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Biodegradation Of Expired Pharmaceutical Raw Materials Using Select Fungal Species

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Abstract
Fungi were isolated from different environmental sources. The fungi were identified morphologically and molecularly: Aspergillus terreus, Aspergillus niger, Penicillium italicum, and Rhizopus arrhizus using the ITS (Internal Transcribed Spacer) molecular diagnostic technique. The ability of fungi to degrade three expired drug raw materials—clopidogrel, paracetamol, and rosuwastatin—prepared by Pioneer Pharmaceuticals, Iraq, Sulaymaniyah, was tested. The highest degradation rate among the pharmaceutical compounds was for Rosuwastatin, reaching 85.2% for Rhizopus arrhizus, followed by Penicillium italicum and Aspergillus niger, with rates of 84.12% and 82.59%, respectively. The fungi under study also played an effective role in the degradation of clopidogrel, as the degradation rates of Aspergillus niger and Rhizopus arrhizus were close at 68.08% and 67.69%, respectively. The lowest degradation rate of paracetamol was observed among the three fungi compared to the previous two. Some secondary metabolites of the fungi under study were analyzed using GC-MS. It was observed that there was an abundance of 6-bromohexanoic acid, 10-undecenyl ester, with an area of 59.20% in Rhizopus arrhizus. While the most abundant secondary metabolite of Penicillium italicum was trichloroacetic acid, undec-10-enyl ester, with an area of 57.77%, the most abundant secondary metabolite of Aspergillus niger was carbonic acid, 2, 2, 2-trichloroethyl cyclohexylmethyl ester, at 46.52%.
Keywords: *Pharmaceutical waste, Biodegradation, Fungi, GC-MS*

1. INTRODUCTION

Pharmaceutical waste, particularly expired raw materials, has become a significant environmental concern due to its persistence, toxicity, and potential to disrupt ecosystems. These materials often contain complex organic compounds that resist natural degradation, leading to their accumulation in soil and water (Kümmerer, 2010). Improper disposal practices, such as incineration and landfilling, exacerbate the issue by releasing harmful byproducts into the environment or leaching contaminants into groundwater. This not only poses risks to biodiversity but also threatens human health through bioaccumulation and water contamination (Boxall, 2004).

Biodegradation presents a promising alternative, employing microorganisms such as fungi to break down complex compounds into simpler, less harmful substances. Fungi are particularly effective due to their unique enzymatic systems, including laccases, peroxidases, and hydrolases, which can degrade a wide range of pollutants (Pointing, 2001). Unlike chemical treatments, fungal biodegradation is eco-friendly, cost-effective, and capable of targeting specific contaminants without generating secondary pollution (Singh, 2006).

Despite these advantages, the application of fungal biodegradation to pharmaceutical waste remains underexplored. Research has primarily focused on industrial pollutants, leaving a significant gap in understanding how fungi can address the challenges posed by expired pharmaceutical raw materials (Rodriguez-Couto, 2009). This study seeks to fill this gap by identifying and optimizing fungal species capable of efficient pharmaceutical waste degradation, contributing to both environmental sustainability and improved waste management practices.

Pharmaceutical raw materials, which include active pharmaceutical ingredients (APIs), excipients, and intermediates, are integral to the production of medications. However, their improper disposal or release into the environment has raised significant concerns due to their persistence, toxicity, and potential to disrupt ecosystems. The environmental impact of these materials varies depending on their chemical composition, usage patterns, and disposal methods. This section

explores examples of commonly used pharmaceutical raw materials and their adverse environmental effects, supported by evidence from scientific studies.

Biodegradation, particularly using fungi, has emerged as a promising solution for mitigating the impact of pharmaceutical waste. Fungi possess unique enzymatic systems capable of breaking down complex organic compounds into less harmful substances, offering an eco-friendly alternative to conventional disposal methods (Pointing, 2001).

Pharmaceutical waste, including antibiotics, analgesics, and hormones, poses a significant environmental hazard due to its persistence in ecosystems and potential to disrupt biological processes. Fungal enzymes have emerged as promising tools for mitigating this issue due to their remarkable ability to degrade complex organic compounds, including pharmaceutical pollutants.

1.1 Pharmaceutical Waste Management

Pharmaceutical waste is generated at various stages of production, distribution, and consumption, including expired raw materials, unused medications, and residues from manufacturing processes. The primary methods for disposing of pharmaceutical waste include incineration, chemical treatment, and landfilling. Incineration is widely used due to its ability to reduce waste volume and destroy hazardous compounds. However, it has significant drawbacks, including the release of toxic gases such as dioxins and furans, which pose environmental and health risks (Kümmerer, 2009). Chemical treatments, such as advanced oxidation processes, are effective in degrading pharmaceutical pollutants but are costly and can generate secondary pollutants (Ahmed et al., 2017). Landfilling, though inexpensive, allows pharmaceutical residues to leach into soil and groundwater, leading to long-term environmental contamination (Verlicchi et al., 2012). These limitations highlight the need for sustainable and eco-friendly alternatives, such as biodegradation, which can address the environmental challenges posed by pharmaceutical waste without generating secondary pollutants.

1.2 Biodegradation Mechanisms

In particular, fungi have demonstrated remarkable capabilities in degrading recalcitrant compounds due to their extracellular enzyme systems. Fungal enzymes, such as laccases, peroxidases, and hydrolases, play a central role in the biodegradation process. Laccases, copper-containing oxidases, catalyze the oxidation of phenolic and non-phenolic compounds, making them effective against pharmaceuticals like antibiotics and endocrine-disrupting chemicals (Rodríguez-Couto & Toca-Herrera, 2006). Peroxidases, including lignin peroxidase and manganese peroxidase, generate reactive radicals that break down complex aromatic structures, facilitating the degradation of pollutants such as NSAIDs and hormones (Dashtban et al., 2010). Hydrolases, on the other hand, target ester and amide bonds in pharmaceutical compounds, enabling their conversion into biodegradable intermediates (Bilal et al., 2017).

