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Biodegradation of the Pyrethroid Pesticide Gamma-Cyhalothrin by Fungi from a Brazilian Cave

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The extensive use of pesticides promoted the need for bioremediation methods, including for pyrethroids. Therefore, biodegradation of gamma-cyhalothrin by fungi from a Brazilian cave known as Gruta do Catão (São Desidério, Bahia, Brazil) was investigated. Experiments were conducted with *Aspergillus ustus* CBMAI 1894, *Talaromyces brunneus* CBMAI 1895, and *Aspergillus* sp. CBMAI 1926 in 2% malt liquid medium with 300 mg L^{-1} gamma-cyhalothrin (25 °C, 130 rpm, 21 days, pH 7.0). All strains biodegraded this insecticide, and the most efficient biocatalyst was *A. ustus* CBMAI 1894 with 50% biodegradation, even though reduced mycelial mass was observed in the presence of gamma-cyhalothrin. A three factor Box-Behnken design was carried out. Temperature and pesticide concentration influenced biodegradation, whereas pH was non-significant. In conclusion, cave fungi can be explored for bioremediation, and future studies should focus on understanding the enzymatic apparatus, physiology, and genetics behind these microorganisms, which can present unique properties for biotechnological applications.

Keywords: *Aspergillus ustus*, *Talaromyces brunneus*, bioremediation, cyhalothrin, insecticide, contamination

Introduction

The production of food that can meet human demand without environmental degradation is one of the greatest challenges for modern society. Since the use of chemical substances has been considered fundamental for the increasing productivity of established agricultural areas, especially regarding the huge losses in conventional agriculture when these substances are not employed.[1,2]

Literature[3-5] showed that less than 0.1% of the applied active ingredients reach their target organisms, therefore 99.9% of these substances have the potential to translocate to undesirable regions, reaching different dimensions of the environment, negatively affecting humans, fauna, and flora.

In 2021, Brazil applied about 721 thousand tons of active ingredients, making this country the world's largest consumer of these products,[6-8] which can be classified according to target organisms (nematicides, insecticides, herbicides, fungicides, rodenticides, acaricides, molluscicides, algicides, etc.) and the chemical groups to which they belong (organochlorines, pyrethroids, organophosphates, carbamates, triazines, etc.).[9]

Throughout history, several active ingredients have been banned because of their high toxicity and/or persistence.[10,11] Organochlorines, the pioneer synthetic pesticides, had their agricultural use banned at Brazil in 1985, and in 1998 these compounds were banned from public health campaigns.[12,13] For this reason, there is an efficiency demand for chemical substances that could replace them, surpassing insect resistance with reduced impacts to humans and the environment. In this perspective, pyrethroids became one of the most widely used class of insecticides.[14]

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Pyrethroids are derived from the natural pesticides pyrethrins, which were traditionally prepared by the trituration of flowers from plants of the genus *Chrysanthemum*, such as *C. cineraiaefolium* and *C. cocineum*.[15] These insecticides have low toxicity levels for mammals, reduced environmental impacts, and small dosage requirement for high insecticidal activity. However, the use of some pyrethroids increased the contamination risk to birds, fish, bees, lobsters, and shrimps.[16-18]

Fungi have been described as efficient biodegraders of polymers from plant origin such as lignin and cellulose, as well as waxes, rubbers, phenols, benzene, toluene, xylene, and xenobiotics in forest environments.[19] Different genera were approached for biodegradation of pyrethroids, with emphasis to *Aspergillus*, *Candida*, *Cladosporium*, *Acremonium*, *Microsphaeropis*, *Westerdykella*, and *Trichoderma* strains.[20-22] For example, *Aspergillus* sp. PYR-P2 from pesticide-contaminated soil biodegraded a pyrethroid mixture composed of cypermethrin, cyfluthrin, and cyhalothrin.[23] *Aspergillus oryzae* M-4 from soil of a tea garden biodegraded beta-cypermethrin and 3-phenoxybenzoic acid in co-culture with the bacterial strain *Bacillus licheniformis* B-1.[24] Moreover, *Acremonium* sp. CBMAI 1676, *Microsphaeropis* sp. CBMAI 1675, and *Westerdykella* sp. CBMAI 1679 from marine environment biodegraded esfenvalerate.[25]

Different alternatives have been approached in the extensive search for sustainable methods and strategies.[26,27] In the context of biodegradation, extreme and unexplored environments such as caves, deep oceans, tropical peats, and deserts have been the target of studies looking for bioactive compounds and microorganisms.[28-31]

Underground environments such as caves can stimulate unique survival strategies in organisms, providing far-reaching improvements in metabolism, which are promoted by the evolutional pressure for adaptability.[32,33] Thus, antimicrobial and enzymatic activities of cave microorganisms are different from elsewhere, constituting an opportunity for novel discoveries, including new communities, species and strains.[34-36]

Little is known about population, distribution and biochemical processes of microorganisms that inhabit cave environments, situation that is not different from the Brazilian scenario, since studies related to the underground microbiota are still incipient.[37-40] Although the use of high-throughput sequencing has been providing insights into the microbial richness and diversity of caves, showing prevalence of Proteobacteria for bacteria, Thaumarchaeota for Archaea, and Ascomycota for fungi.[41] In addition, this exploration has been an interesting initiative for new microbial phenotypes, specially by the use of metagenomic analyses with correlations of active functional genes.[42]

Cyhalothrin is a pyrethroid insecticide allowed for agriculture and constituted by four stereoisomers. Moreover, this active ingredient is available as the product lambda-cyhalothrin composed by a pair of diastereoisomers, in which gamma-cyhalothrin ([(*S*)-cyano-(3-phenoxyphenyl) methyl] (1*R*,3*R*)-3-[(*Z*)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate) is the most active enantiomer.[43,44] In this study was assessed the biodegradation of gamma-cyhalothrin, an enantioenriched commercial formulation.

In this context, literature has shown the importance of biodegradation performed by fungi from caves.[45] So, this study aims the evaluation of strains from Gruta do Catão (São Desidério, Bahia, Brazil) for biodegradation of gamma-cyhalothrin, focusing on the identification of new catalysts for biotransformation of organic compounds and future bioremediation processes.

Experimental

Pesticides, reagents, and solvents

Gamma-cyhalothrin (analytical standard, 98.5%) was obtained from Dr Ehrenstorfer (Augsburg, Germany) and employed for construction of analytical curves for quantification. The insecticide NEXIDE (15% m/v of gamma-cyhalothrin) was generously donated by FMC Química do Brazil (Campinas, Brazil) and used for biodegradation experiments. Reagents and solvents were obtained from Sigma-Aldrich (Missouri, USA) and Synth (São Paulo, Brazil). Formic acid, methanol, and acetonitrile (HPLC grade) were obtained from Panreac Applichem (Barcelona, Spain) and Tedia Co. (Ohio, USA).

