.44 ± 0.01	0.38 ± 0.02	0.3 ± 0.01	0.24 ± 0.02	0.19 ± 0.01	0.06 ± 0	0	0

Table 2: Effect of different concentrations of Rogor (dimethoate 30 EC) on the chlorophyll-*a* content (µg/10mL culture) of four different nitrogen-fixing cyanobacteria.

Organism	Concentrations of Rogor (ppm) in BG-11_0 medium											
	0	0.01	0.02	0.05	0.1	0.2	0.5	1	2	5	10	20
Anabaena variabilis	19.5 ± 1.23	16.85 ± 2.44	13.31 ± 1.85	12.2 ± 0.89	10.63 ± 0.76	7.09 ± 0.53	5.32 ± 0.11	2.66 ± 0	1.77 ± 0	0		
Nostoc muscorum	30.48 ± 3.36	29.26 ± 2.25	26.82 ± 2.26	23.16 ± 3.15	19.5 ± 1.18	17.07 ± 1.83	15.85 ± 1.23	12.19 ± 1.22	8.53 ± 0.86	3.66 ± 0.22	2.44 ± 0.11	0
Calothrix parietina	17.47 ± 1.19	17.47 ± 1.23	15.88 ± 2.23	14.63 ± 1.19	13.41 ± 2.51	12.19 ± 1.13	9.61 ± 0.63	6.1 ± 0.52	4.88 ± 0.25	3.05 ± 0.16	2.44 ± 0	0
Westiellopsis prolifica	14.62 ± 1.18	13.41 ± 0.98	12.19 ± 1.18	12.19 ± 1.11	9.75 ± 0.85	8.53 ± 0.45	5.85 ± 0.25	4.88 ± 0.24	3.66 ± 0	1.22 ± 0	0	0

0 = Without Rogor; Values represent mean of three independent determinations ± S.D.

Table 3: EC_{25}, EC_{50}, EC_{75} and Sub-lethal dose (SL) (ppm) of Rogor on the growth of four different species of cyanobacteria.

Organism	EC_{25} dose	EC_{50} dose	EC_{75} dose	SL dose
Anabaena variabilis	0.02	0.2	10	2
Nostoc muscorum	0.02	0.5	2	10
Calothrix parietina	0.1	0.5	2	10
Westiellopsis prolifica	0.1	0.5	2	2

From the Table 1, the EC_{25}, EC_{50}, EC_{75} and sub-lethal (SL) doses of Rogor on the growth of two selected species of cyanobacteria were calculated (Table 3). Further experiments were carried on to study the effect of EC_{25}, EC_{50}, EC_{75} and SL doses of Rogor on the total carotenoids, proteins and extra-cellular amino acids content (µg/10 mL culture) of *Anabaena variabilis* (Table 4) and of *Calothrix parietina* (Table 5). The per cent decrease over control at 0, 0.02, 0.2, 0.5 and 2 ppm concentration of pesticide Rogor against *Anabaena variabilis* and at 0, 0.1, 0.5, 2, 10 ppm concentrations of Rogor against *Calothrix parietina* on carotenoids, proteins and extra-cellular amino acid content have been represented in the parentheses of Table 4 and Table 5 respectively. The gradual reduction in the content is along with the concentration gradient.

Differential effect of various pesticides on the growth of cyanobacteria has been reported earlier by Hamdi et al. (1970), Ibrahim (1972), Gangawane (1979), Sardeshpande & Goyal (1982), Goyal (1987) and Das (1995). In most of the studies, the magnitude of reduction in chlorophyll-*a* content of the cells was proportional to the concentration of the pesticides. Chlorosis and inhibition of chlorophyll synthesis in higher plants as affected by treatment with herbicides has been reported (Shaw & Swanson 1953, Tiwari et al. 1982). The present findings clearly agree with the earlier reports.

Of the two cyanobacteria, *Calothrix parietina* was comparatively more tolerant to the test pesticide than *Anabaena variabilis* and also than the other two, i.e., *Nostoc muscorum* and *Westiellopsis*

Table 4: Effect of EC_{25}, EC_{50}, EC_{75} and SL doses of Rogor (dimethoate 30 EC) on the total carotenoids, protein and extra-cellular amino acids (µg/10mL culture) of *Anabaena variabilis.*

Concentration of Rogor (ppm)	Total carotenoids at 470nm	Protein at 750nm	Extra-cellular amino acids at 520 nm
0	8.4 ± 0.12	1440 ± 2.88	16.65 ± 1.05
0.02	5.2 ± 0.01 (-38.09)	1027.2 ± 3.03 (-28.67)	14.4 ± 1.25 (-13.51)
0.2	4.0 ± 0.01 (-52.38)	662.4 ± 3.01 (-54.0)	8.25 ± 1.23 (-50.45)
0.5	2.4 ± 0.002 (-71.43)	307.2 ± 2.06 (-78.07)	5.6 ± 0.83 (-66.37)
2.0	0.8 ± 0.0 (-90.48)	172.8 ± 2.01 (-88.0)	1.66 ± 0.06 (-90.39)

Table 5: Effect of EC_{25}, EC_{50}, EC_{75} and SL doses of Rogor (dimethoate 30 EC) on the total carotenoids, protein and extra-cellular amino acids (µg/10mL culture) of *Calothrix parietina.*

Concentration of Rogor (ppm)	Total carotenoids at 470nm	Protein at 750nm	Extra-cellular amino acids at 520 nm
0	12.8 ± 1.88	729.6 ± 3.89	13.6 ± 1.01
0.1	8.8 ± 1.05 (-13.25)	547.2 ± 3.05 (-25.0)	12.0 ± 1.87 (-11.76)
0.5	5.6 ± 0.81 (-56.25)	300.0 ± 4.85 (-58.88)	8.8 ± 1.55 (-35.29)
2.0	3.2 ± 0.35 (-75.0)	240.0 ± 2.85 (-67.10)	6.4 ± 1.05 (-52.94)
10.0	1.2 ± 0.05 (-90.63)	105.6 ± 3.02 (-85.53)	2.0 ± 0.83 (-85.29)

0 = Without Rogor; Values in the parentheses represent the per cent decrease (-) over control.

prolifica. This might be due to the possession of a well defined sheath layer around their trichome. In the present study, *Anabaena variabilis*, although possessed abundant mucilage, was sensitive to Rogor. On the contrary, *Nostoc muscorum* that does not possess a thick slime layer tolerated higher concentration of Rogor, which implies that only presence of additional surface features around the trichome of a cyanobacterium can not be the sole factor responsible for its tolerance at higher doses of the pesticide. Some species might have become resistant, because of routine application of the pesticide in the rice cultivation by local farmers.

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