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Toxicity of parathion-methyl to cells, akinetes and heterocysts of the cyanobacterium *Cylindrospermum* sp. and the probit analysis of toxicity

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Summary

The toxicity of a commercial formulation of the insecticide parathion-methyl to the N_2-fixing filamentous cyanobacterium (blue-green alga) *Cylindrospermum* sp. was studied. A concentration of parathion-methyl of 0.5 ppm caused growth increase in liquid growth media. The minimum inhibitory concentration of parathion-methyl for both types (N_2-fixing and nitrate supplemented) of liquid and solid media was 1.0 ppm. LC_{50} values were: 4.4 ppm (liquid, N_2-fixing), 5.5 ppm (liquid, nitrate supplemented), 3.3 ppm (agar, N_2-fixing) and 4.0 ppm (agar, nitrate supplemented). LC_{100} values for N_2-fixing liquid and both types of agar media were 10.0 ppm, while for the liquid nitrate supplemented medium the LC_{100} was 12.0 ppm. Both akinete (spore) formation and germination were inhibited below the highest permissive concentration of 8.0 ppm, with the insecticide incorporated in the agar media. In soil, the LC_{50} and LC_{100} values for parathion-methyl were 13.6 and 30 ppm, respectively. Both the dehydrogenase activity of heterocysts (monitored by 2,3,5-triphenyl tetrazolium chloride reduction) and the nitrogen concentration of cultures (estimated by the micro-Kjeldahl method) were affected by the insecticide, but the latter (N_2-fixation) was more sensitive. The Kruskal-Wallis H test on the numbers of vegetative cells in the filaments revealed that the insecticide significantly affected the division of vegetative cells. The cyanobacterium could detoxify the growth medium containing high levels (30 and 40 ppm) of the insecticide in short-term exposures at the expense of cell viability.

Key words: Insecticide, parathion-methyl, cyanobacterium, *Cylindrospermum*, toxicity, probit, akinete, N_2-fixation, detoxification

Introduction

Nitrogen-fixing cyanobacteria (blue-green algae) have three types of cells: vegetative cells that carry out oxygenic photosynthesis, thick-walled heterocysts that carry out the oxygen-sensitive nitrogen fixation, and akinetes (spores) that are differentiated from vegetative cells for perennation during the adversity of water-stress. Many of the fresh-water forms are either planktonic in inland waters or the dominant constituents of soil algal flora (Fogg *et al*., 1973; Sauthoff & Oesterreicher, 1994). The rice-field ecosystem provides water-logged conditions in the wet-season and N_2-fixing cyanobacteria release nitrogenous compounds with eventual upgrading of N-fertility of the soil (Padhy, 1985*a*). The utilisation of algal-biofertilisers for rice-cultivation uses *in situ* growth and incorporation of patches of cyanobacteria into the soil by puddling (Padhy, 1985*a*) but in the hot summer months (April to June) cells of cyanobacteria perennate as akinetes, which germinate back to form vegetative cells when the wet-season returns in July (Nichols & Adams 1982; Herdman, 1988).

In the year 1997-98, Indian agriculture consumed 2 000 metric tonnes of technical grade of the insecticide parathion-methyl (Agrawal *et al.,* 1999). Pesticide-cyanobacteria interactions have been reviewed several times recently (Padhy, 1985*b*; Pipe, 1992; Prasad & Vaishampayan, 1994). However, a systematic toxicity study on the three types of cells (vegetative cells, heterocysts and akinetes) of a N_2-fixing cyanobacterium by any pesticide has not been reported. Toxicity studies using soil would also help to assess the situation where a cyanobacterium is exposed to pesticides in agricultural soil. Continuing the line of investigation on akinetes (Panigrahi *et al.*, 1998), toxicity studies of the insecticide parathion-methyl on the vegetative cells, formation and germination of akinetes, dehydrogenase activity and N_2-fixation activity of heterocysts by the rice field cyanobacterium *Cylindrospermum* sp. are reported here. The detoxification of the insecticide by the cyanobacterium is also reported.

Materials and Methods

Biological material

Cylindrospermum sp. (P K Singh strain) was originally isolated from a paddy field in Cuttack and

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is maintained in axenic culture. It is planktonic and filamentous with two polar (terminal) heterocysts separated by 16 ± 2 vegetative cells. It was grown in modified Chu-10 medium with and without a calcium nitrate supplement. The media are referred to as C-N (N_2-fixing medium) and C+N, respectively, which along with other growth conditions are detailed elsewhere (Padhy, 2001).

