BIODEGRADATION OF PROMETRYNE HERBICIDE BY *PHANEROCHAETE CHRYSOSPORIUM* FUNGUS

A.GH. RAHAL[2], F.A. AHMED[1], AND NASHWA A.HASSAN[2]

1 Fac. Agric. Cairo Univ., Giza, Egypt.

2 Soil, Water and Environment Res. Inst., Agric. Res. Center, Egypt.

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Abstract

The persistence and biodegradation of prometryne in the liquid culture of *Phanerochaete chrysosporium* fungus was studied. The fungus was grown in liquid culture treated with different concentrations of prometryne (0,1,10 and 50 ppm) and incubated at 28°C. in shaking incubator. Samples were taken periodically (0, 1,2,4,8,10,15,30 and 45 days) to study the herbicide persistence and degradation process.

Results revealed that the time intervals for 50% disappearance rate of prometryne were 3, 8 and 13 days for 1, 10, and 50 ppm treatments respectively. Prometryne was degraded by the fungus to six by-products having distinct chromatographic properaties, giving R_f values of 0.12, 0.21, 0.32, 0..42, 0.5 and 0.74 on the TLC plates. Those by-products could be identified by IR spectroscopy as different N-dealkylated and hydroxylated derivatives.

INTRODUCTION

The chemical protection of plants is based on the toxic effect of various organic and inorganic compounds on harmful organisms. The chemical means of plant protection (pesticides) are distinguished by their high universality. They can be used to control most pests, diseases and weeds on all agricultural crops and various lands. The use of herbicides is very effective. Many years of experience gained in this field show that herbicides appreciably lower the cost of controlling weeds and faciliate an increases in the yields of agricultural crops.

Different problems are anticipated as a result of the application of the new synthetic pesticides, e.g., the accumulation of these compounds in the soil, pollution of ground water, water drainage and inhibation of soil microbial activities.

Once introduced into the environment, pesticides and other anthropogenic pol-

lutants are subject ed to biological and nonbiological transformation processes. Among the biological processes, microbial metabolism is the primary force in pesticide transformation or degradation. Indeed, it has been established that, in many cases, microbes are more important in the degradation of a pesticide than are physical or chemical mechanisms (Munnecke *et al.*, 1982).

Microorganisms are key agents in the degradation of a vast array of organic pesticide molecules in terrestrial and aquatic ecosystems, because of such processes as aerobic, anaerobic and chemolithotrophic metabolism, fermentation and metabolism via extracellular enzymes. Some pesticides, however, are resistant to microbial degradation and persist longer in the environment. Others are only transformed to intermediates, occasionlly with increased toxicity.

The white rot fungus (*Phanerochaete chrysosporium*) is known by its ability to degrade a wide variety of environmentally persistent compounds, many of which are toxic organopollutants, among these compounds, lignin, chlorinated hydrocarbon pesicides and polycyclic aromatic hydrocarbons (PAHs); (Bumps, 1993; Bumpus *et al.*, 1985; Fernando *et al.*, 1990; Valli and Gold, 1991 and Lamar *et al.*, 1990)

The aim of the present investigation is to study the ability of *Ph. chrysosporium* fungus to degrade prometryne herbicide.

MATERIALS AND METHODS

MATERIALS:

1. Selected herbicide:

Prometryne; gesagard 80% wettable powder (2,4 bis (isopropylamino)-6-(methylthio)-S-triazine) was obtained from Ciba Geigy Company, Cairo, Egypt.

2. Selected fungul strain:

One strain of white rot fungus: *Phanerochaete chrysosporium* 6359 NRRL. was used throughout this investigation to determine its efficiency in the decomposition of prometryne herbicide. This strain was kindly obtained from the culture collection of the Northern Regional Research Center, Peoria, Illinois, USA.

