Influence of Insecticides on Microbial Transformation of Nitrogen and Phosphorus in Typic Orchragualf Soil

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Four insecticides, viz., BHC, phorate, carbofuran, and fenvalerate, were applied at the rate of 7.5, 1.5, 1.0, and 0.35 kg a.i. ha^{-1}, respectively, to investigate their effects on the growth and activities of N_2-fixing and phosphate-solubilizing microorganisms in relation to the availability of N and P in laterite (Typic Orchragualf) soil. Insecticides in general, and BHC and phorate in particular, stimulated the proliferation of aerobic nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms and also their biochemical activities, such as nonsymbiotic N_2-fixing and phosphate-solubilizing capacities, which resulted in greater release of available N (NH_4^+ and NO_3^-) and P in soil. All the insecticides were persistent in soil for a short period of time, and the rate of dissipation was highest for fenvalerate followed by phorate, carbofuran, and BHC, depicting the half-lives ($T_{1/2}$) 8.8, 9.7, 16.9, and 20.6 days, respectively. The insecticides followed first-order reaction kinetics during their dissipation in soil.

Keywords: *Insecticides; microorganisms; N_2-fixation; phosphate solubilization; insecticides residues; soil*

INTRODUCTION

Microorganisms are scavengers in soil. Due to physiological variability, they degrade a great variety of chemical substances including insecticides in soil to derive energy and other nutrients for their growth and metabolism (El-Shahaat et al., 1987; Bhuyan et al., 1993). As a result, the population density of the active microorganisms increases which favorably influences the biological transformation of nutrient elements in soil (Rangaswamy and Venkateswarlu, 1993; Jana et al., 1998). There are many reports regarding the favorable effects of insecticides on the growth and activities of microorganisms in soil (Simon-Sylvestre and Fournier, 1979; Agnihotri et al., 1981; Das et al., 1995). On the other hand, there are some insecticides which exert adverse effect on the growth of soil microorganisms (Moorman, 1989; Martinez-Toledo et al., 1992). However, no definite conclusion can be made on the effect of different insecticides on the growth and activities of microorganisms in soil, since different groups of insecticides exhibit manifold variations in toxicity (Matsumura and Boush, 1971). Keeping the above reports in view, an experiment was conducted in the laboratory (condition) with four insecticides, representing one each from chlorinated hydrocarbon, organophosphate, carbamate, and synthetic pyrethroid groups of insecticide. The selected insecticides were BHC (1,2,3,4,5,6-hexachlorocyclohexane), phorate (*O*,*O*-diethyl-*S*-ethylthiomethyl dithiophosphate), carbofuran (2,2-dimethyl-2,3-dihydrobenzofuranyl-7-*N*-methyl carbamate), and fenvalerate [(*RS*)-cyano (3-phenoxyphenyl) (*RS*)-methyl-4-chloro-α-(1-methylethyl) benzene acetate] and were applied at their recommended field application rates in a *Typic Orchragualf* soil. The objective of the experiment was to investigate the influence of these insecticides on the growth and activities of nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms in relation to the availability of N and P, and also the persistence of insecticidal residues in soil.

MATERIALS AND METHODS

Experimental Methods. Four insecticides, viz., BHC, phorate, carbofuran, and fenvalerate, at the rate of 7.5, 1.5, 1.0, and 0.35 kg a.i. ha^{-1} in the form of 50% wettable powder, 10% granular, 3% granular, and 20% emulsifiable concentrate respectively, were thoroughly mixed with 1 kg of air-dried and sieved laterite soil (≤2 mm) collected from the top soil layer (0−10 cm) of the farm of Vidyasagar University, Midnapore, India. The soil was classified (USDA) as Typic Orchragualf, and the general characteristics are presented in Table 1. The soils treated with individual insecticides were placed separately in earthenware pots, and the water content of the soil was adjusted to 60% of water-holding capacity. To prevent the photodegradation of insecticides in soil, the pots were kept covered with a black polyethylene sheet and were incubated in the dark at 30 °C ± 1 °C for 60 days. All the treatments were replicated three times. During incubation, soil samples were collected at periodic intervals from the replicated pots of each treatment and were analyzed immediately.

