Bull. Environ. Contam. Toxicol. (1988) 41:277–281

Environmental Contamination and Toxicology

Effect of Insecticides and Phenolics on Nitrogen Fixation by *Nostoc linckia*

M. Megharaj, K. Venkateswarlu, and A. S. Rao

Department of Botany, Nagarjuna University, Nagarjunanagar 522 510, Andhra Pradesh, India

The nitrogen-fixing blue-green algae (cyanobacteria) significantly influence the nitrogen economy of temperate and tropical soils (Watanabe and Yamamoto 1971, DaSilva et al.1975). Although the genera Nostoc and Tolypothrix have been particularly implicated in the fixation of significantly large amounts of atmospheric nitrogen (Round 1965), these diazotrophs received little attention in relation to insecticide treatment and the available few reports do not indicate a permanent deleterious effect of insecticides on their nitrogenase activity (Prasad Reddy et al.1984). Very recently, we reported the impact of monocrotophos and quinalphos (Megharaj et al.1986a), cypermethrin and fenvalerate (Megharaj et al.1987a) and phenolic compounds (Megharaj et al.1986b) on the growth of Nostoc linckia (Roth) B & F, a filamentous dinitrogen fixer, in view of the great concern over the environmental hazard of these xenobiotics to the nontarget microorganisms implicated in soil fertility. As it has been well established that the effect of insecticides on nitrogen fixation by cyanobacteria is independent of that on growth (Prasad Reddy et al.1984), an attempt was, therefore, made to determine the influence of four insecticides (monocrotophos, quinalphos, cypermethrin and fenvalerate) and four phenolics (p-nitrophenol (PNP), m-nitrophenol (MNP), 2,4-dinitrophenol (DNP) and catechol) on nitrogen-fixing capacity of N.linckia, isolated from a black soil.

MATERIALS AND METHODS

Pure culture of N.linckia was grown in modified nitrogen-free Chu-10 medium supplemented with trace elements (Megharaj et al.1986a). Stock solutions from technical grade monocrotophos (Dimethyl(E)-1-methyl-2-methyl car-

Send reprint requests to Dr.K.Venkateswarlu, Department of Microbiology, S.K.University, Anantapur 515 003, India

bamoylvinyl phosphate, 74.2% pure from Hindustan Ciba-Geigy Ltd.), quinalphos (O,O-diethyl O-quinoxalin-2-yl phosphorothioate, 86.7% pure from Sandoz (India) Ltd.,), cypermethrin (α-Cyano-3-phenoxyphenyl-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate,92.2% pure from Bharat Pulverising Mills Pvt.Ltd., Bombay) and fenvalerate (Cyano(3-phenoxyphenyl)-methyl 4-chloro-α-(1-methylethyl) benzeneacetate, 93.7% pure from Rallis India Ltd.) were prepared in acetone. Chromatographically pure PNP (JT Baker Chemical Co., Phillipsburg, NJ), MNP (Sigma Chemical Co., St. Louis, US), DNP (Sarabhai Chemicals Ltd., Baroda) and catechol (Bioorganics, Madras) were used to obtain aqueous stock solutions.

The test compounds were added to aliquots of Chu-10 medium taken in test tubes (25- x 150-mm) to provide different concentrations ranging between 5 and 100 ug ml^{-1} as outlined earlier (Megharaj et al. 1986a,b, 1987a). Cell suspension, obtained by breaking the filaments of exponentially growing culture of N. linckia using sterilized glass beads, was inoculated into each tube. Untreated but inoculated samples served as controls. The culture tubes were incubated at room temperature (28 ± 4°C) in a growth chamber in slanted position under continuous fluorescent illumination (200 uE m^{-2} sec^{-1} PPFD). Triplicate samples were withdrawn after 20 days of inoculation for the determination of total nitrogen by micro-Kjeldahl method (Jackson 1971). The amount of nitrogen, if any, present in the chemicals was deducted from the total N in order to determine the quantities of nitrogen fixed during the incubation period.

RESULTS AND DISCUSSION

The effect of graded concentrations of insecticides and phenolics, when supplemented in the nitrogen-free Chu-10 medium, on diazotrophy of N.linckia was recorded in relation to controls. The ability of the cyanobacterium to fix atmospheric nitrogen was affected to varying degrees under the influence of the selected insecticides and phenolic compounds.

Monocrotophos, at all concentrations tested, significantly inhibited nitrogen fixation (Table 1). According to an earlier report (Megharaj et al. 1986a), monocrotophos, even up to 50 ug ml^{-1}, exhibited a pronounced stimulation in the growth of N.linckia. Treatment with quinalphos, at 5 and 10 ug ml^{-1}, also inhibited the nitrogen-fixing activity; the higher concentrations (above 20 ug ml^{-1}) were proved to be lethal to the organism. The above concentrations thus affected the nitrogen-fixing capacity greatly although it has been reported recently that N.linckia significantly metabolized both monocrotophos and quinalphos when supplemented at 50 and 10 ug ml^{-1} culture medium, respectively (Megharaj

et al. 1987b). Concentrations, up to 20 ug ml^{-1} ,of both cypermethrin and fenvalerate significantly stimulated nitrogen fixation. The synthetic pyrethroids at the above levels were also stimulatory to the growth of N.linckia (Megharaj et al. 1987a). Thus, the present data in relation to organophosphates alone conform to the generalization that growth response and nitrogen fixation in non-symbiotic nitrogen fixers (Tu 1978) and cyanobacteria (Prasad Reddy et al. 1984) are independent of each other.

