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# Fungal Utilization of Organophosphate Pesticides and Their Degradation by *Aspergillus flavus* and *A. sydowii* in Soil

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**ABSTRACT.** Fungal species were isolated which utilize organophosphate pesticides, *viz.* phosphorothioic (pirimiphos-methyl and pyrazophos), phosphorodithioic (dimethoate and malathion), phosphonic (lancer) and phosphoric (profenfos) acid derivatives. Pesticide degradation was studied *in vitro* and *in vivo* (soil). *Aspergillus flavus, A. fumigatus, A. niger, A. sydowii, A. terreus, Emericella nidulans, Fusarium oxysporum* and *Penicillium chrysogenum* were isolated from pesticide-treated wheat straw. The number of *A. sydowii* colonies was significantly promoted by 1 mmol/L pirimiphos-methyl, pyrazophos, lancer, dimethoate and malathion when used as phosphorus sources and by pirimiphos-methyl and pyrazophos when used as carbon sources. The number of *A. flavus* colonies increased with 0.5 mmol/L lancer and malathion used as the only carbon sources. *A. sydowii, A. niger, A. flavus, E. nidulans* and *F. oxysporum* grew on, and utilized, 5 pesticides as phosphorus source and showed more than 50 % mass growth. *A. sydowii, A. flavus* and *F. oxysporum* phosphatase hydrolyzed the pesticides suggesting that these species are important pesticide degraders. *A. sydowii* produced higher amounts of the phosphatase than *A. flavus* and *F. oxysporum*. The enzyme was highly active against pyrazophos, lancer and malathion used as the only sources of organic phosphate. *A. flavus* and *A. sydowii* phosphatases efficiently hydrolyzed pesticides at 300 ppm in soil, the degradation at 1000 ppm was lower. Mineralization of 1000 ppm pesticides in soil amended with wheat straw was higher than in nonamended soil. All added pesticides except profenfos were degraded within 3 weeks. Lyophilized adapted biomass of *A. flavus* and *A. sydowii* could thus be used for field biodegradation of these pesticides.

Successive applications of pesticides may result in combinations of pesticide residues in plants or soils, which may cause premature inactivation of a pesticide, crop damage, or the formation of new complex residues. Biodegradation of the residues in soil is therefore highly desirable.

Organophosphate residues in biosphere give rise to concern over their ultimate environmental fate and possible recalcitrance. Cleavage of the inherently stable C–P bond is the central requirement for complete organophosphate mineralization (Schowanek and Verstraete 1990).

Most studies of organophosphate fungicides, herbicides, and insecticides have been devoted to their action on microorganisms (Abdel-Mallek 1984; Hasan 1988; Čerňáková *et al.* 1992; Hasan and Omar 1993; Ismail *et al.* 1995). A few studies on utilization of organophosphate and other pesticides by bacteria have been conducted (Wanner 1994; Čerňáková 1995; McGrath *et al.* 1997) but no studies exist on the biodegradation of organophosphate pesticides by fungi.

We isolated and screened fungi utilizing organophosphate pesticides. Efficient utilizers could subsequently be used along with organophosphate pesticides in soil to serve as phosphate fertilizer for crop production. Several experiments in this direction are running *in vitro* and *in vivo*.

## MATERIALS AND METHODS

*Organophosphate pesticides.* Six pesticides belonging to 4 groups of organophosphate were used:

(*1*) Phosphorothioic acid derivatives included the insecticide pirimiphos-methyl [O,O-dimethyl-O-(2-diethylamino-6-methylpyrimidin-4-yl)phosphorothioate] (*Plant Protection Division*) and the fungicide pyrazophos [O,O-diethyl-O-(5-methyl-6-ethoxycarbonyl-pyrazolo(1,5-*a*)pyrimidin-2-yl)-phosphorothioate] (*Hoechst Orient S.A.A*).

