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Availability of phosphorus and sulfur of insecticide origin by fungi

S.A. Omar
Botany Department, Faculty of Science, Assiut University, Assiut 71516, Egypt

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Abstract

Thirteen fungal species isolated from soil treated with pesticides were tested for their ability to mineralize and degrade three organophosphate insecticides currently used in Egypt (Cyolan®, Malathion® and Dursban®) in liquid media free from phosphorus (P) and sulfur (S). All fungal species grew successfully on the culture media treated with the three used doses of insecticides (10, 50 and 100 ppm active ingredient) but the growth rate varied with the species, the insecticide and the doses. At 10 ppm level, insecticide degradation expressed in term of organic P mineralization (calculated as % of applied P) was the highest with all fungi tested. Organic P mineralization from pesticides was decreased by increasing the dose used to 50 and 100 ppm. The highest amount of P mineralized was observed with Cyolan® followed by Malathion® whilst P mineralization from Dursban® proceeded very slowly. *Aspergillus terreus* showed the greatest potential to mineralize organic P followed by *A. tamarii*, *A. niger*, *Trichoderma harzianum* and *Penicillium brevicompactum* whilst the remaining fungi only moderately mineralized the organic P component of the insecticides tested.

Organic sulfur mineralization by the used fungal species paralleled, to some extent, organic P mineralization. The extracellular protein content of culture filtrates in the presence of various doses of insecticides was also decreased by increasing insecticide concentrations. The extracellular protein was significantly correlated with P and S mineralization ($r = 0.89^{**}$ and 0.64^{**}, respectively) whilst correlation with cell dry mass was not significant ($r = 0.03$ and 0.003) suggesting a direct relationship between pesticide degradation and microbial protein production. The addition of P or S to the growth media enhanced extracellular protein excretion, and increased organic P and S mineralization by the most potent species tested (*A. niger*, *A. tamarii*, *A. terreus* and *T. harzianum*). This increment was significant in most cases, especially at the higher application rates. The relationship between extracellular protein excretion and organic P and S mineralization from insecticides was highly significant with the addition of inorganic phosphorus ($r = 0.96^{**}$ and 0.83^{**}, respectively) or sulfur ($r = 0.85^{**}$ and 0.89^{**}, respectively) to the growth media.

Introduction

The use of insecticides for controlling plant pests is one of the features of modern agriculture for higher crop yield. After application, the insecticides may reach the soil by missing the 'target' or run-off from leaves and stems (Hill & Wright 1978). Treated seeds also represent another source of soil pollution from insecticides. A portion of the applied pesticide may also eventually become incorporated into the soil as a result of root exudation or death of treated plants (Sato & Tanaka 1987).

Beside controlling specific plant pests, insecticides in soil may temporarily or permanently hamper the biodynamics involved in soil fertility, especially if they persist in soil for long periods. Abdel-Mallek et al. (1994) reported an inhibitory effect of Profenfos on urease and nitrate reductase activity in sandy-clay soil. Fenvalerate, at 10 and 50 ppm, suppressed growth, respiration and nitrogenase activity of three free-living non-symbiotic nitrogen fixing bacteria (Omar & Abd-Alla 1992). Nitrification, ammonification and degradation of plant residues was delayed by insecticide application (Sahrawat 1979; Omar 1987, 1991). The

effect of insecticides on acetylene reduction by alfalfa, red clover and sweet clover was examined by Smith et al. (1978) who found that the inhibitory effect of insecticides on C_2H_2-reduction varied with the insecticide used and the plant. Also, the high application rate of several insecticides decreased acetylene reduction in an agricultural sandy loam (Tu 1978). Vesicular-arbuscular mycorrhizal fungi (VAM) enhance plant growth and crop yield mainly by increasing the phosphorus supply to plant (Omar 1995, 1998), and were markedly influenced by pesticide application. The harmful effects of pesticides on root colonization by vesicular-arbuscular mycorrhizal fungi and P transport were well documented (Dodd & Jeffries 1989; Larsen et al. 1996; Kling & Jakobsen 1997). Another disadvantage of pesticide application is their inhibition to plant growth (Bertoldi et al. 1978).

Organophosphate insecticides are an important group of modern pesticides used in agricultural practice, to resist harmful insects that attack crops, because of their high insecticidal activity, broad spectrum and rapid action (Gruzdyev et al. 1983).

Little information is available on the mineralization and degradation of organophosphate insecticides. In this study, thirteen fungal species predominantly found in soil treated with the three insecticides were able to mineralize the organic P and S components of these insecticides were able. The effect of supplementation of the growth medium with elemental P or S, each added singly, on mineralization of organic P and S from insecticides was also examined.

Materials and methods

Insecticides

Three organophosphate insecticides commonly used in Egypt were used in this study. These were Cyolan® [O,O-diethyl N-1,3-dithiolan-2-ylidenephosphoramidate], a systemic insecticide particularly useful in cotton; Malathion® [O,O-dimethyl S-1,2-bis (ethoxycarbonyl) ethyl phosphorothioate], used for controlling aphids; and Dursban® [O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate], usually used to control cotton leaf worm.

Soil experiments

A clay soil taken from a layer at depth of 0 –25 cm in the Botanical Garden of Assiut University. The soil was air dried, passed through a 2-mm sieve and 500 g aliquots (on oven dry basis) placed in polyethylene bags. Insecticides were added at rates of 10, 50 and 100 ppm (a.i.) in addition to controls. After incubation for 4 weeks at 28 °C, soil samples for isolation of fungi were taken using the dilution plate method (Johnson et al. 1959). Czapek's agar medium, containing rose bengal (65 ppm) as a bacteriostatic agent, was used as isolation medium (Smith & Dawson 1944). Plates were incubated at 28 °C for 7 days and the developing fungi were counted and identified.

