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Growth Assessment of Marine-Derived Fungi in the Presence of Esfenvalerate and its Main Metabolites

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Abstract

The growth and biodegradation potential of marine-derived fungi were evaluated by measuring the radial growth of colonies. It was observed that *Penicillium raistrickii* CBMAI 931, *Aspergillus sydowii* CBMAI 935, *Cladosporium* sp. CBMAI 1237, *Microsphaeropsis* sp. Dr(A)6, *Acremonium* sp. Dr(F)1, *Westerdykella* sp. Dr(M2)4 and *Cladosporium* sp. Dr(M2)2 were able to grow and develop in the presence of the pyrethroid insecticide esfenvalerate (S,S-fenvalerate) and its main metabolites (3-phenoxybenzaldehyde, 3-phenoxybenzoic acid, 3-phenoxybenzyl alcohol and 2-(4-chlorophenyl)-3-methylbutyric acid), showing the possibility of esfenvalerate biodegradation by these strains. The presence of technical grade esfenvalerate and its metabolites caused significant growthinhibition, while fungal development was not affected by the presence of the commercial insecticide SUMIDAN 150 SC in the culture medium. This fact might show that the biodegradation of the esfenvalerate in the commercial insecticide is slower than that of the technical grade active ingredient, since slower biodegradation of esfenvalerate would reduce the concentration of phenolic compounds and thus the growth inhibition. Future studies will focus on the quantitative biodegradation analysis of technical grade esfenvalerate and active ingredient in the commercial insecticide.

Assessment of Growth of Marine-derived fungi in the Presence of Esfenvalerate and its Main Metabolites

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Acremonium sp. Dr(F)1 growth in the presence of esfenvalerate on solid culture medium.

Keywords: Fenvalerate; Marine fungi; Biodegradation; 3-Phenoxybenzoic acid; 3-Phenoxybenzaldehyde

Introduction

Synthetic pyrethroids have been developed to improve on the specificity and activity of pyrethrin, the natural insecticide produced by the flowers of pyrethrum species (*Chrysanthemum cinerariaefolium* and *coccineum*). Pyrethrin is known for its instability in light and air, which limits its effectiveness in crop protection. The synthetic pyrethroids were developed to increase the photostability while retaining the potent and rapid insecticidal activity and relatively low acute mammalian toxicity of pyrethrin. There are about 1000 different structures and some of them are very different from the original pyrethrin structure [1,2].

The structural diversity of synthetic pyrethroids was further enhanced by the discovery that the 2,2-dimethylcyclopropanecarboxylic acid moiety of the pyrethrins and most previous synthetic compounds could be replaced by an α-isopropyl phenylacetic acid moiety. This new series of compounds led to the discovery of the commercial insecticide fenvalerate [2].

Fenvalerate is a pesticidal active ingredient composed of four stereoisomers. Originally, a balanced mixture of all four isomers was marketed. However, since the *S,S*-isomer shows the highest insecticidal activity, the synthesis of fenvalerate has been modified to enrich the racemic mixture with the *S,S*-isomer, which is named esfenvalerate [3].

Pyrethroids such as esfenvalerate are esters, with an alcohol and an acid moiety, so that cleavage by esterases is the first step in the biodegradation pathway. Studies available in the literature show that 3-phenoxybenzaldehyde [4,5], 3-phenoxybenzoic acid [5,6], 3-phenoxybenzyl alcohol [7] and 2-(4-chlorophenyl)-3-methylbutyric acid are the main products of pyrethroid biodegradation such as fenvalerate (Figure 1).

Given the capacity of microorganisms to degrade xenobiotics, scientists are exploring the microbial diversity in the search for new catalysts. Marine-derived microorganisms are naturally exposed and adapted to extreme temperature, acidity, high pressure and/or high salt concentration, which are the extreme conditions found in a significant part of the biosphere. Another important characteristic is quick

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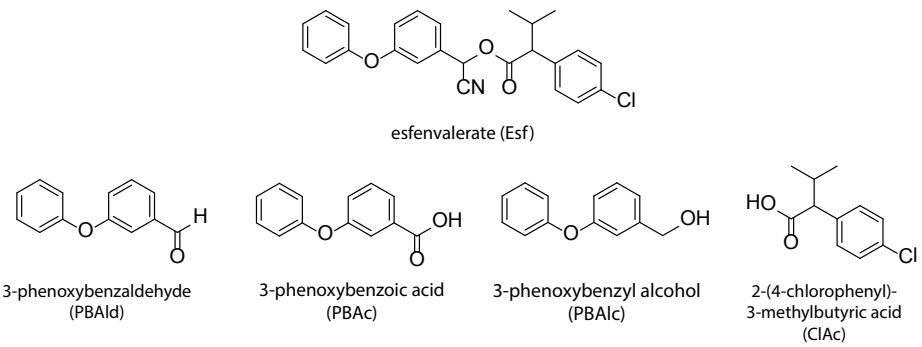

Figure 1: Structure of esfenvalerate and its main metabolites.