(*2*) Phosphorodithioic acid derivatives included the insecticide dimethoate [O,O-dimethyl-5-(N-methylcarbamoylmethyl)phosphorodithioate] (*Kafr El-Zayat*) and the insecticide malathion [O,O-dimethyl-5-(1,2-dicarbethoxyethyl)phosphorodithioate] (*El-Naser Chemical Co*).

(*3*) Phosphonic acid derivative was the herbicide lancer [N-phosphonomethylglycine] (*Monsanto*).

(*4*) Phosphoric acid derivative was the insecticide profenfos [O,O-diethyl-O-(2-chloro-4-bromophenyl)phosphoric acid] (*Ciba Geigy*).

*Isolation and identification of wheat straw-borne fungi utilizing organophosphate pesticides as phosphorus and carbon sources.* The dilution-plate method (Johnson and Curl 1972) was used. Czapek–Dox agar medium (g/L: sodium nitrate 3.0, magnesium sulfate 0.5, potassium chloride 0.5, agar 15.0) was used for isolation of fungi. Potassium dihydrogen phosphate (5 mmol/L) served as a phosphorus source and glucose (50 mmol/L) served as carbon source. When organophosphates were used as sole phosphorus source, inorganic phosphate was replaced by the pesticides at 0.5, 1, 3 and 5 mmol/L. When the pesticides were used as the sole source of carbon, 5 mmol/L glucose was used as control and replaced by pesticides at 0.5, 1, 3 and 5 mmol/L. Rose-bengal was added to the medium as a bacteriostatic agent. Five plates were used for each concentration. The plates were incubated at 28 °C for 1–5 weeks and the growing fungi were counted and identified according to Raper and Fennell (1965) for *Aspergillus*, Booth (1977) for *Fusarium*, Christensen and Raper (1978) for *Emericella* and Pitt (1985) for *Penicillium* species. The average number of colonies per dish was multiplied by the dilution factor to obtain the number of colonies per g of wheat straw.

*Screening of fungal isolates for the ability to utilize organophosphate pesticides in enrichment liquid medium.* The culture medium (Czapek–Dox broth) contained (g/L): glucose 30, $NaNO_3$ 3, $MgSO_4$ 0.5 and KCl 0.5. $KH_2PO_4$ was omitted from the medium and replaced by the organophosphate pesticides in a final concentration of 0.5 mmol/L. The pH of the media was adjusted to 7. 250-mL Erlenmeyer flasks containing 50 mL of a sterilized medium were inoculated with 1 mL of spore suspension of *A. flavus*, *A. niger*, *A. sydowii*, *A. fumigatus*, *A. terreus*, *E. nidulans*, *F. oxysporum* and *P. chrysogenum*. Ammonium sulfate was used in culture media of *A. niger* and *P. chrysogenum* due to the $NaNO_3$ toxicity. The flasks were then incubated at 28 °C on a shaking platform at a frequency of 1.7 Hz. After 7 d, the cultures were filtered and dry mycelial mass was determined. Phosphate release into the culture supernatant was monitored by the method of Olsen *et al.* (1954). Inoculated medium with $KH_2PO_4$ (0.5 mmol/L) served as a standard against which fungal growth on pesticides was scored. Three flasks were used for each treatment and control.

*Activity of phosphatases against different pesticides.* Three isolates from a pesticide-treated liquid medium (*A. flavus*, *A. sydowii* and *F. oxysporum*) were tested further for their ability to produce phosphatase. The optimum conditions of Famurewa and Olutiola (1994) were used. Maximum phosphatase production was determined after 4 d of fungal growth in a medium containing 70 mmol/L phosphate, 5 % glucose and 0.5 % ammonium sulfate. Mycelial mats were extracted by acetate–acetic acid buffer (pH 4.5) or glycine–NaOH (pH 9.6). The homogenate was centrifuged at 10 000 ***g*** for 30 min at 4 °C. Phosphatase activity in pesticide hydrolysis was determined using the organophosphates as sole substrates. The reaction mixture consisted of 2.5 mL of the substrate (1 mmol in 0.1 mol/L sodium acetate–acetic acid buffer, pH 4.5, or glycine–NaOH buffer, pH 9.6) and 0.5 mL of enzyme preparation. After 1 h at 25 °C, the released phosphates were determined as mentioned above. One unit of phosphatase activity (1 nkat) is the amount of the enzyme which produced 1 nmol of phosphorus per second. Specific activity is given in nkat per mg dry mass.

