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Fungi Biodegradation of Sorial Microni Pesticides with Highly Efficient Capacity

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Abstract

Current study investigated the capability of soil fungi to biodegrade the fungicide (Sorial Microni).Among the isolated fungi was isolate No.1 which has been identified to be *Aspergillus terreus* KK1 which has high degradation ability. The efficiency of thisfungus has been tested against different concentrationsof Sorial Microni (0,0.35,0.52,0.9 and 1.1%(w/v). Sorial Micronidegradation time course was tested for a period of (0,7,14,21and 30) days at 27°C. Also, Sorial Microni degradation factors with *Aspergillus terreus* KK1have been optimized that included; temperature, pH, incubation period, nitrogen and carbon sources. Under optimized conditions *Aspergillus terreus* KK1showeddegradation percentage ranged between 76.5 and 93.3 %. The optimal temperature for degradation was 27°C and pH was 6 while the incubation period was 14 days.

Keywords: Biodegradation; Sorial Microni; Pollution

Introduction

Soil and an aqueous environment are natural and preferential sinks for contamination where, their contaminations represent an important concern for human and environmental health [1]. Pollution of the environment is one of the largest concerns to science and the general public life in the last years.

Microbial-based treatments of environments; contamination and industrial effluents offer an economical alternative to existing treatment methods [2]. Bioremediation involves the use of microbes to detoxify and degrade pollutants, which has been received increased attention as an effective biotechnological approach to clean up polluted environments [3]. Biodegradation of pesticide by microorganisms i.e. recombinant strain for degradation would be boon to soil bioremediation processes [4].

Fungal technology was play important role in clean up of contaminated soils holds significant promise since 1985 when the white rot fungus. Phanerochaete chrysosporium was found to be able to metabolize several important contaminated environment [5]. This capacity was later described for other white rot species among them Trametes versicolor. Successful removal of pesticides by the addition of bacteria (bioagumentation) has been also reported for many compounds including, parathion, coumaphos, ethoprophos and atrazine [3].

Generally, pesticides are a group of chemical compounds which classified by physico-chemical characteristics, which will require the development of varied assays. Mineralization and co-metabolism were the main mechanisms for the further degradation of pesticides and their intermediate products, while the group and molecular structure of pesticide determined its degradation behavior of microbial environment, chemical structure, its solubility, molecular orientation, spatial structure, chemical functional groups, intermolecular attraction, and repulsion characteristics effecting the ingestion of pesticides by microorganism. The main research directions of microbial degradation of pesticides were the development of high efficiency pesticide degradation engineered bacteria, the cultivation of mixed bacteria, and the immobilization of degrading bacteria. Researchers began to shift to the construction of efficient engineering bacteria and used the gene recombination technique. In short, the using of microbial agents might be an effective method to eliminate pesticide pollution. Still not clear,the process of pyrethroid degradation in the human body. A large number of

(3-PBA) residues not only caused two pollutions of agricultural products, but also led to pyrethroid pesticides being blocked in biomineralization, which indirectly caused the pesticide residue problems to become more serious and had a threat to food safety or the environment, and/or human health. Although heterologous compounds may be partially or completely decomposed by some microorganisms, they may be resistant to degradation in the environment due to their greater structure, insolubility, and high thermal stability. Therefore, we need to pay attention to this problem of pesticide environmental elimination through searching for potent degrading fungal strain to produce efficient degradation with harmless remediation.

Material and Methods

Collection of soil samples for fungal isolation

Soil and water samples were collected from different localities around (Kafer ELZyyate city) in Gharbia Governorate, Egypt. Pesticides contaminated Soil samples, were collected (at a depth range of about 5-10cm from the top), were air-dried, and sieved through a two mm sieve. The samples were dispensed into sterile polyethylene bags and were brought to the laboratory and stored at 3°C before processing and used *in vitro* for the isolation of the fungi [6]. However, water samples have been brought to Lab. in sterilized glass bottle and cultivated with 24 hours.

Cultural medium

Czapeks-Dox medium was used for the isolation, screening, and cultivation of fungi. All components of each medium were weighed and dissolved in 1000ml of distilled water by stirring and autoclaved at 121°C for 15min. Czapek's-Dox agar medium [7] was composed of (gm/L): Sucrose 30.0, Sodium nitrate 2.00, Potassium dihydrogen phosphate 1.00, Potassium chloride 0.50, Magnesium sulfate 0.50, Ferrous sulfate 0.01g and Agar 15.00g with pH (7.3±0.2). Chloramphenicol 0.05g/l was added as a bacteriostatic agent.

