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Effect of Some Pesticides on Soil Microorganisms

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The widespread agricultural use of pesticides resulted in these chemicals entering soil and water ecosystems (Hill and Wright, 1978). The degradation of xenobiotic compounds by members of the soil microflora is an important means by which these compounds are removed from the environment, thus preventing from becoming pollution problems. Bacteria, actinomycetes and fungi occupy a unique position in biological cycles and are essential for plant growth and soil fertility. Much work has been directed towards understanding the complexity of pesticide microbial interactions in soil. Many studies have employed pure cultures of soil isolates or agar plate counts of soil populations. Microbial communities composed of several numerous species are more likely to be responsible for pesticide biodegradation in soil and rhizosphere environments than are single species. Pesticides applied to soil at planting should persist during the development of plant roots. Therefore, a portion of the pesticide likely interacts with microorganisms in the soil and rhizosphere (Wootton et al. 1993). Herbicides generally appear to have no adverse on the population of total bacteria in soil except for the concentrations exceeding recommended rates. Soil fungi and actinomycetes are not as susceptible to herbicides and insecticides as they are to fungicides (Anderson, 1978). The fungicides fentin acetate and maneb reduced soil yeast populations (Dickinson, 1973).

There are a number of studies that, in general, implicate the involvement of adapted soil microbial populations in accelerated pesticide degradation (Motosugi and Soda 1983; Obrigawitch et al. 1983; Racke and Coats 1987; Rahman and James 1983; Skipper et al. 1986). Some investigations resulted in the identification of microbial isolates which are apparently responsible for the accelerated degradation of individual pesticides (Chaudhry and Wheeler 1988; Racke and Coats 1988; Ramanand et al. 1988). This paper reports the effects of four pesticides on soil microbial activities in a sandy loam soil.

MATERIALS AND METHODS

In this study we examined the response of microbial populations in soils after incorporation of the insecticides and fungicides: Endosulfan 35 WP "6,7,8,9,10-hexachloro-1, 5, 5a, 6, 9, 9a-hexahydro-6-9-methano-2,4,3-benzo-dioxathiepin-3-oxide; Poligor " dimethoate = 0, 0 - dimethyl -S- (N - methyl carbamoyl methyl)

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phosphoro-dithioate" ; Vitavax EC 200, " %37.5 Carboxin: (5,6-dihydro-2-methyl-1,4-oxathin-3-carboxanilide) % 37.5 Thiram: (tetra methyl thiruam disülfide); Systhane 12 E, 125 g cyclobutanil: 2-(4-chlorophenyl)-2-(IH-1,2,4-triazol - 1 - yl methyl) hexane nitrile). The soil samples were collected from experimental plots at the Sütçü Imam Universty Karacasu Campus on September 13, 1995. Separate plots that received no application of pesticides were used as controls. The samples of soil were taken at from soils a depth of 10-15 cm with no history of pesticides treatment. The soil was fine sand with about 2.9 % organic matter and the pH was 6.1. The mineral contents in mg litter^{-1} were as follows: Ca, 572; K, 136; P, 5.8; and Mg, 43.

In the laboratory, plant material and soil macrofauna were removed, and soil samples sieved (<2 mm) and mixed. Insecticides and fungicides at 1 mg/kg were applied to the soil. Controls, with soil only, were included within all tests. The mixed and control samples were set up in 1000 ml-bottles (Beher glass) and incubated at 28±1°C for 20 days. Measurements were done with of the number of actinomycete, spore-forming aerobes, anaerobic bacteria, proteolytic, cellulolytic and total microorganisms, yeast-mold by using the dilution method (Collins et al. 1989; Digrak et al. 1996).

