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PLANT PROTECTION

Microbial Degradation of Pirimiphos-methyl and Carbaryl by Pure Cultures of Two Soil Fungi

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Abstract. Microbial degradation of pirimiphos-methyl and carbaryl was studied in a pure liquid culture media of either *Rhizoctonia solani* or *Fusarium oxysporum*. The detection limits of pirimiphos-methyl and carbaryl by the utilized method were 97 and 75 ppb. High performance liquid chromatographic analysis revealed that both pirimiphos-methyl and carbaryl disappeared in part from the media enriched by *R. solani* or *F. oxysporum*. The amounts of pirimiphos-methyl reached 25.6 and 16.9%, meanwhile, carbaryl amounts were 15.2 and 11.2% of the applied doses after 21 days in the media cultured with *R. solani* and *F. oxysporum*, respectively. The half-life values ($T_{1/2}$) for pirimiphos-methyl and carbaryl were 8.0 and 6.7 days, respectively in the culture amended with *R. solani*, while in case of *F. oxysporum* they were 6.8 and 6.9 days, respectively. The amounts of carbaryl declined with time faster than pirimiphos-methyl. Data showed that *F. oxysporum* was capable of degrading pesticides under investigation much faster than *R. solani*.

Introduction

Several hundred pesticides of different chemical nature are widely used for agricultural purposes. Even though the soil received various pesticides, an active microbial population is still present. This microbial population could either be adapted to these pesticides or capable of degrading it through existing enzymes and may serve as source of nutrients [1,2]. Pirimiphos-methyl and carbaryl are recommended for use against many insect pests of fruits, vegetables and other field crops (Cleoptera and Lepidoptera) [3]. In Saudi Arabia both pesticides are being used against a broad spectrum of insects. Most of organophosphorus and carbamate compounds are very susceptible to chemical alteration [4]. Carbaryl was found to be degraded by mixed microbial cultures isolated from soil and more than 90% of the compound was lost within 3 days of inoculation [5].

Series of studies were conducted to examine the degradation of pesticides in soil [1,2,4,5,6,7]. However, there is no data available on such *in vitro* biodegradation of pesticides by pure cultures of soil fungi. *In vitro* biodegradation of aldicarb and methamidophos by two soil fungi was carried out [8]. Radwan *et. al.*, [8] found that both *Aspergillus niger* and *Rhizoctonia solani* degraded methamidophos more than aldicarb. Also they found that *A. niger* was capable of degrading both pesticides much faster than *R. solani*. Felsot *et. al.*, [9] suggested that the biodegradation of pesticides in soil depends upon the interactions between microbial ecology and physiochemical processes. The process of biodegradation is taking place by stimulating the indigenous subsurface microflora to enhance the decomposition rate [10].

In an effort to compare the capability of pesticide degradation by different microorganisms, the present study was undertaken to investigate the microbial degradation of pirimiphos-methyl and carbaryl by a pure culture of either *R. solani* (Kuhn.) or *F. oxysporum* (Schlecht.) which are widespread in soil.

Materials and Methods

Chemicals

Analytical grade sample of pirimiphos-methyl [*O*-2-Diethylamino-6-methyl pyrimidin-4-yl *O,O*-dimethyl phosphorothioate], (99.0% purity) and carbaryl [1-naphthyl methylcarbamate], (99.2% purity) were supplied by Chem. Service Co., USA. All other chemicals used in this study were of the highest purity grade available.

