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Biodegradation of Difenoconazole Using Fungal-Bacterial Consortia

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Abstract. Pesticides are used all over the world in agricultural operations to reduce the damage of pests and to improve the productivity of agricultural crops. The excessive use of these chemical compounds has led to the deterioration of soil and increase the level of pollution of water and air resources. Moreover, the continuous use and non-target toxicity of pesticides has become a major concern to the agricultural ecosystem that directly hinders the productivity of agricultural products. Therefore, this study aimed to isolate and identify fungal and bacterial species with the ability to biodegrade fungicide via conducting preliminary tests to find out which of them have the ability to grow in a treated medium with a fungicide Difenoconazole, which belongs to the group of the pesticides Triazoles. The fungi and bacteria that succeeded in growing were as follow *Aspergillus flavus*, *Aspergillus ochraceus*, *Bacillus cereus* S1 and *Bacillus cereus* S2 were identified by extracting their genomic DNA and applying the primers ITS and 16s rRNA for both examined fungi and bacteria, respectively. The fungi were laboratory adapted to multiple concentrations reaching the highest concentration of 550 mgL^{-1}, while bacteria reached 1200 mgL^{-1} laboratory to break down the pesticide. The results of the combinations tested as the *B. cereus* S1 and *B. cereus* S2 were the fastest growing in the liquid medium PDB at the concentration of 325 mgL^{-1}. While the combination *A. flavus* and *B. cereus* S1 had the highest biodegrading ability at the same concentration, which led to the disappearance of the pesticide Difenoconazole turbidity in the liquid medium as a result of its consumption by fungal and bacterial isolates. As for the treatment of the pesticide in the solid PDA medium to know its effect on the growth of fungi and the formation of spores, the results revealed that fungal isolates increase their vegetative growth when the concentration increases, the production of fungal spores decreases, and indicates the ability of the fungi to use the pesticide as an energy source.

Keywords. Difenoconazole, Distribution Coefficient, First-order kinetic, Freundlich Isotherm, Langmuir Isotherm, Second-order kinetic.

1. Introduction

Chemical pesticides are among the most important factors in agricultural management due to their role in increasing crop production and improving crop quality by reducing plant disease pathogens and insect, weeds and other harmful organism damage [1]. The excessive use of pesticides leads to their

accumulation in the soil and increased saturation of agricultural soils with pesticides; this has increased global concern about food security worldwide [2].

Moreover, the accumulation of chemical pesticides is considered one of the most problematic issues, which in turn directly influences human health. Some studies have indicated that exposure to pesticides during pregnancy negatively affects fetal growth and, in some cases, may lead to fetal deformities [3] and [4].

[5], indicated that the consumption of chemical pesticides in the world was estimated as two million tons during the past four decades, and Europe constitutes 50% of global consumption, while the United States represents 25% and the rest of the world 25%.

Although pesticides are exposed to chemical processes, including oxidation or reduction, which are two of the most important factors applied in removing pesticides from the environment [6], as well as physical processes, such as photodegradation using ultraviolet radiation [7] and [8], biodegradation via microorganisms can play a vital and effective role in reducing the levels of pollutants in the environment [9].

To lessen the impact of these chemical contaminants, a number of environmental researchers and specialists have turned to bioremediation techniques that employ microbial or fungal agents [10]. The existence of these microbes and their ability to detoxify the environment are the reasons behind this. To lessen the buildup of chemical pesticides or to eliminate them from water or soil, biological treatment methods employ microorganisms like bacteria, fungus, or algae [11].

Soil bioremediation refers to the scientific process whereby microorganisms, such as bacteria, spontaneously decompose organic substances or wastes. When fungi are used for this purpose, it is called mycoremediation. These organisms scavenge for residual contaminants and pesticides and convert them into substances with minimal or no toxicity [12]. The removal of most contaminants using bioremediation technologies is both cost-effective and ecologically benign, as pointed out in [13].

Fungi are multi-celled, ever-present creatures that can adjust to their surroundings. Because of their breakdown or transformation capabilities, they find use in bioremediation procedures, particularly in locations polluted with heavy metals or pesticide residues [14]. Furthermore, they are capable of producing a diverse array of metabolites and enzymes that can degrade complex organic substances including PAHs, dyes, chemical pesticides, and explosives. In addition, fungi have the ability to biosorb, bioaccumulate, or biotransform heavy metals and radioactive isotopes [15,16].

Bioremediation has made extensive use of bacteria because of their fast growth rate and enzyme secretion capabilities, which aid in the detoxification process by converting organic contaminants into inorganic substances like carbon monoxide, water, and methane. references [17,18]. Bacteria have proven useful in breaking down pesticides and reducing their hazardous effects because of their capacity to bioremediate persistent chemical molecules. A few examples of this are the degradation of certain herbicides by specific bacterial strains and combinations [19-21].

DFZ is a pesticide with a chemical structure known as 1-[2-[4-(4-chlorophenoxy)-2-chlorophenyl]-4-methyl-1,3-dioxolan-2-ylmethyl]-1H-1,2 4-triazole. It is a systemic fungicide used to control plant diseases and has eradicating and preventive effects. It acts through penetration and works to prevent reproduction and inhibit growth [22] and [23]. This fungicide is characterized by its high chemical stability, chemical stability to high light, low biodegradability and significant ability to move easily in the environment [24].

Difenoconazole has a direct toxic effect on human health through the accumulation of Difenoconazole residues in the ecosystem and transfer through the food chain [25]. It enters the human body through three routes: skin, inhalation or oral inhalation [26]. Many toxic effects of this pesticide on humans have also been identified, including high binding to blood plasma proteins, potential hepatotoxicity, neurotoxicity and its disorders, birth defects, fetal toxicity, genetic mutations, and skin allergies, while stimulating the formation of benign and malignant tumors in humans [27]. The current study was conducted to identify a fungal-bacterial combination that accelerates the biodegradation of Difenoconazole in soil.

2. Materials and Methods

2.1. Samples Collection

Soil samples were collected from two locations in the northern areas of Basra Governorate in June 2023: from the Bahila area in the Al-Sadiq district close to the West Qurna-1 oil field and from a Date Palm orchard in the Al-Modaina district.

2.2. Isolation and Purification from Soil

The collected soil was mixed separately. Then, 1 gm was taken from each of the oil field soil and agricultural orchard soil samples. This was followed by a series of dilutions using sterile distilled water in 10 mL test tubes for each soil location. Next, 1 mL of the 10^{-3}, 10^{-4} and 10^{-5} dilutions were added to Petri dishes with potato dextrose agar (PDA) media supplemented with the antibiotic chloramphenicol at a concentration of 250 mgL^{-1} to prevent bacterial growth. In the second treatment, 1 mL of the same dilution was added to Petri dishes supplemented with PDA medium without antibiotics to allow the growth of bacteria. Three replicate PDA dishes were prepared for each dilution. The dishes were incubated in an incubator at 35 °C. After 24 hours, the bacteria were purified due to their rapid growth, while the fungi were purified after 3-7 days of isolation [28].

2.3. Examination of the Ability of Fungal and Bacterial Isolates to Grow in the Presence of the Pesticide Difenoconazole

An experiment was conducted to determine which fungal and bacterial isolates were able to grow in the presence of the fungicide Difenoconazole. For fungal isolates, three concentrations of the fungicide Difenoconazole were prepared on PDA media. The concentrations were 750, 500, and 250 mgL^{-1} . Then, a disk 0.5 cm in diameter of fungal growth was placed on each PDA dish. For bacteria, several concentrations of the fungicide Difenoconazole were prepared with N.A. culture media. The concentrations were 600, 700, 800, 900, 1000, 1100, and 1200 mgL^{-1} . One millilitre of the 24-hour-old bacterial suspension was added to the dishes, and the treatments were then transferred to an incubator for 24 hours at 28 °C. The growth of examined fungi and bacteria was followed through a visual comparison with control treatments.

2.4. Identification of Isolates

This process was carried out depending on morphological and microscopic features as follows:

2.4.1. Morphological and Microscopic Identification

Fungal isolates were grown on PDA media and incubated at 25 °C for 7 days. The phenotypic characteristics were examined by measuring radial fungal growth on the back of the dish using the ImageJ program. The following phenotypic characteristics were recorded: the colour of the colony and the pigments; the edges of the colony being curved or straight; fungal growth (rough, smooth, or leathery). The examination was conducted using a compound microscope to observe the phenotypic characteristics of the fungal colony and how it forms vegetative reproductive structures. Microscopic characteristics were according to [29]. The bacteria were grown on N.A culture media and incubated at 30 °C for 24 hours. The size, height, appearance, colour and edge of the bacterial colonies were examined. The bacterial isolates were stained using Gram stain. Microscopic detection was conducted to identify the shape and type of bacterial cells [30].

2.5. Toxicity Effect of Difenoconazole on the Growth of Bacterial Isolates

An experiment was conducted to determine the toxicity of pesticides on bacterial growth by counting the number of bacterial cells at ten different concentrations of the pesticide. Ten concentrations of Difenoconazole were prepared, as shown in Table (1), in sterile 200 ml glass beakers. Then, the N.A medium was added to the beakers under sterile conditions and shaken to homogenize the pesticide. The 10 ml test tubes containing 24-hour-old bacteria in N.B. medium were centrifuged at a speed of 4000 rpm for 10 minutes, the supernatant was removed. After that, 10 ml of nutrient saline solution was added to the tubes and shaken using a vortex device. A series of dilutions were made using the

saline solution that nourishes the bacteria, and 25 microliters of the fourth dilution was added to the dishes containing the NA medium treated with the pesticide. The dishes were incubated at 35 °C. Then, the number of bacterial cells was counted after 24 hours of incubation [31].

Table 1. Pesticide concentrations with the agricultural medium used in the experiment testing pesticide toxicity on bacterial growth.

Concentration mgL^{-1}	Treatment
2000	200 mL N.A + 1600 microliter pesticide
2500	200 mL N.A + 2000 microliters pesticide
3000	200 mL N.A + 2400 microliters pesticide
3500	200 mL N.A + 2800 microliter pesticide
4000	200 mL N.A + 3200 microliter pesticide
4500	200 mL N.A + 3600 microliter pesticide
5000	200 mL N.A + 4000 microliters pesticide
5500	200 mL N.A + 4400 microliter pesticide
6000	200 mL N.A + 4800 microliter pesticide
6500	200 mL N.A + 5200 microliter pesticide

2.6. Experiments on the Effects of Difenoconazole on the Radial Growth Toxicity of Fungal Isolates and Estimation of the Half-Inhibitory Concentration of Difenoconazole

The half-inhibitory concentration (I_{50}) and the effect of the pesticide on radial growth toxicity in fungi were estimated. Ten concentrations of the fungicide were prepared as shown in Table (2). The PDA medium was added to the bottles under sterile conditions, the medium treated with the pesticide was poured into Petri dishes and subsequently left to solidify. A disk 0.5 cm in diameter was taken from the fungi under study growing on PDA media and placed in the centre of the dish. The dishes were incubated at 25 °C. The half-inhibitory concentration was estimated when the growth of examined fungi in control treatment reached the edge of the dish. The fungal growth area of the treatments was also calculated after one month, and the inhibitory area was calculated using the ImageJ program [32].

Table 2. Pesticide concentrations used in the experiment to estimate the half-inhibitory concentration and radio toxicity of fungi.

Concentration mgL^{-1}	Treatment
100	200 mL PDA + 80 microliters pesticide
150	200 mL PDA + 120 microliters pesticide
200	200 mL PDA + 160 microliters pesticide
250	200 mL PDA + 200 microliters pesticide
300	200 mL PDA + 240 microliters pesticide
350	200 mL PDA + 280 microliters pesticide
400	200 mL PDA + 320 microliters pesticide
450	200 mL PDA + 360 microliters pesticide
500	200 mL PDA + 400 microliters pesticide
550	200 mL PDA + 450 microliters pesticide

2.7. Biochemical Tests

It included several tests:

2.7.1. Citrate Utilization Test

Part of the isolate growing in the N.A medium was transferred to the Simmon Citrate agar medium that was prepared. The dishes were kept in an incubator at 30 °C for 7 days, after which the colour of the medium turned blue, which is a positive result of the test [33].

2.7.2. Oxidase Test

This test was performed to determine the ability of bacteria to produce the oxidase enzyme. This was performed by placing a few drops of the oxidase reagent on the surface of a sterile glass slide and then adding to it a portion of a bacterial colony growing in N.A medium at the age of 24 hours, as this was

considered a colour change. Bacterial growth turning dark blue or purple was considered a positive test result [34].

2.7.3. Gelatin Hydrolysis Test

The activated bacterial culture was introduced into the tubes holding the gelatin medium by the stinging method. For seven days, the samples were kept in an incubator set at 30 °C. In a container with ice cubes, the tubes were angled and left at 4 °C for half an hour. Next, the tubes were checked to see if the medium could be observed changing from a solid to a liquid condition; this would suggest that the bacterial isolate could manufacture gelatinase [35].

2.7.4. Catalase Test

The activated bacterial culture was introduced into the tubes holding the gelatin medium by the stinging method. For seven days, the samples were kept in an incubator set at 30 °C. In a container with ice cubes, the tubes were angled and left at 4 °C for half an hour. Next, the tubes were checked to see if the medium could be observed changing from a solid to a liquid condition; this would suggest that the bacterial isolate could manufacture gelatinase [35].

