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BIODEGRADATION OF THE ORGANOPHOSPHORUS INSECTICIDE MONOCROTOPHOS BY *PENICILLIUM CORYLOPHILUM*

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ABSTRACT

Biodegradation of the organophosphorus insecticide monocrotophos by a local strain of Penicillium corylophilum has been cofirmed to be via the production of a thermolabile extracellular oxidizing enzyme. The enzyme is active over a relatively wide range of pH vaules (6-9) with an optimum actiuity at pH 8.0. Moreover, the possibility of using the partially purified enzyme as field inoculum to decontaminate soil from monocrotophos residues was also studied.

INTRODUCTION

Obviously, intensive use of pesticides in modern agriculture leads to contamination of agricultural soils and consequently plants with residues of these chemicals[1,2]. However, microbial degradation of pesticides in the rhizosphere of crops has the potential for protecting susceptible plants from these toxic substances[3,4].

The role of microbial adaptations and biotechnology in promoting fungal degradation of pesticides was reported[5-7]. Moreover, different roles for fungi and bacteria in the biotransformation pathways of malathion, alachlor, atrazine and carbofuran were demonstrated[8]. It was also found that, the mineralization of alkyl-side chains of alachlor and alkyl-amino-side chains of atrazine was mainly due to fungal activity. The mineralization of carbofuran and malathion was mainly due to bacterial activity.

These results indicated that the biodegradation of pesticides in the soil is performed by both fungi and bacteria. On the other hand, the degradation of organsphosphonates by *Penicillium citrinum* was proved[9] and reported that the fungus utilized 2-aminoethylphosphonic acid and 2-oxoalkylphosphonic acids as the sole phosphorus source.

Concerning the route by which soil microflora degrade the pesticides, it was reported[10] that the insecticide dyfonate was first absorbed and metabolized by the mycelia of the tested fungi, and water soluble metabolities were then released to the media. On the other hand, it was found that[11] the degradation of the herbicide propanil by *Fusarium solani* occurred in the culture media by the action of acylamidases enzyme.

The aim of the present investigation is to gain an insight on the mode by which the organophosphorus insecticide monocrotophos is being degraded by a local strain of *Penicillum corylophilum.*

MATERIALS AND METHODS

Test Soil :

The soil used in this investigation was collected at a depth of 10 cm from a cultivated land in Zagazig area, Egypt. The soil samples were air-dried at room temperature and passed through a 2- mm sieves to remove pebbles and debris.

Experimental organism:

A fungal organism was isolated from agricultural soil previously treated with monocrotophos and was found to be able to utilize monocrotophos as a sole carbon source (unpublished data). This organism was identified as *Penicillium corylophilum.* according to procodure of Raper et al[12] and Pitt [13].

Test for the presence of extracellular enzyme:

For confirmation of the presence of extracelluar enzyme capable of degrading the organophosphorus monocrotophos in the cultures of P. corylophilum, the fungus was cultivated at 28°C in a medium (pH 8.0) containing 100 ug monocrotophos /50 ml for 22 days. Complete disappearance of the insecticide in the culture filtrate was ensured by TLC. Mycelia-free filtrates were divided into two sets. One of them was boiled for 10 min and after cooling to room temperature, both sets was supplemented with 100 ug monocrotophos.

The same quantity of the insecticide was added to equivalent amount of sterilized media. All sets of flasks were, then, incubated at 28°C for one hour. Thereafter monocrotophos residues were extracted and estimated.

Determination of the optimum pH of the Degrading Enzyme:

To determine the optimum pH value of the degrading enzyme, partial purification of the enzyme

from 22 day old culture was carried out. Two ml. of 2% (w/v) streptomycin sulphate was added to 50 ml cell-free medium[14]. The precipate collected by centrifugation was discarded. Further purification was achieved using 80% ammonium sulphate[11]. The precipitate was resuspended in 2ml deionized water and was used as a crude enzyme. Crude enzyme was poured in 10 ml conical flask containing 2ml, 0.5 M phosphate buffer of the desired pH value and one mg insecticide in one ml distilled water. Monocrotophos residues were determined after 30 min at 28°C.

Determination of incubation time:

To study the effect of aging on the partially purified enzyme acivity, and the kind of enzymatic reactions occurs (after preliminary trials using various time intervals and different enzyme cofactors including; $NADPH_2$, ATP and FAD). Two sets of flasks cotaining 2 ml phosphate buffers (pH 8.0), 2 ml crude enzyme suspension and 1 mg monocrotophos dissolved in 1 ml distilled water were prepared and 5.0 ug of $NADPH_2$ was added to one set of flasks [15]. Flasks were incubated at 28°C. At zero time and at each time interval the residual insecticide was determined.

