Bull. Environ. Contam. Toxicol. (1988) 41:556–562

Environmental Contamination and Toxicology

Effect of Phorate With and Without Amendments on Soil Microflora

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We had earlier reported the findings on persistence of phorate in soils with and without amendments and its effects on a Pseudomonas sp., in the soils (Venkatramesh et al. 1987). In this paper we report the effects of phorate on other groups of microflora such as fungi, non-symbiotic nitrogen fixers (Azotobacter) and total bacteria.

MATERIALS AND METHODS

Studies were conducted with four soils viz., red loam, laterite, black and alluvial. Details on soil characteristics and method of treatment were reported earlier (Venkatramesh et al. 1987). Phorate (Thimet 10G, Cyanamid India ltd.) was applied to the soils at 50 and 100 ppm a.i. The amendments used were ammonium sulphate (500 ppm-N) and groundnut oil (GN oil) cake powder (1.5 g).

The details on sampling and plating were reported earlier (Venkatramesh et al. 1987). Samples, in triplicate, were plated in appropriate media- Thornton's agar for bacteria and actinomycetes, Base Medium 77 for Azotobacter and Czapeck Dox agar for fungi. Colonies were counted seven days after incubation at room temperature (30°C). All the data were analysed statistically by the factorial method.

RESULTS AND DISCUSSION

The effect of phorate on soil bacteria varied between soils and the treatments recorded very slight variations (Table 1). The overall effect at the end of sixty days was stimulation ranging from 4 to 83% in red and laterite soils while in black soil there was a reduction of 9 to 11% and in alluvial soil there was an 8% increase at 50 ppm and 19% reduction at 100 ppm. This gives an

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interaction effect of 9% stimulation at 50 ppm and 12% stimulation at 100 ppm which, however, is not significant (Table 3). Amending the soils with ammonium sulphate along with phorate reduced bacterial growth in all the soils by 20 to 59% while GN oil cake stimulated growth by 365 to 1035%. All these variations are significant both over control as well as unamended soils where only 100 ppm phorate is present (Table 3).

Actinomycetes were inhibited by phorate in all the soils (Table 1). while in red and alluvial soils this inhibition was concentration dependent, in laterite and black soils the inhibition at 50 ppm was more than at 100 ppm. The inhibitory effect was significant over control in the case of the interactions (Tables 3 & 4). Ammonium sulphate amendment also significantly reduced actinomycete populations while GN oil cake had a stimulatory effect.

In the case of Azotobacter the effects varied between soils from a slight stimulation to a slight reduction. However, none of these variations were significant except the stimulation due to GN oil cake amendment (Tables 2 & 3).

Red and alluvial soils recorded lower fungal numbers (Table 2) in phorate treated soils and the inhibition was greater at higher concentration. Further, the inhibition in red soil at 100 ppm was statistically significant. In laterite and black soils there were both inhibitory and stimulatory effects which were not significant. However, from the interactions it is seen that 100 ppm phorate had significant inhibitory effect on soil fungi (Table 4). Ammonium sulphate amendment increased fungi in red soil significantly, whereas in other three soils there was an inhibition ranging from 12 to 59% which was, however, not significant. The stimulation due to GN oil cake amendment is significant only in red and black soils but not in laterite, while in alluvial soil, there is an inhibition.

Among the fungal species isolated A. niger alone had some treatment response. In black soil it was the only species that survived beyond 25 days and in alluvial and laterite soils, it was the only predominant species.

From this study it is seen that phorate had different effects on the different groups of soil microflora. However, statistical significance is seen only in the case of actinomycetes and fungi, that too for interactions only indicating that the type of of soil, pesticide concentration and time of sampling after treatment influence the e ffect of phorate on soil microflora. The results reported here are generally in accordance with those reported in the literature. Tu (1981) reported that bacteria were not affected by phorate while Sathpathy (1974) and Pandian and Balasubramanian (1978) reported the inhibitory effect of phorate on fungi. However, Pandian and Balasubramanian (1978) reported stimulation of actinomycetes while Kandasami et al. (1975) reported stimulation of Azotobacter. These conradictions may be due to the fact that while our results are for in vitro studies the others are from field trials. Similarly Kandasamy et al. (1975) reported an increase in