*Pesticide degradation in soil.* Clay soil was obtained from the *Botanical Garden, Faculty of Science, Assiut University*. It has the following properties: organic matter 1.64 %, total N 0.12 %, soluble salts 0.42 %, pH 7.5. The soil was air-dried, passed through a 4 mm sieve, and remoistened with sterile distilled water to 20 % to permit good aeration. Amounts of 100 g of nonsterile soil were packed in polythene bags. The following variants were set up in triplicate: soil + pesticides (control I), soil + pesticides + inoculum (test I). Each pesticide was added at 300 and 1000 ppm. The soil was also amended with wheat straw in triplicate: soil + wheat straw + 1000 ppm pesticides (control II), soil + wheat straw + pesticides + inoculum (test II). Inoculations were done with 2 mL of spore suspension (*A. flavus* and *A. sydowii*). The bags were incubated at 25 °C. Weekly, the available phosphorus was extracted with $NaHCO_3$ (0.5 mol/L). Phosphorus in the filtrate was determined by the colorimetric molybdate blue method of Olsen *et al.* (1954). The phosphorus produced by the fungi in test I was compared to the phosphorus produced in control I and that produced in test II was compared to control II.

*Statistical analysis of the results.* Triplicate data of each experiment were analyzed statistically using one-way analysis of variance (PC program).

## RESULTS AND DISCUSSION

*Pesticide utilization as phosphorus source*

The utilization by wheat straw mycoflora of different pesticides containing the carbon-phosphorus bond as sole phosphorus sources is shown in Table IA. *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *A. terreus*, *Emericella nidulans*, *Fusarium oxysporum* and *Penicillium chrysogenum* were able to utilize 0.5 mmol/L organophosphate pesticides as phosphorus source.

*A. flavus* and *A. sydowii* utilized 1 mmol/L phosphorothioic (pirimiphos-methyl and pyrazophos), phosphorodithioic (dimethoate and malathion) and phosphonic (lancer) acid derivatives after 1 week, and 1 mmol/L phosphoric (profenfos) acid derivative after 5 weeks of incubation. *P. chrysogenum* utilized 1, 3 and 5 mmol/L pirimiphos-methyl, dimethoate, and malathion. *A. fumigatus* utilized pirimiphos-methyl and lancer, *F. oxysporum* used malathion and lancer, and *E. nidulans* used lancer in concentration of 1, 3 and 5 mmol/L as sole phosphorus sources.

Screening of fungal species utilizing pesticides showed that more than 75 % species utilized pirimiphos-methyl, dimethoate, malathion and lancer at 1 mmol/L and more than 50 % utilized these compounds at 3 mmol/L (Table IC). More than 50 % utilized pirimiphos-methyl, malathion and lancer at 5 mmol/L. Pyrazophos was utilized by 50 % at 1 mmol/L and less than 25 % at 3 and 5 mmol/L. Profenfos at 1 mmol/L was utilized by 25 % of fungal species after 5 weeks and not utilized before this period. More than 60 % of *A. flavus* and *A. sydowii* isolates utilized 5 mmol/L malathion and lancer as phosphorus sources. *A. sydowii* also utilized all pesticides at 1 mmol/L.