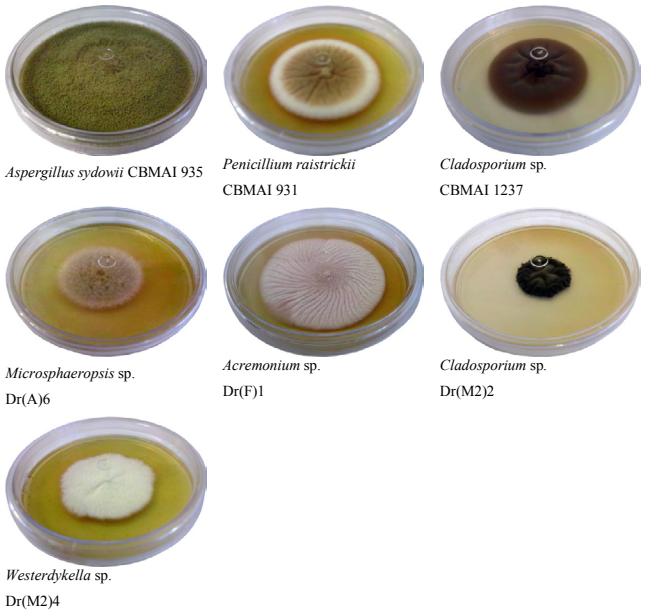

Figure 2: Fungal strains used in this study.

adaptation to environmental change, since marine currents promote rapid temperature and pH alterations. Marine microorganisms may show efficient biodegradation because they possess a unique enzymatic system adapted to highly halogenated and oxygenated compounds, such as the esfenvalerate employed in this study [8]. Thus, marine-derived fungi might have great potential for bioremediation applications and deserve to be studied.

It is also noteworthy that esfenvalerate has been identified as having the potential to accumulate in aquatic sediments [9,10], making the study of its biodegradation in aquatic systems very important.

Marine-derived fungi have already been used in biodegradation processes. Examples are *Aspergillus sclerotiorum* CBMAI 849 and *Mucor racemosus* CBMAI 847, which were capable of metabolizing pyrene to the corresponding pyrenylsulfate and benzo[a]pyrene to benzo[a]pyrenylsulfate [11]. Marine-derived fungi have also been used in the bioremediation of raw textile mill effluents [12], molasses-based raw effluents [13] and the anthraquinone dye, Reactive Blue 4 [14].

Some research on the biodegradation of pesticides by marine-derived fungi has been carried out in this laboratory. The organochlorine insecticide dichlorodiphenyltrichloroethane was biotransformed by *Trichoderma* sp. [15] and the organophosphate insecticide profenofos [16] by the strains *Aspergillus sydowii* CBMAI 935 and *Penicilium raistrickii* CBMAI 931.

The aim of this study was to assess the growth of marine-derived fungi in the presence of esfenvalerate (*S,S*-fenvalerate) and its main biodegradation metabolites [3-phenoxybenzaldehyde, 3-phenoxybenzoic acid, 3-phenoxybenzyl alcohol and 2-(4-chlorophenyl)-3-methylbutyric acid].

Materials and Methods

Pesticides

Esfenvalerate technical grade (92%, EsfTec) and the commercial insecticide Sumidan 150 SC (15% w/v esfenvalerate, EsfCom) were obtained as a gift from IHARABRAS S.A., it is important to note that Sumidan 150 SC also contains 16% w/v xylene. 3-Phenoxybenzaldehyde (98%, PBAld), 3-phenoxybenzoic acid (98%, PBAc), 3-phenoxybenzyl alcohol (98%, PBAlc) and 2-(4-chlorophenyl)-3-methylbutyric acid (98%, ClAc) were purchased from Sigma-Aldrich.

Marine fungi

The fungal strains used in this work were collected from marine sponges on the coast of São Sebastião, São Paulo, Brazil by Prof. Roberto G. S. Berlinck (IQSC-USP). The marine-derived fungi Penicillium raistrickii CBMAI 931 and Aspergillus sydowii CBMAI 935 were isolated from the sponge Chelonaplysilla erecta. The fungal strains Cladosporium sp. CBMAI 1237, Microsphaeropsis sp. Dr(A)6, Acremonium sp. Dr(F)1, Westerdykella sp. Dr(M2)4 and Cladosporium sp. Dr(M2)2 were isolated from Dragmacidon reticulata (Figure 2).

Culture media

Solid medium: Stock cultures of the marine-derived fungi were stored on solid culture medium composed of artificial seawater, agar (20 gL^{-1}), malt extract (30 gL^{-1}) and soy peptone (3 gL^{-1}). The pH was adjusted to 7 with 0.7 M NaOH solution, to avoid spontaneous hydrolysis of esfenvalerate. Artificial seawatercomposition was (1 L): $CaCl_2.2H_2O$ (1.36 g), $MgCl_2.6H_2O$ (9.68 g), KCl (0.61 g), NaCl (30.0 g), Na_2HPO_4 (0.014 mg), Na_2SO_4 (3.47 g), $NaHCO_3$ (0.17 g), KBr (0.1 g), $SrCl_2.6H_2O$ (0.040 g) and H_3BO_3 (0.030 g).