Liquid culture experiments

Soil samples treated with the insecticides revealed 18 fungal species occurring at different frequencies in the insecticide-treated soil. Thirteen species were found to be more resistant, even at the higher application rates (50 and 100 ppm) suggesting the ability of these fungi to degrade insecticides so these were chosen for further studies of their ability to mineralize insecticides.

The isolated fungi were grown on modified Czapek's-Dox medium, free from P and S sources, and contained: $NaNO_3$, 2 g; KCl, 0.5 g; MgO, 0.5 g; glucose, 10 g; $FeCl_3$, 10 mg and 1000 ml distilled water. The medium was dispensed in 100 ml conical flasks with 30 ml in each and sterilized by autoclaving for 20 min at 105 °C. The insecticides were added to the cooled sterilized liquid medium at the same rates used above and individual fungi were inoculated (at rate of 1 ml spore suspension, containing 4×10^3 spores per flask) into the insecticide-amended media in triplicate. The fungal inocula were prepared by growth on Czapek's agar slants for 7 days at 28 °C. Stock spore suspensions of fungi were obtained from slant cultures by adding sterilized water and rubbing the agar surface with a flamed loop. The concentration of spores was assessed using a hemocytometer and by dilution with sterilized distilled water as previously described (Omar 1994). Flasks were incubated in dark at 28 °C for 35 days as static cultures.

Analyses

After incubation, the flasks were filtered through preweighed Whatman No. 1 filter paper to separate the mycelium. Mycelia were washed with twice-distilled water, dried at 80 °C for 24 hr, and weighed. This mycelium was then assayed for P and S digestion with nitric acid (Kothari et al. 1990).

The culture filtrate was made up to its original volume (30 ml) with distilled water and analyzed for P and S mineralized from the degradation of insecticides, and for extracellular protein excretion. Phosphorus in mycelial digests, or culture fluids was estimated using the molybdate blue method (Jackson 1958). Sulfur was determined as barium sulfate according to Black et al. (1965). Extracellular protein was estimated using the method of Lowry et al. (1951) with bovine serum albumin as standard.

Effect of elemental P and S on mineralization of inseticidal organic P and S

To test the effect of utilizable P and S on the release of these elements from organophosphate insecticides, P and S were added to the growth media at concentrations 0, 20, 40, 60, 90 and 120 ppm. KH_2PO_4 was used as P source and $MgSO_4$. 7 H_2O as S source. After autoclaving, media were treated with 10 ppm insecticide and inoculated by 1 ml spore suspension (4 x 10^3 spores), prepared from 7-day-old cultures, of each of *A. niger*, *A. tamarii*, *A. terreus* and *T. harzianum*, each added separately. After incubation at 28 °C for 35 days, mycelia were separated from culture fluid by filtration. Total P and S of mycelia and elemental P, S and extracellular protein in culture filtrate were determined as described before. Total mineralized P and S of pesticide origin in each treatment was calculated by subtracting total estimated metals (in mycelia and culture fluid) from elemental P and S originally applied.

Statistical analysis

Data were subjected to analysis of variances and linear regression using a computer program (Pc Stat).

Results

Results of soil treatment with the three insecticides revealed that the total population of soil fungi recovered in plates declined by insecticide application and by increasing the doses used from 10 to 100 ppm after 4 weeks incubation (Figure 1). However, 13 fungal species were recovered at high frequencies suggesting the ability of these fungi to degrade insecticides and hence their use in the present study. These were *Aspergillus fumigatus, A. flavus, A. niger, A. ochraceus, A. tamarii, A. terreus, Fusarium oxysporum, F. solani,*

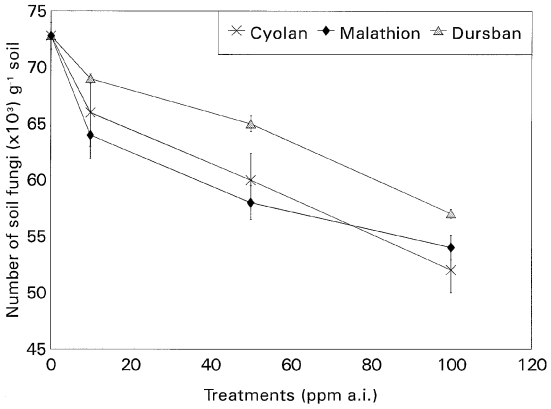

Figure 1. Effect of soil treatment with various doses of Cyolan®, Malathion® and Dursban® on total count of soil fungi after 4 weeks incubation.

Penicillium chrysogenum, P. brevicompactum, P. citrinum, P. funiculosum and *Trichoderma harzianum*.

Liquid culture experiments indicated that mycelial dry weight of the tested fungi varied with species, insecticide and the dose. Inoculation of the growth media by fungi without the addition of insecticides resulted in poor growth of the fungi. Addition of insecticides plus the fungi resulted in greater fungal growth and the dry weight of most fungi was mostly increased by increasing the level of insecticides (Table 1). However, the growth of three fungal species: *A. flavus*, *P. chrysogenum* and *P. funiculosum* was strongly checked by insecticide application even at 10 ppm level.

Extracellular protein production was the lowest for all fungi in media not supplemented with the insecticides (Table 2). The addition of insecticides to the growth media enhanced extracellular protein excretion but it began to decrease by increasing the concentration of insecticides above 10 ppm. In most cases, the highest amount of extracellular protein was excreted from the growing cultures of *A. terreus*, *A. tamarii*, *A. niger*, *P. brevicompactum* and *T. harzianum*.

