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Biodegradation of Petroleum Hydrocarbon and Related Molecular Microbiology Analysis of Some Fungal Species

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ABSTRACT

Petroleum hydrocarbon contamination is one of the main environmental challenges. Bioremediation using fungi (Mycoremediation) is the best method to overcome this problem. Consequently, the current study aimed to investigate the tolerance of four fungal isolates to petroleum hydrocarbon and estimate the level of production of cellulase and Mn peroxidase enzymes involved in petroleum oil biodegradation. The fungal isolates were identified as *Fusarium solani*, *Trichoderma harzianum*, *Aspergillus terreus*, and *Aspergillus flavus* by rDNA sequencing of ITS1 -5.8 S rRNA-ITS2 region. A hydrocarbon tolerance test was conducted using solid minimal media (MM) containing different concentrations of diesel oil, fungal growth rate and dose inhibition response percentage (DIRP) values were recorded. Treated and non-treated Bushnell Haas (BH) broth media were used to test the ability of fungi to secret the target enzymes. The results showed that *T. harzianum* had the highest tolerance towards diesel oil followed by *A. flavus*. Diesel oil induced gene-overexpression for cellulase and Mn peroxidase enzymes. *A. flavus* was the highest producer of cellulase and Mn peroxidase. Finally, all isolates, especially *A. flavus* and *T. harzianum* can adapt to high concentrations of petroleum hydrocarbon. Thus, they can be used successfully in bioremediation.

INTRODUCTION

Currently, petroleum hydrocarbon contamination is one of the main environmental challenges due to its large-scale uses in transportation, industrial fields, crop production, and other sectors. Moreover, an oil spill resulting from a leakage or explosion accident that contains hydrocarbon compounds can alter the soil and water properties in nature. They can transpire through the ecosystems and accumulate in animal and plant tissues, thus causing various toxic effects including cancer induction, mutations, and malfunctioning of respiratory and central nervous systems (Chen *et al.*, 2015 and Wang *et al.*,2018). Hence, remediation of these pollutants becomes an important issue, which needs to be resolved urgently. Different physical, chemical, and biological methods have been reported to salvage the environment from the impact of petroleum spills and hydrocarbon contamination (Asemoloye *et al.*, 2017a). The biological remediation methods using bacteria and fungi have been investigated as the potential degrader of many pollutants and are being extensively studied to evaluate the best removal conditions. In addition, bioremediation methods are low-cost and eco-friendly treatments for the disposal of hydrocarbon-contaminated sites. However, microorganisms can play a significant role in the reduction and decomposition of dangerous pollutants such as heavy metals, pesticides, and petroleum compounds (Chand *et al.*, 2022). The ability of microorganisms to decompose hydrocarbon pollutants depends on the production of certain enzymes that catalyze the bioremediation process. For instance, different types of bacteria have a potential effect as remedial agents for the pollutants in water and soil through their hydrolysis enzymes. These enzymes are effective in getting rid of oil spills and pesticides. In this context, laccases and peroxidase enzymes have a high ability in the hydrocarbon decomposition process, and the analysis of aromatic and phenolic compounds (Yang R. *et al.*, 2016). Compared to bacteria, fungi are more resistant to high concentrations of hydrocarbon toxins and can produce many useful bioactive compounds, such as extracellular enzymes, among other advantages (Treu and Falandysz, 2017). Additionally, various fungal species such as *Aspergillus sp.* and *Rhizopus sp.* are among the most important microorganisms in getting rid of pollutant agents, especially heavy metals and petroleum materials (Hasan, 2014 and Ismail *et al.*, 2022). From an environmental perspective, using potent fungal mixed cultures is a promising strategy for the biodegradation of crude oil (Ang *et al.*, 2015 and Al-Zahrani *et al*, 2022). Consequently, different types of fungi, belonging to the *cladosporium*, *Aspergillus*, *Cunninghamella*, *Penicillium*, *Fusarium*, and *Mucor* have been described to be involved in aliphatic hydrocarbon degradation, as well as in the decomposition of more recalcitrant aromatic hydrocarbons (Filippo Dell' Anno *et al.*, 2021). The high potency of fungi in the clean-up of petroleum pollution is related to