Cave filamentous fungi

The strains of filamentous fungi were obtained previously from an extreme oligotrophic subterranean environment named Gruta do Catão (12°22'6"S, 44°52'3"W) located at the conservation area Lagoa Azul (São Desidério, Bahia, Brazil) under the permit ICMBio/SISBIO (10215 license). A 0.25 m^2 quadrant of soil in two areas of the cave were sampled (Entrance zone and Twilight zone) in the year of 2012. Sediments were collected (0-10 cm depth) in sterile plastic containers. Then, samples were homogenized, sieved with 2 mm mesh, and conserved in refrigerator at 4.0 °C. Strains isolation was carried out by streaking in solid medium of 3% malt agar (30.0 g L^{-1} malt extract, 3.0 g L^{-1} soy peptone, 0.05 g L^{-1} rose

bengal, 20.0 g L^{-1} agar and pH 5.5-6.0) in three replicates. Incubation was performed at 25 °C for 7 and 15 days. Then, each colony was isolated by streaking on solid culture medium for three consecutive times. These procedures are available in the literature.[45,46]

The deoxyribonucleic acid (DNA) extraction was performed as previously described, and then amplified using polymerase chain reaction (PCR) method of the ITS1-5.8S-ITS2 region with the primers ITS-1 and ITS-4. Purification in column was carried out with GFX PCR DNA and Gel Band Purification Kit from GE Healthcare (Chicago, USA). Sequencing was directly performed in an automated sequencer ABI3500XL Series, Applied Biosystems (Waltham, USA).[47]

The DNA sequences alignments were performed with CLUSTAL X[48] and phylogenetic analyses were carried with MEGA 6.0.[49] The consensus DNA sequence was compared with Genbank and CBS. Kimura model was employed for evolutionary distance, and Neighbor-Joining method for phylogenetic three. Bootstrap value of 1000 resamples was used. These strains were identified and deposited by the Brazilian Collection of Environmental and Industrial Microorganisms (CBMAI, WDCM823).[48-53]

The three strains assessed for biodegradation were *Aspergillus ustus* CBMAI 1894, *Talaromyces brunneus* CBMAI 1895, and *Aspergillus* sp. CBMAI 1926.

Spore solution preparation

Fungal spores were taken from solid 7-days cultures grown at 25 °C on 2% malt extract agar. Harvest was performed with aqueous solution of polysorbate 80 (0.05%) filtered through a glass wool for mycelia removal and spores recover into a sterile tube. Concentration was standardized at 10^7 spores mL^{-1} using a Hemacytometer Neubauer chamber from Marienfeld (Lauda-Königshofen, Germany).

Selection of resistant fungal strains for gamma-cyhalothrin biodegradation

Three different culture media were used to evaluate the resistance of fungal strains to the presence of gamma-cyhalothrin. Experiments were performed with commercial Sabouraud dextrose agar (5 g L^{-1} casein enzymatic digest, 5 g L^{-1} enzymatic digest of animal tissue, 40 g L^{-1} dextrose, 15 g L^{-1} agar), commercial potato dextrose agar (4 g L^{-1} potato extract equivalent to 200 g of potato infusion, 20 g L^{-1} dextrose, and 15 g L^{-1} agar) and 2% malt extract agar (20 g L^{-1} malt extract and 15 g L^{-1} agar). Media and agar were from Acumedia (Lansing, USA).

All culture media were sterilized in autoclave AV Phoenix Luferco (Araraquara, Brazil) at 121 °C and 1 atm for 20 min. After cooling until 40-50 °C, gamma-cyhalothrin (300 mg L^{-1}) was added. This mixture was homogenized and poured into Petri plates. Then, spore suspension (10^7 spores) was employed for inoculation at a single central point using a micropipette with 1 μL. Plates were incubated in the absence of light at 25 °C in a biochemical oxygen demand (BOD) incubator model 411D Nova Ética (Vargem Grande Paulista, Brazil), and the radial growths of fungi were determined after 7 days. Plates without gamma-cyhalothrin were used as controls. Data were analyzed by two-way analysis of variance and Tukey's test using RStudio.[54] All experiments were carried out in quadruplicate.

Biodegradation of gamma-cyhalothrin in liquid culture medium

Biodegradation experiments were conducted in 125 mL Erlenmeyer flasks acquired from Corning (Glendale, USA) with 50 mL of liquid culture medium using cotton plugs to enable gas exchange. In each flask, 10^7 spores mL^{-1}, and 300 mg L^{-1} of gamma-cyhalothrin (previously sterilized in autoclave, 20 min, 121 °C, 1 atm) were added to culture media and incubated in orbital shaker Marconi MA830 (Piracicaba, Brazil) for 14 days (25 °C, 130 rpm) in absence of light. Furthermore, pesticide addition at the 3rd day of incubation was also assessed. These experiments were performed in triplicate.

Control trials

Control experiments were carried out to verify the production of natural metabolites under the same experimental conditions, but in the absence of gamma-cyhalothrin (fungal control), avoiding misinterpretation with pesticide byproducts. Moreover, abiotic controls with gamma-cyhalothrin were performed in sterile and uninoculated liquid culture medium for verification of pesticide stability and abiotic degradation at the assessed cultivation conditions. After 14 days at 25 °C and 130 rpm, both controls (fungal and abiotic) were extracted and analyzed by high performance liquid chromatography (HPLC). All experiments were done in triplicate in absence of light.

Fungal growth curve

Three different growth curves were performed: (*i*) spore suspension, 10^7 spores mL^{-1}, added to 2% malt

extract medium; (*ii*) spore suspension, 10^7 spores mL^{-1}, with 300 mg L^{-1} of gamma-cyhalothrin added at day 0 of incubation to 2% malt extract medium; (*iii*) spore suspension, 10^7 spores mL^{-1}, with 300 mg L^{-1} of gamma-cyhalothrin added at the 3rd day of incubation to 2% malt extract medium.

All flasks contained 50 mL of liquid culture medium that were incubated in orbital shaker (25 °C, 130 rpm, 14 days) in the absence of light. For each growth curve, 42 flasks were used since three erlenmeyer flasks were employed *per* day. The mycelia were filtered on a Buchner apparatus with Cytiva Whatman® filter paper 1 (Little Chalfont, United Kingdom), and dry weights were determined after drying at 70 °C for 24 h. The growth curves were constructed by the dry cell weights (g L^{-1}) plotted *versus* incubation time (days). The experiments were conducted in triplicate.

Experimental design

Three factor Box-Behnken design was employed to evaluate the main effects, interaction effects, and quadratic effects of cultivation temperature (15, 25, and 35 °C), culture medium pH (5.5, 7.0, and 8.5) and gamma-cyhalothrin concentration (50, 300, and 550 mg L^{-1}) on biodegradation of gamma-cyhalothrin by the cave fungi strain *Aspergillus ustus* CBMAI 1894. This type of design is the most employed for response surface modelling with more than two factors, when it is supposed that the optimum conditions lie between the assessed ranges.[55,56]

Statistica was used for regression and graphical analysis.[57,58] A design comprising 16 runs was developed, and a non-linear model represented by a second-order polynomial equation was adjusted (equation 1):

$$\hat{y} = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{1,2}X_1X_2 + b_{1,3}X_1X_3 + b_{2,3}X_2X_3 + b_{1,1}X_1^2 + b_{2,2}X_2^2 + b_{3,3}X_3^2 \quad (1)$$

where $\hat{y}$ is the dependent variable gamma-cyhalothrin biodegradation (%); b_0 is the value of $\hat{y}$ if the effects of all independent variables are zero, X_1, X_2, and X_3 are the independent variables temperature (°C), culture medium pH, and gamma-cyhalothrin concentration (mg L^{-1}), respectively; b_1, b_2 and b_3 are the linear coefficients; $b_{1,2}$, $b_{1,3}$ and $b_{2,3}$ are the coefficients of interaction between the independent variables; $b_{1,1}$, $b_{2,2}$ and $b_{3,3}$ are the second-order coefficients.

