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Effects of Some Pesticides on Enzyme Activities in an Organic Soil

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Pesticides are used widely to control a variety of soil pests and often residues of these pesticides or their metabolites are present in soils (DECKER et al. 1965, DUFFY & WONG 1967, TU & MILES 1976). Most studies indicate that pesticides have limited effects on microbial activities related to soil fertility (BOLLEN 1961, TU & MILES 1976). Pesticide effects on soil enzymatic activities have so far received little attention. This paper reports on the effect of some pesticides on the activities of three enzymes, dehydrogenase, phosphatase and urease in an organic soil.

MATERIALS AND METHODS

A well-drained organic soil was collected in early spring from a fallow field and screened through a 2-mm sieve. The soil had the following physical and chemical characteristics: organic carbon 26.8%; Kjeldahl nitrogen 1.94%; pH 7.2; moisture holding capacity 172%, as determined by methods described previously (TU 1970, TU & BOLLEN 1968).

Tests were conducted with 32 pesticides (Table 1). The pesticides used were a minimum of 94% purity. With exception of the fumigants, DD, Telone, Telone C, Telone II and Vorlex, the required amounts of pesticides were applied to the soil using a carrier sand as reported elsewhere (TU 1970). Fumigants were injected directly into the soil. Soil samples treated with streptomycin, p-benzoquinone, $HgCl_2$ or autoclaving were prepared to compare their effects on enzyme activity with those of the pesticides. The activity of soil dehydrogenase was determined by incubating the soil samples at 28°C in a system containing 2,3,5-triphenyltetrazolium chloride (TTC). Formation of 2,3,5-triphenyltetrazolium formazan (TTF) by reduction of TTC was measured using a Coleman model 9 nephocolorimeter at 470 nm (CASIDA et al. 1964). Phosphatase activities were assessed by hydrolysis of p-nitrophenyl disodium orthophosphate (TABATABAI & BREMNER 1969) and urease was appraised by NH_3 evolution resulting from hydrolysis of urea employing a steam distillation method (BREMNER & KEENEY 1966). Soil samples without substrates were included to measure eudogenous formation of enzymatic reaction products. All data were expressed on the oven-dry basis as means of triplicate samples.

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TABLE 1

Effect of different treatments on activity of dehydrogenase.

Treatment	Rates of Application (µg/g)		mg TTF/g soil			
			Low treatment rate		High treatment rate	
			Incubation period (week)			
			1	2	1	2
Control	0	0	9.0	9.5	9.0	9.5
Autoclaving	0	ND**	79.7*	113.7*	ND	ND
Streptomycin	100	200	9.3	13.1*	7.1	12.2*
ρ - Benzoquinone	50	ND	7.0*	9.3	ND	ND
$HgCl_2$	7000	ND	4.0*	6.8	ND	ND
Chlorfenvinphos	5	10	10.3	14.3*	9.8	11.6*
Chlorpyrifos	5	10	10.8	14.7*	9.7	12.6*
Diazinon	5	10	7.9	12.3*	6.4*	10.2
Ethion	5	10	8.1	11.4*	6.9*	9.5
Ethoprop	5	10	9.4	13.0*	6.1*	10.5
Fensulfothion	5	10	8.1	8.1	7.4	11.7*
Fonofos	5	10	8.9	10.8	7.8	7.4
Leptophos	5	10	8.7	12.2*	5.6*	8.5
Malathion	5	10	8.9	8.6	7.6	11.5*
Parathion	5	10	8.3	8.7	8.1	11.0
Phorate	5	10	8.0	9.5	7.9	11.0
Terbufos	5	10	7.3*	11.8*	6.0*	9.1
Thionazin	5	10	8.6	9.6	7.9	11.8*
Triazophos	5	10	7.3*	12.0*	6.7*	9.0
Trichloronat	5	10	7.2*	11.3*	6.6*	10.1
Chlordane	5	10	8.4	11.8*	6.7*	8.3
Dieldrin	5	10	10.3	15.6*	8.9	8.5
Lindane	5	10	8.9	10.5	7.1	9.5
Carbofuran	5	10	9.3	11.3*	8.6	9.6
Metalkamate	5	10	8.9	11.0	8.5	10.9
Oxamyl	5	10	9.6	12.0*	7.9	10.3
Permethrin	5	10	9.4	10.9	8.6	12.7*
DD[a]	150	300	5.3*	8.8	5.3*	8.5
Telone[b]	30	60	5.7*	9.9	4.6*	9.6
Telone-C[c]	30	60	5.0*	8.4	4.0*	7.1
Telone-II[d]	30	60	5.3*	10.0	4.7*	8.4
Vorlex[e]	40	80	8.4	9.6	5.1*	8.8
Captan	5	10	11.2	12.4*	8.9	11.9*
Maneb	5	10	13.0*	17.7*	8.1	12.9*
Thiram	5	10	9.6	9.8	7.5	9.6
2,4-D	5	10	10.6	14.7*	8.7	11.2
Nitrapyrin	30	60	3.9*	8.6	3.4*	6.9

* significantly different from control at p = 0.05.