Enumeration of Microbial Population in Soil Samples. Soil samples were analyzed to enumerate the colony-forming units (cfu) of aerobic nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms following the serial dilution technique and pour plate method (Salle, 1973). For N_2-fixing bacteria, N-free sucrose−calcium carbonate agar, and for phosphate-solubilizing microorganisms, sucrose−tricalcium phosphate agar media were selected for their growth and development (Das and Mukherjee, 1994). The agar plates were incubated at 30 °C ± 1 °C for 7 days, and the cfu on the respective agar plates were counted.

Estimation of Microbiological Activities. The nonsymbiotic N_2-fixing and phosphate-solubilizing capacities of the soils were determined following the methods of Debnath et al. (1994). Nonsymbiotic N_2-fixing capacity was determined by

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Table 1. General Characteristics of the Soil

type of soil	laterite *(Typic Orchragualf)*
sand (%)	44.4
silt (%)	15.0
clay (%)	40.6
water holding capacity (%)	42.0
pH (1:2.5 w/v) in water	5.4
EC[a] (dS m^{-1})	0.33
CEC[b] [cmol(p^+) kg^{-1}]	4.45
organic carbon (g kg^{-1})	2.35
total nitrogen (g kg^{-1})	0.22
C:N ratio	10.68
NH_4^+-N (mg kg^{-1})	22.9
NO_3^--N (mg kg^{-1})	7.1
available P (mg kg^{-1})	1.2
aerobic nonsymbiotic N_2-fixing bacteria (cfu × 10^4 g^{-1} soil)[c]	20.3
phosphate-solubilizing microorganisms (cfu × 10^4 g^{-1} soil)	48.4
nonsymbiotic N_2-fixing capacity (mg g^{-1} soil)	2.3
phosphate-solubilizing capacity (μg g^{-1} soil)	41.3

[a] EC, electrical conductivity. [b] CEC, cation exchange capacity. [c] cfu, colony forming unit.

estimating total N in 50 mL of N-free sucrose−calcium carbonate broth containing 2% sucrose after incubating 1 g of soil in conical flasks at 30 °C ± 1 °C for 15 days (Bremner, 1982; Das and Mukherjee, 1994). Phosphate-solubilizing capacity was determined by estimating soluble P in 15 mL of sucrose−tricalcium phosphate broth containing 1% sucrose after incubating 1 g of soil in culture tubes at 30 °C ± 1 °C for 15 days (Olsen and Dean, 1982; Das and Mukherjee, 1994).

Chemical Analysis. Available N (NH_4^+, NO_3^-) of the soil samples was determined in a potassium chloride extract following the method of Jackson (1973). Available P of the soil was estimated in sodium bicarbonate extract following chloromolybdic acid−stannous chloride method (Olsen and Dean, 1982).

Analysis of Insecticidal Residues. Soil samples were also analyzed for the presence of the residues of BHC, phorate, carbofuran, and fenvalerate following the method as outlined by Das et al. (1995). The residue data were processed to calculate the half-lives ($T_{1/2}$) following the method of Hoskins (1961).

Statistical Analysis. The results were evaluated by analysis of variance (ANOVA), and the statistical significance ($P = 0.05$) of difference between means within factors (insecticides and incubation time) was evaluated using Fisher's protected LSD method (Peterson, 1994).