1.3 Paracetamol

Paracetamol, also known as acetaminophen, is one of the most widely used medications worldwide as a pain reliever and fever reducer, and is a key ingredient in many cold and flu remedies. It is used to relieve mild to moderate pain, such as headaches, muscle aches, backaches, toothaches, and colds, and to reduce fever (Garzón-Posse et al., 2022).

Paracetamol consists of a benzene ring substituted with a hydroxyl group and an acetamide group in the para (1, 4) position, making it an electronically conjugated system (Figure 1). It is a white crystalline substance that melts at 170°C, is well soluble in alcohol and hot water, and is poorly soluble in cold water. It has a molecular mass of 151.165 g/mol, and the pH value of its solution is between 5.5 and 6.5 (Bernal et al., 2017).

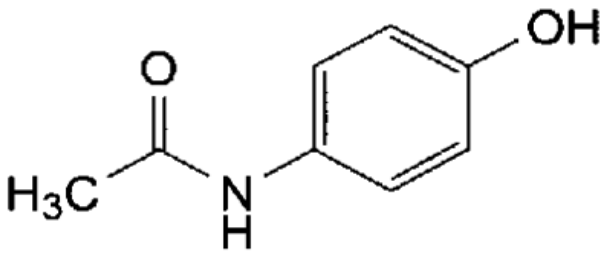

Figure 1: Chemical composition of paracetamol

Paracetamol negatively affects the environment when it enters sewage and rivers, as it is transformed in wastewater treatment plants into toxic compounds such as N-acetyl-para-benzoquinone imine (known as NABQ) and 1,4-benzoquinone, which are suspected of being toxic to the liver and causing genetic mutations (Vieira et al., 2024). The presence of paracetamol in surface water and rivers poses a threat to aquatic life and the environment. It is a drug found in high quantities in water sources due to improper disposal. Furthermore, paracetamol is highly toxic to some animals, such as cats, which lack certain enzymes to break it down, leading to poisoning and death. However, it has a less toxic effect on dogs when used under medical supervision. Therefore, further studies should be conducted to determine the concentration and duration of these toxic compounds in the environment, as well as to improve drug disposal methods to reduce their environmental impact (Futter et al., 2001).

1.4 Clopidogrel

Clopidogrel is an antiplatelet drug used to reduce the risk of heart attacks and strokes, especially in people with a history of cardiovascular disease, unstable angina, or peripheral arterial disease (Sangkuhl et al., 2010). The clear structure of clopidogrel is an organic compound in the thienopyridine class, with the formula C16H16ClNO2S, or more specifically, clopidogrel bisulfate (clopidogrel bisulfate), with the formula C16H18ClNO6S2.The bulky structure contains a thiophene (thiophene) ester and a chlorobenzyl Figure (2). It acts as an irreversible inhibitor of the ADP (P2Y12) receptor on the surface of marine platelets, preventing many of them and the formation of clots.Accordingly, the clear structure of clopidogrel gives it its thrombolytic properties by attracting adenosine diphosphate receptors to the chemical dye, and by identifying the clear transparency of cardiac and cerebral clots (Lestari et al., 2010).

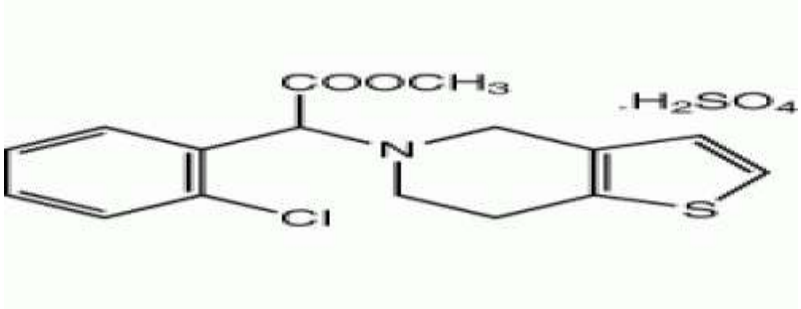

Figure 2: Chemical structure of Clopidogrel

Clopidogrel can indirectly impact the environment through the contamination of water and rivers with pharmaceutical compounds that enter the environment via wastewater or improper drug disposal. Like many drugs, clopidogrel or its active ingredients can leak into aquatic ecosystems, where they can affect aquatic organisms and cause biological disturbances. However, specific studies on the environmental impact of clopidogrel are scarce or unavailable (Roveri et al., 2024).

General research on drug contamination in the environment indicates that anticoagulant drugs and other pharmaceutical compounds can contaminate rivers and waterways, posing a threat to wildlife and human health, especially when they accumulate or interact with other pollutants in

the environment. Therefore, it is essential to dispose of drugs such as clopidogrel in safe ways to reduce environmental pollution (Fouda et al., 2017).

1.5 Rosuvastatin

Rosuvastatin is a statin drug used primarily to lower blood cholesterol and triglyceride levels, thus helping to prevent cardiovascular diseases such as heart attacks and strokes. The rosuvastatin molecule is characterized by the presence of a fluorine atom attached to a phenyl ring, in addition to hydroxyl, amine, and thiophene groups Figure 3, giving it its properties as an inhibitor of the HMG-CoA reductase enzyme responsible for cholesterol synthesis in the liver.