Extraction of gamma-cyhalothrin and its metabolites

The extraction and quantification of gamma-cyhalothrin was performed according to the literature.[59] The fungal cells were filtered using a Buchner apparatus and stirred vigorously in 100 mL of distilled water and ethyl acetate (1:1 v/v) for 30 min. Thereafter, the sample was subjected to a second filtration in Buchner apparatus, and the mycelial extract was added to the enzymatic broth. Then, pH of the aqueous phase was adjusted to 5.0 and the final extract was obtained by a three-step liquid-liquid extraction with 30 mL of ethyl acetate each. The aqueous phase was discarded, and anhydrous sodium sulfate was added to the organic phase, which was filtered and transferred to a 250 mL round bottom flask for evaporation under reduced pressure. The sample was redissolved in 5 mL methanol for analyses.

Quantification of gamma-cyhalothrin by HPLC-UV

Gamma-cyhalothrin was quantitatively determined using a Shimadzu 2010 high pressure liquid chromatographic system (Kyoto, Japan) composed of a LC-20AT pump, a DGU-20A5 degasser, a SIL-20AHT sampler, a SPD-M20A UV-Vis detector, a CTO-20A column oven and a CBM-20A controller with a 25 cm × 4.6 mm Phenomenex C18 column with 5 µm particle size. Analyses were carried out with 0.1% formic acid in deionized water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at 1.0 mL min^{-1} and 40 °C. The injection volume was 10 µL, and detection was performed at 277 nm. The chromatographic analysis was performed as following, from 0 to 17 min: 60% of B isocratic, from 17 to 18 min: 50-90% linear gradient, from 18 to 30 min: 90% of B isocratic.

Analytical curves were obtained using the external standard method with solutions of 500, 1250, 2000, 2750 and 3500 mg L^{-1} gamma-cyhalothrin. The obtained equation was c = (A – 170611)/2556 with coefficient of determination (R^2) = 0.997, where c = gamma-cyhalothrin concentration (mg L^{-1}) and A = gamma-cyhalothrin peak area. Note that samples were re-suspended in 5 mL of methanol after liquid-liquid extraction, being concentrated 10 times.[59] The analytical curve was presented in the Supplementary Information (SI) section.

Method validation

Method recovery and standard deviation were determined using the commercial formulation NEXIDE, which was used in the gamma-cyhalothrin biodegradation experiments, promoting increased similarity with the samples of this study. Therefore, 5 cultures of *Aspergillus ustus* CBMAI 1894 were prepared as described in the sub-section "Biodegradation of gamma-cyhalothrin in liquid culture medium" without pesticide addition. After 7 days of incubation at 25 °C and 130 rpm, these cultures

were sterilized in autoclave (20 min, 121 °C, 1 atm). After cooling, gamma-cyhalothrin addition was performed at 300 mg mL^{-1}, and determination was carried out as described in the sub-section "Quantification of gamma-cyhalothrin by HPLC-UV".

Identification of gamma-cyhalothrin metabolites

The identification of gamma-cyhalothrin metabolites was performed by gas chromatography coupled to mass spectrometry (GC-MS) in a Shimadzu 2010 plus model at electron ionization mode (EI, 70 eV). An Agilent J&W Scientific (Santa Clara, USA) DB5 column (30 m × 0.25 mm × 0.25 µm) was employed in the following temperature program: 90 °C for 4 min, increased to 280 °C at 6 °C min^{-1}, held for 6 min. Injector and interface temperature was maintained at 250 °C with splitless 1 µL injection. Helium was employed as carrier gas at a constant flow of 0.75 mL min^{-1}. Run time was 40 min and the used scan was *m/z* 40-500.[60] Metabolites identification was carried out with NIST 08, 08s, 21, and 107, and Wiley 8 Mass Spectra Libraries employing similarity index.

Results and Discussion

Culture media assessment in gamma-cyhalothrin presence

A culture media screening was performed to check the ability of these cave fungi strains to grow in the presence of 300 mg L^{-1} gamma-cyhalothrin. Three different culture media were evaluated: Sabouraud dextrose agar, potato dextrose agar and 2% malt extract agar. Furthermore, culture media without pesticide addition were used as control. Results are shown in Table 1.

Analysis of variance showed that pesticide addition to the culture medium negatively affected the growth of the investigated strains (*p*-value of $1.53e^{-14}$). Furthermore, Tukey's analysis (95% of confidence level) showed no significant difference between the assessed culture media. Probably because all the evaluated media were rich and complex options, being classically employed for fungi cultivation. Moreover, they were employed in the same pH of 5.6 and temperature of 25 °C.

Thus, biodegradation experiments were performed in 2% malt extract, since satisfactory growth for all strains was observed, with a reduced number of signals in the HPLC-UV analysis due to the presence of the medium components, promoting more reproducible results. Furthermore, malt extract was commonly used in the evaluation of pesticides biodegradation by fungi.[61,62]

Morphological changes were not observed due to the presence of gamma-cyhalothrin in the culture media. The most prominent difference was the reduced growth observed by colony diameter, as can be observed for *Aspergillus ustus* CBMAI 1894 in 2% malt extract medium (Figure 1). This same effect was described for fungi in the presence of different pesticides, like for the pyrethroid esfenvalerate, the organophosphates methyl parathion, profenofos, chlorpyrifos, and the organochlorine dieldrin.[63-65]

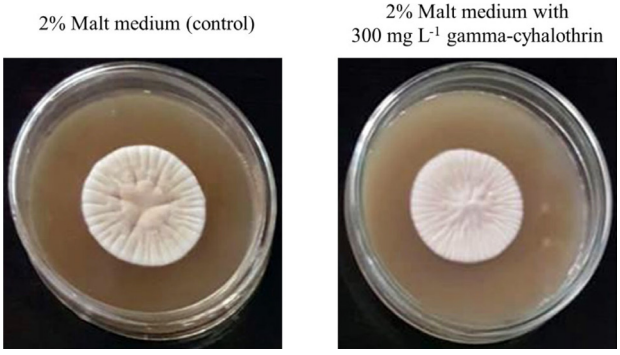


Figure 1. Growth of *Aspergillus ustus* CBMAI 1894 in 2% malt agar medium in the absence and presence of gamma-cyhalothrin.