Fungi isolation and identification

Serial dilutions for soils and water samples were carried out for fungal isolation [8]. Plates containing dox media were inoculated and incubated at 28°C ± 2ºC. Colonies were picked up and transferred to the fresh growth medium. The developed fungi were purified using hyphal tips or the single-spore technique and then transferred to slant dox. The purified fungi were verified, and then fungi strains were selected and identified.

Inoculum preparation

Inoculum was prepared by growing the isolated fungi on sterilized Czapeks-Dox agar petri plates incubated at 28°C for 7 days. Cork porer (9mm diameter) has been used to prepare the fungus discs inoculumwhich used for solid cultures assays. However, inoculum in the form of spore suspension; the fungus spores were harvested by covering the plates with 0.1 saline solution and brushing gently with an inoculum loop. This spore suspension was filtered by pathing through double cheesecloth (Mira cloth filter) to remove hyphal fragments. The spores were diluted with the same solution, vortex and then counting in the hemocytometer. The spore suspension was adjusted to get a final concentration of 107spores/ml.

Screening for pesticides degradation by the isolated fungi

Screening using the solid medium

To analyze the degradation capability of the tested fungi, all tested fungi were cultured on sterilized petri plates containing (Czapeks-Dox) medium supplemented with different concentrations of Sorial Microni : 0, 0.35, 0.52, 0.9 and 1.1% (w/v). In addition to, 0.05 % (w/v) chloramphenicol which was also added to the media to avoid bacterial contamination, and the pH of the medium was adjusted to 7.3 with 1N HCl. The plates were inoculated with 9 mm fungal disc and incubated at 28°C for 7 days. Growth and the tolerance of the fungi to the pesticides were estimated by measuring the colony grown on a growth medium in comparison with the control cultures.

Screening Biodegradation of pesticides using liquid media

The fungal isolates were subjected to quantitative screening. Each 250ml Erlenmeyer's flask containing 100ml of productive Czapek Dox culture medium, supplemented with different concentrations of pesticide and the pH of the medium was adjusted to 7. The flasks were autoclaved, then cooled to room temperature, and chloramphenicol was added as an antibacterial agent. Each flask was aseptically inoculated with 107spores/ml and incubated in a rotatory shaker at 28°C and 130rpm for 7 days. At the end of the incubation period, filtration was carried out using Whatman No. 1. The dry weight was determined, Then, culture filtrates were centrifuged at 10000rpm for 10min, and supernatants were collected to measure the percentage of pesticide degradation using a spectrophotometer at wavelength (λ =300nm).

Determination of pesticide (Sorial Microni) degradation by fungi spectrophotometrically

Sorial Microni was screened over a wide range of wavelength, where 300nm was determined to be the maximum absorbance. So, standard curve has been constructed from Sorial Microni in order to measuring the degradation percentage at 300nm absorbance (Figure 1).

Identification of the isolated fungus with high degradation ability (Isolate No.1)

Fungi isolate No. 1 has been determined to be the best organism able to degrade Sorial Microni. Therefore, pure cultures of the isolatewereprepared and identified by using light microscopy. The identification was based on colony morphology, cultural characteristics, and especially, the morphology of their sporulating structures, according to consult keys given in standard books of mycology [10-14].