3. Microbiological medium:

Nutrient glucose agar medium (Fouda *et al.*, 1960), was used as a maintenance medium for fungal strain, it was also used without agar as a liquid medium for the growth of *Phanerochaete chrysosporium* fungus. It has the following components in g/L:

Glucose	20.0
Peptone	5.0
Yeast extract	5.0
Beef extract	3.0
Agar	20.0

4. Layout of experiment

To obtain an information on the biodegradation of prometryne herbicide by *Phanerochaete chrysosporium* fungus, an experiment was condusted as follows:

Sixty ml. of nutrient glucose medium were dispensed into 96 conical flasks (250 ml. capacity), then the flasks were sterilized at 121°C. for 20 minutes. After sterilization and cooling at room temperature, prometryne was added to the flasks under aseptic conditions to give final concentrations of 1,10, and 50 ppm. Some flasks were kept without prometryne addition as a control. Flasks were inoculated with one ml. of spore suspention of *Phanerochate chrysosporium* fungus and some others were kept without inoculation as another control. All flasks were incubated in a rotary shaker incubator at 28°C. and 150 rpm for 45 days.

At different intervals (0, 1, 2, 4, 8, 10, 15, 30 and 45 days) three flasks were taken from each treatment as well as the control, and percolated individually through 7 layers of thin muslin. Then the liquid phase was taken for prometryne analysis and its by-products.

METHODS

1. Determination of prometryne

a) Extraction method

To determine the amounts of prometryne and its by-products in the percolate, the extraction method mentioned by Abbott *et al.* (1965) was applied.

B. Analysis method

The quantitative analysis of prometryne was achieved by GLC, according to the method of Klaus *et al.* (1974). The by-products were separated by thin layer chromatography (TLC), according the method of Abbott *et al.* (1965), and identified by IR spectra.

RESULTS AND DISCUSSION

1. Persistence of prometryne in the liquid culture of *Phanerochaete chrysosporium*

The data of prometryne persistence in the cultures presented as percent detectable of the initial concentrations are given in Table 1 and illustrated in Fig. 1. These results show considerable disappearance of prometryne during the first week, since 83.36%, 49.98% and 34.34% of the added amounts were not detected in the treatments of 1, 10 and 50 ppm respectively.

Table 1. Persistence of prometryne herbicide in the liquid culture of *Ph. chrysosporium* fungus.

Days after treatment	Reovery of Prometryne (% of control)		
	1 ppm	10 ppm	1 ppm
0	100.00	100.00	100.00
1	80.08	94.34	95.53
2	70.06	88.13	85.22
4	21.26	73.32	80.22
8	16.64	50.02	65.69
10	1.04	40.70	55.88
15	1.04	23.14	43.68
30	1.04	15.70	29.21
45	0.00	0.00	19.29

As shown in Fig. 1, the time intervals for 50% disappearance of applied prometryne concentrations were 3, 8, and 13 days for 1, 10 and 50 ppm respectively.

Disappearance rate of prometryne was slightly lower at the highest rate of application (50 ppm), since 19.29% of the applied dose was detectable as prometryne at the end of the experiment (45 days), whereas no amount was detectable at the same interval when it had been applied at 1 or 10 ppm. However the amounts of prometryne which disappeared were much higher in the case of higher doses of application. The rapid disappearance of prometryne in *Ph. chrysosporium* culture could be attributed to chemical hydrolysis, the biodegradation of executed enzymes by fungus, and/or the ability of this fungus to use this compound as a source of carbon,