Several strategies have been employed for selection of fungi strains for pyrethroids biodegradation, including screening in minimal salt agar medium followed by enrichment culture technique, selection of culturable strains from different sources like marine sponges and Fu Brick tea samples, and evaluation of species related with

Table 1. Radial growth of fungi on three different solid culture media with and without gamma-cyhalothrin (300 mg L^{-1})

Strain	Control (media without gamma-cyhalothrin)			Growth assessment (media with 300 mg L^{-1} gamma-cyhalothrin)		
	Sabouraud dextrose / cm	Potato dextrose / cm	2% Malt extract / cm	Sabouraud dextrose / cm	Potato dextrose / cm	2% Malt extract / cm
Aspergillus ustus CBMAI 1894	3.30 ± 0.17	2.52 ± 0.27	3.40 ± 0.00	0.97 ± 0.06	0.97 ± 0.32	1.15 ± 0.07
Talaromyces brunneus CBMAI 1895	2.50 ± 0.08	2.33 ± 0.06	2.37 ± 0.06	1.90 ± 0.14	1.55 ± 0.21	1.53 ± 0.15
Aspergillus sp. CBMAI 1926	2.42 ± 0.15	2.10 ± 0.10	2.30 ± 0.11	2.03 ± 0.06	1.75 ± 0.07	2.00 ± 0.15

Incubation in absence of light on BOD at 25 °C for 7 days. All experiments were carried out in triplicate.

specific characteristics like entomopathogenicity.[23,66-68] Thus, different approaches can be employed for obtaining an interesting biocatalyst. Here, the assessment of cave fungi strains was performed aiming at unique properties and high efficiency.

Biodegradation of gamma-cyhalothrin

Experiments were compared with abiotic controls and method recovery. Therefore, it was ensured that the analyte was not absorbed or adsorbed by the fungal cells or degraded abiotically. Residual gamma-cyhalothrin concentration and biodegradation in percentage were presented at Figure 2. Numeric data were presented in SI section.

The abiotic control and recovery tests showed that losses and consumed gamma-cyhalothrin in absence of microorganisms were very low or null in absence of microorganisms, 2.7 ± 3.4% and –6.4 ± 1.4% biodegradation, respectively. Moreover, data suggested that higher biodegradation rates were obtained for gamma-cyhalothrin addition at day zero of cultivation, when compared to the addition at the 3rd day after inoculation, although statistical significance was observed only for *A. ustus* CBMAI 1894 (156.9 ± 12.4 mg L^{-1} ≠ 211.4 ± 9.0 mg L^{-1} gamma-cyhalothrin, two-tailed *p* value equals 0.0035). These results indicated that the lag phase is important to biodegradation, and earlier contact of the biocatalyst with the substrate during incubation is an important factor for pesticide consumption.

Among the three investigated cave strains, *A. ustus* CBMAI 1894 presented the highest gamma-cyhalothrin biodegradation, 47.7 ± 4.1%. For this reason, further experiments were performed with this biocatalyst. Pyrethroids are biodegradable compounds, and it is already known the importance of microorganisms for enhanced bioremediation and increased soil quality, including in processes as soil aggregation, formation of symbiotic relationships, decomposition of residues, control of pests and diseases, and mineralization of nutrients.[69]

Few studies about cave microorganisms and its biotechnological contributions, such as in bioremediation, have been described. Although fungi from subterranean environments have been associated with damage at cultural heritage due to their biodegradation capacity,[70,71] which can be used with a defined and interesting purpose, like in this study aiming at pyrethroid biodegradation.

Microbial metabolism has been studied for many years with focus on its roles at detoxification and degradation of contaminants by bacteria and fungi.[22,66,72] In this context, studies about novel species and strains from different environments, as in this work, expands the knowledge about how microorganisms that inhabit extreme habitats, such as caves, can develop ways of optimizing energy obtention and development.

Growth curve

The growth curve of *Aspergillus ustus* CBMAI 1894 was performed with 2% malt extract broth without pesticide (control) and with the addition of gamma-cyhalothrin at 300 mg L^{-1} in the day zero and at the 3rd day of cultivation (Figure 3).

In the analysis of the control curve, it was noted that the maximum biomass was about 7 g L^{-1} with lag phase until day one, followed by exponential phase (days 1-5), stationary phase (5-11 days) and logarithmic decline phase or death (day 11 onwards).

The addition of gamma-cyhalothrin to the culture medium modified the growth curve of *Aspergillus ustus* CBMAI 1894. Biomass reduction was observed, and the lowest value (4.5 g L^{-1}) was obtained with the addition of

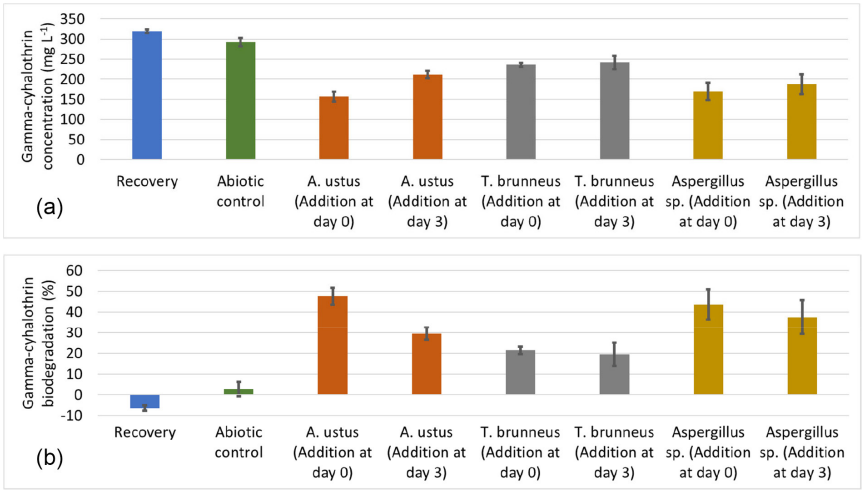


Figure 2. Gamma-cyhalothrin biodegradation by cave fungi strains in (a) residual gamma-cyhalothrin concentration (mg L^{-1}) and (b) biodegradation (%).

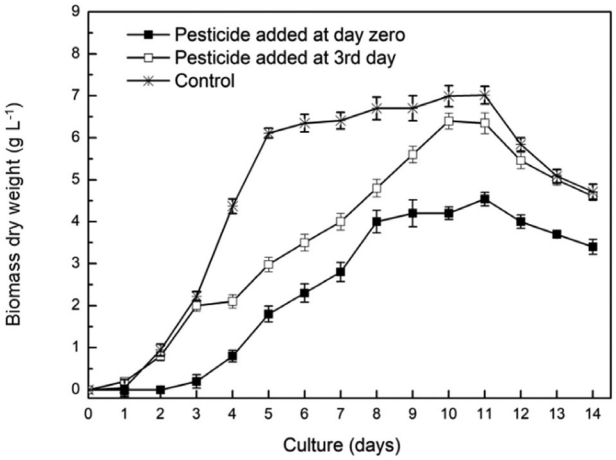


Figure 3. Growth curve of *Aspergillus ustus* CBMAI 1894 during 14 days of cultivation. Culture medium without pesticide (control), culture medium with 300 mg L^{-1} gamma-cyhalothrin added at day zero and at the 3rd day of cultivation. Weight of dry mass after drying at 70 °C for 24 h.

pesticide at day zero, although biodegradation experiments showed that this condition promoted increased gamma-cyhalothrin consumption (sub-section "Biodegradation of gamma-cyhalothrin"). In this case, the duration of lag phase was 3 days (days 0-3), followed by the exponential phase (days 3-8), stationary phase (8-11 days) and logarithmic decline phase or death (day 11 onwards).