*Pesticide utilization as carbon source*

The ability of wheat straw mycoflora to use organophosphate pesticides as a sole carbon source is shown in Table IB. *A. sydowii* utilized pirimiphos-methyl and pyrazophos (phosphorothioic acid derivatives) at 0.5, 1 and 3 mmol/L, dimethoate (phosphorodithioic acid derivative) at 0.5 and 1 mmol/L after 1 week of incubation. Malathion and lancer (0.5, 1 and 3 mmol/L) were used as carbon source by *A. flavus*. Profenfos (0.5 mmol/L) preserved the growth of *A. sydowii* and *A. flavus* after 5 weeks of incubation.

16–33 % of fungal species utilized the pesticides as a sole carbon source (Table IC). More than 85 % of *A. flavus* isolates utilized 3 mmol/L lancer and more than 75 % of *A. sydowii* isolates utilized pirimiphos-methyl, and their colony number increased over the control at 1 mmol/L. Also, *A. sydowii* colony count increased 3-fold compared to control with 1 mmol/L pyrazophos.

*Pesticide utilization in enrichment culture*

In liquid enrichment cultures of eight fungal species supplied with 0.5 mmol/L pesticides (Table II), seven species grew on pirimiphos-methyl and pyrazophos and gave 58–81 and 50–101 % of dry mass, respectively, compared to the control (0.5 mmol/L $KH_2PO_4$). Five species utilized malathion and gave 60–115 % dry mass. Also, four species utilized lancer and dimethoate and gave more than 50 % of dry mass. *A. sydowii* followed by *F. oxysporum*, *A. niger*, *E. nidulans* and *A. flavus* were the best degrading species. *A. fumigatus*, *A. terreus* and *P. chrysogenum* also utilized the pesticides but produced less than 50 % growth mass. Zboinska *et al.* (1992) found that *P. citrinum* in liquid media did not use the herbicide lancer in 0.5 mmol/L. This may be explained by the inhibitory effect of sodium nitrate which was used as a nitrogen source. In our experiments *A. niger* and *P. chrysogenum* grew in a medium containing ammonium sulfate as nitrogen source instead of sodium nitrate.

The enrichment cultures thus utilized organophosphates as a sole source of phosphorus but their metabolism was not accompanied by a detectable release of inorganic phosphate. This may be due to the incorporation of soluble phosphorus into the fungal biomass. Shishkina and Trotsenko (1991) found that the phosphorus residue participates in the synthesis of ATP from ADP as well as in the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate.

*Phosphatase activity against pesticides*

The metabolism of organophosphate pesticides includes oxidation of dithiophosphates to thiophosphates (S–P=S → O–P=S) and thiophosphates to phosphates (O–P=S → O–P=O) by oxidase. Enzymic hydrolysis is probably the most important mechanism for the conversion of organic phosphate to inorganic phosphate in the soil (Brannon and Sommers 1985). The ability of phosphatases to

**Table I.** Isolation of common wheat straw-borne fungi (count per g) utilizing organophosphate pesticides after 1–3 weeks of incubation on solid media at 28 °C