2.7.5. Indole Test

A portion of the bacterial colony growing on the N.A culture medium was removed and placed in a test tube containing 10 mg of sterile peptone water medium. Then, they were mixed and incubated at 30 °C for 24 hours. After the incubation period, drops of Kovaks reagent were added to the test tube. The appearance of a red ring in the middle of the nutrient broth indicated the ability of the bacteria to produce indole and a positive result in the production of tryptophanase [33].

2.7.6. Lactose Fermentation Test

This test was performed to determine the ability of the different bacterial colonies that were grown to ferment the sugar lactose. This test was conducted using MacConky agar medium, which is a selective differential medium. Alternatively, the distinguish between bacteria that can ferment lactose sugar and those that cannot ferment lactose sugar can be checked, because the medium contains crystal violet, which inhibits gram-positive bacteria [37].

2.8. Generation of Selected Organisms with Difenoconazole Concentrations above the Specified I_{50}

This was performed by growing fungi and bacteria at concentrations above the I_{50} specified in section 8.2. Sterile glass bottles were prepared in an autoclave, to which 100 ml of PDB medium was added. Next, 130 microliters of pesticide was added to obtain a concentration of 325 mgL^{-1} . After that, 0.5mM of the bacterial suspension and a 0.5 cm diameter fungal colony were added individually or together for three replicates of each treatment. The glass bottles were then placed in a shaker incubator for 30 days at 28 °C and a speed of 150 cycles/min. The growth percentages of fungi and bacteria were calculated at wavelengths of 618 and 600, respectively, by using a spectrophotometer after 10, 15, 20, 25, and 30 days of experimentation. The measurement was carried out after taking 1 ml of each combination under study, and then the sample was centrifuged. After that, the filtrate was removed. One milligram of sterile distilled water was added to the liquid. The sample was shaken by using a vortex. Then, growth measurements were performed using a spectrophotometer that was calibrated with distilled water. A statistical analysis of the results was carried out to determine the best combination capable of decomposing the pesticide. The combination of laboratory organisms.

3. Results and Discussion

3.1. The Ability of Fungal and Bacterial Isolates to Grow in the Presence of Difenoconazole was Tested

The results of the isolation and purification of the soil from a palm orchard that used fungicides every agricultural season are shown. Moreover, in the soil near the West Qurna-2 oil field, where 4 different fungal isolates were identified based on morphological and microscopic characteristics, 8 bacterial

isolates were also isolated and purified as a final product of the isolation experiment, as shown in Table (3).

Table 3. Appearance and frequency of fungi resulting from the isolation process from soil.

No.	Isolate Fungi	Replaced
1	*Penicillium* spp.	16
2	*Rhizoctonia* sp.	11
3	*Aspergillus* spp.	7
4	*Cladosporium* sp.	1

After isolation and purification from the soil, this test was conducted to determine the ability of the fungal isolates to grow in media supplemented with Difenoconazole. In this study, the isolates were grown in media supplemented with the pesticide. The results of the experiment revealed that none of the fungal isolates were able to grow at a concentration of 750 mgL^{-1} , which is 6 times the concentration of the pesticide recommended by the manufacturer, while the concentrations of 250 and 500 mgL^{-1} were two and four times the fungicide recommended for use by the manufacturer, respectively. Two fungal isolates, namely, *Aspergillus flavus* (FAR) and *Aspergillus ochraceus* (FAN), were able to grow at concentrations of 250 and 500 mgL^{-1} , respectively. The ability of the bacterial isolates to grow in the presence of Difenoconazole was tested. In this test, medium N.A was prepared, and the pesticide was added to prepare concentrations of 600, 700, 800, 900, 1000, 1100, and 1200 mgL^{-1} . All the bacterial isolates were able to grow at the concentrations mentioned above. Only two bacterial isolates were selected for subsequent biological tests because of their ability to grow faster in the culture media treated with the pesticide than in the other bacterial isolates. The two isolates were A1 and B1.

3.2. Bacterial Isolate Identification

Two phenotypically different isolates grown in N.A medium were selected for diagnosis. The selected bacterial isolates that were able to grow very quickly in media supplemented with Difenoconazole, reaching 1200 mgL^{-1}, were subsequently identified as *Bacillus cereus* 1 (A1) and *Bacillus cereus* 2 (B1).

3.2.1. Morphological Tests

A study was conducted on the morphological characteristics of two bacterial isolates that were grown on the solid medium N.A The edge of the A1 isolate grown in N.A culture medium was irregular, and the colonies were relatively large, had a bright brown colour, and were raised above the surface. When the isolate was placed in a refrigerator, the colour of the colony changed to a shiny black colour. Isolate B1 was characterized by irregular edges and large, flat, white colonies. The colonies were rubbery and sticky in texture.

3.2.2. Microscopic Identification

Two bacterial isolates were activated with N.A solid media for 24 hours. The two bacterial isolates were examined after they were stained with Gram stain. The results of the microscopic examination of the cells of bacterial isolate A1 showed that they were gram-negative and had a single red rod-shaped appearance. Microscopic tests of the bacterial isolate B1 revealed that it was also negative for Gram stain and single short bacilli or in the form of short chains.

3.2.3. Biochemical Tests

Figure (1) and Table (4) show the results of the biochemical tests conducted for the two bacterial isolates. The gelatin liquefaction test was positive for bacterial isolate A1, which indicates the ability of the bacteria to produce gelatinase, and through this enzyme, the bacteria can decompose gelatin, starch and citrate. The mobility, oxidase and indole tests indicated the ability of the bacteria to produce tryptophanase. However, the lactose test was negative when the bacteria were grown in MacConkey agar; their growth appeared flat and smooth with regular edges and a pale color due to their inability to ferment lactose, and the catalase test was negative.

With regard to isolate B1, the results of its growth in MacConkey agar showed its ability to produce bright pink colonies with mucous consistency, which indicates the ability of the isolate to ferment the sugar lactose and its ability to produce catalase by forming air bubbles, which are considered evidence of the production of this enzyme. Catalase breaks down hydrogen peroxide (H_2O_2) and releases oxygen. Citrate decomposition, kinetics, oxidase and indole tests were positive, while gelatin decomposition and starch decomposition tests were negative.

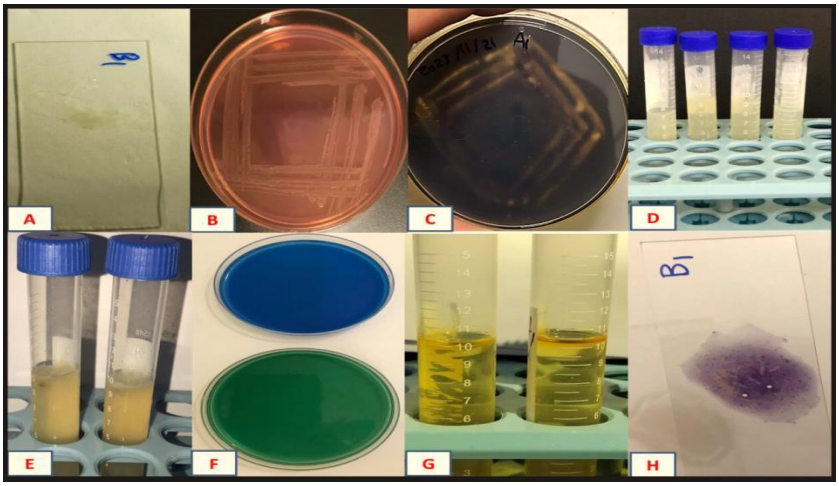

Figure 1. Some biochemical tests to diagnose bacterial isolates selected for analysis of the pesticide Difenoconazole: A: Catalase test B: Lactose fermentation test C: Starch hydrolysis test D: Gelatin liquefaction test E: Motility test F: Citrate hydrolysis test G: Indole test H: Oxidase test.

Table 4. Biochemical tests for the two bacterial isolates under study.

No.	Biochemical Test	A1	B1
1	Lactose fermentation test	-	+
2	Gram stain test	-	-
3	Starch hydrolysis test	+	-
4	Gelatin liquefaction test	+	-
5	Citrate hydrolysis test	+	+
6	Motility test	+	+
7	Indole test	+	+
8	Catalase test	-	+
9	Oxidase test	+	+

The result is positive (+), and the result is negative (-).

3.3. Estimating the Inhibitory and Lethal Concentrations of Difenoconazole in Bacteria

Figures 2 and 3 show the growth results of the bacterial isolates after 24 hours of aerobic incubation at 28 °C. Both *B. cereus* S1 and *B. cereus* S2 had several colonies that were able to grow in NA media supplemented with Difenoconazole in dishes containing several concentrations of the pesticide (2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000 or 6500 mgL^{-1}). The bacteria were able to grow in the culture medium, and the number of bacteria was calculated. Bacterial colonies in each dish, as both isolates were at the same level of growth. The number of bacterial colonies began to decrease with increasing concentrations of fungicide compared to the initial concentrations and the control treatment.

$L.S.D_{0.01}$= 38.79

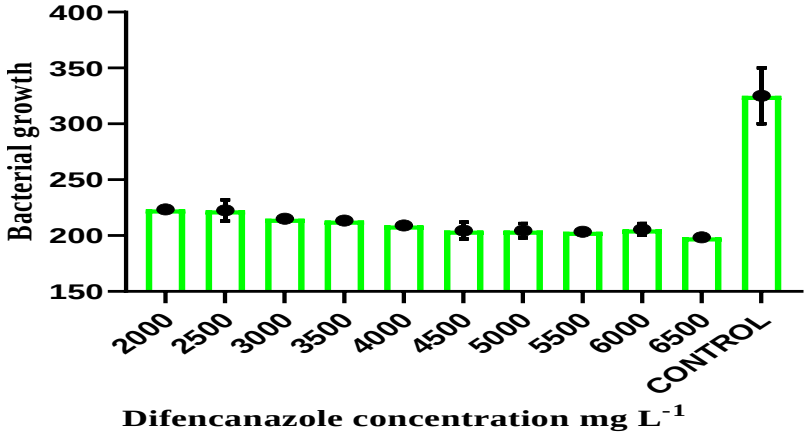

Figure 2. Effect of different Difencanazole concentrations on bacteria *Bacillus cereus* S1.

L.S.D$_{0.01}$= 48.68

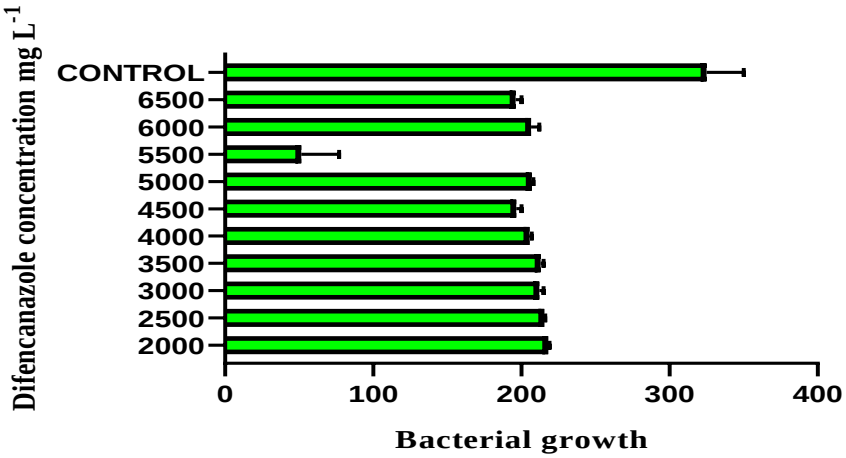

Figure 3. Effect of different Difencanazole concentrations on bacteria *Bacillus cereus* S2.

3.4. Identification of Bacterial Isolates Using the 16S rRNA Gene and Generation of the Genetic Tree
The results extracted DNA from the selected bacterial isolates were shown in Figure (4). The 16SRNA gene was selected for DNA amplification and species identification of examined bacteria, the efficiency of 16SRNA in bacterial diagnosis have been revealed in several studies with many bacteria such as *E. cloacae*, *P. aeruginosa*, and *P. putida* [38,39].
Tables (5 and 6) show the results of similarity of DNA sequences amplified via 16SRNA for the bacterial *B. cereus* A1 and B1 using BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi), the results showed a similarity percentage of 99% for both examined isolates, the A1 isolate of *B. cereus* was similar with the Chinese isolate under the gene accession number KR967395, while the isolate B1 was matched with Chinese isolate under the accession number MN733157. The phylogenetic analysis revealed BLAST search results as shown in phylogenetic trees (Figure 5 and 6).

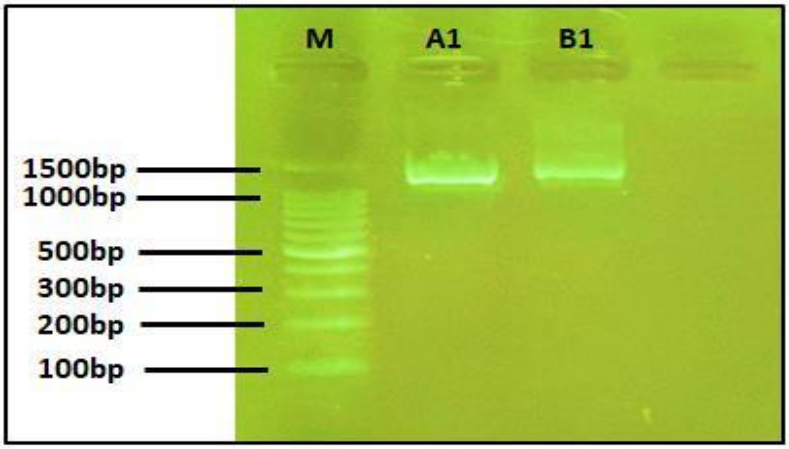

Figure 4. Electrophoresis results of DNA extracted from the bacterial isolates under study.