Mineralization of the insecticidal P by the tested fungi was presented in Table 3. Generally, Cyolan® P was easily mineralized compared with Malathion® but Dursban® P, on the other hand, mineralized more slowly. *A. terreus* was the best P mineralizer in addition to *A. tamarii*, *A. niger*, *T. harzianum* and *P. brevicompactum*. At low application rate (10 ppm) of Cyolan®, an average of 64.5%, 61.3%, 49.3% and 46.9% of the chemical has been degraded by the fungi, respectively, based on P mineralization. The remaining fungal species mineralized small amounts of the

Table 1. Mycelial growth (mg dry weight/30 ml growth medium) of some fungi grown in media supplemented with three levels of organopho-sphate insecticides after 35 days of incubation

Species	Cyolan® (ppm)				Malathion® (ppm)			Dursban® (ppm)		
	0.0	10	50	100	10	50	100	10	50	100
A. fumigatus	9.4± 0.23	57±5.3	67±6.1	62±1.0	33±0.6	51±2.0	61±1.0	20±1.3	20±1.0	17±0.9
A. flavus	8.3± 0.57	15±1.7	17±1.8	12±0.4	18±2.1	8±0.4	0.0±0.0	11±0.4	13 ±1.1	12±0.6
A. niger	13±0.46	88±2.2	78±2.8	153±11.4	79±1.5	88±2.9	134±7.9	48±1.3	58±0.6	73±2.9
A. ochraceus	7.2±0.23	52±4.1	70±0.8	83±0.6	27±1.5	45±1.2	58±1.0	46±1.6	37±2.1	34±1.2
A. tamarii	15.6±0.46	112±11.7	91±2.0	65±1.6	40±2.0	60±2.3	58±1.7	46±0.7	69±2.1	51±1.2
A. terreus	8.2±0.12	70±1.2	89±1.0	74±1.4	56±7.6	54±0.6	64±0.8	39±1.0	51±1.4	36±2.1
F. oxysporum	10.4±0.47	67±2.1	66±2.4	63±1.7	92±3.6	87±2.6	63±1.2	62±3.6	41±2.3	23±1.6
F. solani	13.8±0.42	95±6.6	47±2.1	35±3.2	38±0.6	56±0.6	41±1.9	158±6.6	170±10.4	137±11.2
P. chrysogenum	16.4±0.23	15±0.8	22±2.2	9±1.2	16±0.3	5±0.1	4±0.1	13±1.6	9±0.5	11±1.0
P. brevicompactum	6.2±0.12	43±2.9	76±3.1	83±2.0	34±1.0	34±1.5	60±1.6	38±1.9	39±2.5	28±2.1
P. citrinum	7.0±0.23	39±2.5	51±1.2	86±1.5	32±1.2	59±2.9	71±1.0	20±1.1	20±1.9	35±1.2
P. funiculosum	6.3±0.06	16±1.4	15±0.8	11±1.2	18±0.7	16±1.5	13±0.4	14±1.6	12±0.5	7±0.4
T. harzianum	7.0±0.14	47±3.0	38±1.1	35±1.5	50±1.7	73±4.5	78±1.2	50±1.7	152±13.3	108±5.5

Each value represents the mean ± standard error (SE) of three replicates.

Table 2. Extracellular protein excretion (mg BSA protein/30 ml medium) from cultures of soil fungi grown in media supplemented with three levels of organophosphate insecticides after 35 days of incubation

	Cyolan® (ppm)				Malathion® (ppm)			Dursban® (ppm)		
Species	0.0	10	50	100	10	50	100	10	50	100
A. fumigatus	0.04±0.005	0.22±0.024	0.18±0.035	0.13±0.015	0.28±0.025	0.23±0.026	0.15±0.008	0.18±0.015	0.14±0.009	0.12±0.015
A. flavus	0.03±0.002	0.12±0.009	0.13±0.009	0.09±0.005	0.15±0.010	0.13±0.012	0.14±0.004	0.11±0.008	0.13±0.011	0.09±0.004
A. niger	0.05±0.005	0.38±0.020	0.29±0.026	0.25±0.020	0.32±0.023	0.25±0.014	0.17±0.013	0.27±0.040	0.19±0.012	0.14±0.007
A. ochraceus	0.04±0.003	0.16±0.012	0.12±0.020	0.09±0.007	0.35±0.030	0.24±0.025	0.18±0.010	0.15±0.031	0.11±0.010	0.08±0.006
A. tamarii	0.05±0.007	0.45±0.046	0.23±0.025	0.17±0.023	0.34±0.036	0.28±0.023	0.21±0.023	0.22±0.030	0.18±0.007	0.12±0.004
A. terreus	0.07±0.005	0.53±0.026	0.39±0.052	0.35±0.030	0.43±0.020	0.27±0.035	0.23±0.032	0.25±0.032	0.15±0.008	0.13±0.006
F. oxysporum	0.02±0.004	0.23±0.017	0.17±0.021	0.13±0.034	0.29±0.029	0.13±0.020	0.07±0.001	0.08±0.007	0.05±0.003	0.03±0.002
F. solani	0.02±0.003	0.18±0.010	0.14±0.012	0.10±0.020	0.31±0.015	0.16±0.025	0.11±0.012	0.06±0.003	0.04±0.001	0.04±0.003
P. chrysogenum	0.03±0.002	0.11±0.002	0.08±0.006	0.09±0.006	0.14±0.055	0.09±0.004	0.07±0.001	0.8 ±0.057	0.07±0.008	0.09±0.005
P. brevicompactum	0.07±0.004	0.34±0.030	0.29±0.046	0.25±0.017	0.41±0.026	0.29±0.015	0.20±0.017	0.23±0.032	0.19±0.004	0.11±0.005
P. citrinum	0.03±0.003	0.27±0.026	0.24±0.034	0.18±0.010	0.34±0.015	0.22±0.017	0.17±0.004	0.09±0.004	0.07±0.006	0.04±0.002
P. funiculosum	0.02±0.001	0.13±0.020	0.1 ±0.013	0.08±0.004	0.11±0.005	0.07±0.002	0.06±0.003	0.09±0.003	0.07±0.004	0.05±0.005
T. harzianum	0.04±0.003	0.35±0.040	0.37±0.040	0.32±0.029	0.30±0.020	0.23±0.026	0.19±0.006	0.24±0.023	0.17±0.006	0.13±0.006