Table 1. Effect of Phorate on soil bacteria and Actinomycetes (Counts/g soil)

SOILS	TREATMENTS	COLONY COUNT DAYS AFTER INCUBATION*				
		3	10	25	40	60
RED	Control	125.8 (11.7)	58.0 (11.0)	57.5 (19.7)	51.0 (12.3)	60.1 (13.0)
	50 ppm	158.5 (7.0)	78.4 (8.0)	108.4 (13.4)	70.6 (11.5)	86.9 (16.0)
	100 ppm	228.6 (5.5)	131.4 (6.1)	114.3 (11.5)	70.2 (13.8)	101.7 (14.9)
	100 ppm + $(NH_4)_2SO_4$	75.6 (3.1)	23.1 (0.0)	12.3 (0.0)	5.3 (0.0)	27.5 (0.0)
	100 ppm + GN Oil Cake	2070.8 (81.8)	905.3 (49.2)	349.1 (3.7)	92.1 (0.0)	48.3 (0.0)
LATERITE	Control	187.4 (9.6)	188.7 (16.4)	185.7 (24.3)	181.5 (7.3)	146.0 (4.8)
	50 ppm	169.4 (6.7)	228.5 (6.6)	163.8 (14.2)	182.3 (8.9)	146.0 (6.8)
	100 ppm	163.8 (4.6)	220.3 (12.7)	201.9 (12.5)	199.3 (9.3)	136.4 (8.8)
	100 ppm + $(NH_4)_2SO_4$	193.2 (5.4)	210.9 (7.0)	175.2 (8.9)	89.5 (0.0)	42.1 (0.4)
	100 ppm + GN Oil Cake	2287.8 (121.3)	1779.7 (115.1)	845.5 (145.6)	664.2 (36.5)	489.4 (20.1)
BLACK	Control	52.1 (0.5)	51.1 (0.0)	42.5 (3.2)	55.9 (0.5)	39.5 (1.8)
	50 ppm	71.2 (0.0)	57.8 (0.0)	38.0 (0.0)	21.0 (0.0)	31.5 (0.4)
	100 ppm	71.2 (0.0)	85.2 (0.0)	33.4 (3.2)	42.5 (0.0)	32.5 (1.3)
	100 ppm + $(NH_4)_2SO_4$	65.8 (0.0)	52.6 (0.0)	22.0 (0.9)	11.0 (0.0)	19.1 (0.4)
	100 ppm + GN Oil Cake	843.1 (19.5)	1235.8 (52.1)	274.4 (18.3)	248.3 (1.9)	137.6 (4.4)
ALLUVIAL	Control	160.6 (2.3)	125.2 (1.8)	152.5 (32.7)	102.0 (0.9)	49.9 (0.5)
	50 ppm	172.6 (1.4)	136.6 (0.0)	129.4 (1.7)	130.2 (4.6)	68.5 (5.1)
	100 ppm	155.9 (0.5)	81.6 (0.5)	78.9 (0.4)	94.7 (2.3)	65.3 (0.0)
	100 ppm + $(NH_4)_2SO_4$	193.7 (1.4)	70.2 (0.0)	31.4 (0.4)	39.7 (1.9)	23.3 (0.0)
	100 ppm + GN Oil Cake	1185.8 (14.0)	820.7 (4.6)	226.6 (4.4)	309.1 (4.6)	205.1 (0.0)

* Average of three replicates

Bacteria : $X10^5$

Actinomycetes : $X10^5$ (Numbers within parentheses)

Table 2. Effect of Phorate on soil fungi and Azotobacter (Counts/g soil)