Table 1. Nitrogen fixation by Nostoc linckia under the influence of insecticides

Concentration (ug ml^{-1})	Nitrogen fixed, in % of control, after 20 days			
	Monocrotophos	Quinalphos	Cypermethrin	Fenvalerate
5	75.2±16.1ab	87.9±6.7a	132.1±11.3a	128.9±8.8a
10	78.5±5.4a	71.8±9.4b	126.4±8.8a	111.3±7.6ab
20	78.5±4.0a	0[+]c	102.5±5.1b	102.5±5.0b
50	62.2±8.0bc	0c	93.7±6.3b	84.9±9.1c
100	50.3±5.4c	0c	n.d.	n.d.

[+]No growth
n.d. = not determined
Means (n=3) in each column followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's new multiple range (DMR) test

All the nitrophenols (PNP, MNP and DNP), even at 5 ug ml^{-1} level, significantly inhibited nitrogen-fixing capacity of N.linckia (Table 2). Catechol, only at 5 ug ml^{-1}, stimulated nitrogen fixation. However, in all cases, nitrogen-fixing capacity of the cyanobacterium decreased with increasing concentrations of the phenolic compounds. It is of particular interest to note that the present observation on the capacity of N.linckia to reduce dinitrogen in the presence of four phenolics supports our earlier report on their toxicity to the growth of this organism (Megharaj et al. 1986b), clearly suggesting that the growth and nitrogen fixation are equally affected.

Table 2. Nitrogen fixation by Nostoc linckia as influenced by phenolic compounds

Phenolic compound	Concentration (ug ml^{-1})	Nitrogen fixed, in % of control, after 20 days
PNP	5	74.1±10.5bc
	20	76.6±7.0b
	50	0[+]e
MNP	5	65.2±4.0cd
	10	58.2±6.0d
	20	0e
DNP	5	81.1±5.0b
	20	72.1±8.1bc
	50	0e
Catechol	5	104.5±7.1a
	20	67.2±4.0cd
	50	0e

[+]No growth

Means (n=3) in each column followed by the same letter are not significantly different ($p \leq 0.05$) according to DMR test

The difference in sensitivity of algae to insecticides was attributed to the nature of organism and their ability to accumulate and metabolize insecticides (Kar and Singh 1979). However, it would seem from the present findings that different toxicants might react differently with regard to the nitrogenase activity even in a single species of cyanobacteria, vitally implicated in the natural process of increasing the nitrogen content of soil by virtue of their capacity to fix atmospheric nitrogen. The effect of different insecticides and phenolics on the nitrogen-fixing capability of blue-green algae is, therefore, considered to be significant when these chemicals are applied heavily to control insect pests or accumulated in large amounts in soil.

Acknowledgment. The senior author is grateful to the Council of Scientific & Industrial Research, New Delhi, for financial assistance.

REFERENCES

DaSilva EJ, Henricksson LE, Henricksson E (1975) Effect of pesticides on blue-green algae and nitrogen fixation. Arch Environ Contam Toxicol 33:193-204

Jackson ML (1971) Soil chemical analysis. Prentice-Hall of India Ltd., New Delhi

Kar S, Singh PK (1979) Effects of nutrients on the toxicity of pesticides carbofuran and hexachlorocyclohexane to blue-green alga Nostoc muscorum. Z Allg Mikrobiol 19:467-472
Megharaj M, Venkateswarlu K, Rao AS (1986a) Growth response of four species of soil algae to monocrotophos and quinalphos. Environ Pollut A42:15-22
Megharaj M, Venkateswarlu K, Rao AS (1986b) The toxicity of phenolic compounds to soil algal population and to Chlorella vulgaris and Nostoc linckia. Plant Soil 96:197-203
Megharaj M, Venkateswarlu K, Rao AS (1987a) Influence of Cypermethrin and fenvalerate on a green alga and three cyanobacteria isolated from soil. Ecotoxicol Environ Saf 14:(in press)
Megharaj M, Venkateswarlu K, Rao AS (1987b) Metabolism of monocrotophos and quinalphos by algae isolated from soil. Bull Environ Contam Toxicol 39:(in press)
Prasad Reddy BV, Dhanaraj PS, Narayana Rao VVS (1984) Effects of insecticides on soil microorganisms. In:Lal R (ed) Insecticide microbiology. Springer Verlag, Berlin, pp 169-202
Round FE (1965) The biology of algae. Edward Arnold (publs.) Ltd., London
Tu CM (1978) Effect of pesticides on acetylene reduction and microorganisms in a sandy loam. Soil Biol Biochem 10:451-456
Watanabe A, Yamamoto Y (1971) Algal nitrogen fixation in the tropics. Plant Soil (spl vol) pp 403-413
Received October 13, 1987; accepted March 9, 1988.