Soil samples (20 g soil) were suspended in 180 ml strength Ringer's solution and dispersed with a top drive macerator for 5 min (Yentumi and Johnson 1986). The number of actinomycetes was determined on Bacto-Actinomycete Isolation agar (Difco) with added rifampicin and cycloheximide (50 mg Litter^{-1}) (Athalye et al. 1981); plate count agar was used to estimate total aerobic bacteria and spore-forming aerobes (Collins et al. 1989); the roll tube method (Bradshaw 1992) was used to estimate anaerobic bacteria, using N_2-purged plate count agar. The number of cellulolytic microorganisms was determined in the Skinner medium, gelatin medium for proteolytic bacteria, and malt extract agar for yeast-molds. Three replicate plates were prepared for each dilution.

RESULTS AND DISCUSSION

The response of soil microorganisms to pesticides in pure culture was variable with no consistent relationship evident between the source of the microorganisms and the observed response. Therefore, results from bioassays on microorganisms representive of the dominant isolates occurring at the soil samples grow presented in Tables 1-4. The greatest number of isolates not inhibited by the pesticides occurred among the bacterial and the fungal groups. However, the fungi were the least inhibited (Table 3). The number of isolates which is able to grow on pesticide substrates alone was the highest also for the bacteria. Representative *Actinomycetes* isolates grew on all pesticide compounds. Most of the fungal isolates were not inhibited by any of the pesticides. Overall, endosülfan inhibited growth of the greatest number of microorganisms except for *Actinomycete sp.* These results support previous reports implicating the involvement of individual soil microorganisms in accelerated degradation of several of the pesticides under study

Table 1. Effect of Endosulfan 35 WP (ES) on growth of soil microorganisms

Incubation days		Actinomycete	Spore-forming aerobes	Anaerobic bacteria	Proteolytic bacteria	Cellulolytic bacteria	Total micro-organisms	Yeast-Mold
	Control	$2.5x10^3$	$1.5x10^5$	$1.2x10^4$	$2.2x10^4$	$1.3x10^3$	$3.4x10^6$	$1.2x10^4$
0	ES	$2.4x10^3$	$1.5x10^5$	$1.2x10^4$	$2.1x10^4$	$1.4x10^3$	$3.4x10^6$	$1.2x10^4$
	Control	$3.6x10^4$	$3.1x10^5$	$3.4x10^5$	$1.0x10^4$	$6.1x10^4$	$2.2x10^8$	$2.5x10^5$
5	ES	$3.8x10^4$	$5.2x10^4$	$2.1x10^4$	$1.3x10^4$	$5.6x10^4$	$6.7x10^7$	$9.7x10^3$
	Control	$5.6x10^4$	$2.8x10^5$	$9.2x10^5$	$3.2x10^4$	$2.6x10^4$	$4.6x10^7$	$2.2x10^4$
10	ES	$7.4x10^4$	$1.5x10^4$	$5.5x10^3$	$2.2x10^4$	$1.0x10^4$	$5.9x10^6$	$4.0x10^3$
	Control	$4.5x10^4$	$3.8x10^4$	$5.2x10^5$	$3.0x10^4$	$2.0x10^4$	$3.1x10^6$	$9.5x10^4$
15	ES	$5.7x10^4$	$2.9x10^4$	$2.4x10^4$	$1.4x10^4$	$2.0x10^4$	$1.1x10^6$	$5.4x10^3$
	Control	$4.0x10^4$	$1.5x10^4$	$1.5x10^4$	$2.2x10^3$	$3.8x10^3$	$5.8x10^6$	$4.4x10^4$
20	ES	$4.6x10^4$	$1.3x10^3$	$2.6x10^2$	$1.0x10^3$	$2.4x10^3$	$1.9x10^6$	$3.1x10^2$