Fungal cultures and testing

The fungi used in this study were *Rhizoctonia solani* and *Fusarium oxysporum*. Both fungi were isolated from wilted alfalfa plant with severe crown and root rot. The fungal cultures, 7 days old, were maintained on potato dextrose agar (PDA) medium at 27 °C to serve as a source of inoculum. The fungi were grown in 500 mℓ flasks containing 250 mℓ of Czapek-Dox medium. The pH of this medium was adjusted to 7.0 using phosphate buffer. Inoculation was accomplished by adding one disc (5mm, diameter) of the fungus under aseptic conditions. Pirimiphos-methyl was added to the medium at concentration of 200 *ug*/mℓ just before inoculation, while carbaryl was added at the rate of 120 *ug*/mℓ The flasks were kept at 27 ± 1 °C during a period of 21 days without shaking. At intervals of 1,3,7,10,14 and 21 days of incubation, aliquots (20 mℓ each) were taken and subjected to chromatographic analysis. After 21 days, the mycelial mats were filtered, washed and dried to constant weight at 80 °C for 24 hr., then the weights were recorded. In the same manner, each pesticide was added to fungal free culture media as a control. At each time interval, three replicates were carried out.

Analytical procedure

Aliquots (20 mℓ) of the culture medium, for each time interval, were saturated using sodium chloride and extracted three times with 50 mℓ of methylene chloride. The

combined extracts for each time interval was dried over anhydrous sodium sulphate and rotary evaporated at 40 °C under vacuum to dryness. The residue was dissolved in 5 mℓ of methylene chloride and subjected to clean up prior to High Performance Liquid Chromatographic (HPLC) analysis. Clean up was carried out on silica gel column preconditioned by passing 10 mℓ of methanol through it. The column was eluted with 20 mℓ of methanol and the elute was collected and concentrated to 5 mℓ to be ready for HPLC analysis.

Recovery experiments

Aliquots of the standard solution of either pirimiphos-methyl or carbaryl were added to 20 mℓ portions of the fungal free culture media. The fortification levels were carried out at three levels of each pesticide (2.5, 12.5 and 25 ppm). Each sample was mixed thoroughly and extracted as described above.

Liquid chromatographic analysis and quantification

This was made by a Varian-VISTA-5500 High Performance Liquid Chromatograph (HPLC) equipped with an ultra-violet detector (uv) and a *u* Bondapack C18 stainless steel column (30 x 5 mm I.D.). The HPLC analysis was conducted using an isocratic elution system with methanol at a wavelength of 254 nm. Identification was accomplished by retention time and compared with either pirimiphos-methyl or carbaryl known standard at the same conditions. The peak areas corresponding to each injected sample were compared with that of standard solutions of each pesticide and used for quantification.

Quantification limits

Detection limits for pirimiphos-methyl and carbaryl using the employed method were determined according to the analytical concentrations that produce a chromatographic peak equal to three times of base line noise.

Results and Discussion

The preliminary observation showed that pirimiphos-methyl was not toxic to the tested fungi at the concentration used up to 200 *ug*/mℓ. However, in the case of carbaryl, this concentration showed some toxic effects. To investigate the breakdown of the tested pesticides, pirimiphos-methyl was added at a level of 200 *ug*/mℓ to the pure culture media of each fungus, while carbaryl was added at the concentration of 120 *ug*/mℓ.

Fungal growth experiments

The fungal mass values recorded at 21 days after inoculation were illustrated in Table 1. These values were 3.05 and 8.74 g/flask for pirimiphos-methyl and 0.41 and

0.64 g/flask for carbaryl in the media inoculated with *R. solani* and *F. oxysporum*, respectively. This finding indicates that *F. oxysporum* was able to utilize pirimiphos-methyl more than *R. solani*. In case of carbaryl, the results showed that the fungal growth was less than the obtained growth from the control, and for both fungal strains carbaryl was utilized more by *F. oxysporum* than with *R. solani*.

Table 1. The fungal mass values of *F. oxysporum* and *R. solani* incubated with either pirimiphos-methyl or carbaryl

Treatment	Mycelial mass (g)	
	Fusoxysporum	*Rhizoctonia solani*
Control	0.96	1.69
Pirimiphos-methyl	8.74	3.05
Carbaryl	0.64	0.41

Recovery and quantification limits

The recoveries of pirimiphos-methyl and carbaryl from the pure culture media at various spiking levels are presented in Table 2. The recovery values of pirimiphos-methyl from the pure culture media at the fortification levels of 2.5, 12.5, and 25 *ug*/mℓ were 90.4, 89.0 and 84.6%, respectively. In case of carbaryl, the recoveries at the same fortification levels were 83.6, 77.9 and 76.3%, respectively. These results indicate the applicability of the employed method.