Table 5. Summary of all similarities between *B. cereus* S1 isolate in Basrah with NCBI – BLAST results.

Bacterial species	GenBank accession number	Query coverage	Percentage of sequence identity	Country
Bacillus cereus	KR967395	99	99	China
Bacillus cereus	MH552993	99	98	Bangladesh
Bacillus cereus	MH187637	99	98	China
Bacillus cereus	MH187623	99	98	China
Bacillus cereus	KX694390	99	98	U A E
Bacillus cereus	KU247981	99	98	India
Bacillus cereus	MN087808	99	98	China
Bacillus cereus	OQ726405	99	98	Iraq
Bacillus cereus	OQ726404	99	98	Iraq
Bacillus cereus	MZ895396	99	98	China
Bacillus cereus	ON045775	99	98	China
Bacillus cereus	MN087786	99	98	China

Table 6. Summary of all similarities between B. cereus S2 isolate in Basra with NCBI – BLAST results.

Bacterial species	GenBank accession number	Query coverage	Percentage of sequence identity	Country
Bacillus cereus	MN733157	99	99	China
Bacillus cereus	OQ756357	99	97	China
Bacillus cereus	HQ238536	99	96	China
Bacillus cereus	GQ280806	99	96	South Korea
Bacillus cereus	FJ390480	99	96	China
Bacillus cereus	KY750685	99	96	Indonesia
Bacillus cereus	KY115189	99	96	China
Bacillus cereus	OM432029	99	96	Pakistan
Bacillus cereus	KF376341	99	96	China
Bacillus cereus	MZ314087	99	96	Egypt
Bacillus cereus	MN733157	99	99	China
Bacillus cereus	OQ756357	99	97	China

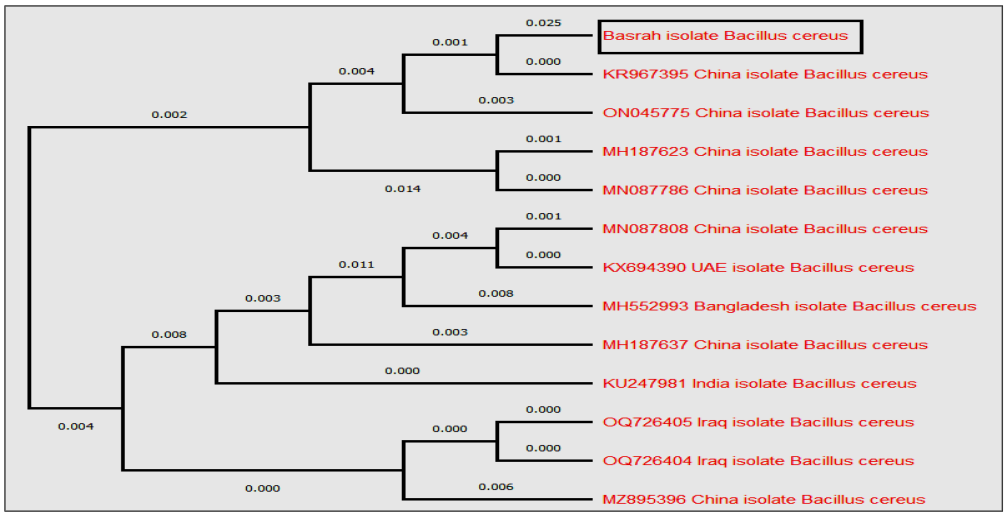

Figure 5. Phylogenetic tree constructed by the neighbour-joining method using 16S sequence for *Bacillus cereus* Basrah isolates with the nearest *B. cereus* published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/samplerecord/).

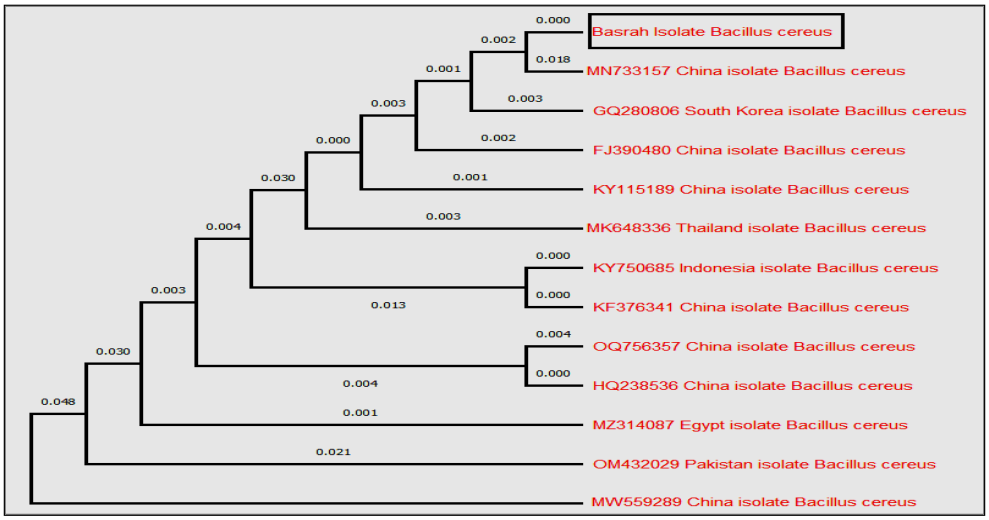

Figure 6. Phylogenetic tree constructed by the neighbour-joining method using 16S sequence for *Bacillus cereus* Basrah isolate with the nearest *B. cereus* published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/samplerecord/).

3.5. Fungal Isolate Identification

Aspergillus flavus (FAR) and *Aspergillus ochraceus* (FAN) have been selected because their ability to grow in Difenoconazole treatment at a concentration of 500 mgL^{-1} .

3.5.1. Morphological and Microscopic Identification

3.5.1.1. Aspergillus flavus

Aspergillus flavus (FAR) was selected for further identification, a pure colony of the fungus is distinguished with white to green colour, with the progress of age turned unto green to olive due to the production of conidial. The edge of the colony appears white, while the reverse growth is pale yellow. The growth diameter of the colonies reached 7 cm after 9 days. The phyllides are arranged in a single row (uniseriate). The length of the phyllides is 8-13 x 4-5 µM, and the phyllides. The conidia are large spherical with thick walls that are 5-12 µM in size, as shown in Figure (7).

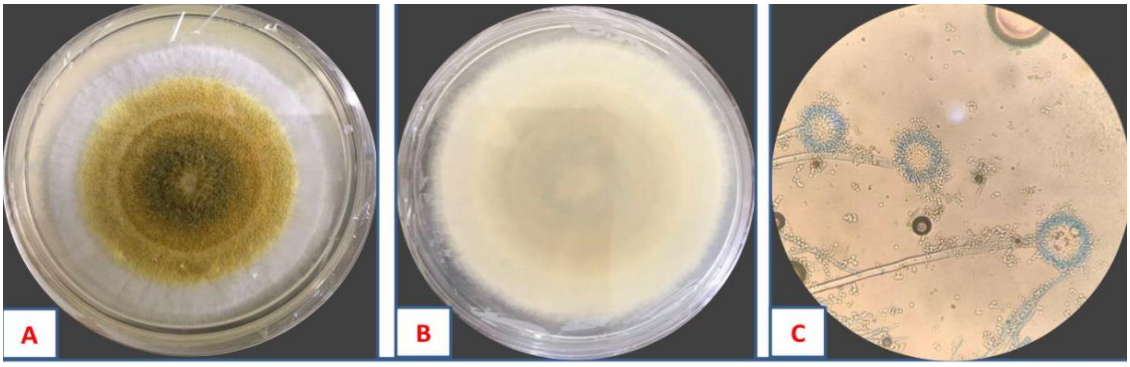

Figure 7. *A. flavus* fungal colony growing on PDA medium at a temperature of 25 °C. A: The front side of the plate B: The back side of the plate C: Microscopic structures of the fungus - conidia that carry conidia spores microscopically (40X magnification).

3.5.1.2. Aspergillus ochraceus

Aspergillus ochraceus (FAN) was isolated from the soil of a date palm orchard, which periodically uses pesticides to control palm pests. A pure colony of the fungus appeared yellow, with white colour edges. Reverse growth of the colony is pale yellow. Growth diameter reaches 6 cm after 9 days. The phyllids are arranged in biseriate rows 6-13 x 2-3 µM long. The conidia are smooth and spherical, their dimensions are 2-4 µM, and sometimes they are slightly rough, as shown in Figure (8).

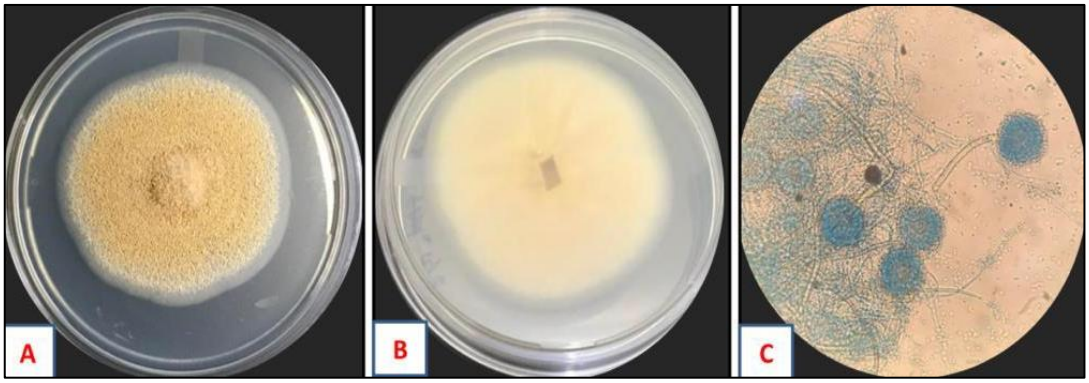

Figure 8. *A. ochraceus* fungal colony growing on PDA medium at a temperature of 25 °C. A: The front side of the plate B: The back side of the plate C: Microscopic structures of the fungus - conidia that carry conidia spores microscopically (40X magnification).

3.5.2. Genetic Diagnosis of Fungi Isolated from Soil and Phylogenetic Tree Construction

The fungal isolates (FAR and FAN), which could destroy and grow in culture media treated with Difenoconazole, were molecularly characterized, as shown in Figure (9). This was performed using the universal molecular code for the internal transcribed spacer (ITS) regions in polymerase chain reaction (PCR). The results showed that there was a high degree of matching between the fungal species mentioned above and between the fungal species registered in the NCBI GenBank. The standard sequences were recorded and deposited in the Global GenBank, and the diagnostic identification of these isolates was performed.

The results of the genetic analysis (Table 7, Figure 10) for the fungus *A. flavus* isolated from the soil in the present study indicated a genetic match rate of 98% with the standard sequences of the Indian isolate under the gene accession number KX898361 of the fungus *A. flavus*. The matching rates for this type were identical to those recorded by [40]. The results of the molecular diagnosis of the fungus *A. ochraceus* had a genetic identity of 98% with the standard sequences of the Indian isolate under the gene accession number OM952173 deposited in GenBank (NCBI) (Table 8, Figure 11). The identity rates for these fungi were identical to those reported by [41].

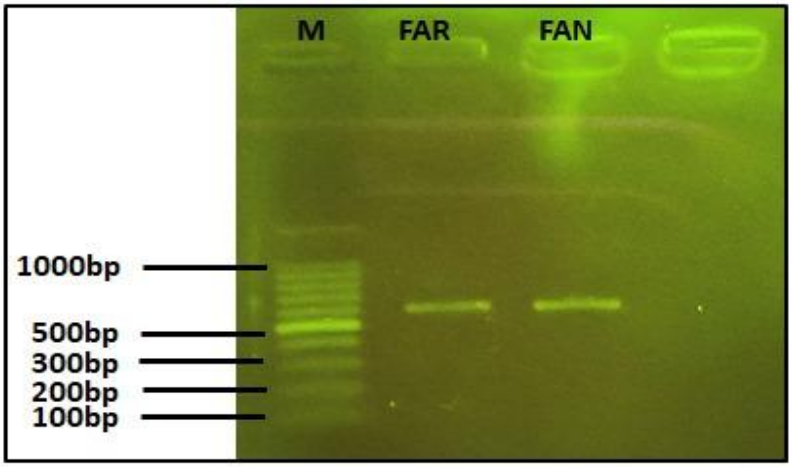

Figure 9. Electrophoresis results of DAN extracted from the fungal isolates under study.

Table 7. Summary of all similarities between the *Aspergillus flavus* isolate in Basra with NCBI – BLAST results.

Fungal species	GenBank accession number*	Query coverage	Percentage of sequence identity	Country
Aspergillus flavus	KX898361	98	98	India
Aspergillus flavus	MH664051	98	97	Pakistan
Aspergillus flavus	OP526902	98	97	Thailand
Aspergillus flavus	MW741887	98	97	Morocco
Aspergillus flavus	OR784318	97	97	Cameroon
Aspergillus flavus	MN031598	97	97	India
Aspergillus flavus	MH716402	97	97	Nigeria
Aspergillus flavus	KY234268	97	97	East Africa
Aspergillus flavus	KY234267	97	97	East Africa
Aspergillus flavus	OR784316	97	96	Iraq
Aspergillus flavus	OR192858	97	96	Iraq
Aspergillus flavus	KR905618	97	96	Turkey

Table 8. A summary of all similarities between the *Aspergillus ochraceus* isolate in Basra with the NCBI – BLAST results.