SOILS	TREATMENTS	COLONY COUNT DAYS AFTER INCUBATION*				
		3	10	25	40	60
RED	Control	66.2 (22.6)	23.1 (26.9)	53.1 (8.5)	30.7 (13.4)	53.8 (30.8)
	50 ppm	65.8 (21.8)	47.0 (17.1)	18.2 (21.5)	22.3 (11.9)	29.3 (15.2)
	100 ppm	97.0 (18.3)	47.7 (9.1)	76.5 (1.9)	40.3 (2.3)	16.0 (7.1)
	100 ppm + $(NH_4)_2SO_4$	86.5 (47.5)	12.5 (11.7)	6.3 (16.0)	12.3 (30.7)	1.9 (76.1)
	100 ppm + GN Oil Cake	954.1 (25.1)	757.6 (6.2)	48.3 (25.5)	122.7 (11.3)	289.5 (7.6)
LATERITE	Control	38.5 (12.6)	63.7 (9.0)	19.4 (3.2)	55.9 (0.8)	43.7 (1.6)
	50 ppm	51.0 (18.4)	71.5 (4.9)	43.7 (1.6)	55.1 (2.0)	23.7 (2.4)
	100 ppm	28.4 (10.5)	74.4 (3.7)	54.2 (3.6)	40.9 (2.0)	30.9 (2.4)
	100 ppm + $(NH_4)_2SO_4$	30.5 (6.3)	93.3 (15.2)	54.2 (1.6)	18.6 (0.4)	4.4 (0.4)
	100 ppm + GN Oil Cake	1417.8 (25.1)	1265.9 (6.2)	323.6 (25.5)	153.9 (11.3)	80.2 (7.6)
BLACK	Control	82.8 (6.3)	73.4 (3.3)	74.5 (3.7)	66.9 (1.0)	60.8 (2.7)
	50 ppm	119.9 (2.4)	103.2 (1.4)	120.3 (1.8)	67.3 (1.9)	63.5 (1.3)
	100 ppm	92.6 (3.9)	115.5 (9.0)	64.9 (4.1)	65.0 (2.4)	64.4 (2.2)
	100 ppm + $(NH_4)_2SO_4$	108.7 (5.4)	106.1 (3.3)	52.1 (1.4)	43.9 (0.0)	62.6 (1.3)
	100 ppm + GN Oil Cake	1023.4 (55.1)	1292.6 (23.2)	1303.2 (8.2)	434.6 (0.0)	332.9 (0.0)
ALLUVIAL	Control	55.6 (12.7)	104.1 (8.7)	55.3 (13.5)	53.6 (6.9)	65.3 (7.5)
	50 ppm	42.5 (15.1)	101.8 (8.7)	36.6 (7.0)	43.4 (2.8)	46.2 (4.2)
	100 ppm	44.8 (10.7)	98.6 (1.8)	40.5 (4.8)	57.3 (5.1)	42.4 (9.3)
	100 ppm + $(NH_4)_2SO_4$	56.0 (9.8)	44.0 (8.3)	34.0 (1.3)	42.9 (0.0)	56.9 (0.9)
	100 ppm + GN Oil Cake	1139.1 (9.8)	779.5 (11.6)	344.2 (10.9)	300.1 (3.7)	382.3 (2.3)

* Average of three replicates
Fungi : $X10^3$ (Numbers within parentheses)
Azotobacter : $X10^2$

Table 3. Two way tables for treatment x interval interactions

INTERVAL / TREATMENTS	3	10	25	40	60	TOTAL
1. Control	525.9	422.9	438.3	390.4	295.5	2073.0
50 ppm	571.8	501.3	439.6	404.0	332.9	2249.6
100 ppm	619.4	518.5	428.4	406.6	338.9	2311.8
100 ppm + $(NH_4)_2SO_4$	335.1	356.7	240.7	145.6	112.0	1190.1$^-$
100 ppm + GN Oil Cake	6387.4$^+$	4741.5$^+$	1695.5$^+$	1313.7$^+$	880.4	15018.5$^+$
2. Control	24.1	29.3	79.8	21.0	20.0	174.2
50 ppm	15.1	14.5	29.7$^-$	25.0	28.4	112.7$^-$
100 ppm	10.5	19.3	27.7$^-$	25.4	25.0	107.9$^-$
100 ppm + $(NH_4)_2SO_4$	10.0	7.8	10.3$^-$	1.9	0.8	30.7$^-$
100 ppm + GN Oil Cake	236.6	221.0	172.0$^+$	43.0	24.5	697.1$^+$
3. Control	243.1	264.3	202.4	207.0	223.6	1140.4
50 ppm	271.2	323.5	218.7	188.1	162.6	1164.1
100 ppm	262.8	336.2	236.1	203.4	153.6	1192.1
100 ppm + $(NH_4)_2SO_4$	281.7	255.9	146.6	117.8	125.7	927.7
100 ppm + GN Oil Cake	4534.4$^+$	4095.6$^+$	2019.3$^+$	1011.3$^+$	1084.9$^+$	12745.5$^+$