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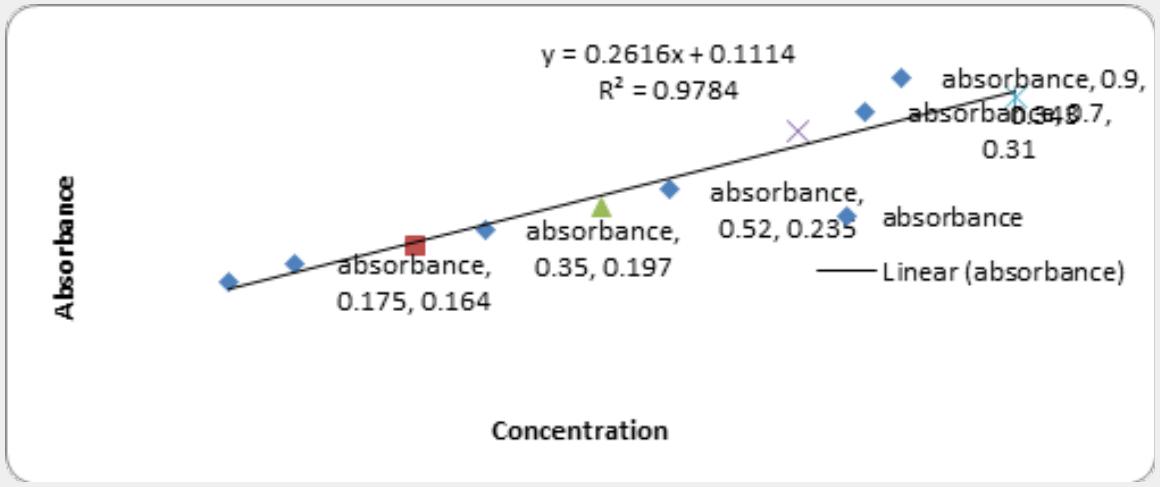

Figure 1:

Molecular Identification of Fungal Strains

The isolated fungal strain (isolate No.1) was identified by 18S rRNA sequence analysis. DNA was extracted from fungal mycelia using the following protocol: 20µl Chelex, 40µl TE buffer and mycelia heated to 95°C for 2min followed by centrifugation at 13.000g for 2min, DNA now in the supernatant was transferred to clean tubes and frozen (-18°C). PCR amplification of the internal transcribed spacer (ITS) region was performed as described by [15] with the following modifications: Amplification for 35 cycles consisting of denaturation for 35 s at 94°C, annealing for 55 s at 55°C and extension at 72°C for 45 s rising with 4 s per cycle. 10 × diluted fungal DNA was amplified using IT Sprimers forward primer 5'-TCCGTAGGTGAACCTGCGG-3' (ITS5) and the reverse primer 5'-TCCTCCGCTTATTGATATGC-3' (ITS4 [15] and ITS4 [16]. PCR products were sequenced by Macrogen (Seoul, Korea), and the sequences aligned in MEGA version 5 (www.megasoftware.net/index.php). The most similar sequences were found by BLAST search in GenBank. The phylogenetic relationship between the isolates and related sequences from GenBank was made by Maximum likelihood in MEGA version 5 [17] and the robustness of the phylogenetic tree tested by 1000 bootstraps. It has been concluded that isolate No.1 is Aspergillus terries KK1.

Optimization growing condition for Sorial Microni biodegradation by selected isolates

Aspergillus terries KK1 was growing in Czapeks Dox liquid medium under different physiological factors that were included different: pH values 2,4,6 and 8; temperatures were at 20, 25, 27 and 30°C, different time periods 7, 14, and 21 days; carbon sources were zero carbon, glucose, fructose, sucrose, maltose, lactose and starch; and nitrogen sources were included zero nitrogen, Sodium nitrate, Ammonium nitrate, urea, Asparagine and Peptone on liquid Czapeks media, under a shaking condition(130rpm).

Sorial MarconiSulfur contents determination by ICP-MS

The sulfur concentrations in samples (after and before degradation with fungus) were determined by means of inductively coupled plasma-mass spectroscopy (ICP-MS) (I CAP, Thermo, Germany). Certified reference materials (Merck, Germany) were included in the analyses. Integra software was used for average and relative standard deviation calculation [18]. **The laboratory accredits from EGAC/ILAC under No. 217006.** The recovery of metals result was within the certified limits.

Results

Fifteen fungal strains have been isolated from soil and three from wastewater samples as shown (Table 1).

The result indicated that isolates (1, 2, 3,4 ,5,11) having ability to degraded Sorial Microni (Table 2). Isolate No. 1 (*Aspergillus terreus* KK1) had a high degrading ability other than other isolates. Therefore, factors affecting on its degradation capability were. This study focused on isolate No. 1 (*Aspergillus terreus* KK1) which had a high ability to degraded Sorial Microni

Soil isolated fungus number one (*Aspergillus terreus* KK1) has performed a maximum ability to grow at different concentrations of used pesticide that was ranged from 0.0 to 1.10 (w/v) (Table 2 & Figure 2).

The result indicated that isolates (1, 2, 3,4 ,5,11) having ability to degraded Sorial Microni, butisolate No. 1 (*Aspergillus terreus* KK1) had a high degrading ability than other isolates. Therefore, factors affecting on its degradation capability were studied (Table 3).