The insecticide:

The desired concentration of a technical grade monocrotophos (74.2% pure, Ciba Geigy Ltd) was dissolved in acetone. The solvent was allowed to evaporate at room temperature and the residues were redissolved in deionized water and then added to the soil.

Insecticidal culture treatments:

Sterilized Czapek-Dox broth without carbon source was prepared (3.0 gm $NaNO_3$, 1.0 gm KH_2PO_4, 0.5 gm $MgSO_4$, 0.5 KCl, 0.01 gm $FeSO_4$ and 1000 ml distilled water). The prepared medium was distributed in sterilized 250 Erlenmeyer flasks. The fasks were inoculated with one ml spore suspensions harvested from 4 days old slants of Czapek-Dox agar containing the insecticide as a sole carbon source. To the culture media, 100 ug of monocrotophos was supplemented in an aqueous solution previously passed through a Millipore filter (pore size 0.22 um) to provide a final culture volume of 50 ml.

Extraction of the insecticide:

Monocrotophos residues were extracted from soil, fungal mycelia and cell free media using a method adopted by Megharaj et al [16]. The insecticide was separated by TLC and determined colorimetrically at 490 nm [17].

Determination of the mycelial dry weight:

The contents of each flask were filtered through previously weighed wathman no. 1 filter paper and the obtained mass was successively washed with distilled water then dried at 70°C in an oven for 24 h. or till constant weight.

Determination of the pH:

The pH values were adjusted using phosphate buffers and was measured according to Richards [18].

RESULTS AND DISCUSSION

The mycelial dry weights and monocrotophos residues in both fungal felts and cell free filtrates in the series of culture plasks were determined.

Results of the experiment (Table 1) revealed that after 2 days of incubation insecticide residues could be detected in the fungal cells, meanwhile, some of the parent insecticide added to the medium was degraded in the cell free cultures. Moreover, a loss of 7.8% of monocrotophos was recorded in the unboiled flasks. Meanwhile, the degradation was not noticed in control treatment or previously boiled cell free cultures. These results support the urgument that degradation of the insecticide monocrotophos by the test fungus was due to thermolabileextracellular enzyme and the secretion of this degrading enzyme started in the lag phase of the fungal growth. The synthesis of pesticides degrading enzymes by microorganisms during the lag phase of growth was previously reported [19-22].

The recovery of monocrotophos in the culture filtrate decreased steadily by increasing the incubation time and a complete loss of monocrotophos in the cell free media was attained after 22 days of incubation. The disappearance of the insecticide may be a reflection of de novo enzyme secretion. The insecticide monocrotophos was found to accumulate in the mycelial cells throughout the course of the experiment. Maximal accumulation (18%) was recorded at the 12th day of incubation, it decreased steadily but did not disappear after that. The fungus might store considerable amount of the insecticide to use it as a sole source of carbon. In accordance with this observation, association of insecticides with the microbial cells was reported by many investigators [23-25].

The adsorption and/or the absorption of insecticides by the mycelial mats was also confirmed before[26]. In the present investigation, the maximal association of the insecticide with the fungal cells was evident during the highest level of the fungal biomass (Table 1), which may indicate that the association of the insecticides to the microbial cells is growth dependant. due to the increase in the surface area to which the compound was adsorbed. Similar result was previously reported[27, 28]. They showed that the insecticides were liable to accumulation in fungal mycelia and a saturation point was reached where more accumulation occurred with a greater amount of mycelia.

Also, as described previously, the association of monocrotophos with the fungus mycelia was growth

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Table (1): Effect of incubation period on growth and degradation of monocrotophos by the soil fungus *P. corylophilum.*

Incubation days	Dry weight g/50ml	% monocrotophos residues		
		Filtrate	Mycelia	Control
0	ND	98	ND	95
2	0.02	93	ND	94
4	0.169	81	5	92
6	0.441	57	11	89
8	0.695	42	13	87
10	0.726	35	14	86
12	0.796	25	18	85
14	0.693	18	13	84
16	0.660	15	12	83
18	0.630	13	11	82
20	0.605	10	10	74
22	0.581	ND	10	

* pH of the culturue media was adjusted at 8.0.
* O: time is the culture medium directly after inoculation.
* ND: not detected under the experimental conditions.
* Each result was a mean of 3 replicates.