Each value represents the mean ± standard error (SE) of three replicates.

organic P component of the used three insecticides. However, the percentage of the insecticide degraded on the basis of P mineralization was decreased by increasing levels of insecticides from 10 to 100 ppm, regardless of the fungal species or insecticide used.

Sulfur mineralization from organophosphate insecticides by the used fungi was closely related to P mineralization (Table 4). Except in case of Dursban®, % of S mineralized was smaller, compared with P, for all tested fungi and the rate of Malathion® S mineralization was the lowest. It is worth mentioning that inorganic P and S were not detected in cultures of *A. flavus*, *P. chrysogenum* and *P. funiculosum* and accordingly these were omitted from the tables. Statistical analyses showed non-significant relationship between cell dry mass and insecticide degradation expressed as % of P or S mineralization (r = 0.03 and 0.003, respectively). On the other hand, the correlation between extracellular protein production and insecticide degradation (expressed as percentage of P and S mineralized) by fungi was highly significant (r = 0.89** and 0.64**, respectively).

Statistical analysis of results (Table 5) revealed that the addition of inorganic P to the growth media significantly stimulated insecticide degradation, with regards

Table 3. Total mineralized P (calculated as% of applied) from organophosphate insecticides by some fungi grown in media supplemented with three levels of the insecticides after 35 days incubation

	Cyolan® (ppm)			Malathion® (ppm)			Dursban® (ppm)		
Species	10	50	100	10	50	100	10	50	100
A. fumigatus	37.3±5.4	16.2±1.2	11.7±0.9	15.7±2.11	18.7±1.61	11.0±0.9	3.4±0.2	4.3±0.3	2.5±0.10
A. niger	49.3±1.9	19.4±1.9	16.9±2.5	22.7±1.8	20.7±1.7	16.9±1.6	6.2±1.4	6.1±0.2	3.5±0.20
A. ochraceus	21.9±1.0	6.0±0.3	4.0±0.2	30.6±1.7	9.7±0.5	12.6±1	4.2±0.2	5.7±0.3	2.7±0.40
A. tamarii	61.3±1.2	16.6±1.8	12.3±1.3	38.0±2.1	23.0±2.3	15.8±0.5	8.9±0.8	8.6±0.2	4.4±0.16
A. terreus	64.5±1.6	62.9±4	46.8±3.7	54.0±2.3	31.5±4.2	26.8±2	16.7±0.7	6.3±0.4	3.5±0.10
F. oxysporum	29.1±2.6	15.9±1.4	9.4±0.8	20.3±0.7	5.5±0.2	3.9±0.2	2.4±0.1	1.7±0.2	1.4±0.10
F. solani	20.0±1.5	15.8±0.6	12.2±0.7	21.7±2.5	6.7±0.3	6.5±0.8	2.0±0.1	3.2±0.1	3.5±0.05
P. brevicompactum	40.3±3.5	36.8±3.2	32.8±4.3	46.3±3.6	26.5±2.5	12.1±1.9	7.8±0.7	7.3±2.2	6.7±0.15
P. citrinum	31.5±3.4	26.9±2.8	17.2±1.5	33.7±2.4	13.1±1.7	9.5±0.7	3.6±0.2	4.2±0.4	2.3±0.09
T. harzianum	46.9±0.7	50.7±1.7	36.2±1.9	40.3±3.1	28.7±2.3	24.7±2.2	12.1±2	8.1±1.1	5.6±0.43

Each value represents the mean ± standard error (SE) of three replicates.

Table 4. Total mineralized S (calculated as % of applied) from organophosphate insecticides by some fungi grown in media supplemented with three levels of the insecticides after 35 days incubation