**A** – *As phosphorus sources*

| Fungal species | Control C : P[a] mmol/L 50 : 5 | Phosphorothioic acid derivatives, mmol/L | | | | | | | | Phosphonic acid derivative | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pirimiphos-methyl | | | | pyrazophos | | | | lancer | | | |
| | | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 |
| Total count | 1540 | 1175 | 1251 | 364 | 114 | 774 | 688 | 63 | 13 | 752 | 789 | 826 | 726 |
| *Aspergillus flavus* LINK | 363 | 350 | 138 | 63 | 25 | 313 | 175 | – | – | 275 | 225 | 263 | 400* |
| *A. fumigatus* FRESENIUS | 150 | 25 | 150 | 25 | 13 | 38 | – | – | – | 13 | 88 | 50 | 75 |
| *A. niger* VAN TIEGHEM | 113 | 150* | 25 | – | – | 100 | – | – | – | 200* | 25 | 25 | 25 |
| *A. sydowii* THOM and CHURCH | 300 | 125 | 825* | 263 | 63 | 113 | 475* | 38 | 13 | 75 | 375* | 400* | 188 |
| *A. terreus* THOM | 13 | 75 | 25* | – | – | 25* | 13 | 25* | – | 25* | 25* | 25* | – |
| *Emericella nidulans* (EIDAM) VUILLEMIN | 13 | – | – | – | – | – | – | – | – | 13 | 13 | 50* | 25* |
| *Fusarium oxysporum* SHELECHT. ex FR. | 25 | – | – | – | – | – | – | – | – | 13 | 13 | 13 | 13 |
| *Penicillium chrysogenum* THOM | 563 | 450 | 88 | 13 | 13 | 135 | 25 | – | – | 113 | 25 | – | – |
| | | Phosphorodithioic acid derivatives | | | | | | | | Phosphoric acid derivative | | | |
| | | dimethoate | | | | malathion | | | | profenfos[b] | | | |
| | | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 |
| Total count | 1540 | 1102 | 740 | 836 | 38 | 1115 | 1301 | 889 | 639 | 400 | 325 | – | – |
| *Aspergillus flavus* LINK | 363 | 363 | 163 | 63 | – | 275 | 163 | 138 | 300 | 100 | 75 | – | – |
| *A. fumigatus* FRESENIUS | 150 | 38 | 38 | 75 | – | 63 | 75 | – | – | – | – | – | – |
| *A. niger* VAN TIEGHEM | 113 | 113 | 75 | 75 | – | 113 | 75 | 25 | 25 | – | – | – | – |
| *A. sydowii* THOM and CHURCH | 300 | 300 | 338* | 400* | – | 300 | 575* | 625* | 288 | 300 | 250 | – | – |
| *A. terreus* THOM | 13 | 50* | 13 | 38* | – | 50* | – | – | – | – | – | – | – |
| *Emericella nidulans* (EIDAM) VUILLEMIN | 13 | 13 | 63* | – | – | 13 | 150* | 50* | – | – | – | – | – |
| *Fusarium oxysporum* SHELECHT. ex FR. | 25 | – | – | – | – | 13 | 13 | 13 | 13 | – | – | – | – |
| *Penicillium chrysogenum* THOM | 563 | 225 | 50 | 175 | 38 | 275 | 250 | 38 | 13 | – | – | – | – |

*Significant increase in fungal count over control at 5 % level.

[a]C : P = glucose as carbon source, $KH_2PO_4$ as phosphorus source.

[b]Profenfos fungi were recovered and identified after 5 weeks of incubation.

**B** – *As carbon sources*

| Fungal species | Control C : P mmol/L 5 : 5 | Phosphorothioic acid derivatives, mmol/L | | | | | | | | Phosphonic acid derivative | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | pirimiphos-methyl | | | | pyrazophos | | | | lancer | | | |
| | | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 |
| Total count | 1025 | 375 | 375 | 225 | – | 1250 | 1000 | 50 | – | 300 | 200 | 150 | – |
| *Aspergillus flavus* LINK | 175 | – | – | – | – | – | – | – | – | 300* | 200* | 150 | – |
| *A. fumigatus* FRESENIUS | 100 | – | – | – | – | – | – | – | – | – | – | – | – |
| *A. niger* VAN TIEGHEM | 125 | – | – | – | – | – | – | – | – | – | – | – | – |
| *A. sydowii* THOM and CHURCH | 300 | 375* | 375*[a] | 225[a] | – | 1250* | 1000* | 50[a] | – | – | – | – | – |
| *Emericella nidulans* (EIDAM) VUILLEMIN | 50 | – | – | – | – | – | – | – | – | – | – | – | – |
| *Penicillium chrysogenum* THOM | 275 | – | – | – | – | – | – | – | – | – | – | – | – |