Fungal species	GenBank accession number*	Query coverage	Percentage of sequence identity	Country
Aspergillus ochraceus	OM952173	98	98	India
Aspergillus ochraceus	KU743890	98	97	Saudi Arabia
Aspergillus ochraceus	ON332163	98	97	China
Aspergillus	MZ452415	98	97	China

Fungal species	GenBank accession number*	Query coverage	Percentage of sequence identity	Country
ochraceus				
Aspergillus ochraceus	FJ427513	97	97	China
Aspergillus ochraceus	OP430536	97	97	China
Aspergillus ochraceus	MT378419	97	97	China
Aspergillus ochraceus	OP237086	97	97	China
Aspergillus ochraceus	ON248273	97	97	China
Aspergillus ochraceus	OM753115	97	96	China
Aspergillus ochraceus	MT529996	97	96	China
Aspergillus ochraceus	OP237391	97	96	China

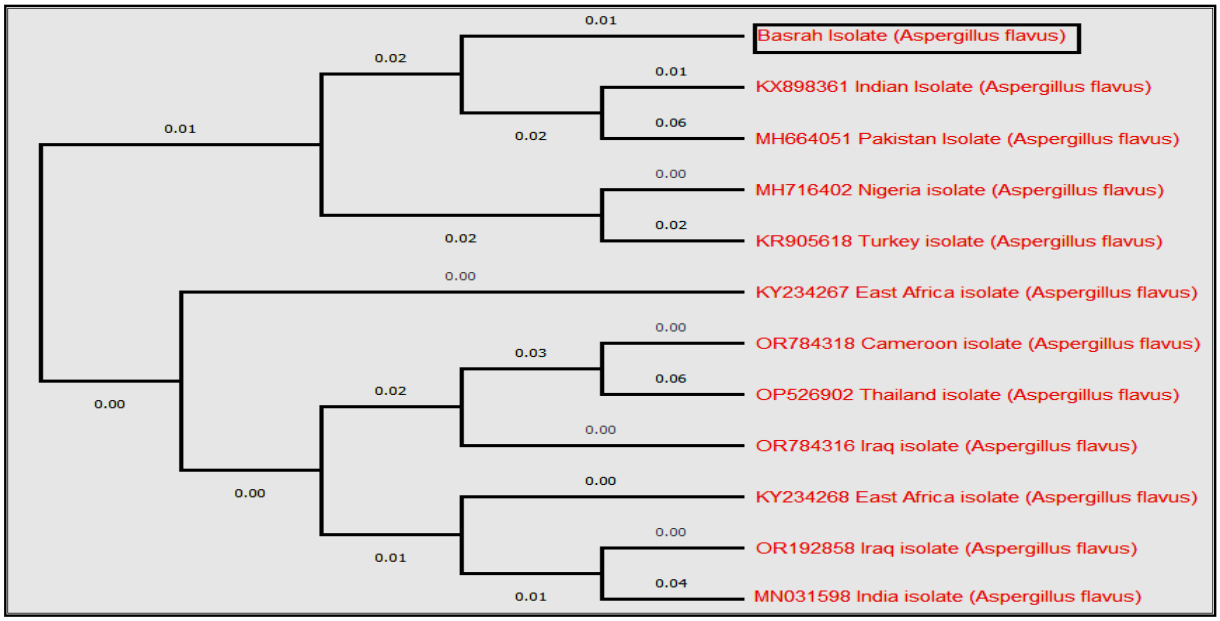

Figure 10. Phylogenetic tree constructed by the neighbour-joining method using ITS sequence for *Aspergillus flavus* Basrah isolates with the nearest *A. flavus* published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/samplerecord/).

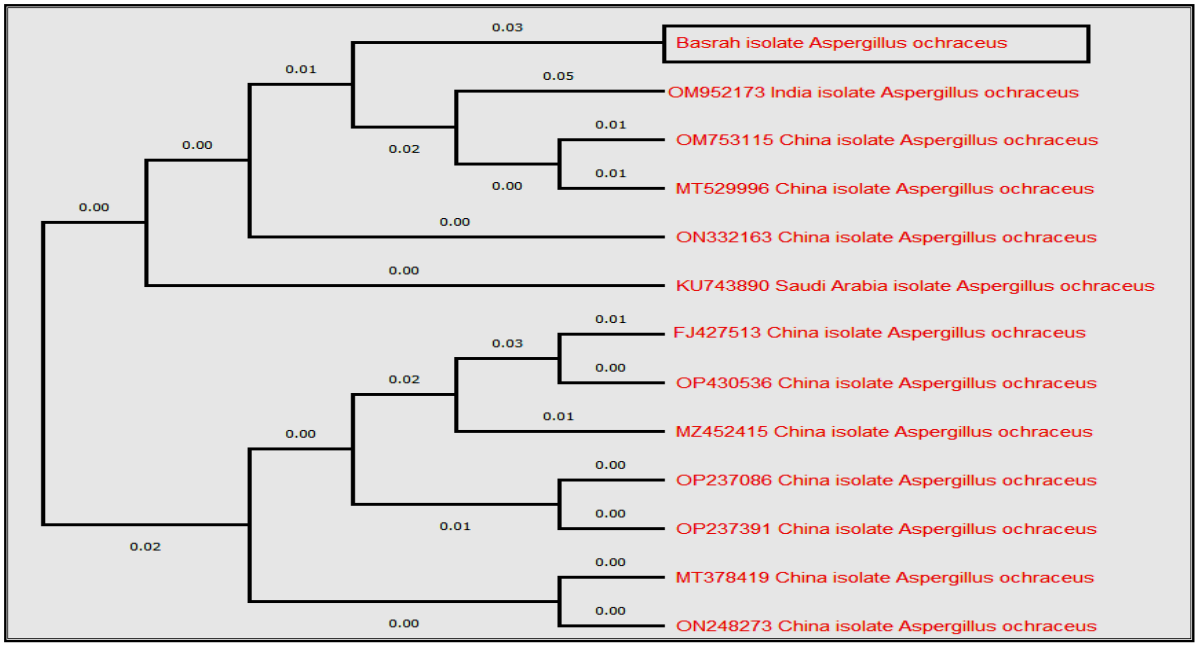

Figure 11. Phylogenetic tree constructed by the neighbour-joining method using ITS sequence for *Aspergillus ochraceus* Basrah isolates with the nearest *A. ochraceus* published in GenBank (https://www.ncbi.nlm.nih.gov/genbank/samplerecord/).

3.5.3. The Effect of the Toxicity of the Pesticide Difenoconazole on the Radiation Poisoning of Isolated Fungi was Studied, and the Half-Maximal Inhibitory Concentration was Estimated

The results of the statistical analysis in Table (9) indicate that there were significant differences in the results of radial growth after 30 days of incubation at 25 °C for the fungus *A. flavus* in the culture media treated with different concentrations of Difenoconazole compared to the non- treated control. The results measured in Petri dishes using the Image J. application showed that the highest radial growth rate of the fungus *A. flavus* was in the 100 mgL^{-1} treatment when the growth percentage in this treatment reached 84.9%. The lowest radial growth percentage of the fungus reached 29.9% at a concentration of 550 mgL^{-1} . Increasing concentrations of Difenoconazole affected the radial growth of the fungus *A. flavus*. However, the fungus was able to grow at high concentrations that reached five times the concentrations recommended by the pesticide manufacturer.

According to the pesticide treatments shown in Table (10) and Figure (12), the growth curve of the fungus in the culture media supplemented with Difenoconazole revealed that there was an effect and fluctuation in the radial growth of the fungus at a concentration of 325 mgL^{-1} , at which point the ability of the fungus to grow began to decrease.

The results of the statistical analysis are shown in Table (11) show the radial growth rates of the fungus *A. ochraceus* growing at 25 °C for 30 days in culture media supplemented with different concentrations of the fungicide Difenoconazole; radial growth was measured using ImageJ. There were significant differences in the ability of the fungus *A. ochraceous* to grow in media supplemented with different concentrations of the pesticide, where the highest growth rate was examined at a concentration of 350 mgL^{-1} and a growth percentage of 80.8%. While the lowest percentage of radial growth of the fungus occurred at a concentration of 300 mgL^{-1} which reached 31.1%.

The results of the pesticide treatments are shown in Table 10 and Figure 13. The growth curve of the fungus in media supplemented with Difenoconazole revealed that there was an effect and fluctuation in the radial growth of the fungus. Additionally, at a concentration of 325 mgL^{-1} , the ability of the fungi to grow began to decrease significantly.

Table 9. Effect of the toxicity of Difenoconazole on the percentage of vegetative growth and formation of fungal spores of the fungus *A. flavus*.

Concentrations mg L^{-1}	Sporulation Number	SD	Growth%	SD	Sporulation%	SD	Probity
100	106.3	6.1	84.9	10.6	10.6	10.6	3.96
150	95.6	11.8	75.4	2.8	2.8	2.8	4.25
200	88.6	12.7	69.7	0.6	0.6	0.6	4.48
250	90	24.3	69.2	9.5	9.5	9.5	4.48
300	80.6	7.5	63.9	4.3	4.3	4.3	4.64
350	71.6	3.8	57.3	7.9	7.9	7.9	4.8
400	85.3	10.7	67.2	1.7	1.7	1.7	4.53
450	62	7.1	49.1	4.4	4.4	4.4	5
500	47	1.4	37.6	4.8	4.8	4.8	5.31
550	37.3	3.2	29.9	5.2	5.2	5.2	5.52
CONTROL	127.3	19	-	-	-	-	-

Table 10. Effect of the toxicity of the pesticide Difenoconazole on the percentage of vegetative growth and fungal spore formation of the *A. ochraceus* isolate.

Concentrations mg L^{-1}	Sporulation Number	SD	Growth%	SD	Sporulation%	SD	Probity
100	43.6	4.7	42.4	5.6	10.6	5.6	5.18
150	55.3	6.5	53.6	6.8	2.8	6.8	4.9
200	51.3	8.3	49.2	3.3	0.6	3.3	5
250	69.6	4.1	67.7	6.6	9.5	6.6	4.53
300	32	2.1	31.1	4	4.3	4	5.47
350	83.3	6.5	80.8	7.1	7.9	7.1	4.12
400	58.3	8.3	57.4	13.2	1.7	13.2	4.8
450	49	8.2	47.4	8.1	4.4	8.1	5.05
500	36.3	2	35.3	3.2	4.8	3.2	5.36
550	66	11.4	63.2	6.7	5.2	6.7	4.64
CONTROL	103.6	10.2	-	-	-	-	-

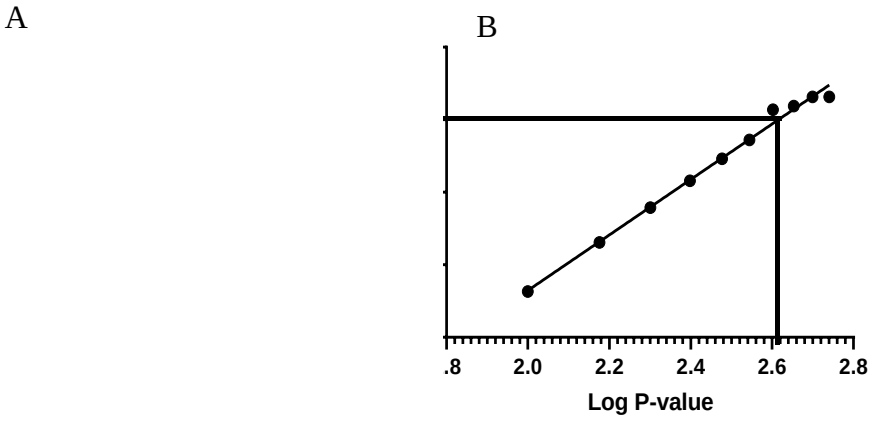

Figure 12. Toxicity assessment of Difenoconazole on FAR sporulation A) before correction B) after correction. I_{50}= 0.431mg L^{-1}.

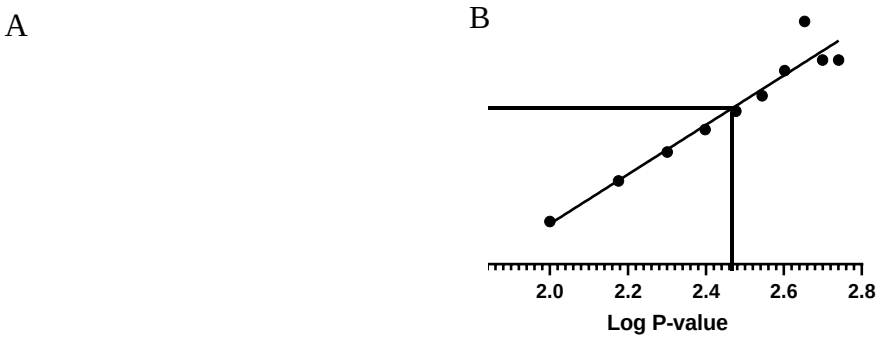

Figure 13. Toxicity assessment of Difenoconazole on FAN sporulation. A) before correction, B) after correction.

