Bull. Environ. Contam. Toxicol. (2005) 75:374–381

DOI: 10.1007/s00128-005-0764-2

Bulletin of Environmental Contamination and Toxicology

Biological Responses of Cyanobacteria to Insecticides and Their Insecticide Degrading Potential

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Received: 20 October 2004/Accepted: 11 June 2005

Application of pesticides, a routine agricultural practice, has its effects on non-target organisms including cyanobacteria (Padhy 1985; Moorman 1989; Adhyo *et al.* 2000). Cyanobacteria contribute significantly towards soil fertility and crop yield (Roger *et al.* 1992; Jha *et al.* 1999). It is therefore necessary to study the effect of pesticides on them. Depending on the type and concentration of pesticides and composition of growth media, pesticide have been found to exert stimulatory/inhibitory or no effect on the growth and nitrogen fixing ability by cyanobacteria into pure culture (Zargar and Dar 1990; Jamilselvam *et al.* 2002). The possible reasons for such conflicting behavior of cyanobacteria under pesticides stress are poorly understood. Further, information on insecticides degradation by cyanobacteria is far from complete (Wright 1978; Megharaj *et al.* 1987). Present communication embodies the observation of cyanobacterial behaviour in pesticide burdened soil, response of three very widely distributed cyanobacteria namely *Westiellopsis, Nostoc* and *Anabaena* to carbofuran, chloropyriphos and endosulfan and degradation of these insecticides by cyanobacteria.

MATERIALS AND METHODS

Commercial endosulfan (Endocel, 35% EC by Excel Industries Limited, Mumbai, India), chloropyriphos (Suban, 20% EC by Sudarshan Chemicals Industries, Kolkata, India) and carbofuran (Furadan, 3% G by FMC India Pvt. Ltd., Kolkata, India) were diluted with distilled water to get stock solution of 10 μgml^{-1} Treatment rates correspond to 1x and 10x recommended field application level of chloropyriphos and endosulfan and 1x and 5x level for carbofuran. Conversion of field application rates to parts ai/10^6 was calculated assuming an even distribution of insecticides in the plough layer (1.8 million kg soil ha^{-1}). This is by necessity a simplification of the natural situation where a concentration gradient determined by the nature of the pesticides, the soil, the spray volume etc. may exist.

Ten to twelve sub-samples of top soil (0-15 cm) taken randomly from vegetable and pulse growing fields with a special soil sampling auger and combined to give one mixed sample of each plot were taken for analysis. The soil samples were thoroughly mixed and allowed to air dry before sieving. The soil samples were stored in a freezer at -5^0C until analysis.

Two sets of four sterile glass vial (diameter 3.5 cm, capacity 30 ml) for each soil

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samples were prepared and inoculated with 6g each of composite soil samples. The first set was kept moist by adding 2 to 2.5 mL of BG_{11} medium and to the second set 10 mL BG_{11} medium was added to create waterlogged conditions. These vials were incubated under continuous illumination of 2000 lux and 30 ± 2^0C temperature for 25 days. Cyanobacterial growth, Acetylene Reduction Assay (ARA), and distributional pattern were measured (Jha *et al.* 2001).

Westiellopsis prolifica, Nostoc hatei and *Anabaena sphaerica* were isolated from vegetable soils during the course of this study and maintained in BG_{11} N free medium. Each stock solution of carbofuran, chloropyriphos and endosulfan were diluted to have 1mL diluted solution contained 0.25 to 2.5 μgml^{-1} insecticides. One mL of diluted insecticide solution was mixed with 49 mL sterilized BG_{11} N free medium dispensed in 100 mL Erlenmeyer flasks. Each experimental flask was inoculated with 2.0 mL of uniform suspension of 10d old culture of each cyanobacterium. The flasks were incubated under continuous illumination of 2000 lux at 30 ± 2^0C for 25 days. All the treatments were run in triplicate. The parameters examined were growth, ARA (Jha *et al.* 2001), Glutamine synthetase (Farnden, KJF and Robertson JG 1969), ammonia excretion (Solerzano 1969) and polysaccharide (Roe 1955).