The detection limits according to the pesticides concentrations that produce peaks equal to three times of baseline noise were 97 and 75 ppb for pirimiphos-methyl and carbaryl, respectively.

Table 2. Percent recovery of pirimiphos-methyl and carbaryl from fungi media after fortification at three levels

Added amounts	% recovered amount ± S.E.	
(ug/mℓ)	Pirimiphos-methyl	Carbaryl
2.5	90.4 ± 5.2	83.6 ± 12.2
12.5	89.0 ± 7.0	77.9 ± 6.6
25.0	84.6 ± 6.6	76.3 ± 3.2

Pesticides amounts recovered from the inoculated media

The results in Table 3 and 4 showed that the amount recovered of pirimiphos-methyl and carbaryl declined with increasing the incubation period in the media of either *R. solani* or *F. oxysporum*. One day after administration of 200 *ug* of pirimiphos-methyl or 120 *ug* of carbaryl /mℓ to the media inoculated with *R. solani* or *F. oxysporum*, the remaining amounts of pirimiphos-methyl were 162.4 and 195.5 *ug*/mℓ, respectively, which accounted for 81.2 and 97.7% of the applied compound. On the other hand, the amounts of carbaryl after the same period were 110.2 and 105.4 *ug*/mℓ, respectively, which accounted for 91.8 and 87.8% of the applied dose. After 7 days, the amounts of pesticides declined to become 105.4 and 102.0 µg of pirimiphos-methyl and 58.6 and

71.9 μg of carbaryl /mℓ in case of *R. solani* and *F. oxysporum*, respectively. These values equal to 52.7 and 51.0% of the initial dose of pirimiphos-methyl, while they were 48.8 and 59.9% in the case of carbaryl, respectively. At the last time point (21 days), the recovered amounts of pirimiphos-methyl were 51.2 μg/mℓ (25.6%) and 33.9 μg/mℓ (16.9%) while those for carbaryl were 18.3 μg/mℓ (15.2%) and 13.4 μg/mℓ (11.2%) from the media amended with *R. solani* and *F. oxysporum*, respectively. Pesticide free fungal media was run to check the background material.

At the end of experiments, it could be concluded that the pure culture of *F. oxysporum* was capable of degrading either pirimiphos-methyl or carbaryl much faster than *R. solani*.

Table 3. Degradation of pirimiphos-methyl by *R. solani* and *F. oxysporum in vitro*

Time Days	Recovered amounts of pirimiphos-methyl			
	Rhizoctonia solani		*Fusarium oxysporum*	
	μg/mℓ	%	μg/mℓ	%
1	162.4±11.0	81.2	195.5±12.9	97.7
3	118.8± 3.9	59.4	175.0±15.1	87.5
7	105.4± 11.2	52.7	102.0± 6.3	51.0
10	97.0± 5.3	48.5	61.8± 0.3	30.9
14	81.3± 5.6	40.7	53.4± 1.2	26.7
21	51.2± 7.3	25.6	33.9± 1.8	16.9

Concentrations are expressed as μg/mℓ. medium.

Table 4. Degradation of carbaryl by *R. solani* and *F. oxysporum in vitro*

Time Days	Recovered amounts of carbaryl			
	Rhizoctonia solani		*Fusarium oxysporum*	
	μg/mℓ	%	μg/mℓ	%
1	110.2±2.0	91.8	105.4±3.9	87.8
3	88.5±1.2	73.7	94.5±1.2	78.8
7	58.6±8.6	48.8	71.9±4.8	59.9
10	44.4±3.6	37.0	46.6±0.7	38.8
14	36.8±2.5	30.7	37.1±0.5	30.9
21	18.3±7.6	15.2	13.4±7.7	11.2

Concentrations are expressed as μg/mℓ medium.