3.5.4. Effect of Difenoconazole Concentrations on the Fungal Sporulation

The results of the statistical analysis in Tables (9), (10) and in Figure (14) show edthat when Difenoconazole was added to the media, it led to a reduction in the fungal sporulation. This was demonstrated by the concentrations used in the radiation growth poisoning test, there were significant differences in the percentage of fungal sporulation. The highest percentage of sporulation was detected at a concentration of 350 mgL^{-1} for both the fungus *A. flavus* and *A. ochraceus* FAN. The number of fungal spores decreased with increasing concentrations of the pesticide. In the experiment, it was observed that the lower number of fungal spores in the treatments was, the greater the vegetative growth of the fungus, indicating that the fungus can grow in the culture media treated with the pesticide and that the fungus has dense mycelia. This dense vegetative growth was observed in the high-concentration treatment group, which indicates that the fungus could utilize Difenoconazole as an additive nutrient. Therefore, natural fungi do not form spores when nutrients are abundant but rather form spores when they are exposed to unsuitable environmental conditions. Through this experiment, it can be concluded that the fungal isolates were able to break down and analyse the pesticide.

These results are in a good accordance with those of some studies confirming that the fungicide Difenoconazole inhibits fungal spore growth, as the pesticide Difenoconazole effectively inhibits fungal spore growth and prevents fungal proliferation [23] and [22].

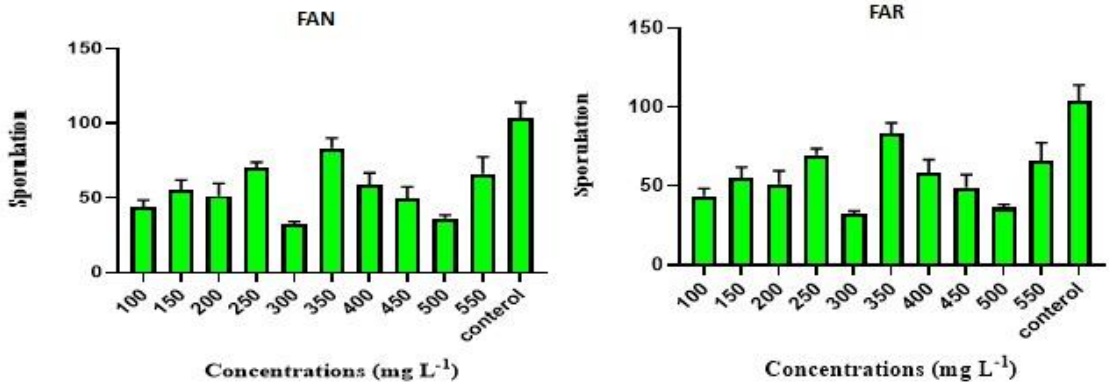

Figure 14. Effect of concentrations of the fungicide Difenoconazole on the spore average of the fungal isolate *A. flavus* (FAR) and *A. ochraceus* (FAN).

3.6. Combinations of Fungal and Bacterial Consortia with Culture Media Treated with Difenoconazole at Concentrations above the Half-Lethal Concentration (I_{50})

This experiment was conducted to determine the ability of several combinations of fungal and bacterial isolates to degrade Difenoconazole. The fungal and bacterial consortia were cultured together and individually in PDB media supplemented with Difenoconazole at a concentration of 325 mgL^{-1} . Figure (15) shows the growth curve of *B. cereus* strain S1 30 days after treatment of the bacterial nutrient medium with Difenoconazole. The strain needed 15 days to be able to secrete enzymes. The strain began the exponential phase after 15 days, and the bacterial growth curve increased from 0.2 on the day of treatment to 2.215 on the twentieth day. As a result, after consuming the nutritional substance represented by carbon atoms, which constitute an essential part of the chemical structure of the pesticide, the bacterial strain began to decline into the lag phase without going through the stationary phase, so the bacterial community reached 1.28 on the thirtieth day.

Figure (16) shows the growth of *B. cereus* S2 bacteria in the culture medium treated with the pesticide. It appeared from the growth curve that 15 days were needed for the bacterial strain S2 to begin consuming the nutrient. The growth curve began to fluctuate and then returned to increase after day 15, reaching a stasis phase after 20 days of treatment. The bacterial density then reached 1.768, and at this stage, the bacterial isolate had depleted the largest possible amount of the nutritional substance present in the medium, which is represented by the carbon atoms of Difenoconazole. After that, a decrease in the curve was observed, which indicates the stage of bacterial decomposition, during which the optical density reached 1.077. In their study,[42] reported the use of the bacteria *Aeromonas* sp. In the analysis of Difenoconazole, the activity of enzymes inside and outside the cell led to the transformation of the pesticide into three main compounds (DIF-TPs 406, DIF-TPs 216, and DIF-TPs 198). They also showed that it undergoes hydroxyl substitution, hydrolysis, cleavage of the ether bond between the benzene rings, and rearrangement. In addition, the byproducts of this pesticide were shown to be less toxic or even nontoxic to three typical aquatic organisms (fish, Daphnia and green algae) than the parent substance Difnoconazole.

When the two bacterial isolates were mixed in one treatment, as shown in Figure (17), the effect of their growth on each other was synergistic, as the two isolates depleted the nutrients in the medium. The growth curve began to increase from day 15 to day 20. After 20 days of treatment, the bacterial density reached a stagnation phase. The density of the bacterial community increased from 0.152 on day 15 to 1.43 on day 20. Then, a growth fluctuation occurred after 25 days of treatment, after which it returned to growth at an increasing rate, as the optical density reached 1.811 nanometres after 30 days of treatment. This was due to the complete dissolution and decomposition of the pesticide in the medium by bacterial enzymes as a result of tolerance and adaptation of the bacterial isolates in the medium, such that they can decompose the pesticide by secreting enzymes and using it as a food substance due to the structure of the pesticide containing carbon atoms.

In this field, [43], stated that the bioremediation process is carried out by enzymatic methods through metabolism, where further degradation of the pesticide into byproducts occurs via complete mineralization and metabolism. Degradation pathways include oxidation, reduction, hydrolysis, dehalogenation, dehydrogenation, condensation, decarboxylation and synthesis.

Figure (18) shows the growth curve of the fungus *A. flavus* in the presence of the pesticide. It was noted from the growth curve that the fungus was characterised by continuous and progressive growth. The growth of these fungi was characterised limited lag phase. This may be due to the ability of fungus to secrete enzymes that help them decompose pesticides. The exponential phase also began on day 15 and continued to increase in an upwards and continuous manner. The fungal community density increased from 0.03 on the first day to 0.719 on the 15th day. Growth continued to increase exponentially, reaching 1.180 after 30 days of treatment with the pesticide. These results confirm the ability of the fungus to grow and benefit from the pesticide as a carbon-rich substance.

Figure (19) did not differ much from Figure (18) in terms of the ability of the fungus *A. ochraceous* to benefit from the pesticide as a primarily carbon-rich source. The results indicated an increase in the fungal community number from 0.003 on the first day to 1.005 after 30 days. This increase undoubtedly confirmed the decomposition of the pesticide and the exploitation of its components in the growth of fungal organisms.

Figure (20) shows the growth curve of the combination of the two fungi *A. flavus* and *A. ochraceus*. The figure shows the ability of both fungi to grow by influencing each other. The fungal community density increased from 0.05 on day 15 to 1.71 after 25 days. The fungal community numbers decreased to 1.41 after 30 days of treatment.

Figure (21) shows the growth of the combination of the fungus *A. flavus* and strain S1. It was evident that there was harmony in growth. In addition, this was confirmed by an increase in the growth curve from 0.07 to 2.24. This represents a significant increase in the fungal and bacterial communities. This may be due to their role in secreting enzymes that break the pesticide's bonds into small molecules, the components of which could be used.

Figure (22) shows that the growth curve was closer to the ideal conditions for bacterial growth. These results reflected the ability of the fungus *A. flavus* and the bacterium S2 to grow together and that each helped the other by secreting enzymes. The growth rate in the exponential phase started at 0.4 on day 15 and increased to 1.61 after 20 days. Then, the curve returned to normal growth in the stationary phase.

Figure (23) also shows the ability of both the fungus *A. ochraceus* and strain S1 to grow together and benefit from the presence of the pesticide primarily as an energy source. The lag phase disappeared, and the combination rapidly increased. The density of both the fungal and bacterial communities increased from 0.07 to 2.02 after 30 days. It was believed that the synergistic effect between *A. ochraceous* and S1 played a role in adapting the combination to grow on the pesticide and benefit quickly.

Figure (24) shows that there was overlapping between the fungi *A. ochraceus* and S2 in terms of rapid growth on the pesticide. The growth of the mixture increased from 0.07 to 2.025 after 30 days. It was also noted that the lag phase disappeared as a result of the two isolates adapting and agreeing to grow in the medium poisoned with the pesticide.

Figure (25) shows the significant overlapping in growth between the fungal isolates of *A. flavus* and *A. ochraceus* and the bacterial strain S1. The first phase of growth disappeared, indicating complete adaptation between the isolates, which led to an increase in the density of the microbial community from 0.05 after inoculation of the medium to 0.83 on day 15. After that, growth increased exponentially to 1.90 on day 20. The combination reached this level of growth, indicating the efficiency of the isolates in exploiting and utilizing the pesticide present in the medium as an energy source.

It was also noted here that there was a similarity with the previous results, as the first growth phase in Figure (26) disappeared, and growth increased from 0.06 to 0.71 on day 15 until growth returned exponentially to 1.8 after 20 days.

A significant compatibility was also observed in the growth of the fungus *A. ochraceous* and the bacterial strains S1 and S2. The increase in growth once the medium was inoculated with the fungal isolate and the two bacterial strains was clear evidence of the state of adaptation of these organisms together in the medium poisoned with the pesticide. The growth increased from 0.08 to 0.8 on day 15, returning to an exponential increase to 1.8 on day 20. Similarly, the combination of the fungus *A. flavus* with strains S1 and S2 increased the growth of the combination over 15 days. Then, it increased to 1.8 on day 20.

The growth results shown in Figure (29) indicate that the adaptation period lasted 10 days, after which the two fungi, *A. ochraceus* and *A. flavus*, strain S1, and strain S2, began to grow together. The growth curve increased from 0.4 on day 10 to 1.5 after 30 days. Although the amount was low compared to the rest of the growth curves, this indicated the possibility of using this combination in treating soil contaminated with Difenoconazole due to the steady and continuous growth of this combination.

Figure (30) shows that individual isolates and combinations were capable of growing in the presence of the pesticide. It was noted that the highest growth rate occurred when using the combination of bacterial strains S1 and S2, as the growth rate reached 0.45/day, followed by the use of strain S1 alone, amounting to 0.39/day, and strain S2, 0.3/day. The combination of strain S2 and the fungus *A. flavus* reached 0.28/day.

The combination of the two fungi *A. flavus* and *A. ochraceus* as well as the two strains of bacteria *B. cereus* S1 and *B. cereus* S2 achieved a growth rate of 0.21/day. The compatibility between these

isolates can indicate the efficiency of their use in the field applications as a result of their ability to secrete various enzymes. This led to effective utilization of the pesticide and achieved a high growth rate per day.

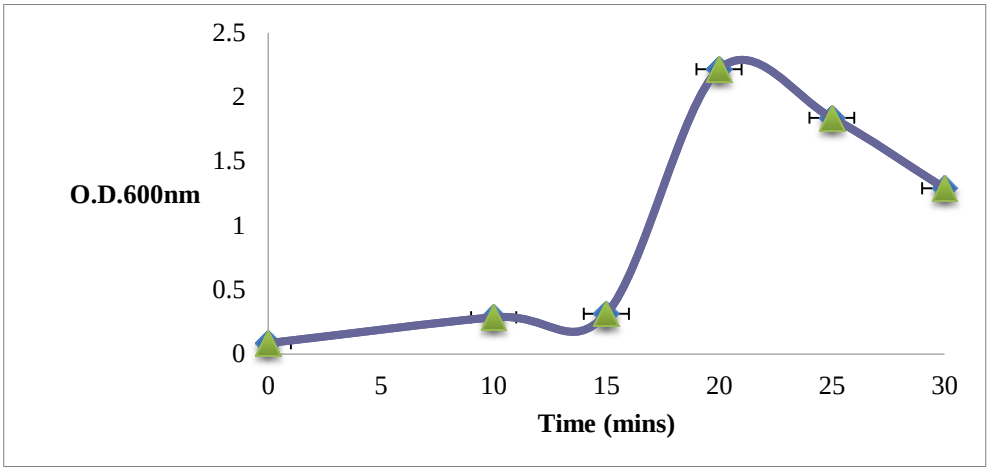

Figure 15. Growth Curve of bacteria isolate *Bacillus cereus* S1.

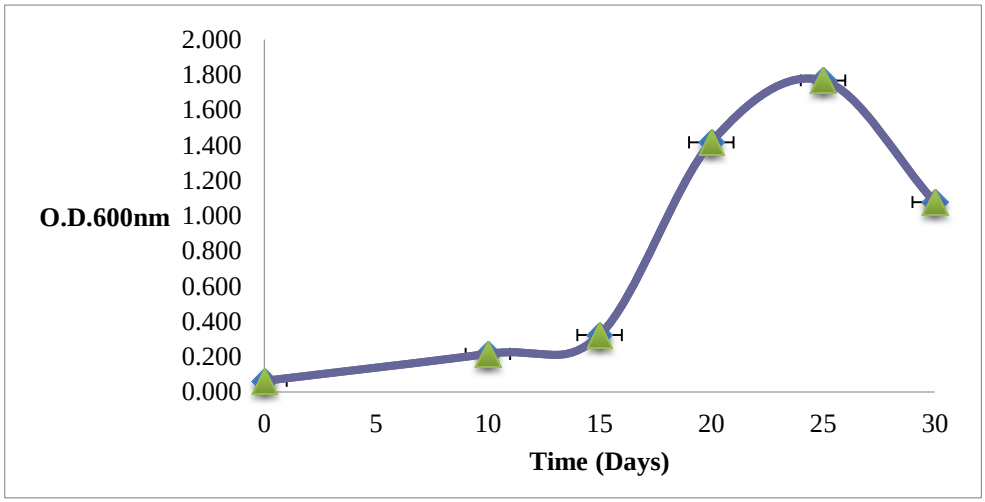

Figure 16. Growth Curve of bacteria isolate *Bacillus cereus* S2.