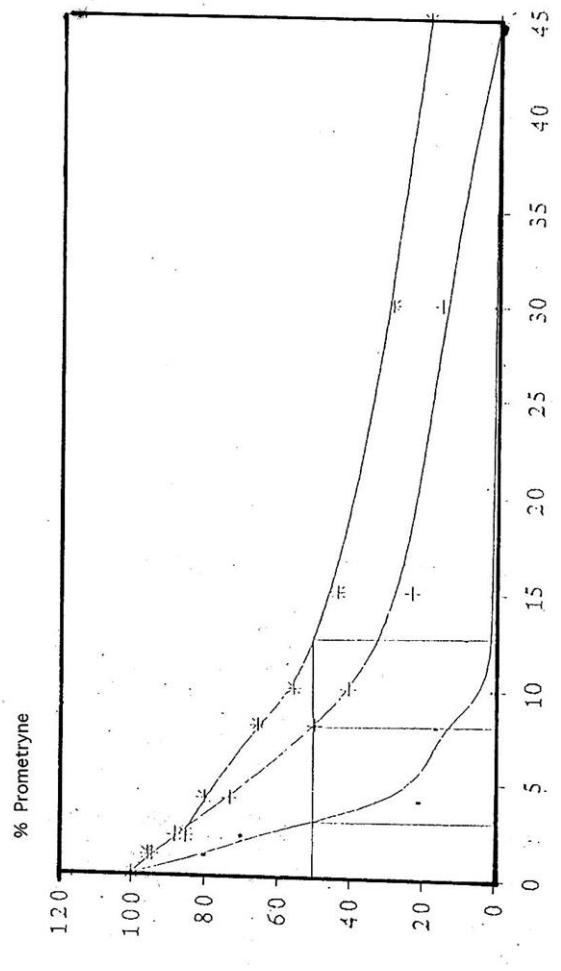

Fig. 1. Persistence of prometryne herbicide in the liquid culture of *Ph. chrysosporium* fungus.

nitrogen or sulphur for nutrition. Similar results were obtained by different investigators using other fungi in biodegradation of triazine herbicides (Kaufman *et al.*, 1965; Bortels *et al.*, 1967; Kaufman and Kearney, 1970 and Murray *et al.*, 1970.

2. Degradation of promtryne by *Ph. chrysosporium*

The transformation of prometryne as a result of its addition to the liquid culture of *Ph. chrysosporium* at rates of 1, 10 and 50 ppm. was investigated by the separation on TLC plates, after 2, 4, 8, 15, 30 and 45 days of incubation, and the identification of the metabolites was achieved by the IR spectrum.

Prometryne and its microbial transformation products exihibited seven distinct chromatographic properties. The R_f values of those compounds are givin in Table 2. Results indicated that prometryne transformation in the liquid culture of *Ph. chrysosporium* began 24 hrs. after its addition to the culture. Therefore, the metabolite appeared as one spot on TLC plate having R_f value of 0.12. Three days later, four metabolites appeared which had moderate polarity with R_f values of 0.21, 0.32, 0.42, and 0.50, and one metabolite of less polarity than prometryne with an R_f value of 0.74 and which disappeared after 30 days from incubation and possibly converted to another polar compound thereafter. Strong evidence for this result was that the spot at R_f value of 0.12 which became deeper at the end of experiment (30 days). This means that, there are some intermediates in the metabolic pathway leading to that compound.

Table 2. Thin layer chromatography (TLC) data for prometryne and its metabolites extracted from the liquid culture of *Ph. chrysosporium*.

Compound	TLC R_f value
Metabolite 1	0.12
Metabolite 2	0.21
Metabolite 3	0.32
Metabolite 4	0.42
Metabolite 5	0.50
Prometryne	0.62
Metabolite 6	0.74

The infrared spectrum of prometryne and its metabolites as shown in Figs. 2 and 3 have different bands of absorption, which indicates the presence of different

compounds of different chemical structures. All separated metabolites as well as prometryne exhibited absorption values in the region of 1555 and 1850 which indicates the presence of C=N groups of the aromatic ring of triazine, which may have persisted without any decomposition. The transformation could occurr on the side groups only, which may be converted to amino groups or hydroxyl groups by dealkylation and/or oxidation of the side groups with addition of hydroxylation of the methyl thio group of prometryne. These results are in agreement with those obtained by Gruzdyev *et al.* (1983).

From the obtained results *Ph. chrysosporium* presented in Fig. 4 may be the same as published by Gruzdyev *et al.* (1983)