their diverse production of catalytic enzymes such as ligninolytic enzymes (Antón-Herrero *et al.*, 2022). These enzymes can decompose hydrocarbons of long chains or multiple rings, breaking down the pollutants into simpler forms and utilizing them as substrates for their growth (Dhagat and Jujjavarapu, 2022). Several ligninolytic enzymes such as cellulase (Cx) Laccases (LaC), manganese peroxidase (MnP), and lignin peroxidases (LiP) are proven to pose the potential for hydrocarbon degradation (Al-Zaban *et al.*, 2021). However, extracellular enzymes produced by fungi during bioremediation are well documented but the genetic basis of regulation of these enzymes production in fungi needs more attention. Until now, the gene regulation at the transcription level of ligninolytic fungal genes is still vague (Al-Zaban et al.,2021), a little research is available. Thus, the genes controlled peroxidase (MnP) and lignin peroxidases (LiP) in *Trichoderma harzianum* detected and expressed as a response to crude oil, the expression of these genes by this strain could be an important part of the factors responsible for its survival in crude oil polluted soil. (Jonathan *et al.*, 2017). A recent study proved that nine genes are responsible for ligninolytic enzyme production; cbh (cbhI.1, cbhI.1, cbhII) lcc, lig (1,2,4and 6) and Mnp were detected and expressed by some fungal species (Al-Zaban *et al.*, 2021)

The current study aimed to identify some isolates of fungal species molecularly by using r DNA sequencing of ITS (Internal transcribed spacer) rRNA, investigate the tolerance of these isolates to petroleum hydrocarbon, estimate the level of production of ligninolytic enzymes (cellulase and Mn peroxidase) involved in petroleum oil biodegradation.

MATERIALS AND METHODS

Fungal isolates

Four fungal isolates were used: *Aspergillus Terreus*, *Aspergillus flavus*, *Trichoderma harzianum*, and *Fusarium solani.*, They were identified previously by morphological and microscopic examination in Plant Pathology and Genetics departments, Faculty of Agriculture, Alexandria University.

Petroleum hydrocarbons

Diesel oil is used as a petroleum hydrocarbon which acts as a sole source of carbon in growth media. It contains 75% aliphatic hydrocarbons and about 25% aromatic hydrocarbons (Gad, 2014).

Molecular identification of fungal isolates

Molecular identification was implemented by amplification of the ITS (Internal transcribed spacer) region of rDNA, ITS1-5.8S-ITS2, using PCR. Genomic DNA extracted from fungal mycelia of four tested isolates grown on a PDA medium for 7 days, using the CTAB method with some modification according to Gontia-Mishra *et al*. (2014). DNA concentration was measured using a NanoDrop spectrophotometer, Maestro Nano® spectrophotometer, (MN-913, Version 02-11, Taiwan). DNA quality was tested by using 1% agarose gel electrophoresis. PCR amplification was performed by using universal primer pair ITS4-F (5′-TCCGTAGGTGAACCTGCCG-3′) and ITS1-R (5′-TCCTCCGCTTATTGATATGC-3′). PCR reactions performed in 25 ul contained 100 ng of genomic DNA,10 uL of 2 X TOP^{TM} simple Dye MIX-n Taq PCR, and 0.5 µM of each primer. PCR condition conducted according to Asemoloye *et al.* (2020b)

PCR product for each sample of the fungal isolate was electrophoresed in 1.5% (w/v) agarose gels, then purified and sent to Macrogen Company, (API3730XL DNA Analyzer, Applied Biosystems) for sequencing analysis. Each sequence aligned with sequences derived from databases by using the BLAST tool (http://www.ncbi.nlm.nih.gov/BLAST/index.html) from from National Centre for Biotechnology Information (NCBI). The phylogenetic tree was constructed based on the rDNA sequence of the tested isolate and the other selected sequences from Gen bank at NCBI using MEGA 11 software and the neighbour-joining method.

Hydrocarbon tolerance assay:

The tested fungal isolates were grown on minimal medium (MM), containing minerals only without carbon source, supplemented with diesel oil in different concentrations (0 ‘‘control”, 5, 10, and 15%; v/v). This assay was implemented to examine the tolerance and adaptation of the fungal isolates with diesel oil. Then, MM medium was inoculated with 5 µl of a solution of 1×10^7 spores/ml at the center of the plate of tested fungi according to Anaisell *et al*. (2014). Each treatment was implemented in sex replicates and the plates were incubated at 30 °C for 8 days. In this regard, the radial growth rate was calculated based on fungal growth extension measurements. Fungal dose inhibition response percentage DIRP calculated according to Al-Zahrani *et al*. (2022).

Enzymes activity assay

Bushnell Haas (BH) broth media supplemented with diesel oil in different concentrations (0 ‘‘control”, 5, 10, and 15 %; v/v). The flasks containing treated media and control were inoculated by a mycelium-water mixture of all fungal isolates and then incubated at 30 °C. This method was conducted according to Asemoloye *et al.* (2020 b) After two weeks cellulase and Mn peroxidase levels were measured by using crude enzyme and expressed as U/ml. Cellulase activity (Cx) was estimated by using Whatman No. 1 filter paper as a substrate then activity was measured as a reducing sugar which oxidized to record the

absorbance at 540 nm according to Remero *et al.* (1999), and Yu *et al*. (2016). Manganese peroxidase (MnP)activity was measured based on oxidation of Mn2+ to Mn 3+. Manganese sulfate and sodium tartrate were used as substrates, while phenol was used as an indicator to monitor the reaction, the optical absorbance was recorded at 460 nm according to Paszcymski *et al*. (1988) and Al-Zaban *et al*.(2021).