	Cyolan® (ppm)			Malathion® (ppm)			Dursban® (ppm)		
Species	10	50	100	10	50	100	10	50	100
A. fumigatus	21.2±1.7	9.0±0.2	6.9±0.2	19.0±1.5	6.8±1.2	7.0±0.4	20.4±1.3	8.1±1.1	8.5±0.4
A. niger	24.7±3.1	19.3±1.7	16.7±2.2	30.2±2.7	15.0±1.0	16.0±1.5	34.1±3.0	16.3±1.7	15.8±1.3
A. ochraceus	13.2±1.2	9.0±0.7	5.4±0.06	14.2±1.2	5.5±0.3	5.9±0.3	16.2±1.0	4.9±0.3	5.1±0.2
A. tamarii	42.3±3.0	22.7±2.9	18.0±1.8	41.8±1.6	20.5±2.0	17.5±1.3	46.0±2.3	15.5±1.4	17.8±2.5
A. terreus	52.0±1.8	33.9±4.8	27.0±2.5	50.9±1.7	22.9±1.7	23.0±2.6	56.3±2.2	24.4±2.7	21.7±3.5
F. oxysporum	10.1±1.0	3.9±0.1	3.0±0.06	10.5±0.9	4.5±0.3	4.5±0.1	16.0±0.9	4.5±0.2	3.8±0.2
F. solani	12.9±0.6	5.4±0.5	4.3±0.3	13.0±0.1	5.0±0.8	5.2±0.2	17.0±1.2	6.0±0.6	3.2±0.1
P. brevicompactum	20.3±1.4	10.8±1.1	9.6±0.5	17.4±1.4	10.2±2.2	7.7±0.5	22.2±2.2	10.7±1.5	7.6±0.3
P. citrinum	13.7±0.5	7.0±0.9	4.6±0.2	14.0±1.2	8.1±0.9	5.7±0.3	24.4±1.4	7.6±0.3	7.2±0.2
T. harzianum	28.4±2.1	29.5±3.0	25.0±2.4	29.0±2.1	16.0±1.7	16.8±1.6	33.7±3.3	21.3±2.5	18.0±2.8

Each value represents the mean ± standard error (SE) of three replicates.

to P and S mineralization, and extracellular protein production by the tested fungi, especially at the higher application rates.

The enhancement of growth media with elemental S at levels equivalent to those of inorganic P exerted also a stimulatory effect on insecticide degradation by fungi (related to P and S mineralized) and extracellular protein excretion (Table 6). However, the effect of S enhancement was more pronounced than P. There were also significant relationships between extracellular protein excretion and total organic P mineralization ($r = 0.96^{**}$ and 0.85^{**}) and insecticidal S release ($r = 0.83^{**}$ and 0.89^{**}) with the addition of inorganic P and S, respectively.

Discussion

The effect of soil treatment with insecticides on population of soil fungi has been well documented. In agreement with our results, reduction in population of soil fungi due to organophosphate insecticides application was reported earlier by some authors (Abdel-Fattah et al. 1982; Abdel-Mallek et al. 1994). However, Gonzalez-Lopez et al. (1992) reported that the presence of 10–300 μg/g of the organophosphate insecticide methidathion in agricultural soil significantly increased fungal populations, suggesting the ability of soil microorganisms to degrade this insecticide. Similar results were obtained by Abdel-Kader et al. (1978) where they found that the organophosphate insecticide Dursban® had no significant effect on the total count

Table 5. Effect of amendment of culture media with various levels of inorganic P on mineralization of organic P and S from the insecticides Cyolan®, Malathion® and Dursban®, added to the growth media at 10 ppm level, by some fungi isolated from insecticide-treated soil

Species	P added (ppm)	Cyolan®[a] Total P mineralized[b]	Cyolan®[a] Total S mineralized[b]	Cyolan®[a] Extracellular protein[c]	Malathion®[a] Total P mineralized[b]	Malathion®[a] Total S mineralized[b]	Malathion®[a] Extracellular protein[c]	Dursban®[a] Total P mineralized[b]	Dursban®[a] Total S mineralized[b]	Dursban®[a] Extracellular protein[c]
A. niger	0	15±1.6	20±1.5	0.43±0.035	10±1.2	15±1.6	0.29±0.031	11±0.5	10±1.1	0.25±0.020
	20	21±3.2	25±0.9	0.46±0.020	11±0.8	20±0.9	0.31±0.029	13±0.6	11±0.5	0.28±0.012
	40	23±2.6	31±2.3	0.48±0.027	13±1.9	27±1.3	0.34±0.017	14±0.4	14±0.7	0.32±0.029
	60	25±2.1	33±2.5	0.51±0.020	14±0.6	29±1.6	0.38±0.038	14±0.9	16±0.9	0.37±0.023
	90	25±2.3	38±0.6	0.53±0.050	15±1.7	32±2.0	0.37±0.026	14±0.6	15±0.4	0.36±0.021
	120	24±1.5	38±1.6	0.55±0.020	15±0.7	34±2.4	0.39±0.022	14±0.2	16±0.3	0.38±0.011
A. tamarii	0	17±2.1	23±0.7	0.47±0.032	11±0.8	20±1.0	0.33±0.012	11±0.4	12±0.2	0.23±0.032
	20	20±1.2	25±2.0	0.50±0.017	16±0.7	22±0.6	0.35±0.018	16±1.2	12±0.7	0.26±0.015
	40	22±1.9	28±2.6	0.53±0.034	18±2.0	25±1.2	0.37±0.023	18±0.9	13±0.5	0.28±0.017
	60	23±2.9	29±1.7	0.55±0.020	18±0.5	28±2.1	0.41±0.021	17±0.8	16±0.3	0.32±0.021
	90	25±1.5	35±2.0	0.55±0.043	17±0.9	31±2.3	0.45±0.017	18±0.4	17±0.9	0.35±0.035
	120	25±1.2	37±1.2	0.54±0.018	18±0.1	33±2.6	0.45±0.015	18±0.6	18±0.6	0.35±0.012
A. terreus	0	21±0.6	28±1.7	0.52±0.020	13±0.8	22±1.8	0.40±0.023	12±1.1	13±1.7	0.26±0.015
	20	25±1.3	31±1.8	0.59±0.015	18±0.5	25±0.9	0.43±0.020	15±0.9	13±0.4	0.28±0.013
	40	27±0.3	32±1.3	0.63±0.043	21±0.6	31±2.9	0.48±0.017	18±0.5	14±1.0	0.33±0.040
	60	29±1.7	36±0.6	0.63±0.035	23±1.0	34±2.2	0.52±0.028	19±1.0	17±0.5	0.37±0.029
	90	30±1.8	38±2.7	0.67±0.011	23±1.7	37±1.0	0.56±0.040	19±0.5	21±1.7	0.38±0.012
	120	30±2.0	38±1.5	0.68±0.033	23±0.4	42±2.2	0.60±0.011	21±1.4	22±1.6	0.42±0.017
T. harzianum	0	20±0.6	29±1.8	0.33±0.031	11±0.5	18±0.6	0.32±0.023	10±0.3	11±0.6	0.19±0.017
	20	21±1.2	32±0.5	0.40±0.016	12±1.1	25±1.3	0.35±0.012	13±1.7	14±1.3	0.23±0.011
	40	23±1.1	35±0.6	0.42±0.020	15±1.4	28±2.5	0.38±0.029	17±0.7	16±0.5	0.27±0.021
	60	27±1.9	38±2.1	0.45±0.012	16±0.8	31±2.1	0.43±0.027	20±0.5	18±1.5	0.32±0.032
	90	26±1.2	39±1.1	0.50±0.017	16±0.5	34±3.0	0.46±0.021	21±0.7	20±0.6	0.35±0.017
	120	26±1.0	40±0.5	0.51±0.018	17±0.7	36±1.6	0.45±0.012	21±0.3	20±1.1	0.35±0.015
LSD 5%		5.1	4.9	0.08	3.0	5.4	0.07	2.3	2.7	0.06
1%		7.0	6.6	0.11	4.1	7.3	0.10	3.1	3.6	0.08