Insecticide

The insecticide parathion-methyl (O,O-dimethyl O-(4-nitro-phenyl) phosphorothioate) is available commercially as Folidol M (a 40% dust) or Metacide (50% EC formulation) (Bayer, India). A 1% Metacide-50 (50% a.i.) in Chu-10 medium was used for all experiments.

Growth studies and akinetes

Growth studies on the cyanobacterium in the presence of various concentrations of parathion-methyl were performed with 10 ml (C+N or C-N media) and 50 ml (C-N medium) liquid cultures. LC_{100} values (concentrations of insecticide giving 100% kill) were measured by collecting cells from 12-day-old cultures by centrifugation, washing with liquid growth medium and plating onto solid medium. The minimum concentration at which no colonies developed was recorded as the LC_{100}. On every 3rd day the growth was checked by measuring both the OD_{660nm} of the cultures and by haemocytometric filament counts. Filaments were measured by an ocular micrometer. The specific growth rate constant (k) was calculated, as described previously (Panigrahi *et al.,* 1998). The data of filament (cell) counts of liquid cultures on the sixth day and colony counts on the seventh day in agar media were further computed for probit analysis by obtaining the percent lethality for each concentration of the insecticide (Newman, 1995). Probit transformations were done with the help of statistical tables (Fisher & Yates, 1963). For solid media (2% agar), 3.6×10^3 filaments or colony forming units (CFU)/ml in aliquots of 0.5 ml were plated and colonies were counted on the seventh, tenth and thirtieth day of plating. When the liquid cultures were 70 days old, about 90% of the vegetative cells had become akinetes. After shaking vigorously and concentrating by decanting off the culture fluid, akinetes were plated in triplicates on both C-N and C+N agar media incorporated with different concentrations of parathion-methyl.

Toxicity studies with soil

Sun-dried garden loam soil (pH 6.5) was distributed in 25 g-lots onto 17 × 150 mm Petri dishes. These soil samples were wetted using aliquots of 15 ml of insecticide solutions (0, 10, 15, 20, 25 and 30 ppm in C-N medium). Cultures of *Cylindrospermum* (1.8×10^3 and 3.6×10^3 CFU/m1) in aliquots of 0.5 ml were placed on to dry Whatman No.1 filter papers in nine or 10 droplets. The filter papers in triplicates were kept in contact with the wet soil samples on Petri dishes. The dishes were illuminated from above. After 3 days, the filter papers were removed and re-soaked for 2 h in large trays with tap water, then transferred on to C-N agar plates on which the filter papers were kept in an inverted position so that cyanobacterial spots touched the nutrient agar. The agar plates were then returned to a normal position after 1 day with filter papers inside them. At the end of 7 days, the colonies were counted.

Dehydrogenase activity and N_2-fixation

TTC (2,3,5-triphenyl tetrazolium chloride) was used to monitor dehydrogenase (reducing) activity of the heterocysts. TTC is reduced in active heterocysts to formazan crystals, which appear as dark bodies under a compound microscope (Kale *et al.,* 1970). For the numbers of vegetative cells between two terminal heterocysts, the Kruskal-Wallis H test was performed. N_2-fixation studies in the presence of different doses of parathion-methyl were conducted in 50 ml C-N cultures; a micro-Kjeldahl apparatus was used to estimate the total fixed nitrogen at intervals of 5 days.

Detoxification of the insecticide

Pellets of the cyanobacterium from actively growing C-N cultures were placed in individual flasks with 0 (control), 30 and 40 ppm parathion-methyl in final volumes of 100 ml. A parallel series of insecticide solutions without the cyanobacterium served as the control. At intervals of 1, 2 and 4 h, aliquots of 12 ml were drawn from each flask and centrifuged (3000 ***g***, 10 min). The supernatants of 30 and 40 ppm treatments were diluted five-fold. The untreated and cyanobacterium-treated supernatants were kept after dilution in culture tubes (in duplicates) for growth of fresh inocula. The collected cyanobacterial pellets were resuspended in 50 ml of fresh C-N medium in duplicates and incubated for growth.