Kinetic analysis of pesticides

The kinetic parameters for the degradation of pirimiphos-methyl and carbaryl were calculated and illustrated in Table 5. The data revealed that the loss of both pesticides in the examined pure culture fungal media could be described by the first order kinetics throughout the entire intervals. The half-life ($T_{1/2}$) of each pesticide in the different pure culture of the fungus was calculated using the following formula [11]:

$$T_{1/2} = 0.693 / K$$

where 0.693 is constant and K is the velocity constant which was obtained by linear regression of terminal linear exponential decline in pesticide concentration.

The disappearance phase of pirimiphos-methyl from *R. solani* and *F. oxysporum* culture media, had rate constants (k) of 0.087 and 0.102 day $^{-1}$, while those of carbaryl were 0.103 and 0.100 day^{-1}, respectively. These values lead to half-life ($T_{1/2}$) values of 8.0 and 6.8 day in the case of pirimiphos-methyl and 6.7 and 6.9 day in case of carbaryl, respectively.

Table 5. Kinetic parameters for the degradation of pirimiphos-methyl and carbaryl in pure culture media of *R. solani* and *F. oxysporum*

Fungus	K (day $^{-1}$)		$T_{1/2}$ (days)	
	Pirimiphos-ethyl	Carbaryl	Pirimiphos-ethyl	Carbaryl
R. solani	0.087	0.103	8.0	6.7
F. oxysporum	0.102	0.100	6.8	6.9

k is the elimination rate constant
$T_{1/2}$ is the half-life value

In the light of these results, it is suggested that the degradation of both compounds was rapid and their persistence was less in the media inoculated with either *F. oxysporum* or *R. solani*. These findings may explain the role of soil fungi in the pesticide degradation. The relatively short half-life values of both pesticides in the media amended with only one fungus could be expected that those pesticides could be degraded rapidly in the soil which has abundance of pesticide-metabolizing microorganisms. Thus the microbial action could accelerate the process of pesticide degradation.

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التحطم البيولوجي لمبيدي بريميفوس ميثايل ، كاربارِيل بواسطة فطرين من فطريات التربة ضمن بيئة اصطناعية

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ملخص البحث. تم دراسة التحطم البيولوجي لكل من مبيد بريميفوس ميثايل وكاربارِيل في البيئة السائلة لفطر *Rhizoctonia solani* وكذلك *Fusarium oxysporum*. وقد تم تقويم طريقة التحليل المستخدمة لكل من المبيدين فكانت القيمة الدنيا لتقدير كل من بريميفوس ميثايل وكاربارِيل باستخدام الطريقة المتبعة هي ٩٧، ٧٥ جزء في البليون على الترتيب. وقد أوضحت نتائج التحليل الكروماتوجرافي انخفاض كمية المبيدين من البيئة المحتوية على الفطرين مع مرور الزمن.

وقد وصلت كميات البريميفوس ميثايل إلى ٢٥،٦ و١٦،٩٪ بعد مرور ٢١ يوما من المعاملة في حالة فطر *R. solani* و *F. oxysporum* على التوالي بينما كانت هذه الكميات في حالة مبيد الكاربارِيل ١٥،٢ و١١،٢٪ بعد مرور نفس الزمن وعلى نفس الترتيب.

وقد تم حساب الوقت الذي تختفي عنده نصف كمية البريميفوس ميثايل فكانت ٨ ، ٦،٨ يوما في حالة فطر، *F. oxysporum* على الترتيب أما في حالة مبيد الكربارِيل فكانت ٦،٧، ٦،٩ يوما، و توضح النتائج أن الكربارِيل يتناقص بمرور الوقت و لكن بمعدل أسرع من تناقص بريميفوس ميثايل.

وعموما توضح النتائج أن فطر *F. oxysporum* له المقدرة على تحطيم المبيدين بدرجة أسرع من فطر *R. solani* في البيئات السائلة.