** ND = not determined.

a. 1,3-dichloropropene,1,2-dichloropropane and related C_3 hydrocarbons mixture.

b. 1,3-dichloropropene and related C_3 hydrocarbons.

c. 1,3-dichloropropene and related C_3 hydrocarbons 85% and chloropicrin 15%.

d. 1,3-dichloropropene 92% and related C_3 hydrocarbons.

e. 1,3-dichloropropene and related C_3 hydrocarbons 80%, and methyl isothiocyanate 20%.

RESULTS AND DISCUSSION

There were increases in dehydrogenase and urease activities after 2 weeks' incubation of untreated control soils (TABLES 1 and 3). The soil dehydrogenase system probably consists of different enzymes or enzyme systems which have a role in the initial stages of oxidation of soil organic matter. With exception of Vorlex, all fumigants, terbufos, triazophos, trichloronat and nitrapyrin inhibited dehydrogenase activity at both concentrations tested after 1 week (TABLE 1). ρ-Benzoquinone and $HgCl_2$ treatments were also inhibitory at that time. Formazan formation was significantly greater with chlorfenvinphos, chlorpyrifos, captan, maneb and streptomycin after 2 weeks. A similar response was observed with diazinon, ethion, ethoprop, leptophos, terbufos, triazophos,

trichloronat, chlordane, dieldrin, carbofuran, oxamyl and 2,4-D at the low concentration, and fensulfothion, malathion, thionazin and permethrin at the high concentration. A significantly greater amount of formazan production was noted in the autoclaved soil. Autoclaving releases organic constituents into the soil which reduce the TTC. The sources of TTC reducing substances are not clear, but could be due to the rupture of soil organic matter, with a release of the fixed constituents during the very rapid heating (KANG & SAJJAPONGSE 1980, LYON & BIZZELL 1910, PAUL & TU 1965). The chemical reaction taking place on reduction of TTC in heat treated organic soil was far in excess of the effect of the pesticide treatments.

The activity of phosphatase reported in Table 2, as indicated by the release of ρ-nitrophenol, is an index of the activity of microflora involved in soil organic phosphate decomposition.

TABLE 2
Effect of different treatments on activities of phosphatase in an organic soil

Treatment	Rates of application (µg/g)		100 µg ρ-nitrophenol released/g soil/2 hr	
			Low treatment rate	High treatment rate
Control	0	0	32.98	32.98
Autoclaving	0	0	3.56*	ND
Streptomycin	100	200	15.13*	12.24*
ρ-Benzoquinone	50	ND**	18.02*	ND
$HgCl_2$	7000	ND	18.87*	ND
Chloffenvinphos	5	10	21.93*	21.76*
Chlorpyrifos	5	10	19.99*	12.92*
Diazinon	5	10	19.21*	15.89*
Ethion	5	10	20.57*	16.66*
Ethoprop	5	10	19.55*	16.66*
Fensulfothion	5	10	23.46*	12.41*
Fonofos	5	10	37.23	21.25*
Leptophos	5	10	18.53*	15.47*
Malathion	5	10	22.44*	17.17*
Parathion	5	10	33.32	23.80
Phorate	5	10	24.48*	19.72*
Terbufos	5	10	19.89*	15.30*
Thionazin	5	10	19.04*	14.96*
Triazophos	5	10	31.62	24.31
Trichloronat	5	10	21.08*	13.09*
Chlordane	5	10	45.56*	25.16
Dieldrin	5	10	24.48*	23.80
Lindane	5	10	26.86*	22.61
Carbofuran	5	10	27.71	26.52
Metalkamate	5	10	28.56	25.84
Oxamyl	5	10	31.79	27.03
Permethrin	5	10	27.54	26.35
DD	150	300	32.47	32.30
Telone	30	60	23.35*	27.54
Telone-C	30	60	18.19*	16.49*
Telone-II	30	60	21.76*	16.49*
Vorlex	40	80	22.95*	20.06*
Captan	5	10	16.66*	11.90*
Maneb	5	10	20.57*	17.85*
Thiram	5	10	21.42*	15.81*
2,4-D	5	10	17.85*	15.64*
Nitrapyrin	30	60	22.95*	19.89*

* Significantly different from control at p = 0.05.

** ND = Not determined.

With the exception of parathion, triazophos, carbofuran, metalkamate, oxamyl, permethrin and DD, the pesticide treatments reduced the release of ρ-nitrophenol after 2 h incubation, while chlordane at 5 µg/g of soil released a greater amount of ρ-nitrophenol. Phosphatase activities generally do not correlate with numbers and respiratory activities of microorganisms in soils (KRAMER & YERDEI 1960, RAMIREZ-MARTINEZ & McLAREN 1966, VUKHRER & SHAMSHIEVA 1968) although activities increase markedly during rapid microbial growth (RAMIREZ-MARTINEZ & McLAREN 1966, LADD & PAUL 1973, ROSS 1965).