The culture medium was sterilized in an autoclave (AV-50, Phoenix, Brazil) at 121°C for 20 minutes and manipulations involving marine fungi were carried out in a laminar flow cabinet (FUV-18, Veco, Brazil). Since the microorganisms used in this study were isolated on

various culture media, 3% malt was used as a nutrient source because it is a rich and appropriate medium for marine microorganisms [17,18].

Growth of marine fungi on solid medium

Radial growth experiments were performed to assess the development inhibition caused by the presence of xenobiotic compounds. Thus, solid culture media were prepared with esfenvalerate technical grade (EsfTec), esfenvalerate commercial insecticide (EsfCom), xylene, 3-phenoxybenzaldehyde (PBAld), 3-phenoxybenzoic acid (PBAc), 3-phenoxybenzyl alcohol (PBAlc) and 2-(4-chlorophenyl)-3-methylbutyric acid (ClAc).

It is noteworthy that, except for the commercial insecticide, all the xenobiotic compounds were predissolved in 100 µL of DMSO per plate to enhance the dissipation of xenobiotic in the culture medium. Xenobiotic was added to the culture medium when the temperature had fallen to 40-50°C to prevent thermal degradation of the added compound. The agar plates were inoculated at a central insertion point and the colony diameters were measured after 7, 14, 21 and 28 days of cultivation in an incubator at 32°C (B.O.D. 411D, Nova Ética) (Figure 3). Each experiment was carried out in triplicate.

The experiments were performed on the following media:

A. 3% Malt: Culture medium (25 mL), without the addition of xenobiotic compounds.

B. 3% Malt+EsfCom (100 mg.L^{-1}): Culture medium (25 mL) with volume of EsfCom (SUMIDAN 150 SC) providing 100 mgL^{-1} active ingredient.

C. 3% Malt+DMSO (100 µL): Culture medium (25 mL) with 100 µL of DMSO.

D. 3% Malt+Xylene (107 mg.L^{-1}, which is the concentration of xylene when 100 mg.L^{-1} active ingredient of EsfCom is added): Culture medium (25 mL) with 3 µL of xylene previously dissolved in 100 µL of DMSO.

E. 3% Malt+EsfTec (100 mg.L^{-1}): Culture medium (25 mL) with 2.5 mg of EsfTec dissolved in 100 µL of DMSO before addition.

F. 3% Malt+EsfTec (100 mg.L^{-1})+Xylene (107 mg.L^{-1}): Culture medium (25 mL) with 2.5 mg of EsfTec and 3 µL of xylene dissolved in 100 µL of DMSO.

G. 3% Malt+ClAc (20 mg.L^{-1}): Culture medium (25 mL) with 0.5 mg of ClAc dissolved in 100 µL of DMSO.

H. 3% Malt+PBAlc (20 mg.L^{-1}): Culture medium (25 mL) with 0.5 mg of PBAlc dissolved in 100 µL of DMSO.

I. 3% Malt+PBAc (20 mg.L^{-1}): Culture medium (25 mL) with 0.5 mg of PBAc dissolved in 100 µL of DMSO.

J. 3% Malt+FBAld (20 mg.L^{-1}): Culture medium (25 mL) with 0.5 mg of PBAld dissolved in 100 µL of DMSO.

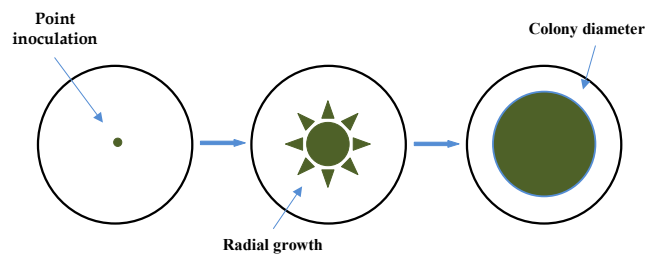

Figure 3: Experiments of colony radial growth.

The diameter percentage was calculated according to the equation 1.

$D\%=(Dx100)/D_{ref}$ (Equation 1)

Where:

D%=colony diameter in relation to the reference (%)

D=colony diameter (cm)

D_{ref}=reference colony diameter (cm)

Results and Discussion

Radial growth experiments of marine-derived fungi, not previously used in any study of pyrethroid biodegradation, were performed in the presence of xenobiotics. These experiments were carried out to assess the growth of these strains in the presence of esfenvalerate and its main metabolites, which are known to be toxic and recalcitrant.

In the experiment with the strain *Microsphaeropsis* sp. Dr(A)6 (Table 1, S.I. 1), it was observed during 7 and 14 days of incubation that the presence of the EsfCom had no significant effect on colony growth (Exp. B, 102% and 104%, respectively), relative to the growth in 3% malt in the absence of the insecticide (Exp. A).

However, when xylene (Exp. D), EsfTec (Exp. E) or xylene plus EsfTec (Exp. F) was added to the medium, a smaller colony, with a 75% diameter of the reference colony diameter (Exp. C) was seen after 7 days of incubation. After 14 days, the growth inhibition was still apparent, but the colony diameter differed from the reference diameter less than after 7 days.