Results

Toxicity in liquid culture

A concentration of 0.5 ppm parathion-methyl caused a growth-enhancement both in C-N and C+N liquid media. In C-N medium, the growth of the cyanobacterium declined progressively in higher concentrations of the insecticide (2, 4, 6 and 8 ppm) and a concentration of 10 ppm was completely toxic (LC_{100}; Table 1). In the C+N medium, a progressive decline in the growth in concentrations 2, 4, 6, 8 and 10 ppm parathion-methyl was noted (Table 1);

12 ppm was the LC_{100} in the C+N medium. The growth enhancing concentration (GEC), minimum inhibitory concentration (MIC), highest permissive concentration (HPC) and LC_{100} in both types of liquid media are shown in Table 3. The computed regression lines for probit (growth) *vs.* $\log_{10}$ concentration are shown in Fig. 1 (liquid medium) and Fig. 2 (agar). Values of LC_{25}, LC_{50} and LC_{75} were calculated and are shown in Table 3. The values of GEC, MIC, HPC and LC_{100} presented in Table 3 are from experimental data. The k values in both media indicate reduction in growth rates of the cyanobacterium due to parathion-methyl (Table 1).

A gradual decrease in filament length was observed with increases in concentration of the insecticide, measured on the sixth day after inoculation (Table 1). Cells became progressively paler with increasing concentrations of the insecticide, as well as during longer incubations at each dose. Furthermore, under insecticide-stress, all filaments invariably had two terminal heterocysts. There was an apparent increase in the ratio of heterocysts:vegetative cells under insecticide stress, as is evident from a decrease in the total number of vegetative cells in the filaments (Table 6). The Kruskal-Wallis H-test of number of vegetative cells in filaments revealed that the test statistic H had the computed value 14.75 (degrees of freedom = 3, i.e. number incubations (4) minus 1). The tabulated H-values are 7.815 (for $P = 0.05$) and 11.345 (for $P = 0.01$) (Table 6). This supports the alternative hypothesis that the insecticide caused a significant difference in the heterocyst:vegetative cell ratio. This also indicates that vegetative cells do not divide frequently under insecticide-stress, a fact supported by the diminishing values of filament length (Table 1).

Toxicity in agar media and akinetes

No growth enhancement of the cyanobacterium was noted in either agar medium (C-N or C+N) dosed with parathion-methyl. The HPC was 8 ppm, while the LC_{100} in both agar media was 10 ppm parathion-methyl (Table 2). At levels below the HPC (2-6 ppm in C-N and 2-8 ppm in C+N medium) intact filaments were visible with a pale green colour on agar plates even up to 45 days after plating, although akinetes were not visible on those agar media, even at the end of 65 days of incubation (Table 2). Thus, sub-lethal doses of parathion-methyl suppressed formation of akinetes of *Cylindrospermum*. In contrast, akinetes were clearly visible on control plates.

Four days after plating akinetes, blue-green

Table 1. *Growth-inhibition of the insecticide methyl parathion on growth of the cyanobacterium* Cylindrospermum *sp. in nitrogen fixing (C-N) and nitrate-supplemented (C+N) liquid media*[a]

Parathion -/Methyl	OD_{660nm} at day 3	Mean cell ml^{-1} at day 3 ($\times 10^3$)	OD_{660nm} at day 6	Mean cell ml^{-1} at day 6 ($\times 10^3$)	OD_{660nm} at day 9	Mean cell ml^{-1} at day 9 ($\times 10^3$)	Average specific growth rate constant (k)	Filament length at day 6 (μ)
In C-N medium								
0	0.050	6.48	0.07	8.53	0.08	12.25	0.120	53.4 ± 14.2
0.5	0.055	7.10	0.07	9.18	0.09	13.15	0.103	--
2	0.045	5.21	0.05	6.45	0.08	7.31	0.009	56.7 ± 12.8
4	0.035	4.12	0.04	4.52	0.06	5.08	0.081	57.2 ± 10.2
6	0.025	3.51	0.03	3.93	0.05	4.12	0.049	33.3 ± 9.6
8	0.025	2.20	0.03	2.20	--	--	--	27.9 ± 8.4
10	--	--	--	--	--	--	--	--
In C+N medium								
0	0.055	7.16	0.07	9.14	0.08	14.12	0.111	58.2 ± 13.8
0.5	0.060	7.85	0.07	10.85	0.08	14.08	0.101	--
2	0.050	6.11	0.07	8.24	0.08	14.00	0.093	55.1 ± 13.0
4	0.045	4.82	0.06	5.24	0.05	8.88	0.080	55.0 ± 9.6
6	0.030	3.92	0.04	4.76	0.04	7.11	0.062	53.7 ± 10.2
8	0.030	2.80	0.04	2.60	0.04	6.22	0.039	45.8 ± 10.8
10	0.030	2.20	0.04	--	0.03	5.33	--	23.8 ± 11.2
12	--	--	--	--	--	--	--	--