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Table 1: Fungi isolates from soil and water samples were collected around Kafer El Zayate Company (KEC) for pesticides.

Type of Sample	Site	Number of Isolates
Soil(S1)	Around the company	Isolate: 1
SoillS2)	Around the company	Isolate: 2
Soil(S3)	Around the company	Isolates: 3, 4, 5, 6
Soil(S4)	Around the company	Isolates: 10, 11
Soil(S5)	Around the company	Isolates: 12, 13, 14, 15
Water (W1)	Wastewater of the company	Isolates: 7, 8, 9

Table 2: Effect of different concentration of Sorial Microni(w/v) on redial growth and dry weight on different isolates.

No. of Isolate	Redial Growth (cm)				
	Different Concentration of Sorial Microni(w/v)				
1	7.7±12	4.4±0.01	3.9±0.01	2.61±0.01	2.2±0.01
2	7.31±0.12	2.3±0.01	1.9±0.001	1.5±0.01	1.1±0.01
3	7.31±0.01	2.72±0.01	2.10.01	1.4±0.02	1.0±0.01
4	6.63±0.01	2.4±0.02	1.7±0.01	1.41±0.03	1.1±0.01
5	6.8±0.02	2.1±0.03	1.9±0.02	1.4±0.02	1.2±0.01
6	6.1±0.03	1.6±0.02	1.2±0.01	1.1±0.02	1±0.01
7	7.31±0.01	2.3±0.02	1.6±0.01	1.3±0.04	1±0.01
8	7.8±0.012	1.4±0.01	1±0.02	0.8±0.01	1±0.01
9	6.2±0.03	2.7±0.04	2.2±0.02	1.8±0.01	1±0.01
10	6.6±0.05	3.3±0.03	1.7±0.01	1.2±0.02	1±0.01
11	7.4±0.01	3.6±0.02	2.39±0.01	1.4±0.01	1±0.01
12	6.31±0.04	2.1±0.02	1.7±0.01	1.03±0.01	1±0.01
13	7.2±0.01	2.12±0.01	1.61±0.01	1.02±0.01	1±0.01
14	6.8±0.01	2.4±0.01	1.9±0.01	1.01±0.01	1±0.01
15	6.7±0.01	2.1±0.01	1.9±0.03	1.1±0.01	1±0.01

Table 3: Degradation of Sorial Microni by different isolates.

Fungal Isolate No.	Degradation of Sorial Microni (%) after 21 days at a different Concentration(w/v)				
	1.1	**0.9**	**1.1**	**0.9**	**1.1**
1	93.0±0.02	93.0±0.01	93.0±0.02	93.0±0.01	93.0±0.02
2	88.3±0.01	68.5±0.02	88.3±0.01	68.5±0.02	88.3±0.01
3	73.1±0.01	78.0±0.02	73.1±0.01	78.0±0.02	73.1±0.01
4	67.6±0.02	75.6±0.01	67.6±0.02	75.6±0.01	67.6±0.02
5	2.9±0.01	0.57±0.01	2.9±0.01	0.57±0.01	2.9±0.01
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	0	0	0	0	0
10	0	0	0	0	0
11	35.9±0.02	39.7±0.01	35.9±0.02	39.7±0.01	35.9±0.02
12	0	0	0	0	0
13	0	0	0	0	0
14	0	0	0	0	0
15	0	0	0	0	0