In the presence of the possible metabolites of biodegradation [PBAc (Exp. I), PBAld Exp. (J), PBAlc (Exp. H) and ClAc (Exp. G)] growth inhibition was also seen, with a colony of around 65% of the reference diameter (Exp. C) after 7 days and 75% after 14 days.

After 21 days, the fungal colony had grown all over the plate in the reference experiment M3 (Exp. A), EsfCom (Exp. B) and M3+DMSO (Exp. C), while in the presence of xylene (Exp. D), EsfTec (Exp. E), xylene and EsfTec (Exp. F), PBAc (Exp. I), PBAld(Exp. J), PBAlc (Exp. H) and ClAc (Exp. G), the colony was smaller than 8.0 cm. At 28 days, all the colonies covered the entire plate surface.

In the experiment with *Westerdykella* sp. Dr(M2)4 (Table 2, S.I. 2), during 7, 14, 21 and 28 days of incubation, the presence of EsfCom (Exp. B) had no significant effect on the diameter of the colony, which remained around 110% of the reference colony size 3% malt (Exp. A), throughout the experiment.

Xylene (Exp. D) and EsfTec (Exp. E) led to smaller colonies of approximately 60% of the reference colony size (Exp. C), throughout the assessed period. It was also observed that in the presence of xylene plus EsfTec (Exp. F), the inhibitory effect was additive, inducing the least-developed colony observed, reaching only 33% of the reference colony diamenter (Exp. C). The presence of ClAc (Exp. G) did not cause any difference from the reference M3+DMSO (Exp. C).

It is important to note that PBAc (I) stimulated the colony growth from 21 days of incubation, resulting in a colony diameter of 124% of the reference M3+DMSO (C).

In the experiment with the strain *Acremonium* sp. Dr(F)1 (Table

Experiment		Time (days)							
		7		14		21		28	
		cm	%	cm	%	cm	%	cm	%
A	M3	4.4 ± 0.3	Ref.	7.4 ± 0.4	Ref.	8.0[c]	Ref.	8.0[c]	Ref.
B	M3+EsfCom (100 mg.L^{-1})	4.5 ± 0.1	102[a]	7.7 ± 0.2	104.0	8.0[c]	-	8.0[c]	-
C	M3+DMSO (100 µL)	4.6 ± 0.1	Ref.	7.5 ± 0.2	Ref.	8.0[c]	Ref.	8.0[c]	Ref.
D	M3+Xylene (107 mg.L^{-1})	3.5 ± 0.2	76[b]	6.6 ± 0.4	88[b]	7.4 ± 0.3	-	8.0[c]	-
E	M3+EsfTec (100 mg.L^{-1})	3.4 ± 0.2	74[b]	7.0 ± 0.4	93[b]	7.6 ± 0.4	-	8.0[c]	-
F	M3+EsfTec (100 mg.L^{-1}) +Xylene (107 mg.L^{-1})	3.5 ± 0.3	76[b]	6.2 ± 0.5	83[b]	7.2 ± 0.5	-	8.0[c]	-
G	M3+ClAc (20 mg.L^{-1})	3.3 ± 0.2	72[b]	5.7 ± 0.3	76[b]	6.4 ± 0.2	-	8.0[c]	-
H	M3+PBAlc (20 mg.L^{-1})	2.8 ± 0.2	61[b]	5.6 ± 0.2	75[b]	6.9 ± 0.2	-	8.0[c]	-
I	M3+PBAc (20 mg.L^{-1})	3.0 ± 0.1	65[b]	5.2 ± 0.1	69[b]	6.8 ± 0.1	-	8.0[c]	-
J	M3+PBAld (20 mg.L^{-1})	2.9 ± 0.4	63[b]	5.8 ± 0.1	77[b]	71 ± 0.3	-	8.0[c]	-

[a]The reference used was experiment A: M3.
[b]The reference used was experiment C: M3+DMSO.
[c]The colony diameter was measured up to 8 cm because of interaction with the edge of the plate at 9 cm.
Data are means of triplicate ± standard deviation.

Table 1: Colony diameter of the fungal strain *Microsphaeropsis* sp. Dr(A)6 in growth experiments.

Experiment		Time (days)							
		7		14		21		28	
		cm	%	cm	%	cm	%	cm	%
A	M3	1.1 ± 0.1	Ref.	2.2 ± 0.4	Ref.	3.4 ± 0.7	Ref.	4.5 ± 1.0	Ref.
B	M3+EsfCom (100 mg.L^{-1})	1.2 ± 0.1	109[a]	2.4 ± 0.1	109,0	3.8 ± 0.2	112	5.0 ± 0.2	111
C	M3+DMSO (100 µL)	1.2 ± 0.2	Ref.	2.5 ± 0.2	Ref.	4.1 ± 0.4	Ref.	5.5 ± 0.6	Ref.
D	M3+Xylene (107 mg.L^{-1})	0.7 ± 0.1	58[b]	1.8 ± 0.1	72[b]	2.5 ± 0.2	61	2.7 ± 0.3	49
E	M3+EsfTec (100 mg.L^{-1})	0.8 ± 0.1	67[b]	1.4 ± 0.4	56[b]	2.6 ± 0.2	63	3.1 ± 0.3	56
F	M3+EsfTec (100 mg.L^{-1}) +Xylene (107 mg.L^{-1})	0.8 ± 0.1	67[b]	1.3 ± 0.4	52[b]	1.7 ± 0.5	41	1.8 ± 0.5	33
G	M3+ClAc (20 mg.L^{-1})	1.2 ± 0.1	100[b]	2.5 ± 0.1	100[b]	4.1 ± 0.3	100	5.2 ± 0.4	94
H	M3+PBAlc (20 mg.L^{-1})	1.0 ± 0.0	83[b]	1.9 ± 0.1	76[b]	3.3 ± 0.3	80	4.5 ± 0.4	82
I	M3+PBAc (20 mg.L^{-1})	1.2 ± 0.3	100[b]	2.7 ± 0.3	108[b]	5.1 ± 0.2	124	6.8 ± 0.3	124
J	M3+PBAld (20 mg.L^{-1})	0.9 ± 0.1	75[b]	1.9 ± 0.1	76[b]	3.9 ± 0.1	95	5.5 ± 0.2	100