RESULTS AND DISCUSSION

Effect on N_2-Fixing Bacteria and P-Solubilizing Microorganisms. Incorporation of insecticides, in general, significantly stimulated the growth of aerobic nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms in soil (Table 2). This supports the reports of earlier workers (Jena et al., 1987; Das and Mukherjee, 1994). Among the insecticides, BHC followed by phorate stimulated the microbial population to the highest extent. Similar observations with chlorinated hydrocarbon and organophosphate insecticides on N_2-fixing bacteria and phosphate-solubilizing microorganisms were recorded by earlier workers (Agnihotri et al., 1981; Ogunseitan and Odeymi, 1985). It was also revealed that the growth of N_2-fixing bacteria was progressively increased up to 15th day under BHC and carbofuran and up to 30th day under phorate and fenvalerate. Similar response was also recorded for phosphate-solubilizing microorganisms up to 15th day under BHC and phorate and up to 30th day under carbofuran and fenvalerate. This gradual increase in the population of N_2-fixing and phosphate-solubilizing microorganisms in soil indicated that these microorganisms were able to utilize the insecticides and/or their degradation products for growth and metabolism (El-Shahaat et al., 1987; Bhuyan et al., 1993).

Effect on N_2-Fixing and P-Solubilizing Capacities. The microbial activities, such as nonsymbiotic N_2-fixing and phosphate-solubilizing capacities of soil (Table 3), were changed concomitantly with the growth of aerobic nonsymbiotic N_2-fixing bacteria and phosphate-solubilizing microorganisms (Table 2), respectively. Incidentally, there was a significant positive correlation ($r = 0.965$) between N_2-fixing bacteria and N_2-fixing capacity. Similar observation was also recorded ($r = 0.984$) for phosphate-solubilizing microorganisms and phosphate-solubilizing capacity of soil. The stimulating influence of different insecticides on N_2-fixation and phosphate solubilization in soil was in accord with previous reports (Nayak and Rao, 1982; Das and Mukherjee, 1994). The higher fixation of atmospheric N_2 and solubilization of insoluble phosphates in soil under phorate and carbofuran on 15th day, and under BHC and fenvalerate on 30th day, pointed out greater microbial activities in soil due to the assimilation of the insecticides for their growth and development. Among the insecticides, BHC followed by phorate stimulated microbial activities to the highest extent.

Effect on Available N and P. The stimulation of growth and activities of N_2-fixing bacteria resulted in greater availability of N in soil (Table 4). The applied insecticides also induced the growth and activities of both ammonifying and nitrifying bacteria which were responsible for the mineralization of organic N to NH_4^+ and oxidation of NH_4^+ to NO_3^-, respectively, resulting in greater accumulation of mineral N (NH_4^+ and NO_3^-) in soil. This was in agreement with the earlier reports (Moorman, 1989; Rangaswamy and Venkateswarlu, 1993). In general, the availability of NH_4^+ nitogen was gradually decreased while that of NO_3^- was progressively increased. Moreover, the soils retained higher amounts of NH_4^+ N than NO_3^- N, indicating that the process of ammonification was faster than that of nitrification (Jana et al., 1998). Among the insecticides, BHC and phorate released more amounts of available N as compared to carbofuran and fenvalerate.

The availability of soluble P in soil treated with different insecticides was significantly increased (Table 4). This pointed out that insecticides in general, and BHC and phorate in particular, augmented the growth and activities of phosphate-mineralizing/solubilizing microorganisms with the resultant increase in available P in soil (Arora and Gaur, 1979). The incessant increase of available P in soils treated with BHC and phorate up to 15th day and with carbofuran and fenvalerate up to 30th day indicated greater stimulation of microbial activities due to the utilization of the insecticides as well as their degraded products for their cellular metabolism (Rajagopal et al., 1984).