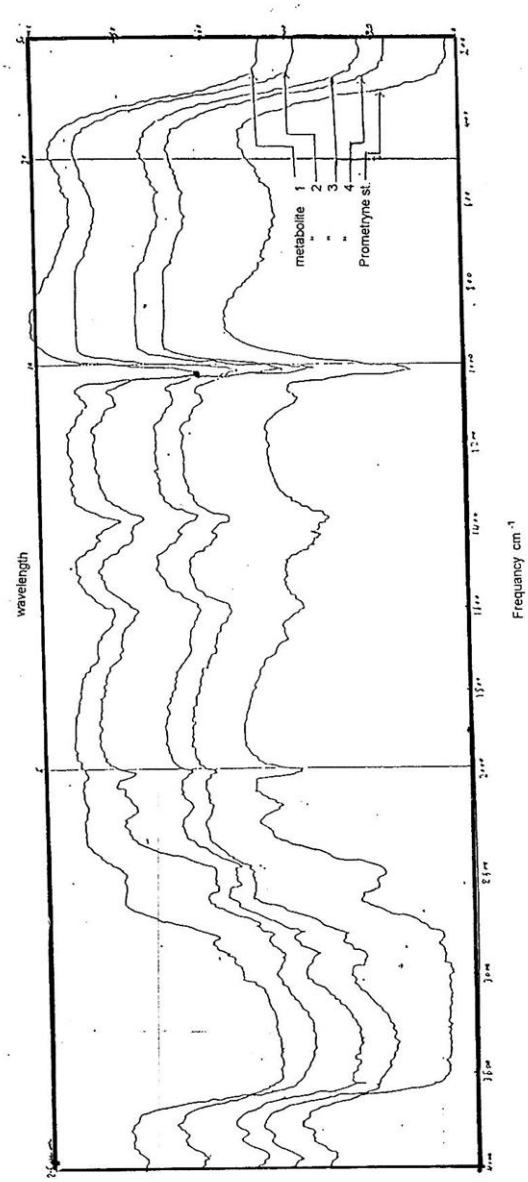

Fig. 2. IR spectral changes in Prometryne upon incubation with *Ph. chrysosporium* fungus.

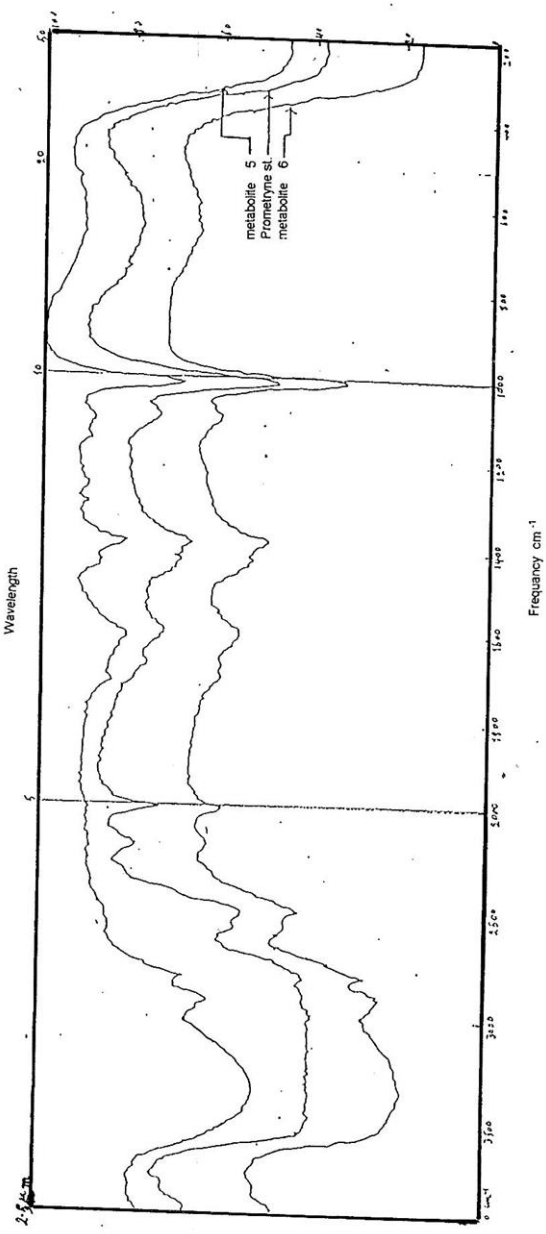

Fig. 3. IR spectral changes in Prometryne upon incubation with *Ph. chrysosporium* fungus.