Table 2. Effect of Poligor on growth of soil microorganisms

Incubation days		Actinomycete	Spore-forming aerobes	Anaerobic bacteria	Proteolytic bacteria	Cellulolytic bacteria	Total micro-organisms	Yeast-Mold
	Control	$2.5x10^3$	$1.5x10^5$	$1.2x10^4$	$2.2x10^4$	$1.3x10^3$	$3.4x10^6$	$1.2x10^4$
0	Poligor	$2.4x10^3$	$1.5x10^5$	$1.3x10^4$	$2.2x10^4$	$1.3x10^3$	$3.0x10^6$	$1.5x10^4$
	Control	$3.6x10^4$	$3.1x10^5$	$3.4x10^5$	$1.0x10^4$	$6.1x10^4$	$2.2x10^8$	$2.5x10^5$
5	Poligor	$4.8x10^4$	$2.7x10^5$	$3.7x10^5$	$2.1x10^4$	$6.3x10^4$	$7.6x10^7$	$2.4x10^5$
	Control	$5.6x10^4$	$2.8x10^5$	$9.2x10^5$	$8.2x10^4$	$2.6x10^4$	$4.6x10^7$	$2.2x10^4$
10	Poligor	$6.1x10^4$	$5.5x10^5$	$9.6x10^5$	$3.8x10^4$	$2.9x10^4$	$8.9x10^7$	$2.0x10^4$
	Control	$4.5x10^4$	$3.8x10^4$	$5.2x10^5$	$3.0x10^4$	$2.0x10^4$	$3.1x10^6$	$9.5x10^4$
15	Poligor	$6.5x10^4$	$5.7x10^5$	$7.7x10^5$	$9.1x10^4$	$4.0x10^5$	$2.4x10^8$	$8.4x10^4$
	Control	$4.0x10^4$	$1.5x10^4$	$1.5x10^4$	$2.2x10^4$	$3.8x10^3$	$5.8x10^6$	$4.4x10^4$
20	Poligor	$4.2x10^4$	$6.7x10^4$	$8.4x10^4$	$6.7x10^4$	$6.4x10^4$	$1.9x10^7$	$5.3x10^4$

Table 3. Effect of Vitavax EC 200 on growth of soil microorganisms

Incubation days		Actinomycete	Spore-forming aerobes	Anaerobic bacteria	Proteolytic bacteria	Cellulolytic bacteria	Total micro-organisms	Yeast-Mold
0	Control	$2.5x10^3$	$1.5x10^5$	$1.2x10^4$	$2.2x10^4$	$1.3x10^3$	$3.4x10^6$	$1.2x10^4$
	Vitavax	$2.5x10^3$	$1.6x10^5$	$1.2x10^4$	$2.2x10^4$	$1.3x10^3$	$3.5x10^6$	$1.3x10^4$
5	Control	$3.6x10^4$	$3.1x10^5$	$3.4x10^5$	$1.0x10^4$	$6.1x10^4$	$2.2x10^8$	$2.5x10^5$
	Vitavax	$2.8x10^5$	$5.0x10^5$	$3.9x10^5$	$2.5 x10^4$	$1.8x10^4$	$4.0x10^6$	$2.6x10^4$
10	Control	$8.6x10^4$	$2.8x10^5$	$9.2x10^5$	$3.2x10^4$	$2.6x10^4$	$4.6x10^7$	$2.2x10^4$
	Vitavax	$4.7x10^5$	$4.3x10^5$	$4.8x10^6$	$3.7x10^4$	$3.0x10^4$	$5.1x10^8$	$2.9x10^4$
15	Control	$4.5x10^4$	$3.8x10^4$	$5.2x10^5$	$3.0x10^4$	$2.0x10^4$	$3.1x10^6$	$9.5x10^4$
	Vitavax	$6.9x10^5$	$1.9x10^5$	$1.6x10^6$	$3.7x10^4$	$2.4x10^4$	$3.4x10^7$	$3.0x10^4$
20	Control	$4.0x10^4$	$1.5x10^4$	$1.5x10^4$	$2.2x10^3$	$3.8x10^3$	$5.8x10^6$	$4.4x10^4$
	Vitavax	$2.3x10^5$	$2.5x10^5$	$2.1x10^5$	$2.5x10^4$	$1.1x10^4$	$2.5x10^7$	$2.4x10^4$