Persistence of Insecticides. The persistence of different insecticides in soil was not the same (Table 5). The rate of dissipation was highest with fenvalerate followed by phorate, carbofuran, and BHC, depicting the half-lives ($T_{1/2}$) of 8.8, 9.7, 16.9, and 20.6 days, respectively. This was in agreement with the reports of Agnihotri et al. (1986) and Das et al. (1995). As microbial degradation is the main cause of dissipation of insecticidal residues in soil, it was clear from the results that soil microorganisms degraded fenvalerate,

Table 2. Influence of Insecticides on the Population of Microorganisms in Soil

	sampling days					
treatments	5	15	30	45	60	mean
	Number of Nonsymbiotic N_2-Fixing Bacteria (cfu $\times 10^4$ g^{-1} soil)					
control	24.4 ± 3.1	26.4 ± 3.6	21.9 ± 3.3	22.9 ± 2.6	19.9 ± 2.3	23.1
BHC	27.9 ± 2.9	40.6 ± 5.2	35.9 ± 3.2	36.6 ± 4.7	30.3 ± 3.3	34.3
phorate	27.2 ± 2.3	36.2 ± 3.6	37.2 ± 4.5	32.8 ± 2.6	30.3 ± 2.1	32.7
carbofuran	22.5 ± 3.9	40.1 ± 4.9	32.1 ± 2.6	29.9 ± 2.1	23.7 ± 2.3	29.7
fenvalerate	22.9 ± 2.2	38.4 ± 3.2	39.9 ± 4.3	27.9 ± 4.2	25.4 ± 2.7	30.9
	LSD ($P = 0.05$) treatment 4.4; sampling date 4.4; interaction ns[a]					
	Number of P-Solubilizing Microorganisms (cfu $\times 10^4$ g^{-1} soil)					
control	61.7 ± 6.3	69.4 ± 4.7	78.6 ± 4.5	62.3 ± 3.8	50.4 ± 9.8	64.5
BHC	78.5 ± 4.2	117.9 ± 10.7	98.5 ± 9.2	94.5 ± 6.7	86.5 ± 5.8	95.2
phorate	77.7 ± 5.2	99.7 ± 8.4	82.1 ± 3.4	78.2 ± 6.8	74.1 ± 3.7	82.4
carbofuran	64.5 ± 4.6	82.1 ± 6.6	95.5 ± 5.7	67.9 ± 7.8	63.1 ± 3.2	74.6
fenvalerate	70.1 ± 4.6	84.7 ± 9.6	95.3 ± 3.9	77.9 ± 5.9	73.6 ± 3.6	80.3
	LSD ($P = 0.05$) treatment 8.2; sampling date 8.2; interaction ns					

[a] ns, not significant.

Table 3. Influence of Insecticides on the Activities of Microorganisms in Soil

	sampling days					
treatments	5	15	30	45	60	mean
	Amount of N_2-Fixed (mg g^{-1} soil)					
control	2.8 ± 0.3	3.0 ± 0.4	2.8 ± 0.2	3.2 ± 0.2	2.7 ± 0.2	2.9
BHC	3.3 ± 0.2	4.3 ± 0.4	4.5 ± 0.2	3.9 ± 0.4	3.5 ± 0.4	3.9
phorate	2.9 ± 0.1	4.2 ± 0.3	3.9 ± 0.3	3.6 ± 0.4	3.5 ± 0.1	3.6
carbofuran	2.9 ± 0.4	3.7 ± 0.3	3.4 ± 0.4	3.5 ± 0.3	3.2 ± 0.2	3.3
fenvalerate	2.9 ± 0.2	3.4 ± 0.2	3.9 ± 0.3	3.5 ± 0.2	3.4 ± 0.2	3.4
	LSD ($P = 0.05$) treatment 0.4; sampling date 0.4; interaction ns[a]					
	Amount of Phosphate Solubilized (μg g^{-1} soil)					
control	48.2 ± 3.2	68.3 ± 5.5	64.2 ± 4.3	46.4 ± 3.8	43.6 ± 4.2	54.1
BHC	69.1 ± 5.2	108.3 ± 5.5	113.4 ± 6.3	74.8 ± 4.8	60.9 ± 3.2	85.3
phorate	55.2 ± 4.3	96.4 ± 4.8	81.8 ± 4.5	73.6 ± 3.2	68.8 ± 4.1	75.2
carbofuran	50.5 ± 5.1	82.6 ± 5.5	79.6 ± 3.9	51.4 ± 2.3	48.2 ± 2.2	62.5
fenvalerate	48.9 ± 3.1	83.7 ± 2.9	88.6 ± 3.7	61.5 ± 3.3	55.4 ± 3.2	67.6
	LSD ($P = 0.05$) treatment 5.5; sampling date 5.5; interaction 12.3					