Table (2): Effect of pH values of growth of *P. corylophilum.* and the degradation of monocrotophos in fungal mycelia and cell free filtrates.

pH values	Dry weight g/50ml	% monocrotophos residues	
		Filtrate (inoculated)	Control (uninoculated)
5.0	0.188	60	77
5.5	0.228	58	78
6.0	0.471	51	80
6.5	0.641	49	85
7.0	0.677	48	89
7.5	0.589	45	89
8.0	0.413	41	87
8.5	0.385	38	83
9.0	0.215	55	70
9.5	0.031	57	64

* pH values were adjusted using phosphate buffers.
* Initial monocrotophos concentration was 100 ug/100ml medium.
* Each result was a mean of 3 replicates.

Table (3): Effect of the pH of the reaction media on the enzyme activity.

pH values	Monocrotophos residues in µg	% Degradation
5.0	966	3.4
5.5	952	4.8
6.0	934	6.6
6.5	926	7.4
7.0	918	8.2
7.5	905	9.5
8.0	891	10.9
8.5	915	8.5
9.0	934	6.6
9.5	971	2.9

* Each result was a mean of 3 replicates

** Initial concentration of monocrotophs was 1mg/ 5ml.

Table (4): Effect of incubation time on monocrotophos degradation by *P. corylophilum* enzyme in presence and absence of $NADPH_2$.

Time (min)	Monocrotophos residues in ug		% Degradation	
	A	B	A	B
0	997	997	-	-
10	935	928	6.2	6.9
20	912	890	8.5	10.7
30	889	863	10.8	13.4
40	884	852	11.3	14.5
50	868	845	12.9	15.2
60	836	806	16.1	19.1

% Degradation = Quantity at zero time - Residue at each time interval x 100/ Quantity at zero time.

Monocrotophos residue at zero time was taken as control

A --> media without $NADPH_2$

B --> media with $NADPH_2$

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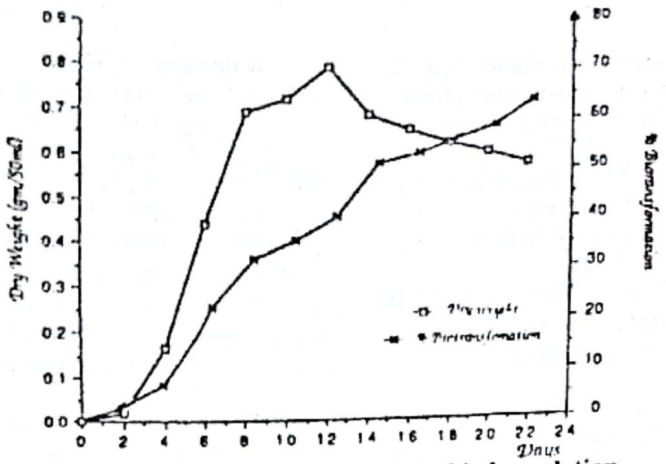

Fig. (1): The relationships between the biodegradation of monocrotophos and the fungal growth.

linked, yet the total biotransformation of the insecticide by the whole culture of *P. corylophilum* which can be calculated by subtracting the summation of monocrotophos residues in the filtrate and mycelia from the residue recovered in the uninoculated control treatment, showed a growth independence (Fig.1). These results are in agreement with that of Flashinski et al [10] whose results indicated that metabolism of dyfonate insecticide by *P. notatum* and *Rhizopus oryzae* was culture-age independent.

Results of table (2) indicated that the rate of hydrolysis of monocrotophos in control treatment was pH- dependent, where it was highly hydrolyzed at high alkaline (pH 9-9.5). On the other hannd, analysis for monocrotophos in fungal filtrate (table 2) also showed that the rate of insecticide breakdown by the test organism was affected mainly by the pH value of the culture media and the maximum activity of biodegradation was recorded at pH (7.5-8.5). In this connection, sensitivity of monocrotophos to pH values in aqueous solution was reported by Lee et al [3] who found that monocrotophos was rapidly decomposed at pH 9.0.

Degradation of insecticides by microbial cell-free media was previously reported by some investigators[25,26].

Results in Table (3) indicated that the crude enzyme was active over a wide range of pH values (6.0-9.0) with optimal activity at pH value 8.0 Alkaline optimal activity of this insecticide degrading enzyme have been also repoted [7,11,28].

Results of Table (4) revealed that the enzyme activity increased by increasing the reaction time and monocrotophos metabolism was stimulated by exogenouly added NADPH2. It is concluted that, the main enzyme responsible for the degradation of monocrotophos is an oxidative stystem. Similar results were obtained before[30].