The data represent the mean ± standard error (SE) of three replicates.

[a] Cyolan®, Malathion® and Dursban® at 10 ppm level contain 36.5, 28.2 and 26.3 μg P/30 ml medium; 75, 58.2 and 27.1 μg S/30 ml medium, respectively.

[b] P and S are expressed in term μg/30 ml medium.

[c] Extracellular protein was measured as mg BSA protein /30 ml medium.

of soil and rhizosphere fungi, but few species were promoted.

The effect of insecticides on biomass production by fungi was fully examined. In disagreement with our findings, a reduction in mycelial biomass production of seven fungal species at doses above 100 ppm of sumi oil (a formulated product of sumithion) was reported by Mahmoud & Omar (1995). Similarly, the organophosphate insecticide selecron at 10 and 50 ppm significantly decreased mycelial dry weight of *Aspergillus fumigatus*, *A. terreus* and *Mycelliophthora thermophila* when grown at 45 °C (Omar et al. 1993). Increasing growth rate of fungi tested here by increasing the doses of insecticides is encouraging and suggested the ability of these fungi to degrade insecticides.

In accordance with the results obtained here (Table 2), Moharram et al. (1994) investigated the effect of the insecticide Selecron on N metabolism of some soil fungi. They reported a disturbance in N metabolism was concomitant to insecticide application. Inhibition in extracellular protein production by insecticide application was also discussed by Abdel-Basset et al. (1992) and Omar et al. (1993). The decrease in extracellular protein excretion by fungi with the addition of insecticides reported here led to the assumption that the effect of insecticides on mycelial growth not related to their effect on extracellular protein production, or insecticides inhibited protein biosynthesis.

In recent years, the extensive literature on the microbial metabolism of naturally-occurring organic compounds has been supplemented by an ever increas-

Table 6. Effect of amendment of culture media with various levels of inorganic S on mineralization of organic P and S from the insecticides Cyolan®, Malathion® and Dursban®, added to the growth media at 10 ppm level, by some fungi isolated from insecticide-treated soil