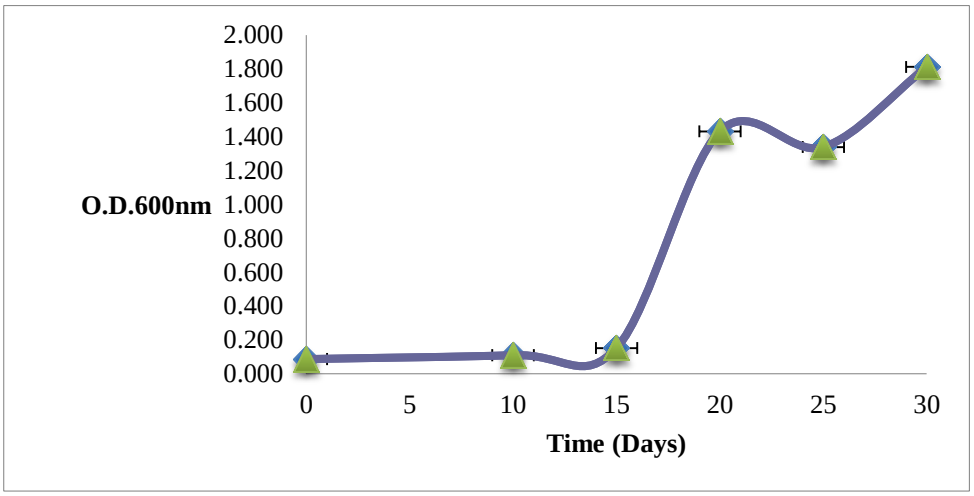

Figure 17. Growth Curve of *Bacillus cereus* S1 + *B. cereus* S2.

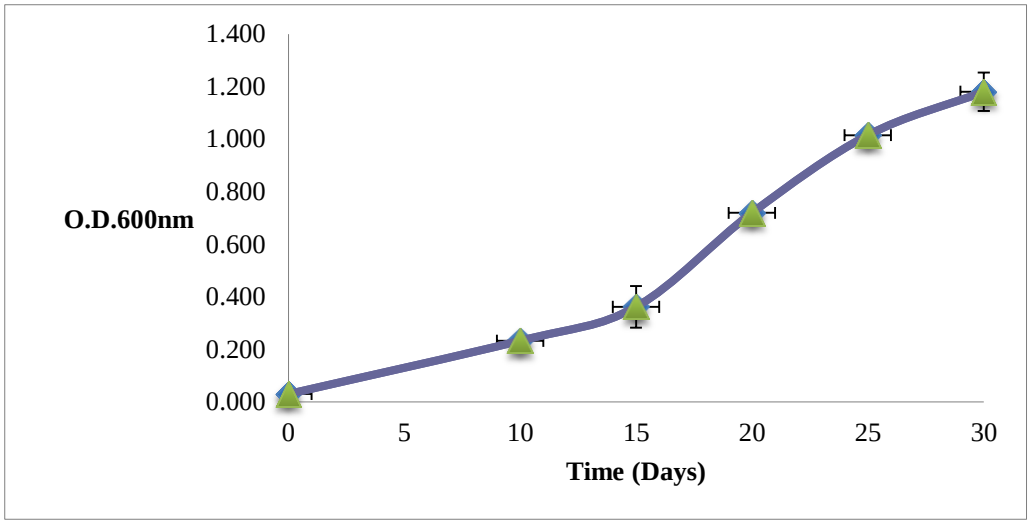

Figure 18. Growth Curve of fungi *Aspergillus flavus*.

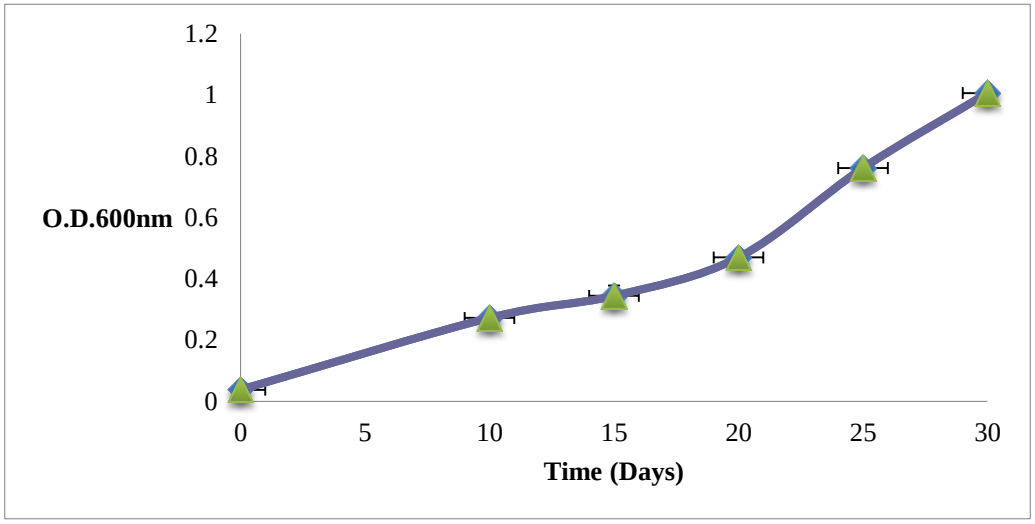

Figure 19. Growth Curve of fungi *Aspergillus ochraceus*.

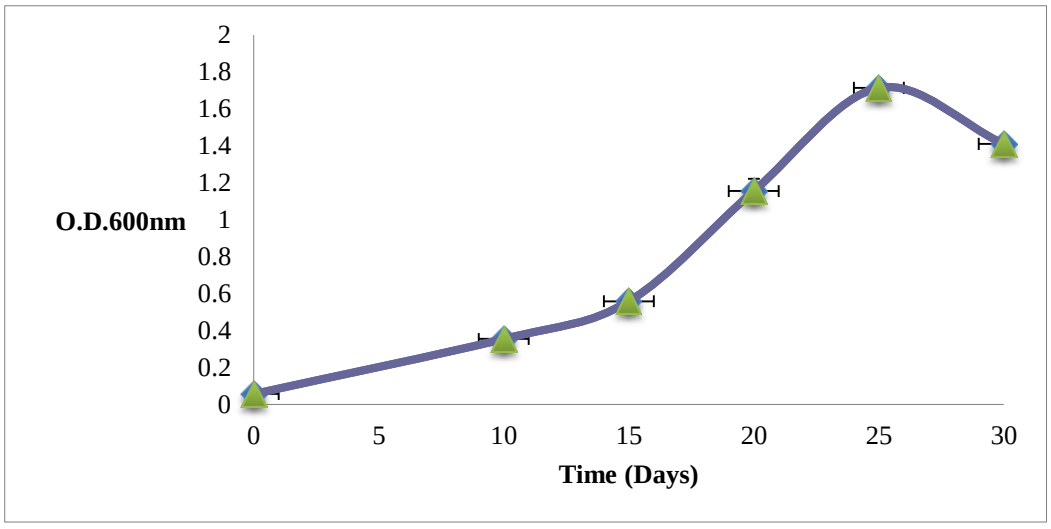

Figure 20. Growth Curve of fungus *Aspergillus flavus* and *Aspergillus ochraceus*.

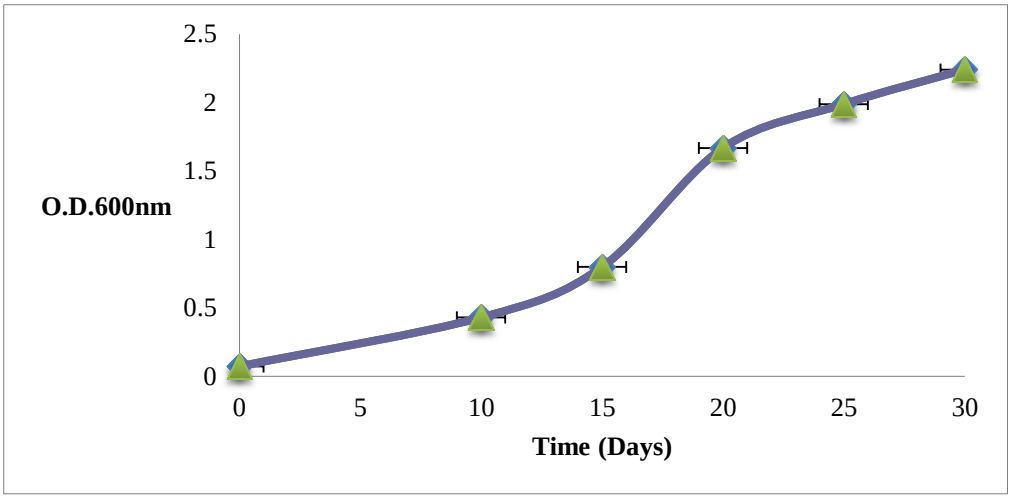

Figure 21. Growth Curve of *Aspergillus flavus* and *Bacillus cereus* S1.

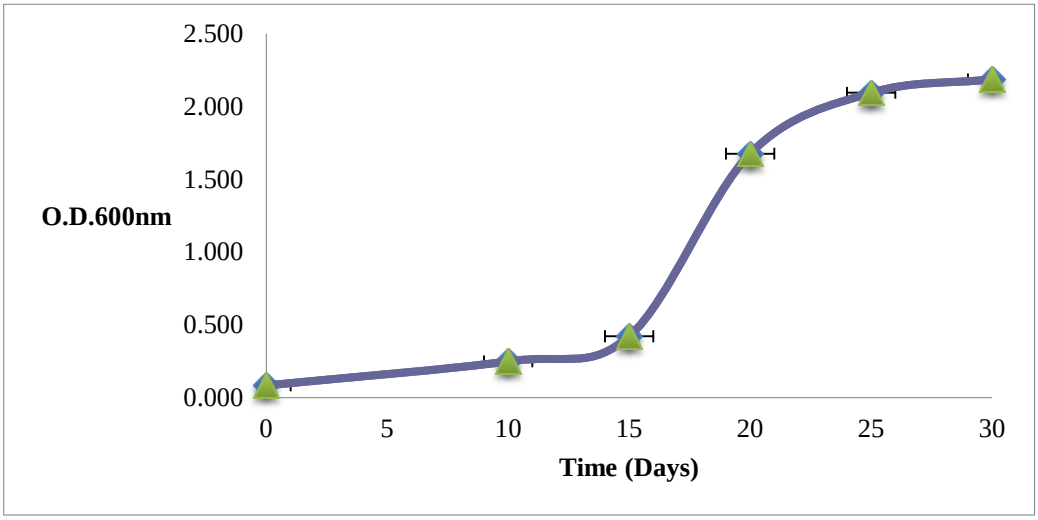

Figure 22. Growth Curve of *Aspergillus flavus* and *Bacillus cereus* S2.

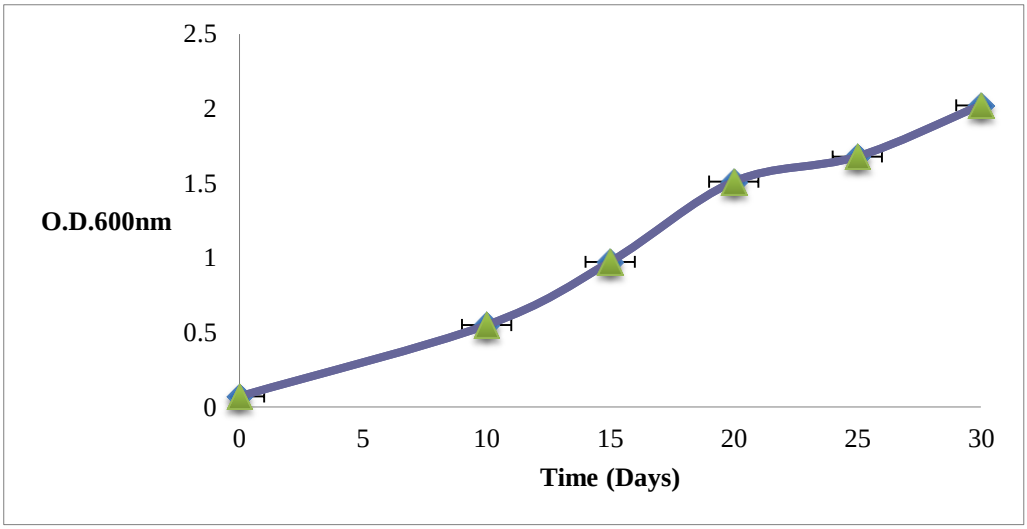

Figure 23. Growth Curve of *Aspergillus ochraceus* and *Bacillus cereus* S1.