[a]The reference used was experiment A: M3.
[b]The reference used was experiment C: M3+DMSO.
Data are means of triplicate ± standard deviation.

Table 2: Colony diameter of the fungal strain *Westerdykella* sp. Dr(M2)4 in growth experiments.

3, S.I. 3), it was observed, at all times up to 21 days of incubation that the presence of EsfCom (Exp. B) slightly stimulated colony growth, resulting in an average diameter of 108% of that in 3% malt in the absence of insecticide (Exp. A).

However, when xylene (Exp. D), EsfTec (Exp. E) or xylene plus EsfTec (Exp. F) was added to the medium, after 7 days a smaller colony was observed, approximately 70% of the size of the reference colony (Exp. C) in the presence of xylene (Exp. D) or EsfTec (Exp. E), and approximately 60% of the reference size in the presence of xylene plus EsfTec (Exp. F). After 14 days of incubation, the growth inhibition was still present, but the colony diameter reduction relative to the M3+DMSO reference was smaller, approximately 90%.

In the presence of the possible metabolites of biodegradation [PBAc (Exp. I), PBAlc (Exp. H) and ClAc (Exp. G)], growth inhibition was not observed, a colony of approximately 100% of the reference diameter (Exp. C) being measured after 7 and 14 days of incubation. After 21 days of incubation, growth stimulation was observed, resulting in a colony diameter of 110% of the reference (Exp. C) diameter.

PBAld (Exp. J) induced significant growth inhibition at the start of the test, resulting in approximately 80, 90 and 100% of the reference colony diamenter after 7, 14 and 21 days of incubation, respectively. After 28 days of incubation, the colony covered the whole plate in all the experiments.

The strain *Penicillium raistrickii* CBMAI 931 (Table 4, S.I. 4) showed no effects on colony growth caused by incubation in the presence of EsfCom (Exp. B), with a diameter of 100% of the reference size (Exp. A) at both 7 and 14 days. This species is fast-growing and thus the colony had filled the agar plate by 21 days on all media.

Xylene (Exp. D), EsfTec (Exp. E) and xylene plus EsfTec (Exp. F), all resulted in a colony with diameter approximately 80% of that of the reference colony (Exp. C), after 7 days. After 14 days, the colony diameter was around 90% of the reference diameter.

In the presence of the possible metabolites [PBAc (Exp. I), PBAld(Exp. J), PBAlc (Exp. H) and ClAc (Exp. G)], some growth inhibition was observed, with a colony of approximately 90% of the reference (Exp. C), after 7 days. After 14 days of incubation, no

Culture medium		Time (days)							
		7		14		21		28	
		cm	%	cm	%	cm	%	cm	%
A	M3	2.3 ± 0.1	Ref.	4.2 ± 0.3	Ref.	6.5 ± 0.2	Ref.	8.0[c]	Ref.
B	M3+EsfCom (100 mg.L^{-1})	2.4 ± 0.1	104[a]	4.6 ± 0.1	109,0[a]	7.0 ± 0.2	108[a]	8.0[c]	-
C	M3+DMSO (100 µL)	2.4 ± 0.1	Ref.	4.5 ± 0.2	Ref.	6.6 ± 0.1	Ref.	8.0[c]	Ref.
D	M3+Xylene (107 mg.L^{-1})	1.8 ± 0.1	75[b]	4.4 ± 0.1	98[b]	6.2 ± 0.2	94[b]	8.0[c]	-
E	M3+EsfTec (100 mg.L^{-1})	1.7 ± 0.2	71[b]	4.1 ± 0.5	91[b]	6.0 ± 0.2	92[b]	8.0[c]	-
F	M3+EsfTec (100 mg.L^{-1}) +Xylene (107 mg.L^{-1})	1.5 ± 0.1	63[b]	4.0 ± 0.2	89[b]	5.6 ± 0.3	85[b]	8.0[c]	-
G	M3+ClAc (20 mg.L^{-1})	2.4 ± 0.1	100[b]	4.6 ± 0.1	100[b]	7.2 ± 0.2	109[b]	8.0[c]	-
H	M3+PBAlc (20 mg.L^{-1})	2.2 ± 0.1	92[b]	4.3 ± 0.2	102[b]	7.2 ± 0.1	109[b]	8.0[c]	-
I	M3+PBAc (20 mg.L^{-1})	2.3 ± 0.1	96[b]	4.4 ± 0.1	98[b]	7.1 ± 0.2	107[b]	8.0[c]	-
J	M3+PBAld (20 mg.L^{-1})	2.0 ± 0.1	83[b]	4.1 ± 0.1	91[b]	6.5 ± 0.2	98[b]	8.0[c]	-

[a]The reference used was experiment A: M3.