Since the main purpose of studying pesticide biodegradation is to find a way to decontaminate soil from these dangerous chemicals, the potentiality of the crude enzyme to degrade monocrotophos in soil was tested. 1mg of the insecticide was mixed with 20 gm sterilized soil. The crude enzyme was added to the soil in an amount of deionized water sufficient to make the field moisture level (19.6%). 6.2% of the applied monocrophos was calculated to be degraded after the investigated period (1 hr.); indicating that the crude enzyme suits well for degradation of this toxic chemical in soil environments.

Low degradation of monocrotophos in soil by the crude enzyme compared to that in vitro might be due to the presence of certain substances in soil that inhibit enzyme active-sites. Furthermore, the degradation of insecticides in soils may be a function of more than one factor such as soil texture[30], soil temperature[31], organic matter content[32,34]. However,, the biodegradation of the insecticide in the soil needs further investigation which will be dealt within another communication. The success in biodegrading these toxic substance at higher rates may make the use of insecticides more safe.

REFERENCES

1. Cabras, P.; Cabitza, F.; Melone, M. and Pirisi, F., Behavior of some pesticides residues on greenhouse tomatoes 2-Fungicides, Acanicides, and Insecticides. **J. Agr. Food Chem.,** 33: 935-937, (1985).
2. Pozo, C.; Salmeroon, V.; Rodelas, B.; Martinez-Toledo, M.V. and Gonzaler-Lopez, J., Effects of the herbicide alachlor on soil microbial activities. **Ecotoxicology**, 3:1, 4-10, (1994).
3. Lee, P.W.; Fukuto, J. M.; Hernandez, H. and Stearns, M.S., Fate of monocrotophos in the environment. **J. Agr. Food Chem.,** 38: 567-573, (1990).
4. Domsch, K.H., **Pesticides in Soils**: microbial decomposition and secondary effects on microorganisms. VCH ver lagsgese schaft mbh; weinheim; Germany (1992).
5. Singh, U.D.; Sethunathan, N. and Raghu, K., Fungal degradation of pesticides. **Handbook of Applied Mycology,** Volume I, soil and plants (edited by Arora, D.K., Bharat, R.; Mukerjj, K.G. and Knudsen, G.R.), New York, USA; Marcel Dekker Inc., pp 541-588 (1991).
6. Cosete, C.M., Biodegradation. Infos-Paris, No. 85, 45-46, (1992).
7. Donnelly, P.K.; Entry, J.A. and Crawford, D.L., Degradation

of atrazine and 2,4- dichlorophenoxyacetic acid by mycorrhizal fungi at three nitrogen concentration in vitro. **Applied and Environmental Microbiology,** 59: 2642-2647, (1993).