[a] OD_{660nm} was 0.2 and mean cell number was 3.0×10^4 ml^{-1} at '0' day. Cell number was determined by multiplying haemocytometric filament count with mean number of cells/filament. Standard deviation values of k day^{-1} are omitted which fluctuate between 0.20 to 0.033 in C-N and 0.024 to 0.045 in C+N medium. Probits of percent lethality against $\log_{10}$ concentration of the insecticide are plotted in Fig. 1. In C-N and C+N medium, the level of 10 ppm and 12 ppm respectively, were LC_{100}, and filaments were not seen at sixth day or further incubation

colonies developed in the control plates which were visible to the naked eye on seventh day. On the tenth day, confluent growth of new filaments occurred in these control plates. Agar plates with the insecticide below the HPC had no colonies even 30 days after plating (Table 2). Thus, parathion-methyl suppressed akinete germination in *Cylindrospermum.*

Toxicity studies in soil

Studies with soil and parathion-methyl stock solutions revealed that *Cylindrospermum* could readily tolerate the concentration of 15 ppm in the soil, despite the observed LC_{100} in liquid culture (C-N medium) of 10 ppm. Both higher and lower inocula could not tolerate a concentration of 30 ppm parathion-methyl in soil (LC_{100}) in a 3-day exposure-period (Table 4). The LC_{50} value in soil was approximately 13.6 ppm (probit computations not shown).

Table 2. *Lethality of in agar media for* Cylindrospermum *and germination of akinetes on parathion-methyl incorporated agar plates (mean of triplicate values)*

Parathion-methyl (ppm)	Number of colonies[a] when filaments plated		Number of colonies[b] when akinetes plated	
	C-N agar	C+N agar	C-N agar	C+N agar
0	187(0)	198(0)	CG	CG
1	179(4.3)	193(2.6)	3	4
2	140(25.2)	162(18.2)	NG	NG
4	90(51.9)	118(40.0)	NG	NG
6	30(84.0)	53(72.3)	NG	NG
8	2(99.0)	8(96.0)	NG	NG
10	--	--	--	--

[a] Numbers in parenthesis indicate percent lethality. Aliquots of 0.5 ml of an inoculum of 3.6×10^3 filaments ml^{-1} were plated. Probits of percent lethality against $\log_{10}$ concentrations of the insecticide are plotted in Fig. 2.
[b] Experiments with akinetes were repeated and data of the second experiment are given. Aliquots of 0.5 ml were plated from a solution of 8×10^3 akinetes ml^{-1}.
CG = confluent growth; NG = no growth

Heterocysts and N_2-fixation

Lysis of vegetative cells in the TTC solution without pre-treatment of parathion-methyl began within 6.5-7 min with complete lysis after an additional period of 3 min. Cells with pre-treatment with 40, 60 and 80 ppm parathion-methyl had delayed initial cell lysis and the delay-period increased with increases of insecticide concentrations (data not given). Further, it was verified that 60 and 80 ppm parathion-methyl did not cause any cell lysis, even up to 8 h of exposure. Thus, the insecticide-pretreatment impaired the entry of TTC into vegetative cells. The penetration of TTC into heterocysts must be through their polar nodules, which are the links to adjacent vegetative cells.

Initial appearance of dark bodies after TTC-exposure in heterocysts without the insecticide pre-treatment took 5 min. It was found that the appearance of dark bodies was delayed by increasing the concentration of parathion-methyl, so that by increasing the incubation period up to 4 h at 60 and 80 ppm parathion-methyl pre-treatment, no dark bodies appeared in the heterocysts (Table 5). Without the parathion-methyl pre-treatment, 70% and 97% of maximum dark body formation appeared after 15 and 30 min of TTC treatment, respectively. In filaments with 40, 60 and 80 ppm parathion-methyl pre-treatment, no dark bodies appeared after 15 min of TTC treatment. At each insecticide concentration, increases in the number of dark bodies were observed by prolonging the TTC treatment from 30 to 60 min. At all concentrations of parathion-methyl used, increasing the period of the insecticide treatment progressively reduced the percentage of active heterocysts, so that after 4 h incubation with higher doses (60 and 80 ppm parathion-methyl), there were no active heterocysts (Table 5). Thus, at levels of the insecticide very much above the recorded LC_{100}

Table 3. *Lethal ranges of* Cylindrospermum *sp. due to parathion-methyl (ppm)*[a]