Another profile was observed when the pesticide was added on the 3rd day. The lag phase lasted one day, followed by an exponential phase (days 1-3). After the addition of gamma-cyhalothrin, a second lag phase at days 3-4 occurred, followed by another exponential phase (days 4-10), then the stationary phase (10-11 days) and logarithmic decline phase or death (day 11 onwards). In this situation, the stationary phase was greatly reduced to one day.

The experiments presented here showed that the highest gamma-cyhalothrin biodegradation occurred when the pesticide was added to the culture medium before the spore suspension. The lag phase lasted 3 days, whereas the addition of pesticide on 3rd day of cultivation induced a one-day lag phase. Thus, it is supposed that this longer lag phase period was crucial for cave-fungal metabolism adaptation, inducing enzyme production for gamma-cyhalothrin biodegradation.

Experimental design

A 2 levels Box-Behnken design with three factors was carried out employing *Aspergillus ustus* CBMAI 1894 for gamma-cyhalothrin biodegradation during 21 days. Results are shown in Table 2.

The analysis of variance (ANOVA), Table 3, showed that the linear effect of temperature and gamma-cyhalothrin concentration were significant for biodegradation levels. Whereas temperature positively affected pesticide biodegradation, gamma-cyhalothrin concentration had a negative effect. Furthermore, culture medium pH did not influence pesticide biodegradation in the assessed range.

These relations were presented in Figure 4. In the generated response surface, higher temperatures and

Table 2. Box-Behnken experimental design and the response factor gamma-cyhalothrin biodegradation by *Aspergillus ustus* CBMAI 1894

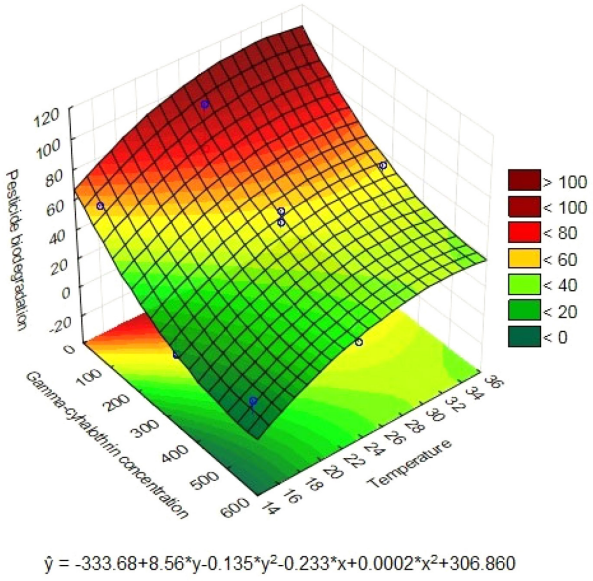
Run	X_1: temperature / °C	X_2: culture medium pH	X_3: gamma-cyhalothrin concentration / (mg L^{-1})	Biodegradation / %
1	15	5.5	300	0
2	15	8.5	300	1.5
3	35	5.5	300	58.8
4	35	8.5	300	32.2
5	15	7.0	50	60.6
6	15	7.0	550	12.6
7	35	7.0	50	89.1
8	35	7.0	550	36.1
9	25	5.5	50	65.4
10	25	5.5	550	14.3
11	25	8.5	50	95.6
12	25	8.5	550	22.5
13[a]	25	7.0	300	46.8
14[a]	25	7.0	300	62.4
15[a]	25	7.0	300	54.6
16[a]	25	7.0	300	38.8

[a]Experiments representing the midpoints were repeated four times to estimate errors.

Table 3. Analysis of variance for Box-Behnken experimental design at 95% confidence level

Data	Df	SS	MS	*F* value	Pr (> *F*)
Temperature	1	2538	2538	24.675	0.015670
Gamma-cyhalothrin concentration	1	6339	6339	61.628	0.004305
Lack of fit	6	1051	175		
Pure error	3	308	102		
Total	15	12377			

Df: degrees of freedom; SS: sum of squares; MS: mean square. $R^2 = 0.8901$.



$\hat{y} = -333.68+8.56*y-0.135*y^2-0.233*x+0.0002*x^2+306.860$

Figure 4. Fitted response surface (at 95% confidence level) for gamma-cyhalothrin biodegradation (%) in function of initial gamma-cyhalothrin concentration (mg L^{-1}) and temperature (°C). The culture medium pH of 7.0 was chosen to create this graphic.

lower gamma-cyhalothrin concentrations were the best combination for pesticide biodegradation.

Kinetics and experimental design of pyrethroids biodegradation by fungi have been presented in some studies, showing the importance of this data for a better overview of the different parameters that can affect a decontamination process.[73] In the literature, factors such as initial pesticide concentration, pH, nutrients, organic matter content, carbon sources, temperature, microbial metabolism, and moisture substantially influenced pesticide biodegradation in both liquid medium and soil.[74-78]

In this study, pH was not statistically significant for the obtained model, and temperature showed a positive correlation with the increasing rates of gamma-cyhalothrin degradation. Although it is known that, tolerance limits of temperature (high or low) are not ideal for microorganisms growth and pyrethroids biodegradation.[79]

Differently from other variables, gamma-cyhalothrin initial concentration showed a negative correlation with biodegradation rates for both linear and quadratic analyses. Other authors[21] also stated that higher pesticide concentrations reduced degradation rates. Since these xenobiotics act as inhibitors for microorganisms by promoting slower growth and adaptation, generating increased lag phase due to the recruitment of more cells for effective beginning of biodegradation.

Metabolites identification

Products of the gamma-cyhalothrin metabolism by cave fungi were identified. The metabolite 2-(3-phenoxyphenyl) acetonitrile was identified for all the evaluated strains with a retention time of 27.1 min and 90% similarity with the spectra library NIST08 (spectra was presented in the SI section). This substance was absent in the abiotic and killed-cells controls, showing that this compound resulted from the biodegradation process.

The metabolite 3-phenoxybenzaldehyde was identified in the GC-MS analyses for all strains with a retention time of 23.6 min and 89% similarity with the spectra library NIST 8 (spectra was presented in the SI section). This compound was confirmed with an authentic standard and its presence was also observed in HPLC-UV analyses, showing that this compound was not a product of thermal degradation during GC-MS analyses. The metabolite 3-phenoxybenzoic acid was identified in HPLC-UV analyses employing an authentic standard (chromatograms were presented in the SI section). Probably, this compound was absent in the GC-MS analyses because of its low concentrations, since carboxylic acids usually require derivatization for better detection in this analysis. A partial biodegradation pathway was proposed (Figure 5).