| | | Phosphorodithioic acid derivatives | | | | | | | | Phosphoric acid derivative | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | dimethoate | | | | malathion | | | | profenfos[b] | | | |
| | | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 |
| Total count | 1025 | 275 | 100 | – | – | 300 | 25 | 25 | – | 125 | – | – | – |
| *Aspergillus flavus* LINK | 175 | 175 | – | – | – | 275* | 25 | 25 | – | 25 | – | – | – |
| *A. fumigatus* FRESENIUS | 100 | 25 | 25 | – | – | 25 | – | – | – | – | – | – | – |
| *A. niger* VAN TIEGHEM | 125 | – | – | – | – | – | – | – | – | – | – | – | – |
| *A. sydowii* THOM and CHURCH | 300 | 75 | 75 | – | – | – | – | – | – | 100 | – | – | – |
| *Emericella nidulans* (EIDAM) VUILLEMIN | 50 | – | – | – | – | – | – | – | – | – | – | – | – |
| *Penicillium chrysogenum* THOM | 275 | – | – | – | – | – | – | – | – | – | – | – | – |

[a]The colonies did not form conidiophores and conidia.
[b]Profenfos fungi were recovered and identified after 5 weeks of incubation.

*continued*

C – Number of fungal species (%) recovered on pesticide-treated media (0.5, 1, 3 and 5 mmol/L) when used as a sole phosphorus and carbon source[a]

| Pesticides | Total fungal species, % | | | | | | | | *A. flavus*, % | | | | | | | | *A. sydowii*, % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | as phosphorus | | | | as carbon | | | | as phosphorus | | | | as carbon | | | | as phosphorus | | | | as carbon | | | |
| | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 | 0.5 | 1 | 3 | 5 |
| Pirimiphos-methyl | 75 | 75 | 50 | 50 | 17 | 17 | 17 | 0 | 96 | 38 | 17 | 7 | 0 | 0 | 0 | 0 | 41 | 275 | 88 | 21 | 125 | 125 | 75 | 0 |
| Pyrazophos | 75 | 50 | 25 | 13 | 17 | 17 | 17 | 0 | 86 | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 37 | 158 | 13 | 4 | 417 | 333 | 17 | 0 |
| Dimethoate | 88 | 88 | 75 | 13 | 50 | 33 | 0 | 0 | 100 | 45 | 17 | 0 | 100 | 0 | 0 | 0 | 100 | 113 | 133 | 0 | 25 | 25 | 0 | 0 |
| Malathion | 100 | 88 | 75 | 63 | 33 | 17 | 17 | 0 | 76 | 45 | 38 | 83 | 157 | 14 | 14 | 0 | 100 | 192 | 208 | 96 | 0 | 0 | 0 | 0 |
| Lancer | 100 | 100 | 88 | 75 | 17 | 17 | 17 | 0 | 76 | 62 | 73 | 110 | 171 | 114 | 86 | 0 | 25 | 125 | 133 | 63 | 0 | 0 | 0 | 0 |
| Profenfos[b] | 25 | 25 | 0 | 0 | 33 | 0 | 0 | 0 | 28 | 21 | 0 | 0 | 14 | 0 | 0 | 0 | 100 | 83 | 0 | 0 | 33 | 0 | 0 | 0 |

[a]Control (100 %) = $KH_2PO_4$ (5 mmol/L) as phosphorus source and glucose (50 mmol/L) as carbon source (extracted from parts A and B).
[b]Profenfos fungi were recovered and identified after 5 weeks of incubation.

**Table II.** Utilization of pesticides (0.5 mmol/L) as a sole phosphorus source by 8 fungal species in liquid medium incubation in shaker at frequency of 1.7 Hz, 28 °C after 7 d[a]