[b]The reference used was experiment C: M3+DMSO.

[c]The colony diameter was measured up to 8 cm because of interaction with the edge of the plate at 9 cm.

Data are means of triplicate ± standard deviation.

Table 3: Colony diameter of the fungal strain *Acremonium* sp. Dr(F)1 in growth experiments.

Experiment		Time (days)							
		7		14		21		28	
		cm	%	cm	%	cm	%	cm	%
A	M3	5.2 ± 0.1	Ref.	7.8 ± 0.2	Ref.	8.0[c]	Ref.	8.0[c]	Ref.
B	M3+EsfCom (100 mg.L^{-1})	5.2 ± 0.2	100[a]	7.9 ± 0.1	101[a]	8.0[c]	-	8.0[c]	-
C	M3+DMSO (100 µL)	5.2 ± 0.2	Ref.	7.9 ± 0.1	Ref.	8.0[c]	Ref.	8.0[c]	Ref.
D	M3+Xylene (107 mg.L^{-1})	4.1 ± 0.1	79[b]	7.1 ± 0.1	90[b]	8.0[c]	-	8.0[c]	-
E	M3+EsfTec (100 mg.L^{-1})	4.0 ± 0.2	77[b]	7.0 ± 0.2	89[b]	8.0[c]	-	8.0[c]	-
F	M3+EsfTec (100 mg.L^{-1}) +Xylene (107 mg.L^{-1})	4.0 ± 0.2	77[b]	6.8 ± 0.2	86[b]	8.0[c]	-	8.0[c]	-
G	M3+ClAc (20 mg.L^{-1})	4.6 ± 0.1	88[b]	7.7 ± 0.1	97[b]	8.0[c]	-	8.0[c]	-
H	M3+PBAlc (20 mg.L^{-1})	4.5 ± 0.1	86[b]	7.6 ± 0.1	96[b]	8.0[c]	-	8.0[c]	-
I	M3+PBAc (20 mg.L^{-1})	4.7 ± 0.3	90[b]	7.7 ± 0.1	97[b]	8.0[c]	-	8.0[c]	-
J	M3+PBAld (20 mg.L^{-1})	4.5 ± 0.1	86[b]	7.7 ± 0.1	97[b]	8.0[c]	-	8.0[c]	-

[a]The reference used was experiment A: M3.

[b]The reference used was experiment C: M3+DMSO.

[c]The colony diameter was measured up to 8 cm because of interaction with the edge of the plate at 9 cm.

Data are means of triplicate ± standard deviation.

Table 4: Colony diameter of the fungal strain *Penicillium raistrickii* CBMAI 931 in growth experiments.

significant difference was observed between the experiments with possible metabolites (Exp. I, Exp. J, Exp. H and Exp. G) and the M3-DMSO reference (Exp. C).

The strain *Cladosporium* sp. Dr(M2)2 (Table 5, S.I. 5) was not affected by the presence of EsfCom (Exp. B), since it showed the same colony diameter as the reference plate M3 (Exp. A) after 7, 14, 21 and 28 days of incubation.

The presence of xylene (Exp. D) and EsfTec (Exp. E) did not produce a significant difference from the reference experiment M3-DMSO (Exp. C), since approximately the same diameter was observed. However, in the presence of xylene plus EsfTec (Exp. F), some growth inhibition occurred, since the colony diameter was about 90% of the reference size (Exp. C) after 7 days, and approximately 80% after 14, 21 and 28 days of incubation.

The possible metabolite ClAc (Exp. G) showed weaker growth inhibition, with a colony of 90% of the reference diameter M3-DMSO (Exp. C) after 7, 14, 21 and 28 days, while PBAc (Exp. I), PBAld (Exp. J) and PBAlc (Exp. H) showed approximately 80% of the reference diameter after 7, 14 ,21 and 28 days.

It is important to note that the strain *Cladosporium* sp. Dr(M2)2 stopped growing after 14 days of development, even on the reference plates. This strain did not grow well or developed properly on the selected culture medium.

In the experiments with *Cladosporium* sp. CBMAI 1237 (Table 6, S.I. 6), it was observed that the commercial insecticide generated growth inhibition, since the observed colony diameters in the presence of EsfCom (Exp. B) were 80% of that of the reference culture on M3 agar (Exp. A).

The presence of xylene (Exp. D), EsfTec (Exp. E) or xylene plus EsfTec (Exp. F) did not affect the growth of the fungus, since approximately the same colony diameter was observed on the reference plate (Exp. C).