The first step in pyrethroids biodegradation generally is the break of the ester bond by carboxylesterases, an enzyme class present in bacteria, fungi, and different animal tissues.[80] Subsequently, a cascade of reactions can produce the most detected metabolite 3-phenoxybenzoic acid, which is usually biotransformed by hydroxylation reactions, e.g., monohydroxylation by the marine-derived fungi *Acremonium* sp. CBMAI 1676[25] and mono- and

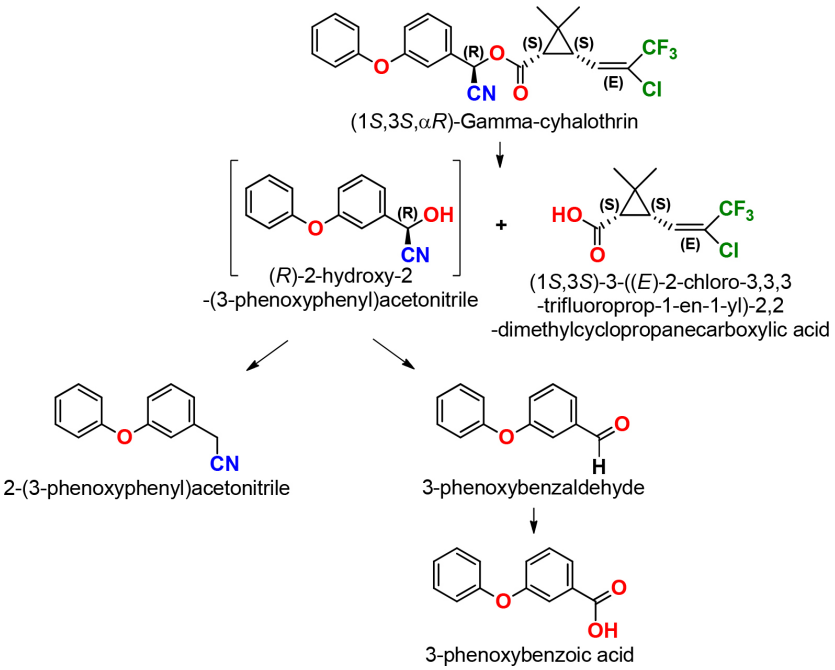


Figure 5. Proposed biodegradation pathway for gamma-cyhalothrin by cave fungi.

dihydroxylation by *Aspergillus oryzae* M-4.[81] Furthermore, phenol, catechol, protocatechuate, benzoic acid and benzylic alcohol were also reported in the literature as 3-phenoxybenzoic acid products.[60,82] However, these hydroxylated and break down metabolites were not observed here for cave fungi. Probably, the production rate of these compounds was lower than their consumption by the employed biocatalysts.

Conclusions

Among the three studied fungi species from Gruta do Catão, *Aspergillus ustus* CBMAI 1894 showed the highest efficiency at gamma-cyhalothrin biodegradation. However, *Talaromyces brunneus* CBMAI 1895 and *Aspergillus* sp. CBMAI 1926 also biodegraded this insecticide, showing potential for bioremediation processes. In the assessed Box-Behnken design with *Aspergillus ustus* CBMAI 1894, temperature positively affected the biodegradation process, whereas increased gamma-cyhalothrin initial concentration presented a negative effect, and pH was not statistically significant at the obtained model. Therefore, these cave fungi strains biodegraded gamma-cyhalothrin and can be explored for bioremediation of contaminated environments. Moreover, future studies on these strains should focus on the understanding of the enzymatic apparatus, physiology, and genetics behind these cave microorganisms, which can present unique properties for biotechnological applications.

Supplementary Information

Supplementary information data (analytical curve obtained by HPLC-UV for gamma-cyhalothrin, numeric results for gamma-cyhalothrin biodegradation by cave fungi strains, 2-(3-phenoxyphenyl) acetonitrile data from gas chromatography-mass spectrometry, 3-phenoxybenzaldehyde data from gas chromatography-mass spectrometry, 3-phenoxybenzoic acid and gamma-cyhalothrin at HPLC-UV analysis) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Fabio R. Rigolin was responsible for the conceptualization, data curation, formal analysis, investigation, methodology, software, project administration, validation, visualization, writing (original draft, review and editing); Carla A. Leite for methodology, supervision, visualization, writing-review and editing; Willian G. Birolli for methodology, supervision, visualization, writing-review and editing; André L. M. Porto for resources and supervision; Mirna H. R. Seleghim for resources, supervision, writing original draft.