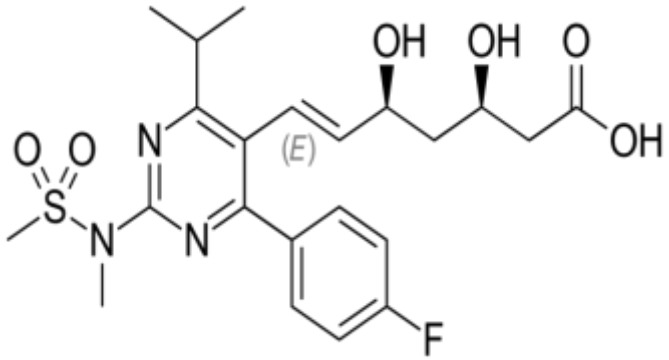

Figure 3: Rosuvastatin chemical composition

Rosuvastatin, like other statins, can accumulate in rivers and lakes, affecting microorganisms and fish, and potentially disrupting the metabolism of aquatic organisms. Some studies suggest that statin drugs (including rosuvastatin) may affect liver and hormonal function in aquatic organisms, particularly fish and amphibians. Even at low concentrations, they may interfere with growth and reproduction in some microorganisms or invertebrates (Sulaiman et al., 2015). Some pharmaceutical compounds, including statins, do not degrade easily in the environment, leading to their accumulation over time (ecoaccumulation). Wastewater treatment processes may not completely remove them, increasing the risk of their reaching drinking water sources or soil. Some studies suggest that drugs may interfere with nutrient cycling in soil by affecting beneficial bacteria (Rathinam & Santhana, 2021).

2. MATERIALS & METHODS:

3.2 Sample Collection:

Expired pharmaceutical raw materials will be collected from manufacturing facilities. Fungal species will be isolated from soil samples and various food sources.

DNA Amplification Diagnostic Kit

A diagnostic kit was utilized for DNA amplification, comprising the components listed in the table below. These materials were supplied by Promega (USA)(Allawi & Hmoshi, 2022). The kit includes the following main components:

Table (1): Main Components of the DNA Amplification Diagnostic Kit

Company Name	Material Name
Promega (USA)	Green master mix
Promega (USA)	Nuclease-free water
Promega (USA)	Agarose gel
Promega (USA)	DNA ladder 1KB
Sigma Company	DNA extraction kit

Nucleic Acid Amplification Using Polymerase Chain Reaction (PCR)

Standard PCR reactions were carried out, beginning with an initial denaturation step at 96°C for 2 minutes to separate the DNA strands, serving as a preparatory phase at the start of the PCR process. This was followed by 35 thermal cycles, with each cycle consisting of three specific steps, each performed at a designated temperature for a particular purpose:

Step 1: Denaturation at 96°C for 2 minutes to separate the double-stranded DNA.
Step 2: Annealing at 95°C for 30 seconds, allowing primers to bind to their complementary sequences on the DNA template.
Step 3: Extension at 45°C for 30 seconds to initiate primer elongation.
A final extension step was performed at 72°C for 1.5 minutes to complete the elongation process.

Table 2: Cycles and Durations for Each PCR Reaction

No.	Stage	Temperature	Time	Cycle Number
1	Initial denaturation	96	2 min.	1
2	Denaturation	95	30 sec.	35
3	Annealing	45	30 sec.	
4	Extension	72	1.30 min.	
5	Final Extension	72	5 min.	1

Steps of DNA Electrophoresis on Agarose Gel

1- Once the agarose had completely solidified, the rubber ends were carefully removed.
2- The gel tray containing the solidified agarose was placed into the electrophoresis tank, and the surface of the gel was gently covered with TAE buffer solution.
3- The gel was left briefly to ensure complete solidification, after which the comb was carefully removed from the gel.
4- The DNA marker (ladder) was prepared by mixing 10 µL of loading buffer with 4 µL of the DNA ladder solution. The mixture was thoroughly blended and then loaded into the designated side well of the agarose gel.
5- A volume of 12 µL from each PCR product sample was loaded into the adjacent wells next to the marker.
6- The electrophoresis device was then powered on after connecting the tank electrodes to a voltage supply set at 100 volts. DNA fragments were separated based on size, migrating from the negative to the positive electrode. The process lasted approximately one and a half hours, allowing clear visualization of the amplified DNA bands.
7- Visualization of the agarose gel was conducted using a UV transilluminator. After electrophoresis, the gel was carefully transferred to the UV imaging system, activated, and images of the gel were captured using a digital camera.

Gas Chromatography-Mass Spectrometry (GC-MS)

The analysis was performed using a model 7022A system integrated with an American-made mass spectrometer (model 5870E). A capillary column (35 m in length, 230 µm internal diameter, and 0.30 µm film thickness) was employed in the DPS_MS prototype, coated with a stationary phase composed of 10% biphenyl and 90% dimethylpolysiloxane. Helium gas of high purity (99.999%) served as the carrier gas, with the flow rate programmed at 70°C, column pressure at 96.1 kPa, and the injection port maintained at 270°C with a flow rate of 550 mL/min (Poole, 2012).

Testing the effectiveness of fungi in degrading pharmaceutical compounds.