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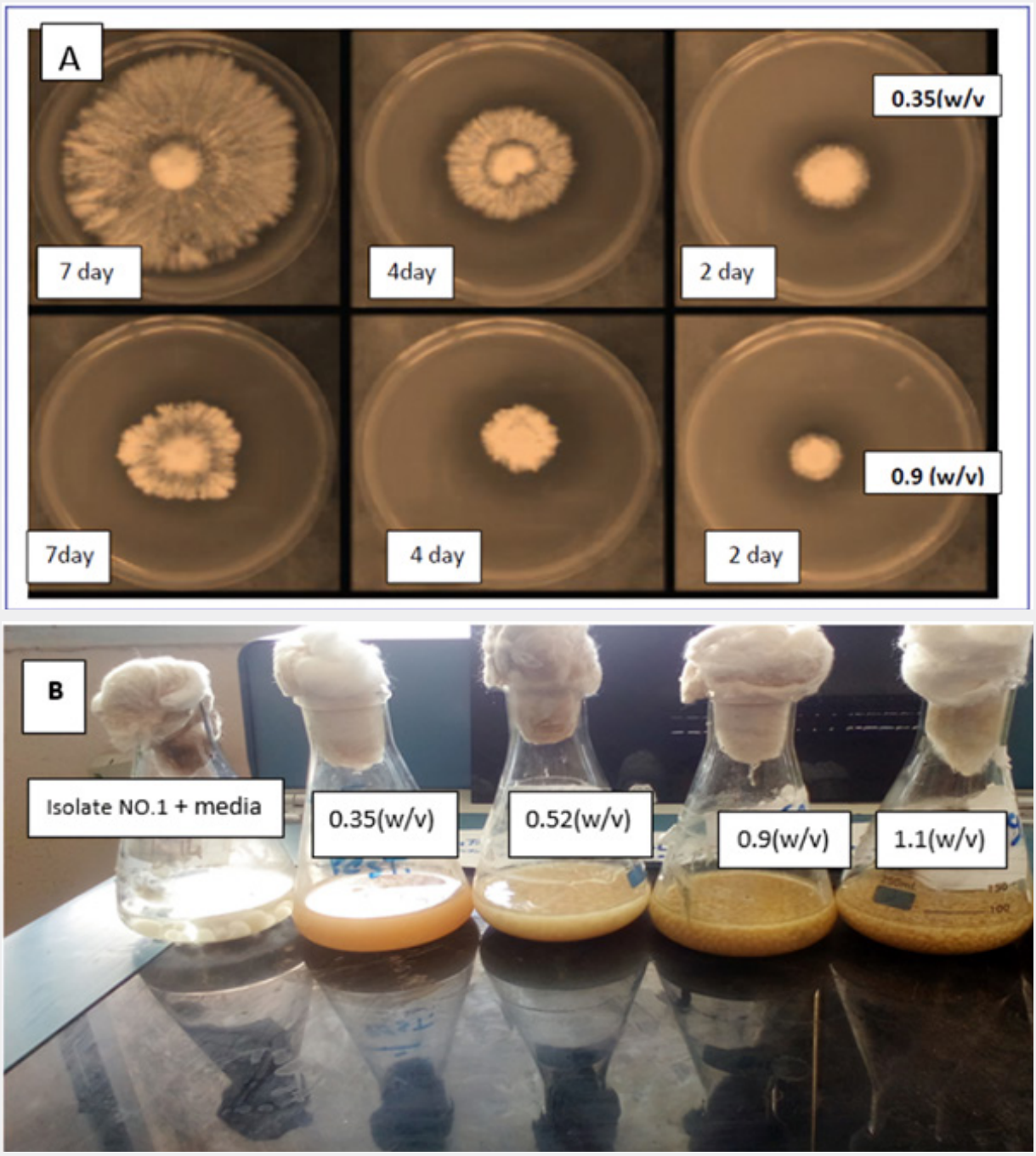

Figure 2: Linear growth and degradation percent of Aspergillus terreus KK1 under different concentration of Sorial Microni(w/v) on solid media (A) and liquid media (B), after 7-day isolate No. 1.

Molecular Identification and phylogenic tree of selected isolate

Fifteen fungal isolates were recovered from the soil and water samples. The fungal isolate designated as isolate 1 was selected and screened based on its potential Sorial Microni degradation abilities for further experiment. The fungus efficiently degraded and metabolized Sorial Microni up to concentrations as high as (1.1%) w/v.

This tree was based on the internal transcribed spacer ITS regions from various fungal type strains as compared with *Aspergillus terreus* KK1 isolate ITS region (OQ519782.1). The tree is rooted using by Maximum likelihood in MEGA version 5

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[17], and the robustness of the phylogenetic tree tested by 1000 bootstraps. It has been concluded that isolate No.1 is Aspergillus terries KK1.

Molecular Identification and phylogenic tree of selected isolate

Sequencing of the highly conserved ITS gene is one of the most commonly used techniques for identifying fungi; it is a powerful, simple, and fast method for determining evolutionary and phylogenetic relationships among fungi. Sequencing of ITS gene has been employed to facilitate the differential identification among the genus Aspergillus terries KK1 [19].