[a] ns, not significant

Table 4. Influence of Insecticides on the Availability of N and P in Soil

	sampling days					
treatments	5	15	30	45	60	mean
	Amount of NH_4^+-N (mg kg^{-1} soil)					
control	20.5 ± 2.1	18.4 ± 2.1	16.3 ± 1.5	15.6 ± 1.8	15.3 ± 1.7	17.2
BHC	31.8 ± 3.2	30.4 ± 3.1	26.2 ± 2.6	24.8 ± 2.4	23.3 ± 3.2	27.3
phorate	34.7 ± 3.5	28.3 ± 2.3	26.2 ± 2.5	24.8 ± 3.4	22.6 ± 2.5	27.3
carbofuran	23.3 ± 2.3	21.2 ± 1.8	19.1 ± 1.6	17.7 ± 2.1	17.7 ± 1.6	19.8
fenvalerate	23.3 ± 2.2	21.9 ± 1.6	21.2 ± 2.1	19.8 ± 1.9	19.8 ± 1.5	21.2
	LSD ($P = 0.05$) treatment 3.1; sampling date 3.1; interaction ns[a]					
	Amount of NO_3^--N (mg kg^{-1} soil)					
control	7.1 ± 1.2	8.5 ± 1.2	9.2 ± 2.1	9.2 ± 1.2	11.2 ± 1.1	9.0
BHC	9.9 ± 1.3	11.3 ± 1.1	12.2 ± 1.3	13.5 ± 1.1	14.2 ± 1.3	12.2
phorate	9.9 ± 1.1	11.3 ± 1.2	12.3 ± 1.1	12.7 ± 1.2	13.4 ± 1.5	11.9
carbofuran	8.4 ± 1.2	9.2 ± 1.2	10.3 ± 1.2	12.1 ± 1.1	12.8 ± 1.1	10.6
fenvalerate	9.2 ± 1.1	9.9 ± 1.2	11.3 ± 1.3	12.8 ± 1.1	12.8 ± 1.2	11.2
	LSD ($P = 0.05$) treatment 1.6; sampling date 1.6; interaction ns					
	Amount of Available P (mg kg^{-1} soil)					
control	0.9 ± 0.2	1.2 ± 0.2	1.3 ± 0.3	0.9 ± 0.2	0.7 ± 0.1	1.0
BHC	1.7 ± 0.2	2.8 ± 0.2	2.4 ± 0.3	1.9 ± 0.2	1.7 ± 0.2	2.1
phorate	1.7 ± 0.3	2.3 ± 0.3	1.9 ± 0.4	1.4 ± 0.2	1.1 ± 0.1	1.7
carbofuran	1.1 ± 0.2	1.2 ± 0.1	1.7 ± 0.2	1.1 ± 0.1	0.9 ± 0.2	1.2
fenvalerate	1.1 ± 0.1	1.5 ± 0.2	1.9 ± 0.2	1.4 ± 0.1	0.9 ± 0.1	1.4
	LSD ($P = 0.05$) treatment 0.3; sampling date 0.3; interaction ns					

[a] ns, not significant

phorate, and carbofuran more favorably as compared to BHC (El-Shahaat et al., 1987; Rache and Coats, 1988). The long persistence of BHC could be attributed to its resistance to biodegradation and/or its ability to form recalcitrant molecules in soil (Edwards, 1966; Kahlon et al., 1990; Kawano et al., 1992). The degradation of all the insecticides was very rapid up to 10th day and except for BHC and carbofuran, no insecticidal residues were detected after 45 days. BHC and carbofuran residues were detected up to 11.73 and 6.52%, respectively, even after 60 days of application of the insecticides in soil. The rate of dissipation of all the