The fungal spore suspension under study was prepared by taking a 5 mm diameter disc from a fresh, 3-day-old fungal culture grown on PDA medium. The fungal disc was placed in a tube containing 10 ml of liquid culture medium (Nutrient Broth). One ml of the spore suspension was then taken and added to another tube containing 9 ml of the same medium. The number of spores was estimated using a hemocytometer (CFU/ml 10^7*1.5). Then, the glass flasks were inoculated with three replicates containing the drug compounds at a concentration of 1000 µg/ml, where 1 ml of spore suspension for each fungus was added to 100 ml of the culture medium (1 ml: 100 ml), and incubated in a shaking incubator at a temperature of 28 ± 2°C, and 150 rpm for 10 days (Alrajeh et al., 2024).

RESULTS

Fungi were isolated from several sources. Several fungi were isolated from soil samples from various places in Mosul, with Rhizopus spp. having the largest percentage (18%) and Fusarium

spp. having the lowest (3%). Fusarium spp. was also recovered from mold-infected cabbage leaves, with the greatest percentage of 3% and the lowest of Caldosprum spp. A. niger, yeasts, and Penicillium spp. were recovered from mold-infected bananas at a rate of 18, 11, and 8, respectively. niger and Alternaria alternata were isolated from cauliflower at 16% each, as was A. flavus at 14%. Rice seeds sold in local markets and consumed by humans were separated, with A. flavus accounting for the highest percentage (14%), and A. fumigatus accounting for the lowest 8%.

Table 3: Isolation of fungal species from soil samples and different food sources.

Percentage %	Repeat it	Isolated fungi	Source
14	9	*A. flavus*	Soil
11	4	*Yeasts*	
16	9	*A. niger*	
3	2	*Fusarium* spp	
18	15	*Rhizopus* spp.	
16	12	*Alternaria alternate.*	
5	5	*A. ochraceus*	
8	4	*A.fumigatus*	
8	4	*Penicillium.spp*	
8	3	*Penicillium.spp*	Orange fruits
1	1	*Caldosprum* spp.	Cabbage leaves
3	1	*Fusarium* spp	
18	2	*A. niger*	Banana
8	1	*Penicillium* spp.	
11	2	Yeasts	
16	1	*Alternaria alternate.*	Kiwi
16	1	*Alternaria alternate.*	Watercress
16	1	*A.Niger*	Mandarin fruits
16	1	*A.Niger*	Cauliflower
16	1	*Alternaria alternate*	
14	1	*A. flavus*	
18	1	*Rhizopus spp.*	option
8	1	*A.fumigatus*	Tomato
18	2	*Rhizopus* spp.	
16	1	*A.Niger*	
11	3	Yeasts	Apples
14	2	*A. flavus*	Rice
8	2	*A.fumigatus*	
11	2	Yeasts	Bread
16	1	*Alternaria alternate*	
14	2	*A. flavus*	
8	1	*A.fumigatus*	
16	2	*A.Niger*	
100			Total isolates

After growing the fungi on PDA medium for seven days, the shape of the fungal colonies and the shapes of the conidiophores of each fungus were observed under a light microscope. Then, the fungi were identified according to the taxonomic keys of Pitt and Hocking (Figure 4).

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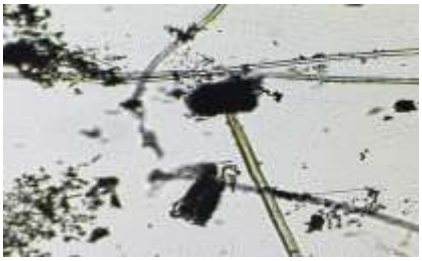
Rhizopus arrhizus fungus of soil

Rhizopus arrhizus fungus

Penicillium italicum spp., a

Penicillium italicum fungus of



Aspergillus niger rice sample

Aspergillus niger rice specimen

Figure 4: The shape of Rhizopus arrhizus, Penicillium italicum, and Aspergillus niger under microscope and morphologically

The PCR reaction products were submitted with the resulting package's primer, and the genes were read using Hitachi's 3130 Genetic Analyzer Device (Figures 5 & 6).