Parathion-methyl (ppm)	GEC	MIC	Computed partially lethal values[b] LC_{25}	LC_{50}	LC_{75}	HPC	LC_{100}
Liquid media							
C-N	0.5	1.0	2.2 (0.34)	4.4 (0.64)	7.4 (0.87)	8.0	10.0
C+N	0.5	1.0	3.3 (0.52)	5.5 (0.74)	8.0 (0.9)	10.0	12.0
Agar media							
C-N	--	1.0	2.0 (0.3)	3.3 (0.52)	5.6 (0.75)	8.0	10.0
C+N	--	1.0	2.5 (0.39)	4.0 (0.6)	6.5 (0.81)	8.0	10.0

[a] Growth enhancing concentration (GEC), minimum inhibitory concentrations (MIC), highest permissive concentrations (HPC) and lethal concentration (LC_{100}) were obtained from growth studies. LC_{25}, LC_{50} and LC_{75} were computed from Figs 1 and 2.
[b] Numbers in parenthesis indicate the corresponding $\log_{10}$ concentrations computed from Figs 1 and 2

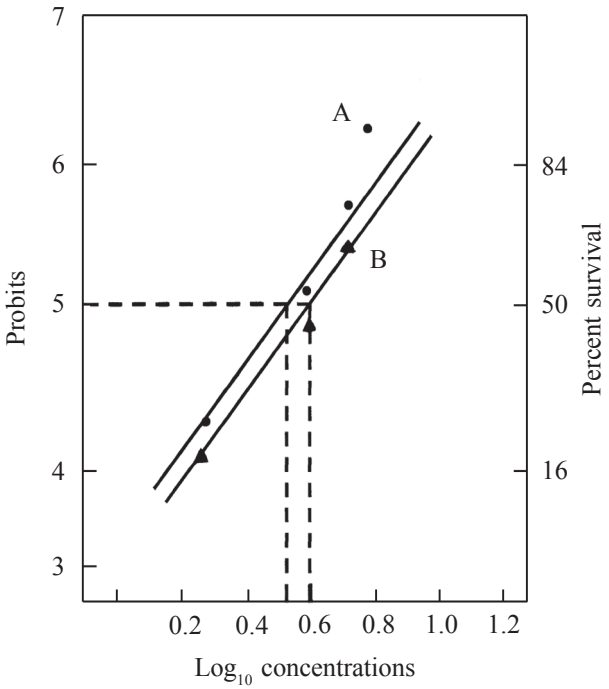

Fig. 1. Probits of percent lethality values plotted against $\log_{10}$ concentrations of the insecticide parathion-methyl in toxicity studies of *Cylindrospermum* sp. in liquid cultures. Line A (solid circles) for C-N medium and line B (solid triangles) for C+N medium. LC_{25}, LC_{50} and LC_{75} values for liquid media were extrapolated from the lines A and B. Percent survival of cells in cultures can be read from back-transformations of probits on the right hand scale.

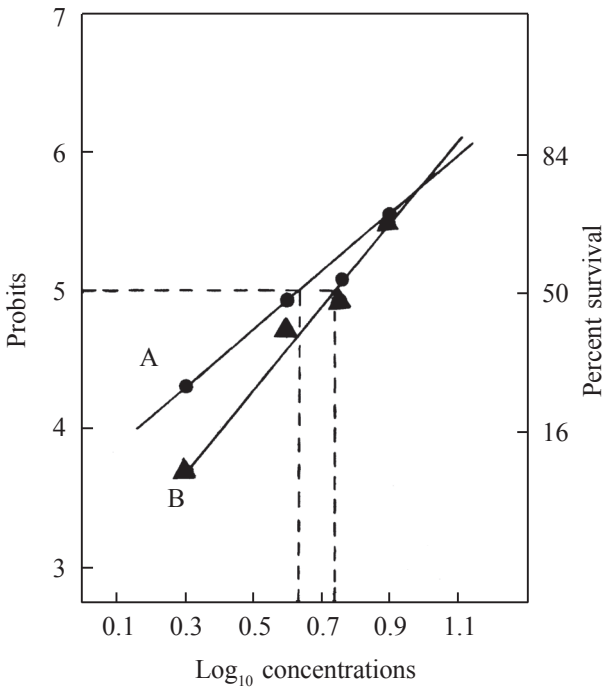

Fig. 2. Probits of percent lethality values plotted against $\log_{10}$ concentrations of the insecticide parathion-methyl in toxicity studies of *Cylindrospermum* sp. in agar media. Line A (solid circles) for C-N agar and line B (solid triangles) for C+N agar. LC_{25}, LC_{50} and LC_{75} values for agar media were extrapolated from the lines A and B. Percent survival of colonies can be read from back-transformations of probits on the right hand scale.