Experiment		Time (days)							
		7		14		21		28	
		cm	%	cm	%	cm	%	cm	%
A	M3	1.9 ± 0.2	Ref.	3.0 ± 0.1	Ref.	3.0 ± 0.1	Ref.	3.0 ± 0.2	Ref.
B	M3+EsfCom (100 mg.L^{-1})	2.0 ± 0.2	105[a]	2.9 ± 0.1	97[a]	3.0 ± 0.2	100[a]	3.0 ± 0.2	100[a]
C	M3+DMSO (100 µL)	2.0 ± 0.1	Ref.	2.9 ± 0.2	Ref.	3.0 ± 0.2	Ref.	3.0 ± 0.2	Ref.
D	M3+Xylene (107 mg.L^{-1})	2.0 ± 0.2	100[b]	2.8 ± 0.1	96[b]	2.8 ± 0.1	93[b]	2.8 ± 0.1	93[b]
E	M3+EsfTec (100 mg.L^{-1})	1.9 ± 0.1	95[b]	2.7 ± 0.2	93[b]	2.8 ± 0.2	93[b]	2.8 ± 0.2	93[b]
F	M3+EsfTec (100 mg.L^{-1}) +Xylene (107 mg.L^{-1})	1.8 ± 0.2	90[b]	2.4 ± 0.1	83[b]	2.4 ± 0.1	80[b]	2.4 ± 0.1	80[b]
G	M3+ClAc (20 mg.L^{-1})	1.9 ± 0.2	95[b]	2.6 ± 0.1	90[b]	2.6 ± 0.1	87[b]	2.6 ± 0.1	87[b]
H	M3+PBAlc (20 mg.L^{-1})	1.8 ± 0.1	90[b]	2.4 ± 0.1	76[b]	2.5 ± 0.1	83[b]	2.5 ± 0.1	83[b]
I	M3+PBAc (20 mg.L^{-1})	1.8 ± 0.2	90[b]	2.4 ± 0.1	83[b]	2.4 ± 0.1	80[b]	2.4 ± 0.1	80[b]
J	M3+PBAld (20 mg.L^{-1})	1.7 ± 0.1	85[b]	2.3 ± 0.1	79[b]	2.4 ± 0.1	80[b]	2.4 ± 0.1	80[b]

[a]The reference used was experiment A: M3.

[b]The reference used was experiment C: M3+DMSO.

Data are means of triplicate ± standard deviation.

Table 5: Colony diameter of the fungal strain *Cladosporium* sp. Dr(M2)2 in growth experiments.

Experiment		Time (days)							
		7		14		21		28	
		cm	%	cm	%	cm	%	cm	%
A	M3	2.2 ± 0.2	Ref.	4.8 ± 0.3	Ref.	6.9 ± 0.4	Ref.	8.0[c]	Ref.
B	M3+EsfCom (100 mg.L^{-1})	1.8 ± 0.1	82[a]	3.8 ± 0.1	79[a]	5.5 ± 0.2	80[a]	7.0 ± 0.1	-
C	M3+DMSO (100 µL)	1.9 ± 0.1	Ref.	3.9 ± 0.1	Ref.	5.8 ± 0.2	Ref.	7.1 ± 0.4	Ref.
D	M3+Xylene (107 mg.L^{-1})	2.0 ± 0.2	105[b]	4.0 ± 0.3	102[b]	5.9 ± 0.3	98[b]	7.2 ± 0.2	101[b]
E	M3+EsfTec (100 mg.L^{-1})	2.0 ± 0.0	105[b]	4.1 ± 0.1	105[b]	6.3 ± 0.1	109[b]	6.9 ± 0.8	97[b]
F	M3+EsfTec (100 mg.L^{-1}) +Xylene (107 mg.L^{-1})	2.0 ± 0.1	105[b]	4.0 ± 0.3	102[b]	5.8 ± 0.2	100[b]	6.8 ± 0.4	96[b]
G	M3+ClAc (20 mg.L^{-1})	1.6 ± 0.2	84[b]	3.5 ± 0.2	90[b]	5.1 ± 0.1	88[b]	6.7 ± 0.1	94[b]
H	M3+PBAlc (20 mg.L^{-1})	1.2 ± 0.1	63[b]	2.8 ± 0.1	72[b]	4.5 ± 0.3	78[b]	6.4 ± 0.2	90[b]
I	M3+PBAc (20 mg.L^{-1})	1.6 ± 0.1	84[b]	3.4 ± 0.1	87[b]	5.4 ± 0.1	93[b]	6.7 ± 0.6	94[b]
J	M3+PBAld (20 mg.L^{-1})	1.0 ± 0.2	53[b]	2.8 ± 0.2	72[b]	4.8 ± 0.2	83[b]	7.0 ± 0.4	98[b]

[a]The reference used was experiment A: M3.

[b]The reference used was experiment C: M3+DMSO.

[c]The colony diameter was measured up to 8 cm because of interaction with the edge of the plate at 9 cm.

Data are means of triplicate ± standard deviation.

Table 6: Colony diameter of the fungal strain *Cladosporium* sp. CBMAI 1237 in growth experiments.