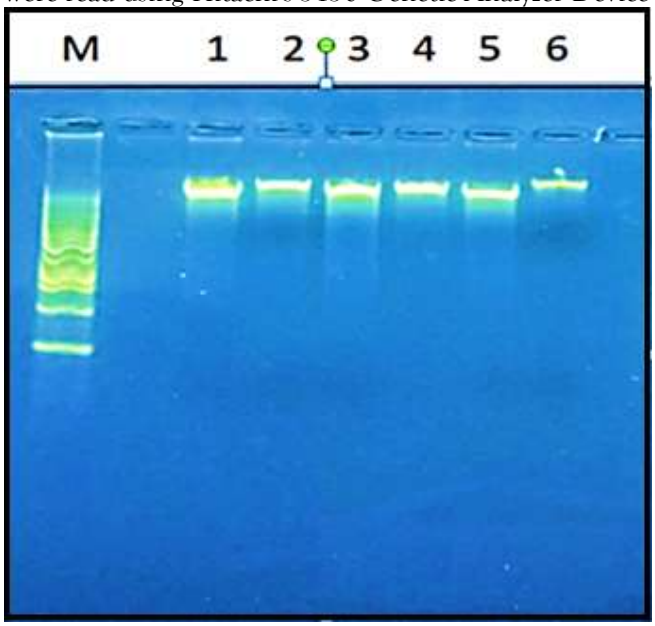


Figure 5: DNA Diagnosis

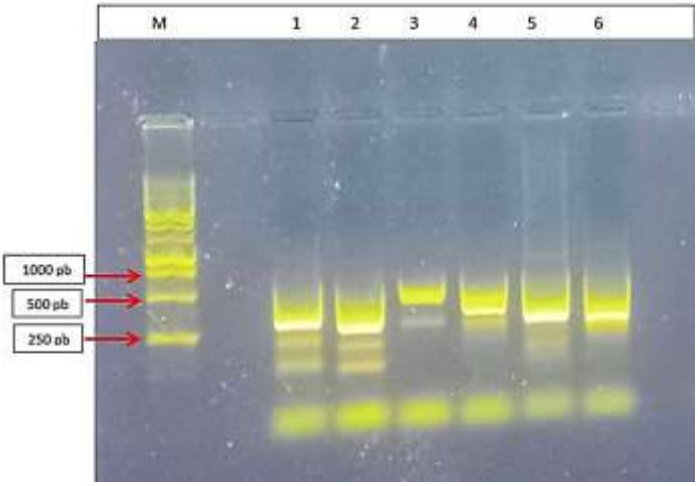


Figure 6: Nucleic Acid Amplification Using Polymerase Chain Reaction (PCR) Steps of DNA Electrophoresis on Agarose Gel

1.Rhizopus arrhizus fungus soil sample

1 aaggccgccc ttaccttagg gtttcctctg gggtaagtga ntgcttctac actgtgaaaa
61 tttgactgag agactcagac tggtcatggg tagacctatc tggggtttga tcgatgccac
121 tcctggtttc aggagcaccc ttcataataa acctagaaat tcagtattat aaagtttaat
181 aaaaaacaac ttttaacaat ggatctcttg gttctcgcat cgatgaagaa cgtagcaaag
241 tgcgataact agtgtgaatt gcatattcag tgaatcatcg agtctttgaa cgcagcttgc
301 actctatggt ttttctatag agtacgcctg cttcagtatc atcacaaacc cacacataac
361 atttgtttat gtggtaatgg gtcgcatcgc tgttttatta cagtgagcac ctaaaatgtg
421 tgtgattttc tgtctggctt gctaggcagg aatattacgc tggtctcagg atcttttct
481 ttggttcgcc caggaagtaa agtacaagag tataatccag caactttcaa actatgatct
541 gaagtcaggt gggattaccc gctgaactta agcatatcaa taagcggagg aaaaactgtc
601 ncctnnatct tgaagagcct nntntaattt cccntctgnn ngatgaaatc cgntacanat
661 cgnanaatnt ccgntggnan aatacatccc cctnggacaa aactaacgct canntgcnaa
721 ancccgntg gctacaggag tagatnccca gatantccac accgtannnn tntcnatttc
781 tnannanntn cnncttcatn natgggcctc cncgggcttc gtc//.

2.Penicillium italicum fungus of cabbage leaf sample

1 cgctgtttat ttaccacgtt gcttcggcgg gcccgcctta actggccgcc ggggggctca
61 cgccccggg cccgcgcccg ccgaagacac ccccgaactc tgcctgaaga ttgtcgtctg
121 agtgaaaata taaattattt aaaactttca acaacggatc tcttggttcc ggcatcgatg
181 aagaacgcag cgaaatgcga tacgtaatgt gaattgcaaa ttcagtgaat catcgagtct
241 ttgaacgcac attgcgcccc ctggtattcc ggggggcatg cctgtccgag cgtcattgct
301 gccctcaagc ccggcttgtg tgttgggccc cgtcctccga ttccgggggа cgggcccgaa
361 aggcagcggc ggcaccgcgt ccggtcctcg agcgtatggg gctttgtcac ccgctctgta
421 ggcccggccg gcgcttgccg atcaacccaa atttttatc caggttgacc tcggatcagg
481 tagggatacc cgctgaactt aagcatatca ataancggag gaaaatcggg cggtgagcga
541 gctcatgcgt cctctctagt ctactgngaa acccttcggc tcnccgaaga aatgcattgg
601 aaactcggga gacttgagtg catannacga cagtgnaact ccacgtgtag cgnaaaatgc
661 gtacananat gnnanaacac ctntggcnaa ngctgctgtc tgntctgcac tgacgctcac
721 gctcgaacgc acgggtagcg aacacgatta gatnccctgg gnantccang ncntanncga
781 cgatntctaa gntgttgnaa ncgttncnnn ccntccntgc tgcatctnnc nntctanatg
841 natcnncctg ngnnnnnccg nccncnngnt gtnannnnnt aattgattat.

3.Aspergillus niger rice sample

1 gggttctgnn cctnngtgtc nnncngcata cctgtatatt gnctcctgtg tgtaccgcgg
61 gaagagacga cgtaagatat cggggaagcg cctaagcact tcggaccccg gaacgccggn
121 nnnccaacac gaacaatgtc ggaacgcaag gggaatgaga nggttgaatg caatctgtca

181 aatctttcaa caatggatct cttggttccg gccctctgaa ctccgggggg caaggccgtt
241 tctaatgcga tttgctcatg tccgtgaatc atcgtggctt tgaccgccca ttgcgccccc
301 tggaattccg gggggcatgc tcgtccgagc gggattgaag ccctcaagcc cggcttgtgt
361 tttgttccgg ggcccctggc tcccgggggа cggggttgaa aggcaacgga gagcccgagc
421 tcgatcctcg ggcgaatggg gntttgactt atgctctgtc ggattggcan naacctgccg
481 acgttttcca accattcttt ccaggttgac ctcggatcag gtagggatac ccgctgaact
541 taagcatatc aataagcgga ggaacccctc cgctcccccc tcgattgtct tttgctatca
601 gcgaaatctt gngtgcaaan aanaaagtgn atctnctnct gtcctntnat atgcacaata
661 tantgagaaa cacagncncn gnnnnntctc tnttctgnta ctgacnctna tctgataagg
721 cgtatcatcn tnagtactac tnncntngtc ctcctcgcc//.