References

1. Reyna, E. F.; Braga, M. J.; Morais, G. A. S. In *Uma Jornada pelos Contrastes do Brasil: Cem Anos de Censo Agropecuário*; Vieira-Filho, J. E. R.; Garques, J. G., eds.; Ipea: Brasília, 2020, p. 173. [Link] accessed in January 2024
2. Mesnage, R.; Séralini, G. E.; *Front. Public Health* **2018**, *6*, 411830. [Crossref]
3. de Freitas, L. M.; Bonfatti, R.; de Vasconcellos, L. C. F.; *Saúde em Debate* **2022**, *46*, 224. [Crossref]
4. Kaur, H.; Garg, H. In *Pesticides - Toxic Aspects*; Larramendy, M. L.; Soloneski, S., eds.; IntechOpen: Rijeka, 2014, ch. 8. [Crossref]
5. Sabik, H.; Jeannot, R.; Rondeau, B.; *J. Chromatogr. A* **2000**, *885*, 217. [Crossref]
6. Albuquerque, A. F.; Ribeiro, J. S.; Kummrow, F.; Nogueira, A. J. A.; Montagner, C. C.; Umbuzeiro, G. A.; *Environ. Sci.: Processes Impacts* **2016**, *18*, 779. [Crossref]
7. Panis, C.; Kawassaki, A. C. B.; Crestani, A. P. J.; Pascotto, C. R.; Bortoloti, D. S.; Vicentini, G. E.; Lucio, L. C.; Ferreira, M. O.; Prates, R. T. C.; Vieira, V. K.; Gaboardi, S. C.; Candiotto, L. Z. P.; *Front. Public Health* **2022**, *9*, 787438. [Crossref]
8. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA), *Relatórios de Comercialização de Agrotóxicos*, https://www.gov.br/ibama/pt-br/assuntos/quimicos-e-biologicos/agrotoxicos/relatorios-de-comercializacao-de-agrotoxicos, accessed in January 2024.
9. United States Environmental Protection Agency (US EPA), https://www.epa.gov/expobox/exposure-assessment-tools-chemical-classes-pesticides, accessed in January 2024.
10. Pannu, R.; Kumar, D.; *Biocatal. Agric. Biotechnol.* **2023**, *48*, 102630. [Crossref]
11. Viera, M. S.; Ferronato, G.; Abreu, H. D. F.; Prestes, O. D.; Adaime, M. B.; Zanella, R.; *J. Braz. Chem. Soc.* **2022**, *33*, 790. [Crossref]
12. Guida, Y.; de Carvalho, G. O.; Capella, R.; Pozo, K.; Lino, A. S.; Azeredo, A.; Carvalho, D. F. P.; Braga, A. L. F.; Torres, J. P. M.; Meire, R. O.; *Environ. Pollut.* **2021**, *271*, 116359. [Crossref]
13. Oliveira, A. H. B.; Cavalcante, R. M.; Duaví, W. C.; Fernandes, G. M.; Nascimento, R. F.; Queiroz, M. E. L. R.; Mendonça, K. V.; *Sci. Total Environ.* **2016**, *542*, 254. [Crossref]
14. Tang, W.; Wang, D.; Wang, J.; Wu, Z.; Li, L.; Huang, M.; Xu, S.; Yan, D.; *Chemosphere* **2018**, *191*, 990. [Crossref]
15. Umetsu, N.; Shirai, Y.; *J. Pestic. Sci.* **2020**, *45*, 54. [Crossref]
16. Yang, Y.; Ma, S.; Liu, F.; Wang, Q.; Wang, X.; Hou, C.; Wu, Y.; Gao, J.; Zhang, L.; Liu, Y.; Diao, Q.; Dai, P.; *Pest. Manage. Sci.* **2020**, *76*, 978. [Crossref]
17. Ullah, S.; Li, Z.; Zuberi, A.; Arifeen, M. Z. U.; Baig, M. M. F. A.; *Environ. Chem. Lett.* **2019**, *17*, 945. [Crossref]
18. Hołyńska-Iwan, I.; Szewczyk-Golec, K.; *Medicina* **2020**, *56*, 582. [Crossref]
19. Conceição, A. A.; Cunha, J. R. B.; Vieira, V. O.; Pelaéz, R. D. R.; Mendonça, S.; Almeida, J. R. M.; Dias, E. S.; de Almeida, E. G.; de Siqueira, F. G. In *Biology of Macrofungi*; Singh, B. P.; Lallawmsanga; Passari, A. K., eds.; Springer: Cham, 2018, p. 361. [Link] accessed in January 2024
20. Bhatt, P.; Huang, Y.; Zhan, H.; Chen, S.; *Front. Microbiol.* **2019**, *10*, 1778. [Crossref]
21. Cycon, M.; Piotrowska-Seget, Z.; *Front. Microbiol.* **2016**, *7*, 218351. [Crossref]
22. Singh, S.; Mukherjee, A.; Jaiswal, D. K.; Pereira, A. P. A.; Prasad, R.; Sharma, M.; Kuhad, R. C.; Shukla, A. C.; Verma, J. P.; *Sci. Total Environ.* **2022**, *829*, 154561. [Crossref]
23. Kaur, P.; Balomajumder, C.; *Environ. Pollut.* **2020**, *261*, 114073. [Crossref]
24. Zhao, J.; Chi, Y.; Xu, Y.; Jia, D.; Yao, K.; *PLoS One* **2016**, *11*, e0166796. [Crossref]
25. Birolli, W. G.; Alvarenga, N.; Seleghim, M. H. R.; Porto, A. L. M.; *Mar. Biotechnol.* **2016**, *18*, 511. [Crossref]
26. Braga, C. S.; Bessegato, G. G.; Maestre, K.; Espinoza-Quiñones, F. R.; Alves, H. J.; da Silva, L. C.; Eising, R.; Baricatti, R. A.; *J. Braz. Chem. Soc.* **2023**, *34*, 1284. [Crossref]
27. Ahmad, T.; Rafatullah, M.; Ghazali, A.; Sulaiman, O.; Hashim, R.; Ahmad, A.; *J. Environ. Sci. Health, Part C: Environ. Carcinog. Ecotoxicol. Rev.* **2010**, *28*, 231. [Crossref]
28. Chávez, R.; Fierro, F.; García-Rico, R. O.; Vaca, I.; *Front. Microbiol.* **2015**, *6*, 154260. [Crossref]
29. Hui, M. L. Y.; Tan, L. T. H.; Letchumanan, V.; He, Y. W.; Fang, C. M.; Chan, K. G.; Law, J. W. F.; Lee, L. H.; *Antibiotics* **2021**, *10*, 682. [Crossref]
30. Kozlov, G.; Alekseev, E.; Chermenskaya, T.; *Biocatal. Agric. Biotechnol.* **2022**, *42*, 102378. [Crossref]
31. Garcia, A. C. F. S.; Birolli, W. G.; Araújo, B. R.; Marques, C. G.; Barbosa-Junior, A. M.; Diniz, L. E. C.; Porto, A. L. M.; Romão, L. P. C.; *J. Braz. Chem. Soc.* **2021**, *32*, 1874. [Crossref]
32. Tomczyk-Żak, K.; Zielenkiewicz, U.; *Geomicrobiology* **2015**, *33*, 20. [Crossref]
33. Adam, D.; Maciejewska, M.; Naômé, A.; Martinet, L.; Coppieters, W.; Karim, L.; Baurain, D.; Rigali, S.; *Antibiotics* **2018**, *7*, 28. [Crossref]