PCR amplification of the DNA samples from the selected isolate generated a PCR product of expected size (1391bp). The analysis of ITS gene of the fungal isolate Aspergillus terries KK1was sequenced and compared with available ITS in the NCBI Gen Bank database website (http://www.ncbi.nlm.nih.gov/GenBank/index.html) using BLAST. The NCBI database showed highest percentage of similarity being 99.66% Aspergillus terries KK1 under accession no. (OQ519782.1). Gene bank nucleotide database using the blast-n algorithm revealed significant matches with hi max score of 1982, zero e-value, and 99.66% nucleotide identity for isolate KK1.The phylogenetic tree (Figure 3) shows high genetic relationship between the Egyptian fungal Aspergillus terries which strongly confirm its identity as Aspergillus terries KK1.

Table 4: Growth factors Optimizing conditions for Sorial Microni degradation percentage with *A.terrus* KK1.

Growth Factors	Optimum Factor	Sorial Microni (w/v)	
		0.35	0.9
		Degradation Percentage	
Carbon source	Sucrose	69.0±0.01	89.2±0.02
pH	6	**72.6±0.02**	**89.2±0.02**
Nitrogen source	Sodium nitrate	75.8±0.02	91.5±0.02
Time course	14 days	**71.6±0.05**	**93.0±0.01**
Temperature	27 °C	72.8±0.02	94.0±0.01

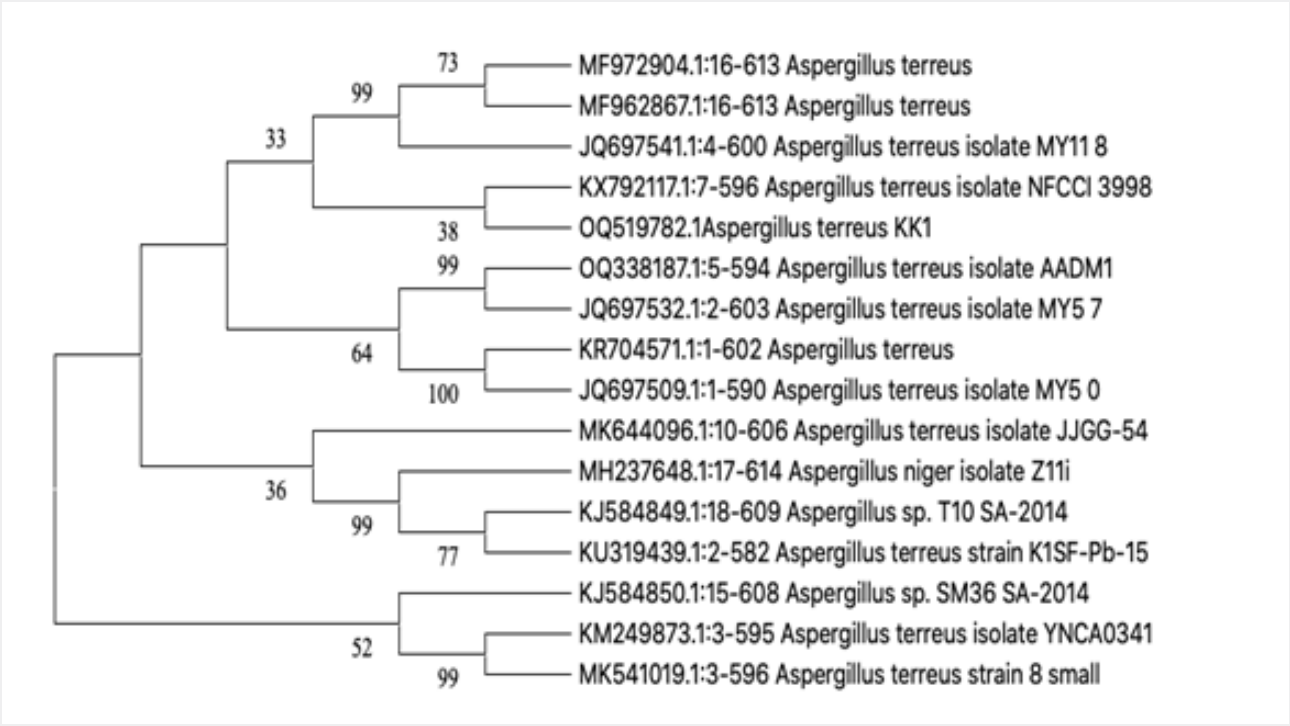

Figure 3: A maximum likelihood phylogenetic tree with 1000 bootstrap replicates is constructed.