Species	S added (ppm)	Cyolan®[a] Total P mineralized[b]	Cyolan®[a] Total S mineralized[b]	Cyolan®[a] Extracellular protein[c]	Malathion®[a] Total P mineralized[b]	Malathion®[a] Total S mineralized[b]	Malathion®[a] Extracellular protein[c]	Dursban®[a] Total P mineralized[b]	Dursban®[a] Total S mineralized[b]	Dursban®[a] Extracellular protein[c]
A. niger	0	15±1.6	20±1.5	0.43±0.035	10±1.2	15±1.6	0.29±0.031	11±0.5	10±1.1	0.25±0.020
	20	23±0.7	26±1.5	0.48±0.031	15±0.6	19±1.0	0.35±0.020	12±0.7	11±0.7	0.30±0.015
	40	24±1.2	32±0.6	0.53±0.034	19±1.0	23±1.3	0.42±0.017	15±0.9	15±0.6	0.34±0.029
	60	28±1.7	39±2.1	0.57±0.017	20±1.2	27±1.2	0.47±0.032	17±1.3	17±0.5	0.39±0.028
	90	27±1.0	41±1.0	0.61±0.040	23±0.9	31±1.4	0.50±0.031	18±0.5	21±1.5	0.43±0.027
	120	28±0.4	43±0.9	0.59±0.020	23±0.9	35±1.2	0.53±0.017	18±0.8	20±0.8	0.45±0.011
A. tamarii	0	17±2.1	23±0.7	0.47±0.032	11±0.8	20±1.0	0.33±0.012	11±0.4	12±0.2	0.23±0.032
	20	20±0.9	30±1.1	0.52±0.012	12±0.5	22±0.8	0.37±0.040	13±1.2	11±0.3	0.28±0.017
	40	25±2.0	36±2.0	0.56±0.026	15±0.7	26±0.9	0.40±0.015	16±1.0	14±1.0	0.33±0.029
	60	26±1.3	40±2.5	0.58±0.034	19±0.6	32±0.5	0.45±0.020	20±0.4	19±0.3	0.40±0.030
	90	27±1.1	45±1.3	0.61±0.021	22±1.1	34±2.3	0.51±0.023	21±0.9	22±1.8	0.42±0.015
	120	28±1.0	47±0.7	0.64±0.043	23±1.4	36±1.0	0.56±0.040	21±1.8	22±0.4	0.46±0.023
A. terreus	0	21±0.6	28±1.7	0.52±0.020	13±0.8	22±1.8	0.40±0.023	12±1.1	13±1.7	0.26±0.015
	20	23±0.5	33±0.8	0.57±0.017	16±0.5	26±0.6	0.47±0.020	16±1.2	13±0.6	0.31±0.011
	40	26±2.0	37±0.9	0.62±0.029	19±1.0	31±1.2	0.52±0.029	19±1.6	15±5.6	0.35±0.029
	60	28±2.3	40±1.3	0.64±0.017	24±2.0	43±2.9	0.56±0.017	21±0.9	18±2.0	0.41±0.027
	90	29±0.7	45±1.7	0.68±0.023	24±0.4	46±1.7	0.60±0.032	22±0.2	20±0.6	0.43±0.017
	120	31±1.3	49±1.6	0.71±0.018	24±1.1	45±4.2	0.62±0.038	22±0.5	22±1.3	0.46±0.038
T. harzianum	0	20±0.6	29±1.8	0.33±0.031	11±0.5	18±0.6	0.32±0.023	10±0.3	11±0.6	0.19±0.017
	20	22±1.7	31±0.7	0.37±0.021	13±1.2	24±0.9	0.43±0.035	15±0.8	12±0.5	0.25±0.015
	40	25±2.3	39±2.1	0.41±0.023	17±0.7	29±1.3	0.47±0.034	18±1.5	15±0.3	0.31±0.023
	60	28±0.9	43±2.5	0.48±0.047	20±1.0	31±3.2	0.52±0.012	20±0.5	16±1.1	0.38±0.035
	90	26±0.7	45±1.0	0.53±0.025	22±1.1	35±5.3	0.59±0.043	21±1.2	19±1.0	0.41±0.023
	120	27±1.2	47±0.9	0.57±0.040	23±1.2	38±2.1	0.61±0.021	22±1.4	21±1.1	0.43±0.029
LSD 5%		3.9	4.3	0.08	2.9	5.8	0.08	2.8	4.4	0.07
1%		5.3	5.8	0.11	3.8	7.8	0.11	3.9	6.0	0.09

The data represent the mean ± standard error (SE) of three replicates.
[a] Cyolan®, Malathion® and Dursban® at 10 ppm level contain 36.5, 28.2 and 26.3 μg P/30 ml medium; 75, 58.2 and 27.1 μg S/30 ml medium, respectively.
[b] P and S are expressed in term μg/30 ml medium. [c] Extracellular protein was measured as mg BSA protein /30 ml medium.

ing number of reports on the degradation of synthetic organic chemicals including pesticides. These studies, which have been carried out with pure cultures of isolated microorganisms, with mixed microbial populations such as those which occur in soil and, in some cases, with microbial enzymes, have revealed that many of these chemicals are susceptible to biodegradation by microbes. The degree of degradation observed varied from one compound to another. Some molecules can be utilized as sole sources of carbon and energy for the growth of a particular organism leading in some, but not all, cases to the complete metabolism of the substrate. Other compounds were only partially degraded to non-metabolizable products while some are apparently completely resistant to microbial attack. This variation in susceptibility to microbial metabolism is not surprising since degradation of compounds of non-biosynthetic origin is presumably due to a low specificity of one or more catabolic enzymes of the organisms.

On the other hand, microbial metabolic alteration of insecticides was classified by Matsumara & Benezet (1978) into two main categories, enzymatic and non-enzymatic. However, the purpose by which microbial activities contribute to the overall alteration of insecticidal molecule by non-enzymatic mechanisms were not deeply investigated and apparently are of a minor role (Tweedy et al. 1970; Matsumara & Benezet 1978). Our results largely agree with such interpretations. There was a significant correlation between insecticides degradation, based on percentage of mineralized organic P and S and extarcellular protein (r = 0.89** and 0.64**, respectively) but not significantly correlated with microbial dry mass (Tables 1, 2, 3 and 4). Glenn & Gold (1985) suggested that extracellular protein is responsible for the biodegrada-

tion of organic matter. Also, culture filtrates of fungi including extracellular protein as crude enzyme preparations are routinely used for enzymatic degradation of organic compounds including organophosphate compounds (Tarafdar et al. 1988; Abd-Alla 1994a, b; Rana et al. 1996). Enzymatic degradation of bromoxynil by *Streptomyces felleus* was discussed by Neuzil et al. (1988). In agreement with results of the present study, Tarafdar et al. (1988) found no significant correlation between biomass production and organic P mineralization by fungi isolated from desert soils. The ability of *Trichoderma viride* to degrade organophosphate insecticides was discussed by Matsumura & Boush (1966, 1968). Biotransformation pathway of some pesticides in soil has been demonstrated by Levanon (1993). He reported that side chain mineralization of Alachlor and Atrazine was found to be mainly due to fungal activity. On the other hand, he found that the mineralization of carbofuran and malathion was mainly due to bacterial activity. Bujacz et al. (1995) studied the utilization of organophosphonates by a wildtype strain of *Penicillium notatum*. Results indicated that the fungus may play an important role in biodegradation of organophosphonates. However, the mould failed to metabolize the organophosphonates tested when they were used as sole carbon or nitrogen sources. Methylparathion degradation by *Pseudomonas* sp. A_3 have been reported by Ramanthan and Lalithakumari (1996). The bacterium detoxified 99% of 1 mM Methylparathion in 48 hr.