| Pesticides | *A. flavus* | | *A. fumigatus* | | *A. niger* | | *A. sydowii* | | *A. terreus* | | *E. nidulans* | | *F. oxysporum* | | *P. chrysogenum* | | Average growth[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | GM | G | GM | G | GM | G | GM | G | GM | G | GM | G | GM | G | GM | G | GM | G |
| $KH_2PO_4$ | 370 | 100 | 365 | 100 | 260 | 100 | 87 | 100 | 210 | 100 | 260 | 100 | 370 | 100 | 253 | 100 | 272 | 100 |
| Pirimiphos-methyl | 250 | 68 | 210 | 58 | 181 | 70 | 68 | 78 | 170 | 81 | 200 | 77 | 265 | 72 | 58 | 23 | 175 | 65 |
| Pyrazophos | 200 | 54 | 30 | 8 | 263 | 101 | 54 | 62 | 170 | 81 | 160 | 62 | 248 | 67 | 154 | 61 | 160 | 59 |
| Dimethoate | 175 | 47 | 103 | 28 | 120 | 46 | 51 | 59 | 60 | 29 | 135 | 52 | 255 | 69 | 144 | 57 | 130 | 48 |
| Malathion | 220 | 60 | 100 | 27 | 178 | 68 | 100 | 115 | 66 | 31 | 120 | 46 | 271 | 73 | 156 | 62 | 151 | 56 |
| Lancer | 195 | 53 | 160 | 44 | 157 | 60 | 53 | 61 | 55 | 26 | 156 | 60 | 290 | 78 | 109 | 43 | 147 | 54 |
| Profenfos | 85 | 23 | 18 | 5 | 0 | 0 | 15 | 17 | 100 | 48 | 100 | 39 | 77 | 21 | 0 | 0 | 49 | 18 |
| Average growth[c] | 188 | 51 | 104 | 28 | 150 | 58 | 57 | 65 | 104 | 49 | 145 | 56 | 234 | 63 | 104 | 41 | – | – |

[a]GM = growth mass (mg/50 mL medium), G = growth related to control (%). *A. niger* and *P. chrysogenum* grown in a medium containing ammonium sulfate instead of sodium nitrate (toxic for the two species) as nitrogen source. Each value represents the mean of three replicates.
[b]For the 8 fungal species.
[c]On 6 pesticides.

participate in the hydrolytic detoxification of pesticides was assessed. *A. flavus*, *A. sydowii* and *F. oxysporum* were investigated for the production of cellular and extracellular phosphatase and their activity in hydrolyzing the organophosphate pesticides was determined (Table III). The fungal species were able to produce both acid (EC 3.1.3.2) and alkaline (EC 3.1.3.1) phosphatases, the production of alkaline phosphatases being higher. They produced also both extra- and intracellular phosphatases, the intracellular activity being higher.

**Table III.** Alkaline phosphatase activity of the fungal species hydrolyzing pesticides at 25 °C

| Pesticides | *A. flavus* | | | *A. sydowii* | | | *F. oxysporum* | | |
|---|---|---|---|---|---|---|---|---|---|
| | P produced µmol/h | EA[a] | specific activity nkat/mg | P produced µmol/h | EA | specific activity nkat/mg | P produced µmol/h | EA | specific activity nkat/mg |
| Pirimiphos-methyl | 890 ± 10 | 247 | 25 | 1150 ± 12 | 319 | 32 | 820 ± 8 | 231 | 23 |
| Pyrazophos | 1270 ± 14 | 353 | 35 | 2170 ± 21 | 603 | 60 | 1240 ± 11 | 344 | 34 |
| Dimethoate | 900 ± 16 | 250 | 25 | 1170 ± 18 | 325 | 32 | 780 ± 7 | 217 | 22 |
| Malathion | 1090 ± 30 | 303 | 30 | 1670 ± 17 | 464 | 46 | 920 ± 13 | 256 | 26 |
| Lancer | 1060 ± 18 | 294 | 29 | 1820 ± 14 | 506 | 51 | 1060 ± 9 | 294 | 29 |
| Profenfos | 600 ± 11 | 167 | 17 | 920 ± 12 | 256 | 26 | 600 ± 14 | 167 | 17 |

[a]Enzyme activity, phosphorus (in nkat) released by 1 mL extract.