NOTE:

1. Bacteria
   $SE_{m+/-}$ = 234.1
   $CD_{0.05}$ for interval = 649.8
   $CD_{0.05}$ for treatment= 649.8
   $CD_{0.05}$ for treatment x interval= 469.3

2. Actinomycetes
   $SE_{m+/-}$ = 17.0
   $CD_{0.05}$ for soil = 54.0
   $CD_{0.05}$ for interval = 47.1
   $CD_{0.05}$ for treatment= 47.1
   $CD_{0.05}$ for treatment x interval= 36.0

3. Azotobacter
   $SE_{m+/-}$ = 166.7
   $CD_{0.05}$ for soil = 530.5
   $CD_{0.05}$ for interval = 462.8
   $CD_{0.05}$ for treatment= 462.8
   $CD_{0.05}$ for treatment x interval= 353.5

'-' indicates significant reduction over control and
'+' indicates significant stimulation over control

fungal numbers. Another interesting observation in the present study is that the microbial populations, though stimulated initially by GN oil cake amendment, were reduced after ten to twenty days. This could be due to the degradation of phorate and accumulation of phorate sulfoxide (Venkatramesh et al. 1987). This metabolite is

Table 4. Two way table for soil x treatment interactions

TREATMENTS / SOILS	CONTROL	50 PPM	100 PPM	100 PPM+ $(NH_4)_2SO_4$	100 PPM+ GN Oil	TOTAL
1. Red	67.6	55.8	51.7	3.9	134.7	313.7
Laterite	62.4	43.2	48.0	21.7	438.5	613.8
Black	5.9	0.9	4.5	1.4	96.2	108.9
Alluvial	38.3	12.9	3.7	3.7	27.6	86.2
Total	174.2	112.8	107.9	30.7	697.0	
2. Red	102.3	87.5	38.6	182.0+	457.0+	867.4
Laterite	27.2	29.4	22.2	23.9	75.7	178.4
Black	16.9	8.9	21.6	11.4	86.5+	145.3
Alluvial	49.3	37.8	31.8	20.3	38.3	177.5
Total	195.7	163.6	114.2-	237.6	657.5+	

NOTE:

1. Actinomycetes $CD_{0.05}$ for soil x treatment = 37.0
2. Fungi $CD_{0.05}$ for interval = 60.6
   $SE_{m+/-}$ = 21.8 $CD_{0.05}$ for treatment = 60.6
   $CD_{0.05}$ for soil x treatment = 71.1
   $CD_{0.05}$

'-' indicates significant reduction over control and
'+' indicates significant stimulation over control

known to be toxic to microorganisms (Le Patourel and Wright 1976). These authors also reported the degradation of phorate by A. niger in pure culture and it is seen in this study that A. niger was not affected by the accumulation of phorate sulfoxide.

The addition of fertilizer amendments influenced the microbial populations significantly and also influenced the effect of phorate on them. There are very few studies on the interaction of pesticides with fertilizers and the effect of such interactions on the soil microflora. Based on the findings reported here it appears logical to strongly recommend such studies considering the high fertilizer inputs in modern farm practices.

Acknowledgment. MVR is grateful to Dr. M.S. Mithyantha, Dr. S.C. Tripathi and Dr. J.C. Edward, Rallis Agrochemical Research Station, Bangalore for their valuable advise during the course of this work.

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Received November 24, 1987, Accepted March 25, 1988.