Gas Chromatography - Mass Spectrometry (GC-MS)

The GC-MS analysis of the soil sample infected with *Rhizopus arrhizus* revealed several compounds, with 6-Bromohexanoic acid, 10-Undecenyl ester being the most abundant at 59.20%, followed by Undecanoic acid at 28.64% and Borane, Butyldiethyl at 11.81%. A small amount (0.35%) of 2-Propenoic acid, Oxiranylmethyl ester was also detected.

Undecanoic acid is a naturally occurring fatty acid that biodegrades easily and poses minimal environmental risk. In contrast, brominated organic compounds such as 6-Bromohexanoic acid, 10-Undecenyl ester are more persistent in the environment, may accumulate in the food chain, and could be toxic to aquatic life and disrupt hormonal systems. Oxiranylmethyl ester, while generally biodegradable and of low toxicity, can still negatively affect aquatic organisms if present in high concentrations (Table 4 and Figure 7).

Table 4: Rhizopus arrhizus fungus soil sample (GC-MS).

Peak	R. Time	Area %	Name Compound
1	18.627	28.64	Undecanoic acid
2	19.792	0.35	2-Propenoic acid, Oxiranylmethyl ester
3	20.463	59.20	6-Bromohexanoic acid, 10-Undecenyl ester
4	20.635	11.81	Borane, Butyldiethyl

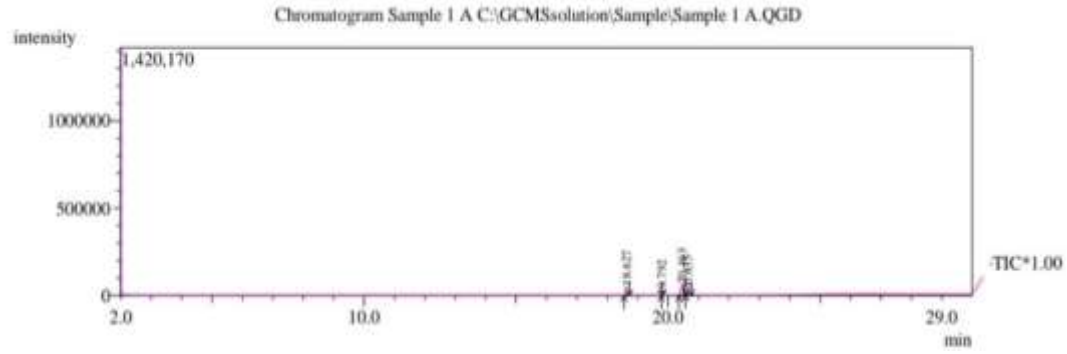


Figure 7: Gas Chromatography - Mass Spectrometry of Rhizopus arrhizus fungus soil samples.

The GC-MS analysis of the cabbage leaf sample infected with *Penicillium italicum* revealed several chemical compounds. The most abundant was Trichloroacetic acid, undec-10-enyl ester (57.77%), followed by 11-Bromoundecanoic acid (25.47%) and 2,2-Bioxirane (11.68%). The concentrations of these compounds differed from those reported in earlier studies.

Trichloroacetic acid is known to have phytotoxic effects, causing stress to terrestrial plants and contributing to environmental degradation such as desertification. It also shows short-term toxic effects on aquatic plants by affecting cellular pigmentation, although not considered a long-term threat at typical environmental concentrations. Additionally, Trichloroacetic acid has been detected in various environmental media such as surface water, rivers, fog, and clouds, indicating its potential impact on environmental sustainability (Table 5 and Figure 8).

Table 5: Penicillium italicum fungus of cabbage leaf sample (GC-MS).

Peak	R. Time	Area %	Name Compound
1	3.249	2.97	Methane, diazo
2	5.606	0.83	1-3- Cyclopentadiene, 1, 2-dimethyl
3	13.365	1.27	4- Fluoro-3 (trifluoromethyl) benzaldehyde
4	18.611	25.47	11-Bromoundecanoic acid
5	20.440	57.77	Trichloroacetic acid, undec-10-enyl ester
6	20.615	11.68	2, 2 - Bioxirane

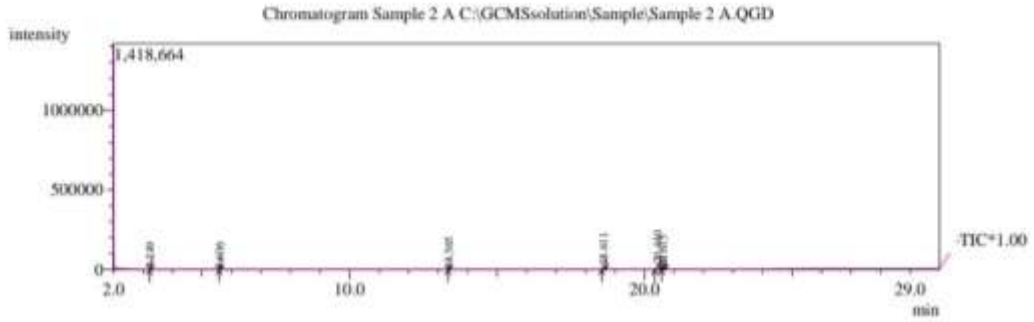


Figure 8: Gas Chromatography - Mass Spectrometry of Penicillium italicum fungus of cabbage leaf samples.