Table 4. *Colony-counts on C-N agar plates, of* Cylindrospermum *after contact of filaments with wet soil with parathion-methyl for 3 days* [a]

Parathion-methyl (ppm)	Number of colonies in C-N agar	
	Higher inoculum	Lower inoculum
0	CG	225(100)
10	CG	208(92.4)
15	CG	128(56.8)
20	183	73(32.0)
25	33	21(9.3)
30	0	0

[a] Higher inoculum: 3.6×10^3 filaments or CFU ml^{-1}; lower inoculum:1.8×10^3 filaments or CFU ml^{-1}
Number in parenthesis represents percent survival. Values are means of triplicate colony counts. CG: confluent growth

level, the dehydrogenase activity was only impaired by the insecticide.

Nitrogen-fixation studies revealed that the total N-content of cultures in the presence of parathion-methyl at levels below the HPC decreased with increasing concentrations of the insecticide (Table 6). Thus, the N_2-fixing activity of heterocysts was less readily affected at insecticide levels below the HPC.

Table 5. *Dehydrogenase activity of heterocysts of* Cylindrospermum *sp.*

Parathion-methyl (ppm)	Duration of TTC treatment (min)	Incubation of filaments in insecticide solution before TTC-treatment (h)		
		2	3	4
		Percentage of dark-bodies in heterocysts due to TTC		
0 (control)	15	70	ND	ND
	30	97	ND	ND
40	15	0	0	0
	30	24	20	12
	60	52	52	22
60	15	0	0	0
	30	15	15	0
	60	17	12	0
80	15	0	0	0
	30	8	6	0
	60	8	5	0
		Time of initial appearance of dark bodies in heterocysts due to TTC (min)		
0 (control)		5	5	5
40		20	30	31
60		23	28	0
80		22	26	0

ND = not determined

Detoxification

At 30 and 40 ppm parathion-methyl, the growth medium derived from cyanobacterial treatments for 2 and 4 h periods supported better growth of fresh inocula than supernatants with shorter or without any cyanobacterial pre-treatment (Table 7). However, such growth was far lower than in the control medium. It was observed that the cyanobacterium-treated insecticide-solutions were less toxic to fresh inocula than the untreated-stocks, indicating a detoxification. It was found that the pellets (filaments) from 30 and 40 ppm insecticide solutions collected after 1, 2 and 4 h treatments had progressively diminished growth (Table 7). This indicated that *Cylindrospermum* could detoxify the medium at short period exposures at the expense of viability of cells, using stock solutions which were three to four times greater (more toxic) than the LC_{100} level (10 ppm).

Table 6. *Effect of parathion-methyl on nitrogen fixation by* Cylindrospermum *sp.*[a,b]

Parathion-methyl (ppm)	Total mg N fixed at different days			
	5	10	15	20
0	0.57	0.85	1.92	2.3
	(17.1)	(17.4)	(16.8)	(17.8)
2	0.51	0.73	1.15	1.86
	(16.8)	(16.5)	(16.3)	(16.7)
4	0.42	0.66	0.93	1.22
	(16.2)	(16.1)	(15.9)	(14.7)
6	0.31	0.48	0.52	0.71
	(16.4)	(15.6)	(14.8)	(14.9)
8	0.25	0.24	0.24	0.28
	(16.4)	(16.1)	(16.2)	(16.3)

[a] Total N-content of the inoculum was 0.23 mg. Data presented are means of triplicate values
[b] Number in parenthesis indicates average number of vegetative cells between the two terminal heterocysts of a filament (values are mean of nine observations from triplicate cultures, for which the Kruskal-Wallis H-test was performed)