Soil urease has attracted a great deal of attention due to the increasing use of urea as a fertilizer. In soil urea is rapidly hydrolyzed to ammonium carbonate by urease activity resulting in formation of nitrate and ammonia. The effects of these pesticides on the activity of urease in an organic soil are summarized in TABLE 3. With exception of triazophos, all treatments

TABLE 3
Effect of different treatments on activity of urease in an organic soil.

Treatment	Rates of Application (µg/g)		mg(NH_4^+-N)/g soil			
			Low treatment rate		High treatment rate	
			Incubation period (weeks)			
			1	2	1	2
Control	0	0	7.33	7.58	7.33	7.58
Autoclaving	0	ND**	1.26*	1.55*	ND	ND
Streptomycin	100	200	6.87*	7.68*	7.14	7.98*
ρ-Benzoquinone	50	ND	7.26	7.73*	ND	ND
$HgCl_2$	7000	ND	7.39	7.68*	ND	ND
Chlorfenvinphos	5	10	7.56	7.73*	6.30*	7.86*
Chlorpyrifos	5	10	7.49	7.71*	6.29*	7.90*
Diazinon	5	10	7.71*	7.73*	6.55*	7.83*
Ethion	5	10	7.68*	7.75*	6.67*	7.83*
Ethoprop	5	10	7.61*	7.78*	6.80*	7.78*
Fensulfothion	5	10	7.78*	7.81*	6.87*	7.78*
Fonofos	5	10	7.39	7.61	6.27*	7.73*
Leptophos	5	10	7.49	7.61	5.86*	7.78*
Malathion	5	10	6.62*	7.73*	5.79*	7.76*
Parathion	5	10	7.51	7.73*	5.96*	7.76*
Phorate	5	10	7.49	7.78*	7.02*	7.83*
Terbufos	5	10	7.58	7.83*	6.06*	7.78*
Thionazin	5	10	7.36	7.78*	6.94*	7.76*
Triazophos	5	10	7.51	7.63*	7.07	7.56
Trichloronat	5	10	7.68*	7.81*	6.86*	7.78*
Chlordane	5	10	7.19*	7.66*	6.25*	7.31
Dieldrin	5	10	7.17*	7.68*	6.08*	7.66*
Lindane	5	10	7.46	7.51	6.75*	7.71*
Carbofuran	5	10	7.36	7.61	6.30*	7.81*
Metalkamate	5	10	7.41	7.76*	6.50*	7.73*
Oxamyl	5	10	7.19*	7.68*	6.18*	7.88*
Permethrin	5	10	7.24	7.78*	6.45*	7.76*
DD	150	300	7.17*	7.71*	6.77*	7.36
Telone	30	60	7.17*	7.68*	6.89*	7.61
Telone-C	30	60	7.51	7.71*	6.52*	7.34
Telone-II	30	60	7.51	7.73*	6.20*	7.24
Vorlex	40	80	7.02*	7.73*	6.33*	7.49
Captan	5	10	5.71*	7.74*	5.24*	7.83*
Maneb	5	10	6.79*	7.71*	6.19*	7.68*
Thiram	5	10	4.58*	7.68*	4.21*	7.81*
2,4-D	5	10	7.39	7.68*	6.01*	7.63*
Nitrapyrin	30	60	6.99*	7.76*	6.11*	7.66*

* Significantly different from control at p = 0.05.
** ND = Not determined.

inhibited urease activity at 10 µg/g after the first week of application. An inhibitory effect was also apparent with malathion, chlordane, dieldrin, oxamyl, DD, Telone, Vorlex, captan, maneb, thiram and nitrapyrin at the low application rate during the same period. An ephemeral effect of the pesticides followed by recovery was observed after 2 weeks' incubation. The control sample showed that urease activities increased 3.4% after 7 additional days' incubation at 28°C. A stimulatory effect on urease activity was evident with treatments of some organophosphorus insecticides e.g. diazinon, ethion, ethoprop, fensulfothion and trichloronat at the low application rate for 2 weeks. Limited activity occurred in the autoclaved soil. This behavior has been noted in some of our previous work (TU 1978, 1979, 1980a, 1980b). Urease is deactivated by prolonged heating of the soil, but may subsequently be reactivated owing to the metabolic activities of the surviving and germinating spores (KOEPF 1954, 1955). Streptomycin at 100 µg/g had inhibited urease activity slightly after 1 week incubation, but activity was greater than that of control after 2 weeks with streptomycin at both application rates and with the inhibitors, ρ-benzoquinone or $HgCl_2$. Because an active microbial population continually contributes to the exoenzyme fraction, any pesticide-induced changes in the microflora during the course of the experiment are reflected in the time related assays.

Soil high in organic matter provides a ready substrate on which a wide range of chemicals that reach soils may be adsorbed (EDWARDS 1966). It appears that enzyme activities in field soils decreased temporarily after the addition of some pesticides.

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