The GC-MS analysis of rice samples infected with Aspergillus niger revealed several chemical compounds. The most abundant was Carbonic acid (46.52%), followed by Undecanoic acid, 10-bromo (32.43%), and 2-Amino-1,3-Propanediol (18.23%). These results differ from previous studies, which reported lower or varying percentages for similar compounds (Table 6 and Figure 9).

Table 6: Aspergillus niger rice sample (GC-MS).

Peak	R. Time	Area %	Name Compound
1	3.230	2.82	Dimethyl sulfoxonium formylmethylide
2	18.579	32.43	Undecanoic acid, 10-bromo
3	20.413	46.52	Carbonic acid, 2,2,2-trichloroethyl cyclohexylmethyl ester
4	20.581	18.23	2-Amino-1, 3 -3-Propanediol

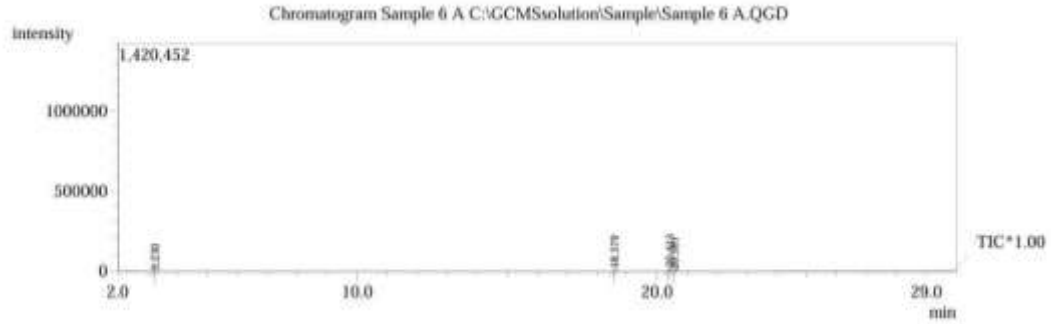


Figure 9: Gas Chromatography - Mass Spectrometry of Aspergillus niger rice samples.

Testing the effectiveness of fungi in degrading pharmaceutical compounds using a spectrophotometer

The results of Table 7 presented in Figure 10 of the spectrophotometer measurement of the Rhizopus arrhizus fungus showed that the drug with the highest degradation rate was Rovastatin, at 82.2%, followed by Clopidogrel, at 67.69 %, paracetamol, 55.82% and then, at 14.8089%, with all of them compared at Standard 0%.

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Table 7: Soil sample for drug degradation percentages of Rhizopus arrhizus fungi

Name Of Drag	repetition			ppm rate	Remaining %	Degradation%
	R1	R2	R3			
Paracetimol	596.258	408.918	320.416	441.864	44.18	55.82
Rosuvaststin	151.455	149.699	143.114	148.089	14.80	85.2
Clapidogrel	325.754	327.964	315.588	323.102	32.31	67.69
Standard	1000	1000	1000	1000	100	0

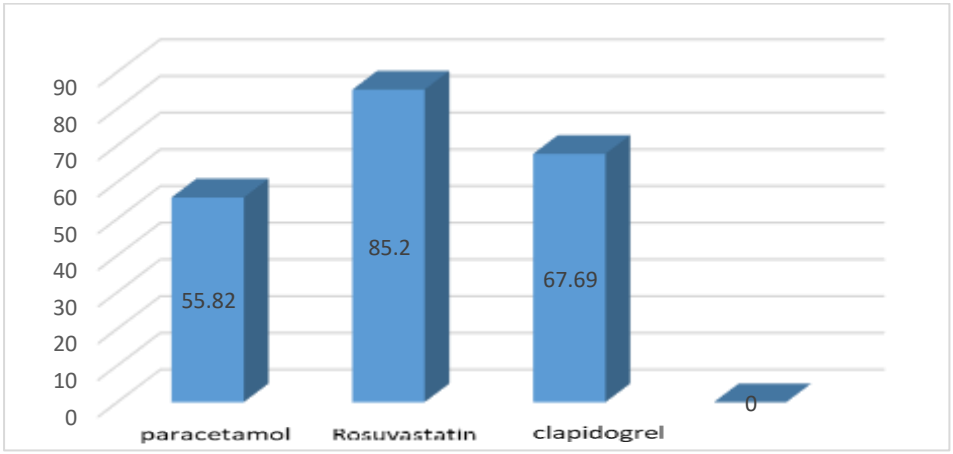

Figure 10: A graph showing the percentages of drug degradation

The results of Table 8 presented in Figure 11 of the measurement in the spectrophotometer of the Penicillium italicum fungus showed that the most expired drug with the highest percentage of destruction is Rosuvaststin, with a percentage of 84.11%, followed by Paracetamol with a percentage of 57.33%, then clapidogrel with a percentage of 53.08% when comparing them all at the standard 0%.