Table 5. Persistence of Insecticide Residues in Soil

	BHC		phorate		carbofuran		fenvabrate	
sampling days	residues (mg kg^{-1} soil)	dissipation (%)	residues (mg kg^{-1} soil)	dissipation (%)	residues (mg kg^{-1} soil)	dissipation (%)	residues (mg kg^{-1} soil)	dissipation (%)
0 (1 h)	3.24 ± 0.07	0	0.69 ± 0.03	0	0.46 ± 0.02	0	0.16 ± 0.01	0
5	2.46 ± 0.03	24.07	0.41 ± 0.01	40.58	0.31 ± 0.01	32.61	0.011 ± 0.01	31.25
10	1.29 ± 0.02	60.19	0.23 ± 0.03	66.67	0.18 ± 0.01	60.87	0.07 ± 0.02	56.25
15	1.19 ± 0.03	63.27	0.21 ± 0.02	69.56	0.16 ± 0.03	65.21	0.05 ± 0.01	68.75
30	0.89 ± 0.03	72.53	0.11 ± 0.02	84.06	0.13 ± 0.01	71.74	0.02 ± 0.01	87.50
45	0.49 ± 0.02	84.88	0.02 ± 0.01	97.10	0.06 ± 0.01	86.96	0.004 ± 0.001	97.50
60	0.38 ± 0.03	88.27	ND[d]	-	0.03 ± 0.01	93.48	ND	-
DL[a]	0.01		0.002		0.01		0.002	
$T_{1/2}$[b] (days)	20.6		9.7		16.9		8.8	
r[c]	−0.961		−0.979		−0.978		−0.994	

[a] DL, detection limit. [b] $T_{1/2}$, half-life. [c] r, correlation coefficient. [d] ND, not detected.

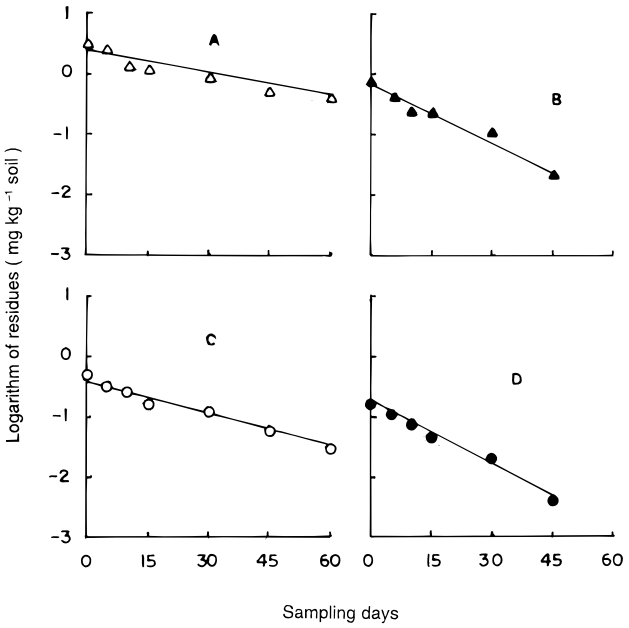

Figure 1. Linear graphs of first-order reaction kinetics of the insecticidal residues in soil. The linear graphs represent A(△,△), BHC; B(▲,▲), phorate; C(○,○), carbofuran; and D(●,●), fenvalerate.

insecticidal residues in soil followed first-order reaction kinetics (Figure 1).

The results of the present investigation thus clearly indicated that application of different insecticides in general, and BHC and phorate in particular, brought about significant stimulations of growth and activities of beneficial microorganisms which, in turn, transformed the nutrient elements into more available forms in soil. It was also revealed that the greater microbial activities led to the greater degradation of the insecticidal residues resulting in rapid dissipation in soil.

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