Insecticide residues were measured in 10 ml cultural filtrate on 10th and 25th days of incubation. Chloropyriphos residues were extracted by shaking culture filtrate (10 mL) with 20-40 mL of hexane and 400 mg of sodium sulphate for 10 min. 2-3 µL extract was injected in a Nucon gas chromatograph 5700 equipped with NI63 detector and a metal column (2ml x 1/8" OD) packed with OV-17 chrom WHP 80/100 mesh. The operating conditions were carrier gas flow 30 mL min^{-1}, column temperature 180^0C, injector temperature 210^0C and detector temperature 250^0C. Under these conditions, the retention time for chloropyriphos was 9.03 min., recovery was around 88.0 per cent and detection limits was 0.114 $\mu g\ ml^{-1}$. Carbofuran residues were estimated by colorimetricallty as developed by Mithyantha and Perur (1974).

Endosulfan residues in 10 mL culture filtrate were extracted in 60 mL separatory funnels with 20 mL hexane. The extracts were passed through an absorbent column containing 4 cm. layer of anhydrous sodium sulphate and concentrated to about 0.5 mL under reduced pressure. Finally, the residues were dissolved in 5-10mL hexane for analysis through Nucon GC 5700 with NI63 detector 1.5% OV-17 nitrogen as the carrier gas @ 30 mL min^{-1} on 80-100 mesh CHW column and temperature of oven, injector and detector were 210^0C, 220^0C and 270^0C respectively. Under these conditions recovery was around 81.6 per cent and detection limits was 0.086, and 0.105 μgml^{-1} for α and β endosulfan, respectively.

RESULTS AND DISCUSSION

Preliminary studies on cyanobacterial growth, ARA and distributional pattern (Table 1) in vegetable field soils and pulse fields soils indicated that cyanobacterial nitrogen fixation (ARA) was comparatively more sensitive to the pesticide stress than their growth, the effect being more pronounced under moist than waterlogged conditions. The above observation was more evident in vegetable soils having 0.141 $\mu g\ g^{-1}$ organochlorine insecticides residue. Maximum growth (10.1 to 13.9 µg chl.a g^{-1} soil) occurred in waterlogged situation. Vegetable soils exhibited less growth

(5.21 to 11.5 μg chla g^{-1} soil) and ARA (0.66 to 5.4nmol C_2H_4 h^{-1} g^{-1} soil) than pulse soils in both situations. ARA was negligible (0.66 to 3.0nmol C_2H_4 h^{-1} g^{-1} soil) in vegetable soils under moist condition.

Table 1. Growth and ARA of cyanobacteria in vegetables and pulses field soil

Sl. No.	Vegetable field soil		Pulse field soil	
	Waterlogged	Moist	Waterlogged	Moist
1	10.4	6.1	13.6	8.6
	(5.4)	(1.6)	(22.3)	(12.8)
2	10.8	6.7	12.3	8.4
	(4.5)	(0.9)	(15.0)	(8.0)
3	11.8	5.9	12.6	8.7
	(2.2)	(0.66)	(15.3)	(08.6)
4	12.3	7.4	13.9	7.9
	(1.0)	(0.88)	(14.8)	(12.4)
5	10.1	5.3	13.2	8.8
	(3.4)	(2.3)	(20.0)	(8.6)
6	11.5	8.8	13.4	9.1
	(1.8)	(1.0)	(19.0)	(10.4)
7	10.1	5.2	11.9	8.4
	(2.6)	(3.0)	(15.1)	(11.8)
8	12.5	7.8	12.8	8.0
	(4.2)	(2.8)	(14.8)	(12.4)

* Figures in parentheses indicate ARA values.

** Values: μg g^{-1} soil for chl. a (growth) and n mol $C_2H_4h^{-1}g^{-1}$ soil for ARA.

*** Organochlorine residues (HCH, DDT & Endosulfan) in vegetable soils and pulse soils were 0.141 & 0.046 μg g^{-1}.

Distributional pattern of cyanobacteria revealed that *Westiellopsis prolifica* dominated in vegetable soils with a rated sure of 490 and 330 in water logged and moist conditions respectively followed by *Nostoc hatei*. The trend was just reverse in pulse soils having domination of *Nostoc hatei* followed by *Westiellopsis prolifica.* The rated score of non-heterocystous cyanobacteria were quite higher in vegetable soils than in pulse soils. Cyanobacterial diversity was more in pulse soils. Due to space problem data on distributional pattern is not shown.

The most interesting observation was that the vegetable soils had very low nitrogenase activity (ARA), although, heterocystous cyanobacteria prevailed in it. This raises questions about the source of nitrogen for the growing cyanobacteria. Could the cyanobacterial activity of the soil be held responsible for making additional nitrogen available through the degradation of nitrogen containing insecticides? The above assumption is further substantiated by the fact that vegetable growers of this state use to apply 10-12 types of pesticides in brinjal crop in one growing season. To get the answer of this question, a laboratory experiment with the cyanobacterial isolates of vegetable soils were conducted in pure culture with carbofuran, endosulfan and chloropyriphos treatments. Dissipation of these insecticides in culture media was also measured.

