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Effect of Carbofuran, Carbaryl, and Their Metabolites on the Growth of *Rhizobium* sp. and *Azotobacter chroococcum*

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Rhizobia and Azotobacter play a conspicuous role in nitrogen cycle of the soil. Seeds of legume crops are coated with rhizobial cells in association with insecticides and fungicides to have effective nodulation and seed protection. In addition, rhizobial and azotobacter cells may be exposed to pesticides and their metabolites present in the soil. Carbamate insecticides, carbofuran and carbaryl are widely used for crop protection. Carbofuran is metabolized to 3-hydroxycarbofuran and 3-keto-carbofuran, carbaryl to 1-naphthol in soil (Rajagopal et al. 1984) and these metabolic products are known to effect some biological processes more than parent compounds (Lee 1976; Bollag and Liu 1971). The present investigation was aimed at studying not only the effects of carbofuran and carbaryl but also their metabolites viz. 3-hydroxycarbofuran, 3-ketocarbofuran and 1-naphthol on the growth of Rhizobium sp. and Azotobacter chroococcum in liquid cultures.

MATERIALS AND METHODS

Rhizobium sp. was isolated from healthy root nodules of mung plants on yeast extract mannitol agar. Rhizobium sp. was later transferred to synthetic liquid medium with sodium glutamate (1.1 g/l) as nitrogen source (Bergersen 1961) for studying the effect of chemicals. A. chroococcum was isolated on nitrogen-free medium from Trombay Experimental Field Station soil according to the procedure described by Clark (1965). The effect of chemicals on A. chroococcum was studied both in Clark's nitrogen -free and nitrogen-containing culture media.

Carbofuran, 3-hydroxycarbofuran, 3-ketocarbofuran, carbaryl and 1-naphthol dissolved in alcohol were added separately to 50 ml liquid culture medium in 150 ml Erlenmeyer flask to obtain normal field application rate and 10-times field application rate. Untreated controls received alcohol only. The ethanolic content of liquid culture medium was 0.5%. Bacterial cells grown overnight were inoculated into treated and untreated liquid culture media and incubated at 28°C on rotary shaker

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(100 rev/min). Each treatment was replicated twice. At each sampling period, aliquots of culture media were removed aseptically. The growth was determined turbedimetrically by measuring optical density (O.D.) in Spectronic 20 spectrometer at 420 and 540 nm for Rhizobium sp. and A. chroococcum respectively.

RESULTS AND DISCUSSION

Carbofuran, carbaryl or their metabolites at normal and 10-times the normal field application rate had no effect on the growth of Rhizobium sp. (Table 1). Different authors reported both

Table 1. Effect of carbofuran, carbaryl and their metabolites on the growth of Rhizobium sp.

Treatment	ppm	Growth (O.D.) Hours incubation			
		8	12	20	36
Untreated	-	0.19	0.43	1.00	1.50
Carbofuran	0.5	0.25	0.49	0.95	1.60
	5.0	0.23	0.45	0.95	1.60
3-hydroxy-	0.5	0.22	0.44	0.97	1.60
carbofuran	5.0	0.22	0.42	1.00	1.60
3-keto-	0.5	0.23	0.47	1.00	1.50
carbofuran	5.0	0.23	0.45	1.00	1.60
Carbaryl	2.5	0.20	0.47	1.00	1.70
	25.0	0.19	0.49	1.10	1.60
1-naphthol	2.5	0.19	0.48	1.10	1.60
	25.0	0.21	0.47	0.90	1.80

innocuous and inhibitory effects of carbofuran and carbaryl on different strains of rhizobium and these aspects had been reviewed (Rajagopal et al. 1984). Carbofuran had no effect on the growth of R. melilotii and R. japonicum but inhibited the growth of R. leguminosarum and R. trifolii (Lin et al. 1972). Different strains of rhizobia isolated, and R. trifolii developed resistance to carbaryl (50 ppm) after serial transfers and the resistance developed was stable (Gupta and Shirkot 1981). It appears that sensitivity of rhizobia to carbofuran and carbaryl varies with species and strain.

The growth of A. chroococcum in nitrogen-containing culture medium was not affected by carbofuran and 3-hydroxycarbofuran at 0.5 and 5 ppm concentrations (Table 2). 3-Ketocarbofuran had no effect at 0.5 ppm, however, at 5 ppm the growth of A. chroococcum was delayed and normal growth comparable to untreated controls reached by 48 h incubation. There was no change in the growth of A. chroococcum with carbaryl or 1-naphthol

at 2.5 ppm. However, there was a delayed growth of bacterium at 25 ppm of both the chemicals.

Table 2. Effect of carbofuran, carbaryl and their metabolites on the growth of A. chroococcum in nitrogen-containing culture medium.

Treatment	ppm	Growth (O.D.) Hours incubation			
		10	20	24	48
Untreated	-	0.01	0.09	0.28	0.30
Carbofuran	0.5	0.01	0.13	0.31	0.30
	5.0	0.01	0.11	0.29	0.29
3-hydroxycarbofuran	0.5	0.01	0.09	0.29	0.28
	5.0	0.01	0.06	0.26	0.31
3-ketocarbofuran	0.5	0.01	0.06	0.23	0.30
	5.0	0.01	0.02	0.02	0.32
Carbaryl	2.5	0.01	0.09	0.29	0.30
	25.0	0.01	0.03	0.10	0.36
1-naphthol	2.5	0.01	0.11	0.29	0.30
	25.0	0.01	0.04	0.12	0.27

Table 3. Effect of carbofuran, carbaryl and their metabolites on the growth of A. chroococcum in nitrogen-free culture medium.

Treatment	ppm	Growth (O.D.) Hours incubation		
		24	36	48
Untreated	-	0.06	1.10	4.40
Carbofuran	0.5	0.05	0.37	0.72
	5.0	0.04	0.27	0.44
3-hydroxycarbofuran	0.5	0.12	0.46	0.55
	5.0	0.04	0.18	0.37
3-ketocarbofuran	0.5	0.10	0.65	0.64
	5.0	0.05	0.26	0.22
Carbaryl	2.5	0.04	0.45	1.00
	25.0	-	0.06	0.45
1-naphthol	2.5	0.08	0.79	2.45
	25.0	0.03	0.20	1.00

The growth of A. chroococcum in nitrogen-free culture medium was inhibited by carbofuran, 3-hydroxycarbofuran and 3-keto-carbofuran and the inhibition was more with higher concentration

(Table 3). Carbaryl and 1-naphthol were both inhibitory to the growth of A. chroococcum, the effect being more prominent with carbaryl. The sustained inhibitory effect of carbofuran, carbaryl and their metabolites on the growth of A. chroococcum in nitrogen-free culture medium as compared to nitrogen-containing culture medium indicate that these chemicals may be interfering with the nitrogen-fixing mechanisms of the bacterium. The toxic effect of carbofuran and carbaryl on the growth of A. chroococcum and A. vinelandii had been reported (Rodell et al. 1977; El-Houseiny et al. 1985). Our investigations indicate that among the metabolites, 3-ketocarbofuran is more inhibitory than the parent compound. However, the impact of these metabolites in a soil milieu on the activities of Azotobacter is dependent on the extent of the transformation of the parent compounds in soil.

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