8. Levanon, D., Roles of fungi and bacteria in the mineratization of the pesticides atrazine, alachlor, malathion and carboforan in soil. **Soil biology and biochemistry,** 25: 8, 1077-1165 (1993).
9. Zboinska, K.; Maliszwska, I.; Lejczak, B. and Kafarzi, P., Degradation of organophosphontes by Penicillium citrinum. **Letters in applied microbiology,** 15: 269-272, (1992).
10. Flashinski, S.J. and Lichtenstein, E.P., Environmental factors affecting the degradation of dyfonate by soil fungi; **Can. J. Microbiol.,** 21: 17-25, (1975).
11. Lanzilotta, R.JP. and Pramer, D., Herbicid transformation. II. Studies with and acylamidase of Fusarium solani. **Appl. Microbiol.,** 19: 307-313, (1970).
12. Raper, K. and Thom, C., **A manual of Penicillia.** Williams and Wilkins, Baltimore, U.S.A., (1949).
13. Pitt, J.I., **The Genus Penicillium** and its teleomorphic states Eupenicillium and Talaromyces. Academic Press (London) Ltd., (1979).
14. Kearney, P.C., Purification of an enzyme responsible for hydrolysing phenylacarbamats. **J. Agr. food Chem.,** 13: 564, (1965).
15. Karasawa, T.; Ikoma, S.; Yamakawa, K. and Nakamura, S., Adetised growth medium for Clostridium difficile. **Microbiology,** 141, 371-375, (1995).
16. Megharaj, M.; Venkaleswalu, K. and Rao, A.S., Microbial degrdation and algal toxicity of monocrtophos and quinophos in flooded soil. **Chemosphere,** 17: 1033-1039, (1988).
17. Megharaj, M.; Venkaleswalu, K. and Rao, A.S., Metabolism of monocrotophos and quinolphos by algae isolated from soil. **Bull. Environ. Contamitoxicol.,** 34: 251-256, (1987).
18. Richards, L.A., **Diagnosis and improvement of saline and alkali soils,** U.S. Dept. Agric. Handbook No. 60, (1954).
19. Wallnofer, P.R. and Bader, J., Degradation of urea herbicides by cell free extract of Bacillus. **Appl. Microbiol.,** 19: 714-717, (1970).
20. Thompson, C.; Movva, N.; Tizard, R.; Crameri, R. and Davies, L., Characterization of the herbicide resistance gene bar from Streptomyces hygroscopicus. **EMBO. J.,** 6: 2519-2523, (1987).
21. Sahid, I.B. and Yap, M.P., Effects of two acetanilid herbicides on microbial populations and their cellulolytic activities. **Bulletin of environmental contaminationn and toxicology,** 52: 61-68, (1994).
22. Tu, C.M., Effect of three newer pesticides on microbial and enzymatic activities in soil. **Bulletin of environmental contamination and toxicology,** 49: 120-126, (1992).
23. Miyazaki, S.; Bruch, G.M. and Matsumura, F., Microbial degradation chlorobenzilate. **J. Agr. Food. Chem.,** 18: 87-91, (1970).
24. Salama, A.M.; Mostafa, I.Y. and El-Zawawy, Y.A., Insecticide and soil microorganisms. III. Fate of C- 14 labelled dipterex as affected by two nodule forming Rhizobium species. **Acta Bid. Acad. Sci. Hung.,** 26: 1-7, (1975).
25. Chakrabarty, S.K. and Anjan, G., Degradation of hexachlor by m soil junge; **Chemosphere,** 23: 1, 99-105, (1991).
26. Ou, Li-Tse and Sharma,A.J., Degradation of methyl parathion by a mixed bacterial culture and Bacillus species isolated from different soils. **J. Agr. Food. Chem.** 37: 1514-1518 (1989).
27. Ko, W.H. and Lockwood, J.L., Accumulation, concentration of chlorinated hydrocarbon pesticides by microorganisms in soil. **Can. J. Microbiol.,** 14: 1075-1078, (1968).
28. Katayama, A. and Kuwatsuka, S., Microflora in soils with long-term application of paraquat. **Journal of Pesticide Science,** 17: 137-138, (1992).
29. Malkomes, H.P., Comparison of the effects of herbicides with different modes of action on soil microbial activity and soil fungi under laboratory conditions. **Zeitschrift-fur pflainzeinkr ankheiten und pflanzenschutz. Sonderheft,** 13, 377-386.
30. Katayama, A. and Matsumura, F., Degradation of organochlorine pesticides, paricularly endosulfan, by Trichoderma harzianum. **Environmental Toxicology and Chemistry,** 12: 1059-1065, (1993).
31. Lee, P.W., Fate of Fenvalerate (pydrin insecticide) in the soil environment. **J. Agr. Food Chem.,** 23: 993-998, (1985).
32. Johnson, D.B. and Cox, B.L., Aerobic soil metabolism and residues uptake inplants of an organophosphrus carbamate insecticide. **J. Agr. Food Chem.,** 33: 252-259, (1985).
33. Fuhreman, T.W. and Lichtenstein, E.P., A comparative study of the persistance and metabolism of 6- carbon- 14 insecticides in soils and plants. **J. Agr. Food Chem.,** 28: 446-452, (1980).
34. Krueger, J. P.; Butz, R.G.; Atallah, Y.H. and Cork, D.J., Isolation and identification of microorganisms for the degradation of Dicamba. **J. Agr. Food. Chem.,** 37: 534-538, (1989).

التكسير الحيوي للمبيد الحشري مونوكروتوفوس بواسطة فطر بنسليوم كوريلوفيليوم

جمال حسن ربيع

قسم النبات كلية العلوم - جامعه الزقازيق - مصر

يهدف هذا البحث الي دراسة كيفية تكسير المبيد الحشري مونوكروتوفوس بواسطة عزلة محلية من فطر البنسيليوم. وقد وجد أن الفطر يكسر المبيد الحشري عن طريق افراز انزيم مؤكسد الي الوسط الخارجي . وبدراسة تأثير تركيز ايون الهيدرجين علي نشاط الانزيم وجد أن هذا الإنزيم يعمل في مدي واسع من تركيز ايون الهيدرجين ويكون في أعلي نشاط عند تركيز ايون الهيدرجين(٨) .

وقد اثبتت التجارب انه يمكن استعمال الانزيم بعد تنقيته جزئيا للتخلص من بقايا المبيد الحشري مونوكروتوفوس في التربة الزراعية.