Carbofuran, chloropyriphos and endosulfan was found to have a stimulatory effect on the growth of *W. prolifica* and *N. hatei* at 0.5, 0.25 and 0.25 μgml^{-1} (recommended level of field application) respectively (Table 2). A significant decrease in growth of both these cyanobacteria was found at 2.5 μgml^{-1}. *Anabaena sphaerica* was more susceptible and either failed to grow at this level or showed mild growth. Cyanobacterial ARA significantly decreased in carbofuran treatment at both the levels. This differential effect of carbofuran on growth and ARA was not perceptible with endosulfan and chloropyriphos.

Stimulation in ARA was also found at 2.5 μgml^{-1} level of chloropyriphos and endosulfan, a level showing mild decrease in growth. Highest ARA (10.8 n mol C_2H_4 $h^{-1}mL^{-1}$ culture) was found in *N. hatei* at 2.5 μgml^{-1} of endosulfan. The trend was just reverse for glutamine synthetase, an enzyme responsible for assimilation of cyanobacterial fixed nitrogen. Glutamine synthetase (GS) activity was significantly augmented in carbofuran treatment but significantly decreased in endosulfan and chloropyriphos treatment. Due to lower GS activity, ammonia release was increased in endosulfan and chlorloropyriphos containing culture. However, significant decrease in ammonia release in all three cyanobacteria at both the levels was found in carbofuran treatment.

Insecticides stress had a pronounced effect on the production of polysaccharides by the cyanobacteria. Such effect was more pronounced in chloropyriphos and endosulfan treatment, where a concentration dependent increase was observed. In carbofuran treatment, significant reduction at higher level was found. Such significant augmentation in polysaccharide content of cyanobacteria under endosulfan and chloropyriphos stress at higher level may be due to devoid mechanism. To draw definite conclusion however, analysis of adsorbed and intracellular pesticide content of cyanobacteria is needed.

The dissipation of endosulfan, chloropyriphos and carbofuran in uninoculated media on day 25 was 18.1, 27.3 and 30.0 per cent respectively (Table 3a & 3b). Such dissipation might be due to hydrolytic action (Brown *et al.* 1966). Inoculation of media with *W. prolifica* and *N. hatei* resulted in rapid reduction in the content of endosulfan, chloropyriphos and carbofuran.The *N. hatei* had more degrading ability in comparison to *W. prolifica*. The initial concentration of 2.03 μgml^{-1} endosulfan in *N. hatei* containing media significantly reduced to 1.61 and 1.10 μgml^{-1} causing a loss of 20.7 and 45.9 per cent on day10 and day25 of incubation respectively. *W. prolifica* were able to reduce the content upto 37.8 per cent. Detection of endosulfan sulphate in inoculated media on day25 might be due to oxidation of α and β isomer of endosulfan into endosulfan sulphate by these two cyanobacteria.

The average initial concentration of chloropyriphos in the medium on 0 day was 2.10μgml^{-1} which was significantly reduced to 1.2 and 0.71 μgml^{-1} in *N. hatei* growing media on day10 and day 25, respectively. The dissipation rate was 42.9 to 66.2 per cent. *W. prolifica* significantly reduced the 2.2 μgml^{-1} of initial level into 1.0 μgml^{-1} at the end of observation period. Maximum dissipation by this cyanobacterium was 54.6 per cent.

Carbofuran dissipated more rapidly than endosulfan and chloropyriphos in cyano-

Table 2. Effect of pesticides on growth, ARA, glutamine synthetase activity ammonia excretion and polysaccharide content of cyanobacteria.