Table 4. Effect of Systhane 12 E on growth of soil microorganisms

Incubation days		Actinomycete	Spore-forming aerobes	Anaerobic bacteria	Proteolytic bacteria	Cellulolytic bacteria	Total micro-organisms	Yeast-Mold
0	Control	$2.5x10^3$	$1.5x10^5$	$1.2x10^4$	$2.2x10^4$	$1.3x10^3$	$3.4x10^6$	$1.2x10^4$
	Systhane	$2.5x10^3$	$1.5x10^5$	$1.2x10^4$	$2.2x10^4$	$1.3x10^3$	$3.5x10^6$	$1.3x10^4$
5	Control	$3.6x10^4$	$3.1x10^5$	$3.4x10^5$	$1.0x10^4$	$6.1x10^4$	$2.2x10^8$	$2.5x10^5$
	Systhane	$3.7x10^4$	$2.0x10^5$	$3.0x10^6$	$4.3 x10^4$	$5.6x10^5$	$3.1x10^7$	$1.0x10^5$
10	Control	$5.6x10^4$	$2.8x10^5$	$9.2x10^5$	$8.2x10^4$	$2.6x10^4$	$4.6x10^7$	$2.2x10^4$
	Systhane	$6.4x10^5$	$4.0x10^5$	$7.0x10^5$	$3.4x10^4$	$2.5x10^5$	$4.8x10^8$	$1.1x10^5$
15	Control	$4.5x10^4$	$3.8x10^4$	$5.2x10^5$	$3.0x10^4$	$2.0x10^4$	$3.1x10^6$	$9.5x10^4$
	Systhane	$9.3x10^5$	$3.4x10^5$	$4.0x10^6$	$2.5x10^3$	$2.4x10^5$	$3.8x10^7$	$4.5x10^4$
20	Control	$4.0x10^4$	$1.5x10^4$	$1.5x10^4$	$2.2x10^3$	$3.8x10^3$	$5.8x10^6$	$4.4x10^4$
	Systhane	$5.9x10^5$	$2.4x10^5$	$6.2x10^5$	$7.0x10^3$	$2.7x10^4$	$2.4x10^7$	$2.8x10^4$

(Chaudhry and Ali 1988; Karns et al. 1986; Stevens et al. 1990).The spore-forming aerobes, anaerobic, proteolytic and cellulolytic bacteria grew on poligor, vitavax, and systhane. In untreated soil (control) the number of microorganisms was usually found to be low, except for endosulfan-treated soils.

An *Achromobacter* sp. that was capable of utilizing carbofuran as the sole source of nitrogen has been reported to exhibit hydrolas activity (Karns et al. 1986). Microbial metabolism is an important process for degrading pesticides in the soil environment. Biochemical research on the pesticide metabolism over the last three decades has primarily been aimed at identifying the microorganisms, metabolites, and enzymes associated with a specific pesticidal compound. With advances in modern microbial genetics, some insight is developing into the evolutionary events that occur during the pesticide adaptation process.

The bacterial isolates were able to degrade carbaryl and carbofuran, especially in the absence of an additional nitrogen source $(NH_4)_2HPO_4$ (Rajagopal et al. 1984). There are reports on added nitrogen retarding the degradation of the other nitrogenous pesticides (Campacci et al. 1977; Cook and Hutter 1981). These observations demonstrate the diversity of metabolic pathways available to bacteria for metabolism of the pesticides. Particularly noteworthy is the finding that the some bacterium (*Bacillus*) affected both nitro group reduction and hydrolysis of methyl parathion and parathion. Occurrence of nitro group reduction hydrolysis of methyl parathion and parathion in a complex, and dynamic soil system, especially, under flooded conditions, is not uncommon (Adhya et al. 1987; Sharmila et al. 1989). Some of the earliest work on microbial adaptation to herbicide molecules was conducted on the phenoxyalkanoic acid herbicides, which include 2,4-D, 2,4,5-T and MCPA. Stimulation of pesticides by warm, moist soil conditions and by additions of organic matter; the correlation between the rate of pesticides and build up of aerobic soil bacteria.

Our work showed that estimations of soil microbial group might be useful to assess long-term side effects of pesticides in soils. Finally, we confirmed that poligor, vitavax and synthane had minimal effects on soil microorganisms though endosülfan was apparently toxic for soil microorganisms.

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