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Growth factors for Sorial Microni biodegradation employed withA.terrus KK1 were investigated (Table 4). Sucrose was provedto be the best carbon source which performed 69.0±0.01 and 89.2±0.02 degradation percentage for 0.35 and 0.9 (w/v) of Sorial Microni. pH of 6, Sodium Nitrate and 27°C were the most suitable for A.terrus KK1 to degrade Sorial Microni at 0.5 and 0.9 (w/v). It became clear that the best growing time was 14 days which performed 71.6 and 93 degradation percentage at 0.35 and 0.9 (w/v) concentration of Sorial Microni.

Sulfur contents of Sorial Microni after and before degradation as determined by ICP-MS

Table 5 is indicating the degradation of Sorial Micronisulfur (70% from Sorial Microni) by isolate No. 1 (*Aspergillus terreus* KK1) in liquid shaked culture of 130 speed of 130rpm for 14day at 27°C and pH(6).

Table 5: *Aspergillus terreus* KK1Sorial Microni Sulfur uptake.

Sorial Micronii Sulfur Concentration (%)	Efficiency of Sulfur Consumed by *Aspergillus terreus* KK1)
0.35	29.4±0.01
0.52	38.87±0.01
0.9	55.44±0.01
1.1	60.53±0.01

Discussion

Microbial degradation of chemical compounds in the environment is an important route for the removal of its removing. The biodegradation of pesticides is often complex processes and involves a series of biochemical reactions. Although many enzymes efficiently catalyze the biodegradation of pesticides, [20].

Fungi degrade pesticides by introducing minor structural changes to the pesticides rendering it nontoxic and are released to soil, which will be susceptible tofurther biodegradation by bacteria [21]. Fusarium oxysporum, *Aspergillus terreus*, and Penicillium chrysogenum were used to degrade various pesticides pesticides in laboratory conditions [22]. The obtained results have showed that *Aspergillus terreus* and Fusarium oxysporum accelerated the decomposition of all pesticides and had the greatest effect when compared with Penicillium chrysogenum. Current results explain that the percentage of Sorial Microni degradation by *Aspergillus terreus* KK1 reached high ability through optimizing its growing conditions. The effect of pH on fungus degradation ability revealed a continuous increase from pH 3 to 6 with the maximum at pH 6. Further increase in pH declined the activity . Some reports have shown that many bacterial/ fungal strains achieved maximum pesticides degradation at neutral and alkali pH values [23,24]. Previous reports stated that the optimum values of pH enhance the activity of the fungus enzyme which causing the increase the degradation of the pesticide [25,26].

Additionally, the degrading of Sorial Microni increased with increasing the incubation periods but at the same time decreased with increasing initial concentrations of Sorial Microni and this almost agree with [27]. Fungal degradion of Profenofos, Diazinon and Malathion increased with increasing incubation period but at the same time decreased with increasing initial concentrations of insecticides [27]. The obtained results indicated that the isolate No. 1 (*Aspergillus terreus* KK1) was able to degrade Sorial Microni at different concentration up to 1.1 w/v. Likewise, [28] found that Isoproturon pesticide did not serve as an energy or nutrient source for the fungi used in their experiment. In contrast, [29] found that an Acremonium strain could utilize the organophosphate chlorpyrifos as a source of both carbon and nitrogen. Since the Mortierella strain cannot utilize diuron as a N or C source it seems plausible, that the degradation process is co-metabolically mediated by enzymes excreted by the fungus during growth leading to successive demethylation through the metabolite.

Difference in temperature influence the ability of fungi in degradation round up. The percentages of degradation increase with the increase in temperature reaching the optimum state at 27°C for (94.0%). The increase after this became opposite, the degradation ability of the fungus decreases with the increase of temperature, and this might be agree with the result in [29] who indicated that the increases in temperature more 40°C cause the total damage of the fungal enzymes.

Conclusion

Environmental pollution caused by pesticides affects the ecosystem services of soil, water resources, and the health of plants, microorganisms, animals, and humans. Therefore, there is a need to develop suitable environmentally friendly strategies to remove pesticides from polluted environments. A review of the data presented in this article, as well as data from around the world are highlighting the role and importance of fungi in the biodegradation of various pesticides.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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