Pesticide	Carbofuran			Chloropyriphos			Endosulfan			LSD
Concentration	0.0	0.5	2.5	0.0	0.25	2.5	0.0	0.25	2.5	(P= 0.05)
Westiellopsis prolifica										
Growth	4.2	5.8	4.0	4.5	5.4	3.0	4.8	5.5	4.0	0.12
ARA	4.6	3.5	2.4	5.2	6.8	8.0	4.5	6.7	8.4	0.36
GS activity	13.0	14.3	15.9	13.7	12.3	10.8	18.0	15.3	12.4	0.91
Ammonia excretion	133	126	121	126	136	142	122	127	133	12.5
Polysaccharide	7.0	7.3	6.0	8.4	9.8	10.6	8.0	10.1	11.6	2.79
Nostoc hatei										
Growth	3.8	4.9	3.2	4.04	4.3	3.0	4.2	4.9	3.6	0.91
ARA	7.1	5.3	4.0	6.7	7.3	9.1	7.3	9.7	10.8	1.59
GS activity	12.4	15.0	17.1	14.4	12.0	10.0	16.0	14.3	12.9	2.02
Ammonia excretion	122	114	105	124	128	133	140	145	152	7.73
Polysaccharide	10.4	11.0	8.6	10.8	14.6	16.0	11.1	15.0	17.0	1.08
Anabaena sphaerica										
Growth	3.9	3.0	1.1	4.8	2.7	0.0	4.0	1.9	0.0	2.11
ARA	7.3	6.0	1.4	8.1	9.1	0.0	9.1	7.4	0.0	0.83
GS activity	15.3	17.0	6.7	15.7	14.0	0.0	16.3	13.0	0.0	2.03
Ammonia excretion	126	111	6.3	116	122	0.0	128	136	0.0	12.11
Polysaccharide	9.3	8.9	2.6	8.9	10.8	0.0	9.1	12.4	0.0	1.25

Values : $\mu g\ mL^{-1}$ chlorophyll for growth, nmol $C_2H_4\ h^{-1}\ mL^{-1}$ for ARA, $\mu g\ \gamma$ GHA $mL^{-1}\ min^{-1}$ for Glutamine synthetase (GS) n mol mL^{-1} for ammonia excretion and $\mu g\ mL^{-1}$ for polysaccharides.

Table 3a. Scavenging/degradation of endosulfan by cyanobacteria.

Pesticides Organism	Endosulfan											
	0 day				10 day				25 days			
	αE	βE	ES	Total	αE	βE	ES	Total	αE	βE	ES	Total
Westiellopsis prolifica	1.4	0.64	0.0	2.04 (100)	1.22	0.48	0.0	1.70 (16.7)	0.72	0.34	0.21	1.27 (37.8)
Nostoc hatei	1.4	0.62	0.0	2.03 (100)	1.20	0.41	0.0	1.61 (20.7)	0.49	0.45	0.16	1.10 (45.9)
Control	1.4	0.63	0.0	2.03 (100)	1.34	0.59	0.0	1.93 (5.0)	1.2	0.46	0.0	1.66 (18.1)
LSD(P=0.05)							0.534					

Table 3b. Scavenging/degradation of chloropyriphos and carbofuran by cyanobacteria.

Pesticides Organism	Chloropyriphos			Carbofuran		
	0 day	10 day	25 day	0 day	10 day	25 day
Westiellopsis prolifica	2.2 (100)	1.7 (22.7)	1.0 (54.6)	2.0 (100)	1.4 (30.0)	0.8 (60.0)
Nostoc hatei	2.1 (100)	1.2 (42.9)	0.71 (66.2)	1.9 (100)	1.2 (36.8)	0.6 (68.5)
Control	2.2 (100)	1.9 (13.6)	1.6 (27.3)	2.0 (100)	1.7 (15.0)	1.4 (30.0)
LSD (P=0.05)		0.321			0.245	

* αE : α endosulfan, βE: Endosulfan, ES: Endosulfan sulphate
* Dose : 2.5ppm
* Figures in parenthesis indicate dissipation percentage.

bacterial inoculated medium. The initial concentration of 2.1μgml^{-1} was significantly reduced to 1.2 and 0.6 μgml^{-1} causing a reduction of 36.8 and 68.5 per cent in *N. hatei* growing media on day10 and day25, respectively. Dissipation was slow and less in *W. prolifica* growing media. Degradation of parathion (Zuckerman *et al.* 1970) and monocrotophos and quinalphos (Meghraj *et al.* 1987) by micro algae and cyanobacteria was also reported. Thus, these findings proved our assumption that cyanobacteria might have derived nitrogen by degrading nitrogen containing insecticides like carbofuran in vegetable soils. Further, degradation of endosulfan by cyanobacteria has of great agricultural significance because of its spraying application and dominance of cyanobacteria on soil surface due to phototrophic nature.

Acknowledgments. We thank to the Department of Biotechnology, New Delhi, India, for finance, Dean, FBS&H for facilities.

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