Table 8: Drug degradation by Penicillium italicum fungi

Name Of Drag	repetition			ppm rate	Remaining %	Degradation%
	R1	R2	R3			
Paracetimol	445.74	384.37	450.262	426.793	42.67	57.33
Rosuvaststin	155.406	152.333	169.015	158.918	15.89	84.11
Clapidogrel	346.97	569.296	491.504	469.256	46.92	53.08
Standard	1000	1000	1000	1000	100	0

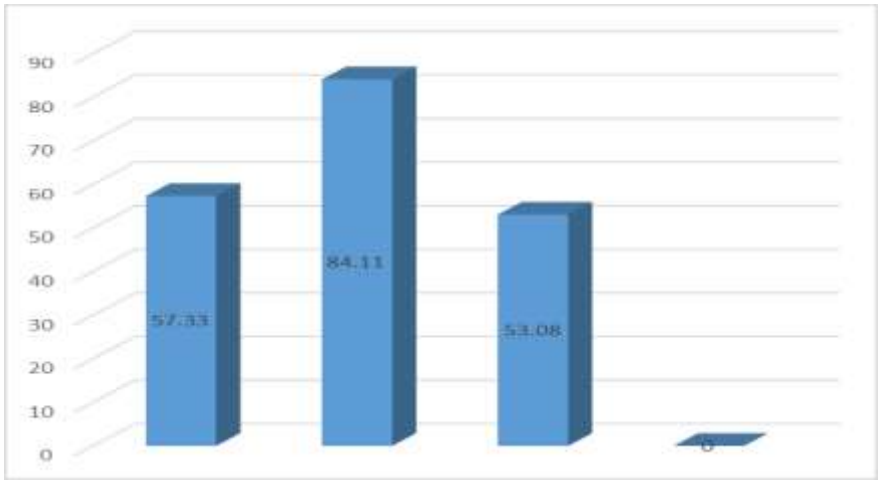

Figure 11: A graph showing the percentages of drug degradation

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The results of Table 9 presented in Figure 12 for the Aspergillus niger showed that the most expired drug with the highest destruction rate is Rosuvaststin, with a percentage of 82.59%, followed by clopidogrel with a percentage of 68.08 %, then paracetamol with a percentage of 58.73 % when comparing them all at the standard 0% (F &T).

Table 9: Percentage of drug degradation by Aspergillus niger

Name Of Drag	repetition			ppm rate	Remaining%	Degradation%
	R1	R2	R3			
Paracetimol	377.91	430.236	443.802	417.316	41.73	58.73
Rosuvaststin	211.598	103.165	207.647	174.136	17.41	82.59
Clapidogrel	358.462	298.792	300.56	319.271	31.92	68.08
Standard	1000	1000	1000	1000	100	0

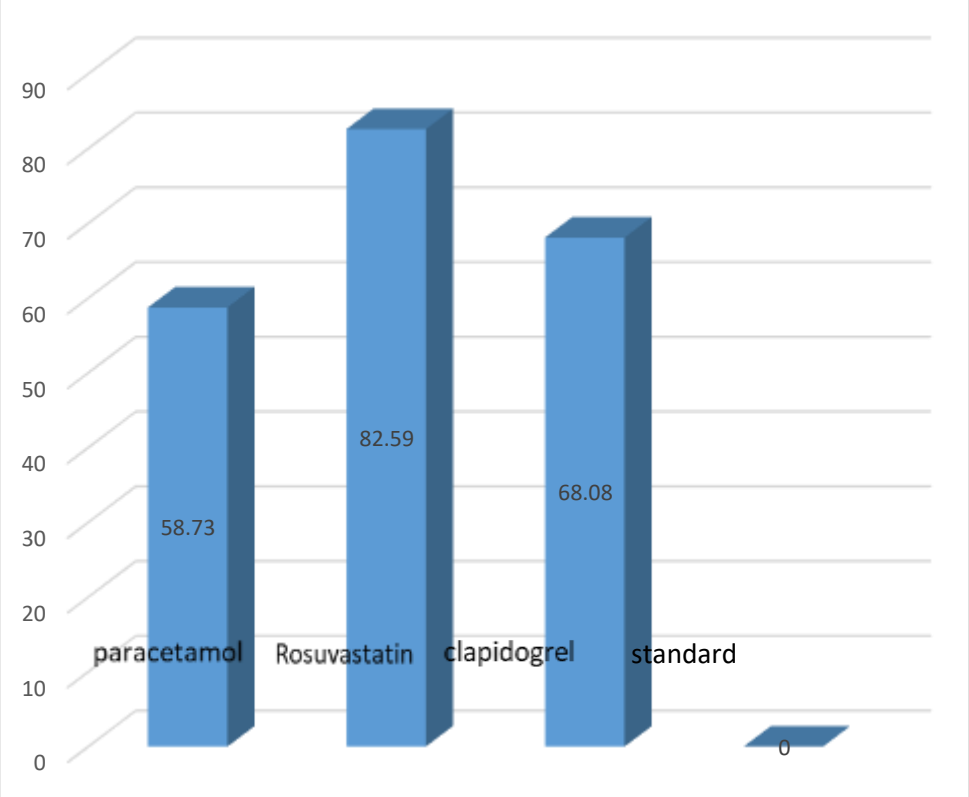

Figure 12: A graph showing the percentages of drug degradation

DISCUSSION

The highest degradation rate among the pharmaceutical compounds was for Rosuvastatin, reaching 85.2% for Rhizopus arrhizus, followed by Penicillium italicum and Aspergillus niger, with rates of 84.12% and 82.59%, respectively. The fungi under study also played an effective role in the degradation of clopidogrel, as the degradation rates of Aspergillus niger and Rhizopus arrhizus were close at 68.08 and 67.69%, respectively. The lowest degradation rate of paracetamol was observed among the three fungi compared to the previous two.

Secondary metabolites are organic compounds produced by organisms (especially plants, fungi, and bacteria) that are not directly necessary for survival or growth but perform important environmental and defensive functions. Some of them stimulate enzymatic activity in microorganisms, leading to the breakdown of complex organic compounds as part of biodegradation (Keller, 2019). Some organisms (bacteria, fungi) secrete secondary metabolites that help break down complex pharmaceutical compounds(Demain, 1999).

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