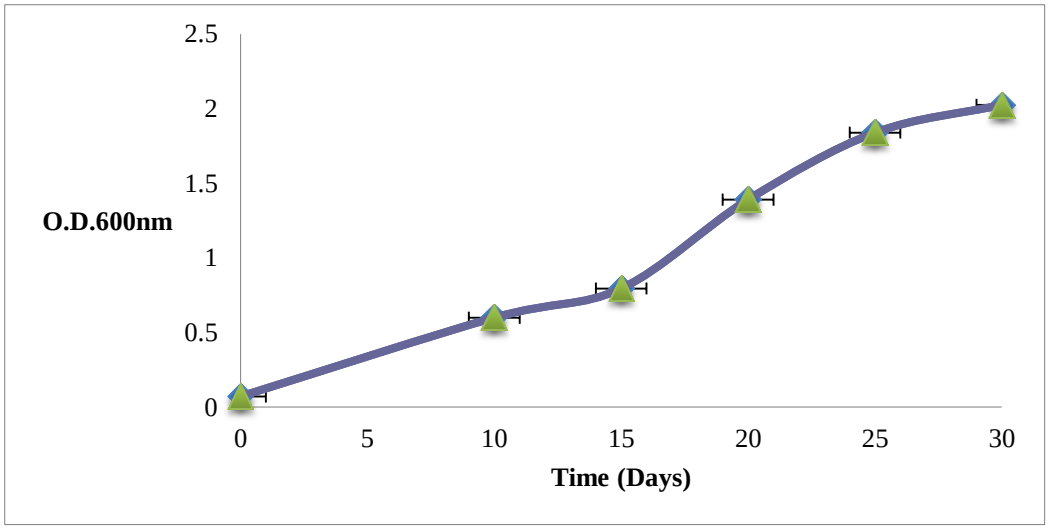

Figure 24. Growth Curve of *Aspergillus ochraceus* and *Bacillus cereus* S2.

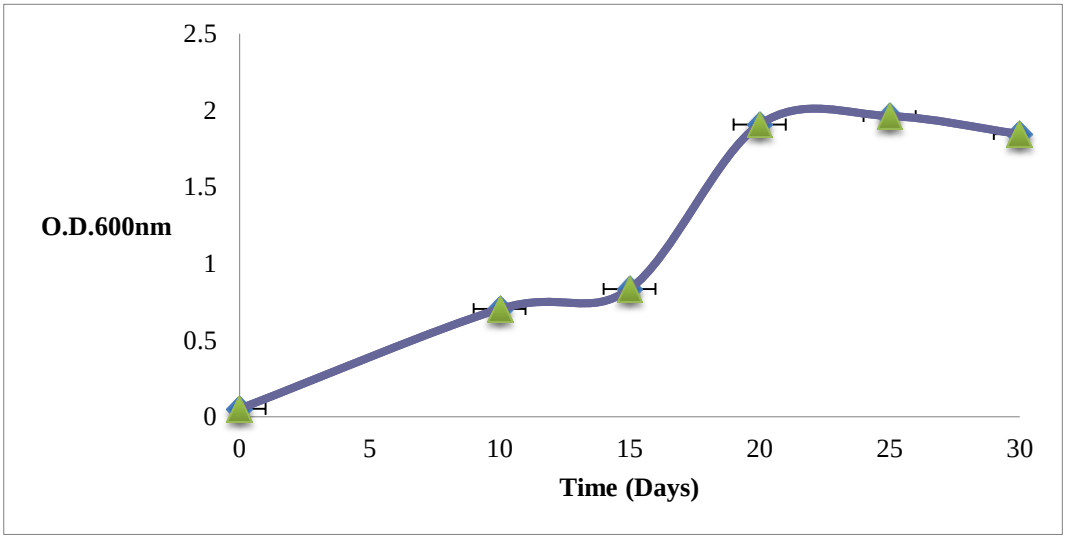

Figure 25. Growth Curve of Aspergillus flavus + A. ochraceus and Bacteria cereus S1.

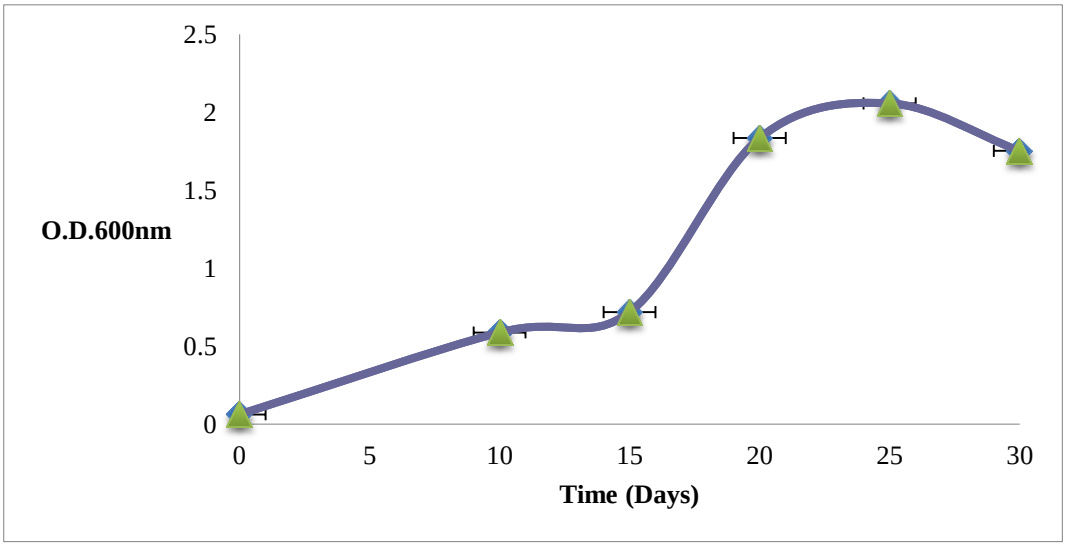

Figure 26. Growth Curve of Aspergillus flavus + A. ochraceus and Bacillus cereus S2.

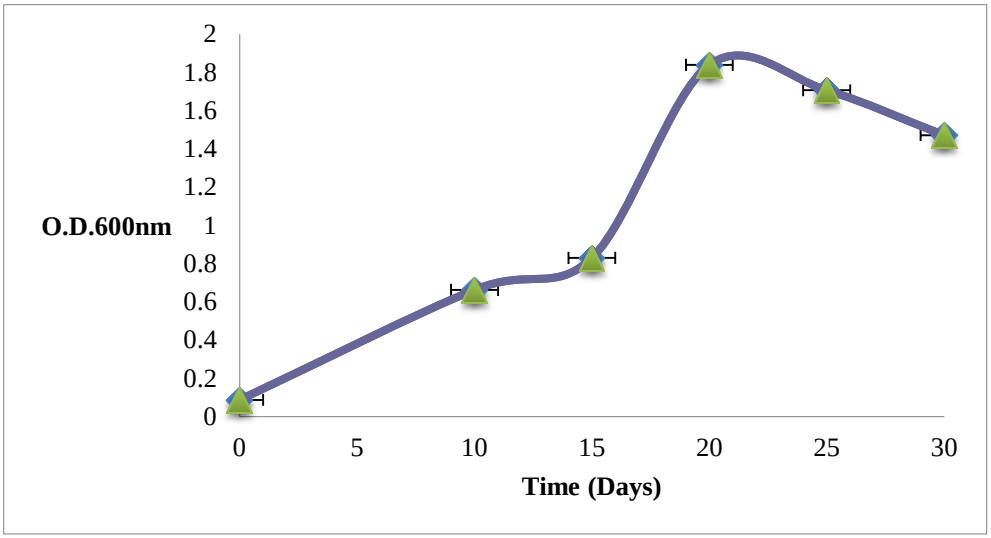

Figure 27. Growth Curve of *Aspergillus flavus* and *Bacillus cereus* S1+ *B. cereus* S2.

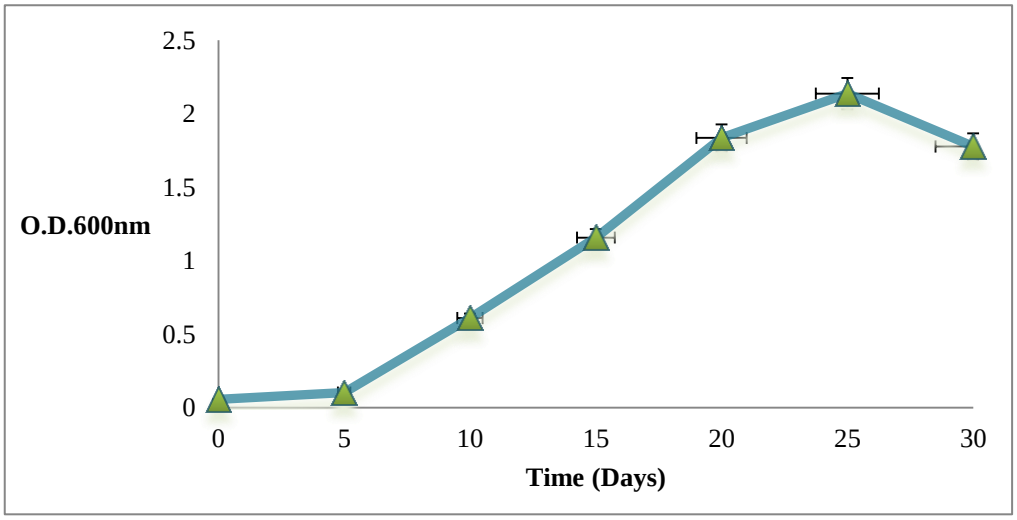

Figure 28. Growth Curve of Aspergillus ochraceus and Bacillus cereus S1+ B. cereus S2.

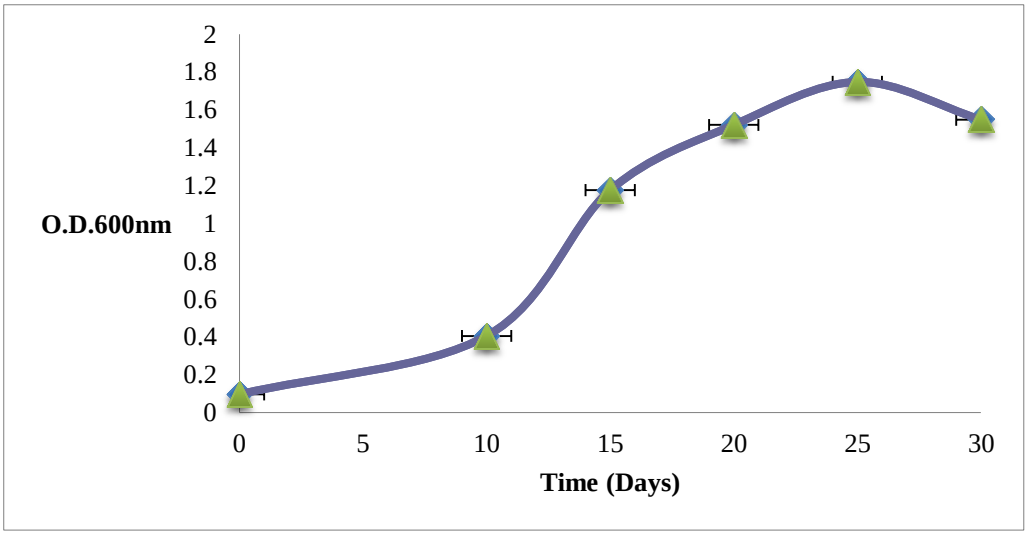

Figure 29. Growth Curve of Aspergillus flavus+ A. ochraceus and Bacillus cereus+ B. cereus.

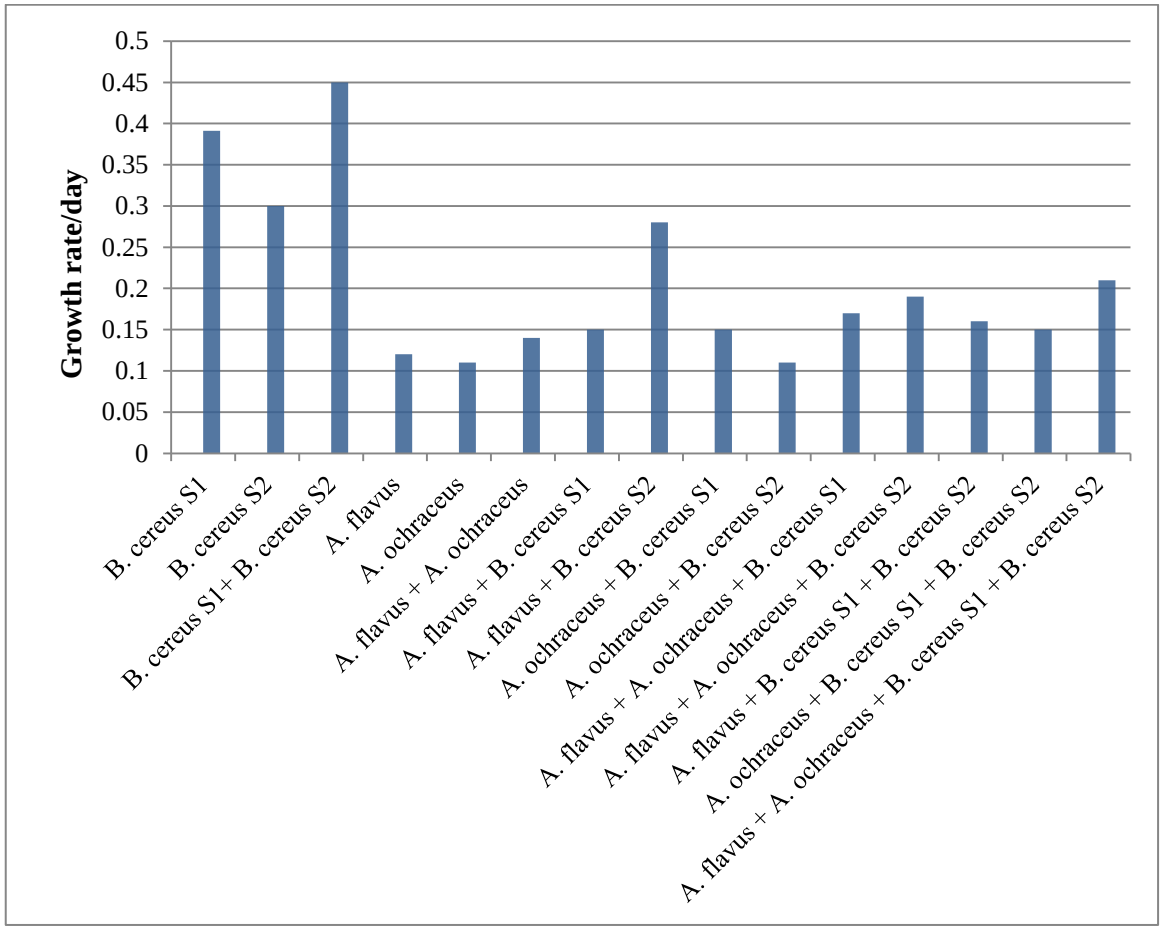

Figure 30. Growth rate of different isolates in liquid media supplemented with Difencanazole over 30 days.