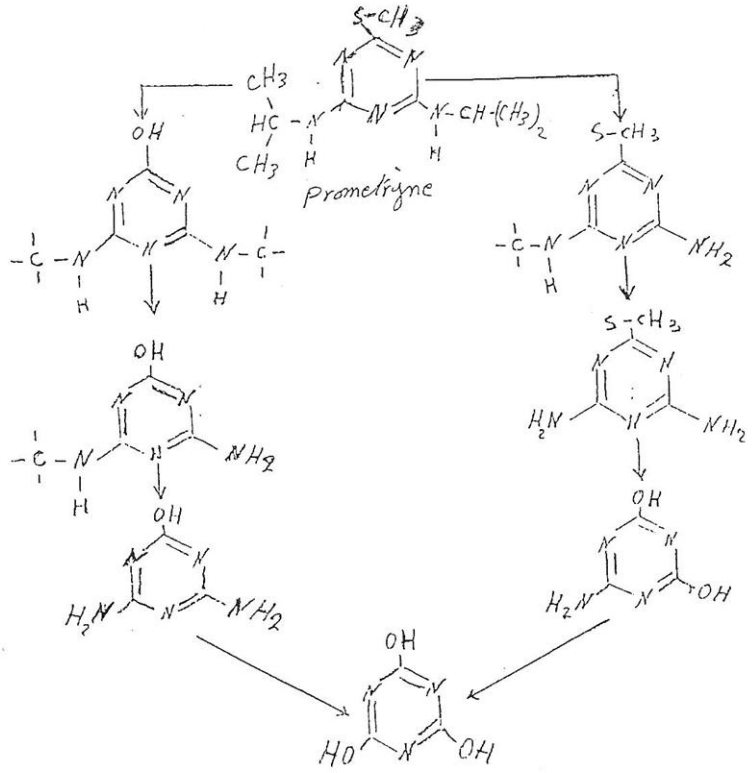

Fig. 4. Proposed partial pathways of prometryne decomposition by *Ph. chrysosporium* as noteced by Gruzdyev *et al.* (1983).

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التحطيم الحيوى لمبيد الحشائش البروموترين بواسطة فطر العفن الابيض (فنيروكيت كريزوسبوريم)

أحمد غنيم رحال[1]، فؤاد عبد الرحيم أحمد[2]، نشوى عبد العليم حسن[1]

[1] قسم بحوث الميكروبيولوجيا الزراعية – معهد بحوث الأراضى والمياه والبيئة – مركز البحوث الزراعية – مصر.

[2] قسم الكيمياء الحيوية الزراعية – كلية الزراعة – جامعة القاهرة.

يتضمن البحث دراسة معدل بقاء البروموترين ونواتج تحطيمه فى المزارع السائلة لفطر العفن الابيض (الفنيروكيت) حيث تم انماء الفطر فى مزارع سائلة معاملة بتركيزات مختلفة من المبيد (١٠,٠ ، ٥٠ جزء فى المليون) وحضنت المزارع على ٢٨° م بجهاز الربج الميكانيكى.

أخذت عينات على فترات (صفر ، ١، ٢، ٤، ٨، ١٠، ١٥، ٣٠، ٤٥ يوما) لتقدير كميات المبيد المتبقية ونواتج هدمة.

أسفرت النتائج المتحصل عليها على الآتى:

١ – الزمن اللازم لاختفاء ٥٠٪ من جرعة المبيد المضاف كانت (٣، ٨، ١٣ يوما) للتركيزات (١,٠ ، ١٠,٠ ، ٥٠ جزء فى المليون) على التوالى.

٢– قام الفطر بتحطيم المبيد الى ٦ مركبات مختلفة فصلت على الواح الفصل الكروماتوجرافى للطبقة الرقيقة وقدرت معاملات فصلها كالتالى (١٢,٠ ، ٢١,٠ ، ٣٢,٠ ، ٤٢,٠ ، ٥٠,٠ ، ٧٤,٠).

٣ – أمكن تمييز نواتج الهدم باستخدام التحليل بطيف الاشعة فوق الحمراء الى مشتقات البروموترين بنزع مجموعة الالكيل من على ذرة النتروجين وأخرى باضافة مجاميع هيدروكسيلية.