34. Ghosh, S.; Kuisiene, N.; Cheeptham, N.; *Biochem. Pharmacol.* **2017**, *134*, 18. [Crossref]
35. Held, B. W.; Salomon, C. E.; Blanchette, R. A.; *PLoS One* **2020**, *15*, e0234208. [Crossref]
36. Nuankaew, S.; Chuaseeharonnachai, C.; Preedanon, S.; Somrithipol, S.; Saengkaewsuk, S.; Kwantong, P.; Phookongchai, S.; Srikitikulchai, P.; Kobmoo, N.; Wang, X. C.; Zhang, Z. F.; Cai, L.; Suetrong, S.; Boonyuen, N.; *J. Fungi* **2022**, *8*, 825. [Crossref]
37. Zgonik, V.; Mulec, J.; Eleršek, T.; Ogrinc, N.; Jamnik, P.; Ulrih, N. P.; *Microorganisms* **2021**, *9*, 2326. [Crossref]
38. Alonso, L.; Pommier, T.; Simon, L.; Maucourt, F.; Doré, J.; Dubost, A.; Trân Van, V.; Minard, G.; Valiente Moro, C.; Douady, C. J.; Moënne-Loccoz, Y.; *Environ. Microbiol. Rep.* **2023**, *15*, 80. [Crossref]
39. Mazina, S. E.; Gasanova, T. V.; Kozlova, E. V.; Popkova, A. V.; Fedorov, A. S.; Bukharina, I. L.; Pashkova, A. S.; Larionov, M. V.; Abdullayev, R. R.; Isaev, V. U.; *Life* **2023**, *13*, 164. [Crossref]
40. Vanderwolf, K. J.; Malloch, D.; Mcalpine, D. F.; Forbes, G. J.; *Int. J. Speleol.* **2013**, *42*, 77. [Crossref]
41. Bontemps, Z.; Alonso, L.; Pommier, T.; Hugoni, M.; Moënne-Loccoz, Y.; *Sci. Total Environ.* **2022**, *816*, 151492. [Crossref]
42. Kato, N.; Arini, G.; Silva, R. R.; Bichuette, M. E.; Bitencourt, J. A.; Lopes, N.; *J. Braz. Chem. Soc.* **2024**, *35*, 1. [Crossref]
43. Budak, T. B.; Ayyıldız, M. F.; Chormey, D. S.; Bakırdere, S.; *Environ. Monit. Assess.* **2020**, *192*, 273. [Crossref]
44. Giddings, J. M.; Barber, I.; Warren-Hicks, W.; *Ecotoxicology* **2009**, *18*, 239. [Crossref]
45. de Paula, C. C. P.; Montoya, Q. V.; Rodrigues, A.; Bichuette, M. E.; Seleghim, M. H. R.; *J. Cave Karst Stud.* **2016**, *78*, 208. [Crossref]
46. Paula, C. C. P. D.; Montoya, Q. V.; Meirelles, L. A.; Farinas, C. S.; Rodrigues, A.; Seleghim, M. H. R.; *Ann. Acad. Bras. Cienc.* **2019**, *91*, e20180583. [Crossref]
47. Aamir, S.; Sutar, S.; Singh, S. K.; Baghela, A.; *Plant Pathol. Quarantine* **2015**, *5*, 74. [Crossref]
48. *Clustal X*, version 2.0; University College Dublin, Dublin, Ireland, 2007. [Link] accessed in January 2024
49. *MEGA*, version 6.0; Pennsylvania State University, Pennsylvania, USA, 2013. [Link] accessed in January 2024
50. Thompson, J. D.; Gibson, T. J.; Plewniak, F.; Jeanmougin, F.; Higgins, D. G.; *Nucleic Acids Res.* **1997**, *25*, 4876. [Crossref]
51. Tamura, K.; Dubley, J.; Nei, M.; Kumar, S.; *Mol. Biol. Evol.* **2007**, *24*, 1596. [Crossref]
52. Kimura, M.; *J. Mol. Evol.* **1980**, *16*, 111. [Crossref]
53. Saitou, N.; Nei, M.; *Mol. Biol. Evol.* **1987**, *4*, 406. [Crossref]
54. *RStudio*, version 1.4; Posit Software PBC Inc.; Boston, MA, USA, 2011. [Link] accessed in January 2024
55. Nakasima-López, S.; Nakasima-López, M. O.; Madrigal Estrada, K. F.; Beltrán Salomón, E. In *Design of Experiments for Chemical, Pharmaceutical, Food, and Industrial Applications*; Carrillo-Cedillo, E.; Rodríguez-Avila, J.; Arredondo-Soto, K.; Cornejo-Bravo, J., eds.; IGI Global: Hershey, 2019, p. 270. [Crossref]
56. Das, A. K.; Dewanjee, S. In *Computatacional Phytochemistry*; Sarker, S. D.; Narhar, L., eds.; Elsevier: Amsterdam, 2018, p. 75. [Crossref]
57. Demir Delil, A.; Gülçiçek, O.; Gören, N.; *Int. J. Environ. Res.* **2019**, *13*, 861. [Crossref]
58. *Statistica*, version 12.0; Statsoft Inc.; Tulsa, OK, USA, 2013. [Link] accessed in January 2024
59. Birolli, W. G.; Vacondio, B.; Alvarenga, N.; Seleghim, M. H. R.; Porto, A. L. M.; *Chemosphere* **2018**, *197*, 651. [Crossref]
60. Birolli, W. G.; Arai, M. S.; Nitschke, M.; Porto, A. L. M.; *Pestic. Biochem. Physiol.* **2019**, *156*, 129. [Crossref]
61. Rajendran, R. K.; Lee, Y. W.; Chou, P. H.; Huang, S. L.; Kirschner, R.; Lin, C. C.; *Chemosphere* **2020**, *240*, 124876. [Crossref]
62. Birolli, W. G.; Souza, L. I.; Porto, A. L. M.; Rodrigues-Filho, E. In *Pyrethroids: Exposure, Applications and Resistance*; Ruijten, J., ed.; Nova Science Publishers: Hauppauge, 2020.
63. Birolli, W. G.; Alvarenga, N.; Vacondio, B.; Seleghim, M. H.; Porto, A. L. M.; *J. Microb. Biochem. Technol.* **2014**, *6*, 260. [Crossref]
64. Soares, P. R. S.; Birolli, W. G.; Ferreira, I. M.; Porto, A. L. M.; *Mar. Pollut. Bull.* **2021**, *166*, 112185. [Crossref]
65. Birolli, W. G.; Yamamoto, K. Y.; Oliveira, J. R.; Nitschke, M.; Seleghim, M. H. R.; Porto, A. L. M.; *Biocatal. Agric. Biotechnol.* **2015**, *4*, 39. [Crossref]
66. Birolli, W. G.; Porto, A. L. M.; *Environ. Sci. Pollut. Res.* **2023**, *30*, 38394. [Crossref]
67. Hu, K.; Deng, W.; Zhu, Y.; Yao, K.; Li, J.; Liu, A.; Ao, X.; Zou, L.; Zhou, K.; He, L.; Chen, S.; Yang, Y.; Liu, S.; *MicrobiologyOpen* **2019**, *8*, e776. [Crossref]
68. Muthabathula, P.; Biruduganti, S.; *Curr. Microbiol.* **2022**, *79*, 46. [Crossref]
69. Buckley, M.; *American Academy of Microbiology Colloquia Reports*; Tucson, Arizona, Washington, 2008. [Link] accessed in January 2024
70. Cuzman, O. A.; Luvidi, L.; Colantonio, C.; Raio, A.; Taiti, S.; *Int. Biodeterior. Biodegrad.* **2023**, *181*, 105605. [Crossref]
71. He, D.; Wu, F.; Ma, W.; Gu, J. D.; Xu, R.; Hu, J.; Yue, Y.; Ma, Q.; Wang, W.; Li, S. W.; *Int. Biodeterior. Biodegrad.* **2022**, *171*, 105406. [Crossref]
72. Fang, Y.; Xu, W.; Zhang, W.; Guang, C.; Mu, W.; *Appl. Microbiol. Biotechnol.* **2022**, *106*, 6915. [Crossref]
73. Bhatt, P.; Zhang, W.; Lin, Z.; Pang, S.; Huang, Y.; Chen, S.; *Microorganisms* **2020**, *8*, 593. [Crossref]
74. Malla, M. A.; Dubey, A.; Kumar, A.; Vennapu, D. R.; Upadhyay, N.; Pradhan, D.; Pradhan, R. C.; Yadav, S.; *Environ. Sci. Pollut. Res.* **2022**, *29*, 77418. [Crossref]

75. Huang, Y.; Chen, W. J.; Li, J.; Ghorab, M. A.; Alansary, N.; El-Hefny, D. E.; El-Sayyad, G. S.; Mishra, S.; Zhang, X.; Bhatt, P.; Chen, S.; *Environ. Res.* **2022**, *214*, 113940. [Crossref]
76. Birolli, W. G.; da Silva, B. F.; Rodrigues Filho, E.; *Environ. Res.* **2022**, *215*, 114388. [Crossref]
77. Chen, W. J.; Zhang, W.; Lei, Q.; Chen, S. F.; Huang, Y.; Bhatt, K.; Liao, L.; Zhou, X.; *Environ. Res.* **2023**, *236*, 116619. [Crossref]
78. Dar, M. A.; Kaushik, G.; *Biocatal. Agric. Biotechnol.* **2022**, *42*, 102362. [Crossref]
79. Zhang, H.; Zhang, Y.; Hou, Z.; Wang, X.; Wang, J.; Lu, Z.; Zhao, X.; Sun, F.; Pan, H.; *Int. Biodeterior. Biodegrad.* **2016**, *106*, 53. [Crossref]
80. Bhatt, P.; Bhatt, K.; Huang, Y.; Lin, Z.; Chen, S.; *Chemosphere* **2020**, *244*, 125507. [Crossref]
81. Hu, K.; Li, J.; Zhao, T.; Zhou, Q.; Li, Q.; Hu, X.; Han, G.; Li, S.; Zou, L.; Liu, S.; *J. Environ. Manage.* **2023**, *325*, 116626. [Crossref]
82. Tang, J.; Hu, Q.; Liu, B.; Lei, D.; Chen, T.; Sun, Q.; Zeng, C.; Zhang, Q.; *Can. J. Microbiol.* **2019**, *65*, 795. [Crossref]

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