Conclusions

Bioremediation is based generally on the idea that different organisms remove pollutants from the environment through the process of biodegradation. In the soil, there are many fungi and bacteria that have a major role in bioremediation due to their strong structures and metabolic capabilities that enable them to break down pollutants and turn them into less harmful or harmless compounds such as carbon dioxide and water and use them in the process of metabolism and growth. The results of laboratory adaptation process were carried out for 30 days and then released into the contaminated environment to conduct the degradation process, adjusting the factors that affect it such as temperature, pH, moisture content and nutrients. The isolates of the fungi (*Aspergillus flavus* and *Aspergillus ochraceus*) and bacteria (*Bacillus cereus* S1 and *Bacillus cereus* S2) diagnosed via molecular technique have proven to be able to degrade the fungicide Difenoconazole with concentrations up to three times the concentration recommended by the manufacturer of the pesticide 125, 225 and 325 mgL^{-1} . These isolates can be used to treat more stable pollutants in the environment such as petroleum hydrocarbons. In the field, multiple microbial consortia can be used to mineralise specific pesticides at a faster rate. Compared to other treatment processes such as burning, thermal disposal, land cultivation, etc., bioremediation has a better future in technology development to remove pollutants *in situ*.

References

[1] H. I. Owamah, T. O. Akpoedafe, S. C. Ikpeseni, E. Atikpo, H. O. Orugba, and S. Oyebisi, 'Spatial distribution of heavy metals in groundwater around automobile workshops in a popular Niger-Delta University town, Nigeria', J. Eng. Appl. Sci., vol. 70, no. 1, p. 79, 2023.

[2] T. S. Telles, G. M. de C. Barbosa, G. H. Merten, T. Pellini, E. J. Didoné, and M. de F. Guimarães, 'Soil governance as a requirement for agricultural land conservation: a historical overview', Rev. Ciência Agronômica, vol. 54, p. e20218315, 2023.
[3] J. Chen, Y. Liu, P. Diep, and R. Mahadevan, 'Harnessing synthetic biology for sustainable biomining with Fe/S-oxidizing microbes', Front. Bioeng. Biotechnol., vol. 10, p. 920639, 2022.
[4] P. Singh, G. Singh, A. Singh, and V. K. Mishra, 'Persistence of pesticides and their impacts on human health and environment', in Pesticides in the Natural Environment, Elsevier, 2022, pp. 139–162.
[5] S. Sindhu and A. Manickavasagan, 'Nondestructive testing methods for pesticide residue in food commodities: A review', Compr. Rev. Food Sci. Food Saf., vol. 22, no. 2, pp. 1226–1256, 2023.
[6] W. Liu, Y. Li, Y. Wang, Y. Zhao, Y. Xu, and X. Liu, 'DFT insights into the degradation mechanism of carbendazim by hydroxyl radicals in aqueous solution', J. Hazard. Mater., vol. 431, p. 128577, 2022.
[7] P. Mazellier, E. Leroy, J. De Laat, and B. Legube, 'Degradation of carbendazim by UV/H 2 O 2 investigated by kinetic modelling', Environ. Chem. Lett., vol. 1, pp. 68–72, 2003.
[8] A.-J. Hong, J. Lee, Y. Cha, and K.-D. Zoh, 'Propiconazole degradation and its toxicity removal during UV/H2O2 and UV photolysis processes', Chemosphere, vol. 302, p. 134876, 2022.
[9] S. Sudarshan et al., 'Impact of textile dyes on human health and bioremediation of textile industry effluent using microorganisms: current status and future prospects', J. Appl. Microbiol., vol. 134, no. 2, p. lxac064, 2023.
[10] M. Z. Mumtaz, M. Ahmad, H. Etesami, and A. Mustafa, 'Mineral solubilizing microorganisms (MSM) and their applications in nutrient availability, weathering and bioremediation', Front. Microbiol., vol. 14, p. 1101426, 2023.
[11] R. J. Fayyad, S. N. Muslim, and A. N. M. Ali, 'Application strategies for using fungi and algae as bioremediators: a review', Plant Arch., vol. 20, no. 1, pp. 788–792, 2020.
[12] J. P. Verma, D. K. Jaiswal, and R. Sagar, 'Pesticide relevance and their microbial degradation: a-state-of-art', Rev. Environ. Sci. Bio/Technology, vol. 13, pp. 429–466, 2014.
[13] S. Dugal and M. Gangawane, 'Metal tolerance and potential of penicillium species for use in mycoremediation.', J. Chem. Pharm. Res., vol. 4, no. 5, pp. 2362–2366, 2012.
[14] A. Vaksmaa, S. Guerrero-Cruz, P. Ghosh, E. Zeghal, V. Hernando-Morales, and H. Niemann, 'Role of fungi in bioremediation of emerging pollutants', Front. Mar. Sci., vol. 10, no. March, pp. 1–21, 2023, doi: 10.3389/fmars.2023.1070905.
[15] K. Chaudhary, S. Agarwal, and S. Khan, Mycoremediation and Environmental Sustainability, vol. 2, no. May. 2018. [Online]. Available: http://link.springer.com/10.1007/978-3-319-77386-5
[16] A. Thacharodi et al., 'Bioremediation of polycyclic aromatic hydrocarbons: An updated microbiological review', Chemosphere, p. 138498, 2023.
[17] O. Schoefs, M. Perrier, and R. Samson, 'Estimation of contaminant depletion in unsaturated soils using a reduced-order biodegradation model and carbon dioxide measurement', Appl. Microbiol. Biotechnol., vol. 64, pp. 53–61, 2004.
[18] E. Chanika et al., 'Isolation of soil bacteria able to hydrolyze both organophosphate and carbamate pesticides', Bioresour. Technol., vol. 102, no. 3, pp. 3184–3192, 2011, doi: 10.1016/j.biortech.2010.10.145.
[19] M. Geetha and M. H. Fulekar, 'Bioremediation of pesticides in surface soil treatment unit using microbial consortia', African J. Environ. Sci. Technol., vol. 2, no. 2, pp. 36–45, 2008.
[20] J. H. Werren, 'Symbionts provide pesticide detoxification', Proc. Natl. Acad. Sci., vol. 109, no. 22, pp. 8364–8365, 2012.
[21] J. Randika, P. Bandara, H. S. M. Soysa, H. A. D. Ruwandeepika, and S. K. Gunatilake, 'Bioremediation of Pesticide-contaminated Soil: A Review on Indispensable Role of Soil Bacteria.', J. Agric. Sci. (Sri Lanka), vol. 17, no. 1, 2022.
[22] Y. Li, X. Ma, and G. Lu, 'Systematic investigation of the toxic mechanism of difenoconazole on protein by spectroscopic and molecular modeling', Pestic. Biochem. Physiol., vol. 105, no. 3, pp. 155–160, 2013.
[23] E. Rekanović, M. Mihajlović, and I. Potočnik, 'In vitro sensitivity of Fusarium graminearum (Schwabe) to difenoconazole, prothioconazole and thiophanate-methyl', Pestic. i fitomedicina, vol. 25, no. 4, pp. 325–333, 2010.
[24] R. H. Bromilow, A. A. Evans, and P. H. Nicholls, 'Factors affecting degradation rates of five triazole fungicides in two soil types: 1. Laboratory incubations', Pestic. Sci., vol. 55, no. 12, pp. 1129–1134, 1999.
[25] L. Pan et al., 'The fungicide difenoconazole alters mRNA expression levels of human CYP3A4 in HepG2 cells', Environ. Chem. Lett., vol. 15, pp. 673–678, 2017.

[26] S. H. Abd-Alrahman et al., 'Exposure to difenoconazole, diclofop-methyl alone and combination alters oxidative stress and biochemical parameters in albino rats', Int. J. Clin. Exp. Med., vol. 7, no. 10, p. 3637, 2014.
[27] D. I. Voiculescu, D. L. Roman, V. Ostafe, and A. Isvoran, 'A cheminformatics study regarding the human health risks assessment of the stereoisomers of difenoconazole', Molecules, vol. 27, no. 15, p. 4682, 2022.
[28] N. Ali, M. Khanafer, and H. Al-Awadhi, 'Indigenous oil-degrading bacteria more efficient in soil bioremediation than microbial consortium and active even in super oil-saturated soils', Front. Microbiol., vol. 13, p. 950051, 2022.
[29] S. Indunil Chinthani et al., 'Morphological approaches in studying fungi: collection, examination, isolation, sporulation and preservation', Mycosphere, vol. 11, no. 1, pp. 2678–2754, 2020, doi: 10.5943/MYCOSPHERE/11/1/20.
[30] Z. M. Hussein and L. A. Naser, 'Phenotypic and Molecular Detection of Escherichia Coli in Patients with Diabetic Foot Infections in Basrah, Iraq', Cent. Asian J. Med. Nat. Sci., vol. 4, no. 3, pp. 216–228, 2023.
[31] H. A. Jasim, R. S. Ibrahim, B. A. Abbas, B. Ali, Q. Najim, and A. Ali, 'Isolation and identification of some bacterial species from dialysis unit in Basrah', in AIP Conference Proceedings, 2022, vol. 2386, no. 1.
[32] S. Hrichi et al., 'Effect of seasonal variation on the chemical composition and antioxidant and antifungal activities of Convolvulus althaeoides L. leaf extracts', Arab. J. Chem., vol. 13, no. 6, pp. 5651–5668, 2020.
[33] E. Goldman and L. H. Green, Practical handbook of microbiology. CRC press, 2015.
[34] A. J. Garabawi, J. K. Al-Faragi, and K. Y. Zakair, 'Detection of the most important pathogenic bacteria affect external organs of Cyprinus carpio in Wasit province', Iraqi J. Agric. Sci., vol. 53, no. 5, pp. 1115–1122, 2022.
[35] B. A. Forbes, D. F. Sahm, and A. S. Weissfeld, Diagnostic microbiology. Mosby St Louis, 2007.
[36] R. R. Yali, A. F. Almansoory, and W. H. Al-Tamimi, 'Isolation and identification of bacteria from contamination by hydro-sites to produce AgNPs', Marsh Bull., vol. 18, no. 1, 2023.
[37] B. Jung and G. J. Hoilat, 'MacConkey medium', in StatPearls [Internet], StatPearls Publishing, 2022.
[38] A. M. M. Essa, A. M. Reyad, T. E. Radwan, and W. M. Ibrahim, 'Biodegradation of the organophosphorus insecticide diazinon by Pseudomonas aeruginosa isolated from agricultural drainage ditches', Egypt. J. Bot., vol. 56, no. 1, pp. 353–370, 2016.
[39] Z. M. Kabir et al., 'Isolation and characterization of molybdate-reducing Enterobacter cloacae from agricultural soil in Gwale LGA Kano State, Nigeria', J. Environ. Microbiol. Toxicol., vol. 7, no. 1, pp. 1–6, 2019.
[40] K. I. Hassan, 'MOLECULAR TECHNIQUES FOR DETECTION OF AFLATOXIGENIC ASPERGILLUS FLAVUS AND DETERMINE THEIR AFLATOXIN IN PISTACHIOS.', IRAQI J. Agric. Sci., vol. 54, no. 6, pp. 1773–1783, 2023.
[41] F. A. Alsalabi, Z. U. Hassan, R. F. Al-Thani, and S. Jaoua, 'Molecular identification and biocontrol of ochratoxigenic fungi and ochratoxin A in animal feed marketed in the state of Qatar', Heliyon, vol. 9, no. 1, 2023.
[42] X. Chen et al., 'Highly efficient in-situ cleaner degradation of difenoconazole by two novel dominant strains: Microflora diversity, monoclonal isolation, growth factor optimization, intermediates, and pathways', Chemosphere, vol. 310, p. 136863, 2023.
[43] P. Kumar and S. G. Sachan, 'Exploring microbes as bioremediation tools for the degradation of pesticides', in Advanced oxidation processes for effluent treatment plants, Elsevier, 2021, pp. 51–67.