Discussion

The growth enhancing effect observed in the present study indicated a detoxification of the pesticide and its co-metabolism, which was confirmed by the increased growth of insecticide-pretreated cells in fresh media. Values of LC_{100} were found to be 10 and 12 ppm in C-N and C+N media, respectively, indicate that non-target adverse effects of the insecticide were more comparable to other insecticides, such as cypermethrin (Mohapatro *et al.,* 1998) or carbaryl and carbofuran (Panigrahy & Padhy, 2000; Padhy & Mohapatra, 2001). LC_{100} values were as follows: cypermethrin, 500 ppm in both C-N and C+N liquid media; carbaryl, 100 and 120 ppm in C-N and C+N liquid media respectively; carbofuran, 2 000 and 3 000 ppm in C-N and C+N liquid media, respectively. Parathion-methyl was reported to cause toxicity to the cyanobacterium *Gloeocapsa* (isolated from Turkey) giving a 50% reduction in growth at 10 ppm during short term exposures (Tozum-Calgan & Sivaci-Guner, 1993). The present study with soil indicated that in natural soil-water environments, a pesticide would be less toxic than in pure cultures in the laboratory. This is probably due to adsorption of pesticides to soil organic matter and/or detoxification by resident exoenzymes (Anderson, 1978; Gray *et al.,* 1978; Paul & Clark, 1996). The recommended field dose of parathion-methyl is 1.5 kg ha^{-1}, which upon extrapolation to laboratory conditions corresponds to 15 to 20 ppm, a level higher than the LC_{100} reported here for the insecticide in pure cultures and lower than that in soil studies. Parathion-methyl is therefore more toxic and less safe than many other pesticides that have higher LC_{100} levels (Padhy, 1985*b*; Padhy & Mohapatra, 2001) in view of its non-target effects on cyanobacteria. Pesticide toxicity studies with soil are not frequently measured with these organisms.

Probit analysis of toxicity data yielded LC_{25}, LC_{50} and LC_{75} values that quantify partially lethal doses against the observed partially lethal ranges, MIC and

Table 7. *Detoxification of parathion-methyl by the cyanobacterium* Cylindrospermum *sp.* [a]

Duration of insecticide treatment prior to separation of growth medium from filaments (h)	Growth of fresh inocula[b] in supernatants of insecticide solution (ppm)			Growth of sedimented, insecticide[b, e] pretreated filaments in fresh C-N medium		
	0	30[c]	40[d]	0	30	40
0	0.35	0.21	0.04	0.15	0.15	0.15
1	0.35	0.21	0.06,	0.15	0.13	0.11
2	0.35	0.24	0.095	0.15	0.12	0.08
4	0.35	0.26	0.105	0.15	0.08	0.05

[a] Mean of duplicate values. Experiments were repeated and data of the second experiment are given
[b] Growth (OD_{660nm} of culture) on tenth day after inoculation
[c] Diluted five-times (initial 30 ppm, final 6 ppm)
[d] Diluted five-times (initial 40 ppm, final 8 ppm)
[e] 50 ml C-N media, OD_{660nm} on tenth day of incubation

HPC. As extreme values computed by the probit method such as LC_{05} and LC_{95} are not statistically dependable, they were not computed here.

At sub-lethal concentrations in agar media both formation and germination of akinetes of the cyanobacterium were suppressed. Both these processes individually require suitable combinations of growth or climatic parameters (Nichols & Adams, 1982; Herdman, 1988). The presence of a toxic chemical should alter the physiological balance entailing failures of both processes. Earlier studies with the fungicides, 2-PAM (Pyridinium-2-aldoxime, methyl iodide) and MEMC (methoxyethyl mercuric chloride), suppression of akinete formation and germination were recorded with the same cyanobacterium (Panigrahi *et al.*, 1998). This could create a disturbing condition in life cycles of similar N_2-fixing cyanobacteria, when a residual insecticide such as MEMC is used.

The reducing activity of heterocysts (which lack PS II activity and do not liberate O_2) in the present study was less readily affected by the insecticide, probably due to the delay in the penetration of the insecticide and TTC into heterocysts, which have thick walls. A high concentration of the insecticide (five times more than the LC_{100} levels obtained) caused an impairment of the heterocyst dehydrogenase activity. The process of N_2-fixation was found to be highly sensitive initially, as the levels below the HPC impaired the process, resulting in a significant reduction in the total N-content of cultures. But at longer incubation times, detoxification probably caused a progressive increase in the total N-content.

The detoxification of the insecticide by the cyanobacterium was definitely due to co-metabolism of the toxic chemical, as insecticide pre-treated cells grew better in fresh media compared with untreated cells. It has been shown earlier that cyanobacteria whose prokaryotic vegetative cells conduct oxygenic photosynthesis possess the enzyme systems to add oxygen and hydroxyl groups during catabolism of these xenobiotic compounds (Padhy, 1985*a*; Paul & Clark, 1996). It has been shown that 2,6-^{14}C-parathion was degraded to p-nitrophenol in flooded soils in India (Reddy & Sethunathan, 1983).

The agricultural importance of this study is that parathion-methyl may be more damaging than previously considered. At soil-water interfaces, a higher LC_{100} level of the insecticide in this study indicated less hazardous conditions for these soil microbes. Formation and germination of akinetes of the cyanobacterium were impaired at partially lethal doses and this finding should be verified with soil studies. However, the detoxification capability of the cyanobacterium should help its survival in nature.