Dechlorination of tetra-chloroisophthalonitril (TNP) in soil was studied by Sato and Tanaka (1987). Also, Balajee and Mahadevan (1990) found that a strain of *Azotobacter chroococcum* efficiently dechlorinated 2,4-D. The release of chloride ion during degradation of Pentachlorophenol by the white rot fungus *Phanerochaete chrysosporium* was discussed by Aiken & Logan (1996).

Numerous studies have been made on C mineralization from organophosphate insecticides. Getzin (1967) showed indirectly that soil microorganisms degraded diazinon to $^{14}CO_2$, with 35% of the labeled ring carbon released as CO_2 in 20 weeks. Flshinski & Lichtenstein (1974) studied metabolism of (ethoxy-^{14}C) and (ring-^{14}C)-fonofos (as dyfonate) by soil fungi. They found that *Mucor plumbeus* and *Rhizopus arrhizus* were most active in degrading this insecticide. Methyl parathion (MPa) degradation in soil was studied by Ou et al. (1983) using ring-labeled [^{14}C] MPa. Results from disappearance of ^{14}C activity indicated that MPa could be rapidly mineralized in soil at 100 KPa, while mineralization at 1.5 MPa was slower.

Information on organic sulfur mineralization is not widely available. Formation of sulfur from pesticide degradation was reported by Moje et al. (1964). Lukens & Sisler (1958) reported the formation of significant amounts of carbon disulfide from captan in the presence of yeast cells.

Pesticides may be transformed or concentrated but not mineralized within the microbial cell. Kristufek et al. (1987) found that *Streptomyces felleus* took up more 95% of the initial amount of bromoxynil from the solid or liquid medium containing 100 μg of the herbicide per ml during a 5-d incubation: 50% of the amount taken up was degraded and 45% deposited in the cell (90% in the cytoplasm and 10% in the cell wall). Ko & Lockwood (1968b) found that fungal and actinomycete mycelia added to soil containing dieldrin, DDT and PCNB accumulated these compounds to levels above ambient concentration. Miller et al. (1967) suggested that the microflora of flooded Cranberry bog was responsible for the breakdown of parathion to aminoparathion and two unidentified metabolites. Partial degradation of parathion to aminoparathion, *p*-nitrophenol and *p*-aminophenol was also discussed by Lichtenstein & Schulz (1964). Conversion of parathion to aminoparathion in soil by an isolate of *Penicillium waksmani* was examined by Rao & Sethunanthan (1974).

Concentration of pesticides within the microbial cells may be a serious problem since these are released into the environment after cell lysis. Also, biotransformation of pesticides may lead to the formation of compounds more toxic than the original ones (Krueger & O'Brien 1959). Ko & Lockwood (1968a) found that DDT was rapidly converted to DDD in submerged soil amended with alfalfa residue. In nutrient media, they found that two of 10 bacteria and two of 10 actinomycetes were completely inhibited by 10 ppm DDT, whereas four of 10 bacteria and six of 10 actinomycetes were inhibited by 10 ppm DDD. Conversion of the organic constituents of pesticides to the mineral forms, which is not fully examined, is essential for complete detoxification of pesticides. Results of our study therefore appear to be important and encouraging.

It has been suggested that pesticides may be degraded photochemically through the microbial absorption of light energy and transmitting it to the insecticidal molecule (Matsumara & Benezet 1978). The

effect of light in the present study was avoided since the study was carried in dark.

The effect of metal ions on mineralization of organic P was examined by Abd-Alla (1994a, b) who reported that Mg^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+}, and Ca^{2+} increased P mineralization. In accordance with our findings, an increase in organic P mineralization at higher phosphate concentration was reported by several authors (Lantos & Ivanovics 1954; Warren 1968; Dobozy & Hammer 1969). Our results could be explained on the basis of increasing extracellular protein production which was significantly correlated to insecticide degradation, measured as inorganic P and S mineralized, (r = 0.96** and 0.83**, respectively). Other suggestions are that metallic P added to the growth medium is essential for enzymatic activity involved in insecticide mineralization i.e. as an energy source, and the synthesis of phospholipids and nucleic acids. The promotive effect of sulfur added on insecticide degradation may be also contribute to enhanced utilization in microbial cells for synthesis of vitamins, sulfolipids, amino acids and protein. Correlation between P and S mineralization from insecticide degradation and extracellular protein which was highly significant (r = 0.85 and 0.89, respectively) suggested a direct relationship between insecticide degradation and extracellular protein excretion. The role of extracellular protein in biodegradation of organic compounds was also demonstrated by Glenn & Gold (1985).

Results of the present study may fit the findings of others on the ability of some fungi to degrade pesticides, and introduce some new information on mineralization of organic constituent of pesticides. These active fungi could be used as inocula for this purpose in area in which these or related pesticides are used. These results will also be of great benefit, since the period of persistence of pesticides reaching the soil from treated plants or added directly to the soil may be shortened. This will decrease the side effects of pesticides on the non-target microorganisms that play an important role in soil fertility. Similarly, the elemental products of microbial mineralization of pesticides would be helpful for increasing soil fertility. This would lead to decrease in the use of phosphatic and sulfatic fertilizers and minimize the cost of crop production. Other important advantages of microbial degradation and mineralization of pesticides are the removal of these toxicants from the natural environment, maintenance of natural equilibrium and decreasing the hazardous effects on health of animals and mankind.

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