The enzymes hydrolyzed the pesticides suggesting that these species may play an important role in the degradation of pesticides. *A. sydowii* phosphatase was highly active against pyrazophos followed by lancer and malathion. Profenfos was more resistant to phosphatase degradation. This may be explained by the chlorobromophenol products which may inhibit phosphatase activity. Famurewa and Olutiola (1994) suggest that the phosphatase may possess cysteine residues or disulfide bridges.

*Pesticide degradation in soil*

Since the conditions in soil are much more complex than those in synthetic media, the ability for pesticide degradation in nonsterile soil was investigated. Two isolates of phosphatase-producing fungi were tested further for their ability to hydrolyze the organophosphates in soil.

**Table IV.** Biodegradation of pesticides in soil (20 % moisture content) by *A. flavus* and *A. sydowii* after 1 week at 25 °C

| Pesticides | Conc. ppm | Net change in phosphorus produced, µg/g soil[a] | |
|---|---|---|---|
| | | *A. flavus* | *A. sydowii* |
| Pirimiphos-methyl | 300 | 36.8 ± 4.5 | 43.3 ± 3.9 |
| | 1000 | 13.5 ± 1.4 | 7.5 ± 0.9 |
| Pyrazophos | 300 | 29.3 ± 2.3 | 25.3 ± 3.0 |
| | 1000 | 14.5 ± 0.8 | 12.5 ± 1.5 |
| Dimethoate | 300 | 25.0 ± 1.2 | 25.0 ± 2.0 |
| | 1000 | 5.0 ± 0.6 | 10.0 ± 1.0 |
| Malathion | 300 | 31.5 ± 3.3 | 26.5 ± 2.2 |
| | 1000 | 11.3 ± 1.1 | 8.8 ± 1.2 |
| Lancer | 300 | 45.0 ± 2.2 | 65.0 ± 4.2 |
| | 1000 | 7.0 ± 0.3 | 7.5 ± 0.8 |
| Profenfos | 300 | 41.5 ± 3.2 | 39.5 ± 3.1 |
| | 1000 | 1.8 ± 0.4 | 4.3 ± 0.6 |

[a]Net phosphorus produced = phosphorus produced in test (nonsterile soil + pesticides + inoculum) − phosphorus produced in control (nonsterile soil + pesticides).

Soluble phosphorus increased distinctly under the action of *A. flavus* and *A. sydowii* (Table IV). Even in nonsterile soil these two species were effective in hydrolyzing pesticides. Phosphatases produced by *A. flavus* and *A. sydowii* hydrolyzed 300 ppm pesticides more than 1000 ppm. The mineralization of 1000 ppm pesticides in amended soil with wheat straw was higher than in nonamended soil (Table V). All added pesticides except profenfos were degraded by the end of week 3 (*data not shown*).

*A. flavus* and *A. sydowii* are to our knowledge the first fungi isolated from wheat straw capable of degrading organophosphate pesticides and utilizing these compounds as sole phosphorus and carbon sources by releasing phosphorus from these pesticides through the action of their phosphatases. These strains could be beneficial as a fungal inoculum for efficient hydrolysis of pesticides.

**Table V.** Biodegradation of a high pesticide level (1000 ppm) in soil amended with wheat-straw after 1 week at 25 °C

| Pesticides | Net change in phosphorus produced, µg/g soil[a] | |
|---|---|---|
| | *A. flavus* | *A. sydowii* |
| Pirimiphos-methyl | 26.0 ± 2.0 | 28.0 ± 2.3 |
| Pyrazophos | 76.0 ± 4.0 | 55.0 ± 5.0 |
| Dimethoate | 20.0 ± 1.8 | 21.0 ± 2.2 |
| Malathion | 47.5 ± 3.3 | 37.5 ± 2.4 |
| Lancer | 35.0 ± 1.8 | 22.0 ± 2.1 |
| Profenfos | 14.0 ± 1.0 | 13.0 ± 0.9 |

[a]Net phosphorus produced = phosphorus produced in test (nonsterile soil + wheat straw + pesticides + inoculum) − phosphorus produced in control (nonsterile soil + wheat straw + pesticides).

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