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References

Agrawal A, Narain S, Sen S. (Eds) 1999. *The Citizens' Fifth Report. Part II: Statistical Database*, pp. 14-18. New Delhi: Center for Science and Environment.

Anderson J R. 1978. Pesticide effect on non-target soil micro-organisms. In *Pesticide Microbiology*, pp. 313-533. Eds I R Hilland and S J L Wright. London, UK: Academic Press.

Fisher R A, Yates F. 1963. *Statistical Tables for Biological, Agricultural and Medical Research,* 6th Edn. Edinburgh, UK: Oliver and Boyd. 875 pp.

Fogg G E, Stewart W D P, Fay P, Walsby A E. 1973. *The Blue-Green Algae.* London, UK: Academic Press. 459 pp.

Gray T R G, Jones J G, Wright S J L. 1978. Microbiological aspects of the soil, plant, air and animal environment. In *Pesticide Microbiology*, pp. 8-77. Eds I R Hill and S J L Wright. London, UK: Academic Press.

Herdman M. 1988. Cellular differentiation: Akinetes. In *Cyanobacteria*, *Methods in Enzymology,* pp. 222-232. Eds L Pecker and A N Glazer. New York, USA: Academic Press.

Kale S R, Bahal M, Talapasayi E R S. 1970. Walls development and tetrazolium chloride reduction in heterocysts of the blue-green alga *Anabaena ambigua Experientia* **26**:605.

Mohapatro P C, Panigarhi S, Padhy R N. 1998. Effects of two common insecticides on akinetes formation and germination of the cyanobacterium *Cylindrospermum* sp. *Fresenius Environmental Bulletin* **7**:80-84.

Newman M C. 1995. *Quantitative Methods in Aquatic Ecotoxicology*. Boca Raton, Florida: Lewis Press. 276 pp.

Nichols J M, Adams D G. 1982. Akinetes. In *The Biology of Cyanobacteria*, pp. 587-412. Eds N G Carr and B A Whitton. Berkeley, USA: University of California Press.

Padhy R N. 1985*a*. Cyanobacteria employed as fertilizers and waste disposers. *Nature (London)* **317**:475-476.

Padhy R N. 1985*b*. Cyanobacteria and pesticides. *Residue Reviews* **95**:1-44.

Padhy R N. 2001. Monitoring of chemical fertilizers on toxicity of two carbamate insecticides to the cyanobacterium *Anabaena* PCC 7120. *Microbios* **106**:165-175.

Padhy R N, Mohapatra K. 2001. Toxicity of two carbamate insecticides to the cyanobacterium *Anabaena* PCC 7120 and computation of partial lethal concentrations by the probit method. *Microbios* **106**:81-95.

Panigrahi S, Mohapatro P C, Padhy R N. 1998. Fungicides impair formation and germination of akinetes of the cyanobacterium *Cylindrospermum* sp. *Fresenius Environmental Bulletin* **7**:74-79.

Panigrahy K C, Padhy R N. 2000. Toxicity of carbamate pesticides to cells, heterocysts and akinetes of the cyanobacterium *Cylindrospermum* sp. *Algological Studies* **99**:95-115.

Paul E A, Clark F E. 1996. *Soil Microbiology and Biochemistry,* 2nd Edn. San Diego: Academic Press. 453 pp.

Pipe A E. 1992. Pesticide effects on soil algae and cyanobacteria. *Reviews of Environmental Contamination Toxicology* **127**:95-170.

Prasad A B, Vaishampayan A. 1994. Prospects of using

pesticide resistant cyanobacterial mutants as viable bio-N-fertiliser and a source material for genetic engineering studies. In *Biology and Application of Nitrogen Organisms*, pp. 47-70. Eds A B Prasad and A Vaishampayan. Jodhpur: Scientific Publisher.

Reddy B R, Sethunathan N. 1983. Mineralization of parathion in the rice rhizosphere. *Applied Environmental Microbiology* **45**:826-829.

Sauthoff W, Oesterreicher W. 1994. Does a highly intensive plant production induce changes in the algal soil flora? In *Effects of a Long-Term Application of Plant Protection Products Used in Different Intensities and Development of Assessment Criteria*, pp. 107-191. Eds G Bartels and T Kampmann. Berlin: Blackwell.

Tozum-Calgan S R D, Sivaci-Guner S. 1993. Effects of 2,4-D and methyl parathion on growth and nitrogen fixation in cyanobacterium, *Gleocapsa. International Journal of Environmental Studies* **43**:307-311.