The presence of PBAc (Exp. I) and ClAc (Exp. G) caused growth inhibition; the colony diameter observed being approximately 85% of the reference diameter after 7 days and approximately 90% after 14 days of growth. PBAlc (Exp. H) also inhibited the growth of this strain, the colony diameter being about 60, 70, 80 and 90% of the reference M3+DMSO (Exp. C) after 7, 14, 21 and 28 day of incubation, respectively.

PBAld (Exp. J) showed a growth inhibition that fell markebly over the time, with a colony measuring 50, 70, 80 and 100% of the reference diameter on M3-DMSO (Exp. C) after 7, 14, 21 and 28 days, respectively.

In the experiments with the strain *Aspergillus sydowii* CBMAI 935 (S.I. 7), it was not possible to measure the colony diameter, since secondary colonies spread over the plate as the spores dispersed. However, it was possible to note that, in the presence of the possible esfenvalerate metabolites [ACl (Exp. G), FBAlc (Exp. H), FBAc (Exp. I), and FBAld(Exp. J)], even though the fungus had spread all over the plate, the colony was visibly less dense than the reference on M3-DMSO (Exp. C).

Several particular characteristics were observed in the fungal growth in the presence of the xenobiotic compounds under study. Generally, it was observed that the possible metabolite ClAc, showed weaker inhibition effects than the other possible metabolites (PBAc, PBAld and PBAlc), while some strains, such as *Westerdykella* sp. Dr(M2)4 and *Acremonium* sp. Dr(F)1 did not exhibit any inhibitory effects at all for this possible metabolite, with colonies of approximately 100% of the reference diameter.

The presence of the compound FBAlc caused significant growth inhibition in all the strains [*Microsphaeropsis* sp. Dr(A)6, *Westerdykella* sp. Dr(M2)4, *Penicillium raistrickii* CBMAI 931, *Cladosporium* sp. Dr(M2)2, *Cladosporium* sp. CBMAI 1237 and *Acremonium* sp. Dr(F)1], but it was also observed that this inhibition decreased over time, showing that FBAlc and any other toxic compound generated by its degradation may have been consumed, thus reducing the growth inhibition.

For the FBAld experiments, the strains [*Microsphaeropsis* sp. Dr(A)6, *Westerdykella* sp. Dr(M2)4, *Acremonium* Dr(F)1, *Penicillium raistrickii* CBMAI 931, *Cladosporium* sp. Dr(M2)2 and *Cladosporium* sp. CBMAI 1237] showed growth inhibition, which decreased considerably during the period of incubation, as observed in the FBAlc experiments.

PBAc caused growth inhibition in most of the tested fungi [*Microsphaeropsis* sp. Dr(A)6, *Penicillium raistrickii* CBMAI 931, *Cladosporium* sp. Dr(M2)2 and *Cladosporium* sp. CBMAI 1237]. However, no growth effects were observed for the strain *Acremonium* sp. Dr(F)1 and even growth stimulation was observed in the *Westerdykella* sp. Dr(M2)4 culture, possibly because of the use of this compound as a carbon source or enzyme effector.

The inhibition caused by ClAc, FBAld, FBAlc and FBAc may be due to the formation of phenolic compounds, which are known for their disinfectant action [19,20], these have been described as metabolites of bacterial biodegradation [6,21] and thus might be produced by fungi too.

Except by *Cladosporium* sp. CBMAI 1237, all the tested strains were significantly inhibited in the presence of esfenvalerate, xylene and esfenvalerate plus xylene. Growth inhibition by esfenvalerate and xylene can be attributed to the formation of phenolic metabolites by biodegradation, which would produce toxic effects. It was observed that the commercial insecticide SUMIDAN 150 SC did not cause growth inhibition effects, suggesting that the biodegradation of esfenvalerate in the commercial insecticide is slower than that of the technical grade compound. A more efficient biodegradation would generate a higher concentration of phenolic compounds, which are the probable cause of growth inhibition.

Another possibility is that the polysaccharides present in the commercial insecticide stimulate the fungal growth and compensate the growth inhibition generated by phenolic metabolites. However, this is not very likely, since a rich medium (malt 3%) was used in these experiments.

Conclusions

The marine fungal strains *Penicillium raistrickii* CBMAI 931, *Aspergillus sydowii* CBMAI 935, *Cladosporium* sp. CBMAI 1237, *Microsphaeropsis* sp. Dr(A)6, *Acremonium* sp. Dr(F)1, *Westerdykella* sp. Dr(M2)4 and *Cladosporium* sp. Dr(M2)2 were grown in the presence of esfenvalerate and its main metabolites, showing the potencial of esfenvalerate biodegradation by these strains. It was observed that technical grade esfenvalerate and its metabolites had inhibitory effects on growth, reducing the size of the colony.

However, the fungal development was not affected by the presence of the commercial insecticide SUMIDAN 150 SC in the culture medium, possibly showing that the biodegradation of esfenvalerate in the commercial insecticide is slower than that in the technical grade compound, since a slower biodegradation of esfenvalerate reduces the concentration of phenolic compounds and thus the growth inhibition. Future studies will focus on the quantitative biodegradation analysis of technical grade esfenvalerate and active ingredient in the commercial insecticide.

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