RESULTS AND DISCUSSION

Using fungi to clean the environment from petroleum spills is one of the best-used approaches because it is rapid, safe, and effective as we mentioned previously. Therefore, this study was conducted to test the ability of some fungal species to tolerate high concentrations of petroleum hydrocarbon and produce ligninolytic enzymes to biodegrade these components.

Molecular identification of the tested isolates of fungal species

The tested fungal species were identified previously by morphological and microscopy assays. In the current study, molecular identification was implemented to confirm the species of the fungal isolates based on ITS1-5.8S rRNA-ITS2 regions. ITS is considered a DNA barcoding method that has been widely used in the identification of fungi (Chen *et al*.,2023). For this purpose, the sequence of these regions for all fungal isolates was compared to the reference sequences in the NCBI Gene bank (Table 1). ITS region sequence distinguished by accuracy and sensitivity, is considered a reliable method for molecular identification. As a result, molecular identification is achieved based on these regions for fungi at the species level (Florez *et al.*,2007; Gehlot *et al.,* 2011 and Lakhani *et al*., 2016). It is worthy to mention that *Trichoderma* spp. identification proved the accuracy of ITS, this region is extremely polymorphic and non-coding with adequate taxonomic parts which enable isolating sequences at the species level (Haque *et al*.,2020). *Fusarium oxysporum* isolates identified by using ITS indicated that it is an indispensable method for identification (Singha *et al*.,2016). Recently, *Trichoderma* spp strains isolated from wetland soil were identified by using the ITS molecular method successfully (Saravanakumarand and wang,2020). The ITS region has been widely used to identify *Aspergillus* spp., while it is not polymorphic enough to identify the isolates in a narrow taxonomic range. Therefore, it is better to use another marker besides the ITS marker, such as B-tubulin and Calmodulin. Identification of *Aspergillu*s spp. is considered a challenge and needs another advanced method (Gautier *et al*., 2016 and Qi *et al.*,2024)

The tested fungal isolates *Fusarium solani* PP979702, *Trichoderma harzianum* PP979703, *Aspergillus terreus* PP979704, and *Aspergillus flavus* PP979705 showed 100%, 99.66%,100%, and 99.27% identities respectively with the reference sequences listed in Table 1. The results confirmed that the fungal isolates were *Fusarium solani, Trichoderma harzianum, Aspergillus terreus, and Aspergillus flavus*. Phylogenetic trees were constructed by using the tested fungal sequences of ITS1-5.8S rRNA-ITS2 and the other fungal species sequences from the NCBI Gene bank. Phylogenetic analysis demonstrated the relationships among *Fusarium* spp. (Figure 1). The neighbour–joining tree was divided into three main groups, the sequence of interest of *Fusarium solani* isolate tested in this study was closely related to *Fusarium proliferatum* strain F3 and clustered in one group with *Fusarium euwallaceae* FD31HYFL isolate and *Fusarium falciforme* CBC475.67. The phylogenetic tree in Figure 2 demonstrates the relationship between ITS sequences of *Trichoderma spp.* dataset and the tested isolate. The results revealed that the sequence of interest of the tested isolate of *Trichoderma harzianum* was closely related to *Trichoderma virens* CS109339, this result is in agreement with the obtained results by Lieckfeldt *et al*. (1998). The phylogenetic tree of *Aspergillus* species in Figure 3 showed that the tested isolate of *Aspergillus terreus* was closely related to *Aspergillus oryzae*, and the tested isolate of *Aspergillus flavus* segregated in one group close to *Aspergillus oryzae*, *Aspergillus terreus*, *Aspergillus nidulans* ATCC10074 and *Aspergillus versicolor* ATCC9577.

Table 1: Identification of fungal species by comparing the DNA sequence of ITS1- 5.8 S rRNA-ITS2 with reference sequences in Gene bank

No.	Fungal species	Th tested fungal isolates Accession no.	Gene bank fungal isolates Accession no.	Identity
1	*Fusarium solani*	PP979702	MH191237.1	100%
2	*Trichoderma harzianum*	PP979703	MT635323.1	99.66%
3	*Aspergillus terreus*	PP979704	MZ268151.1	100%
4	*Aspergillus flavus*	PP979705	DQ198161.1	99.27%

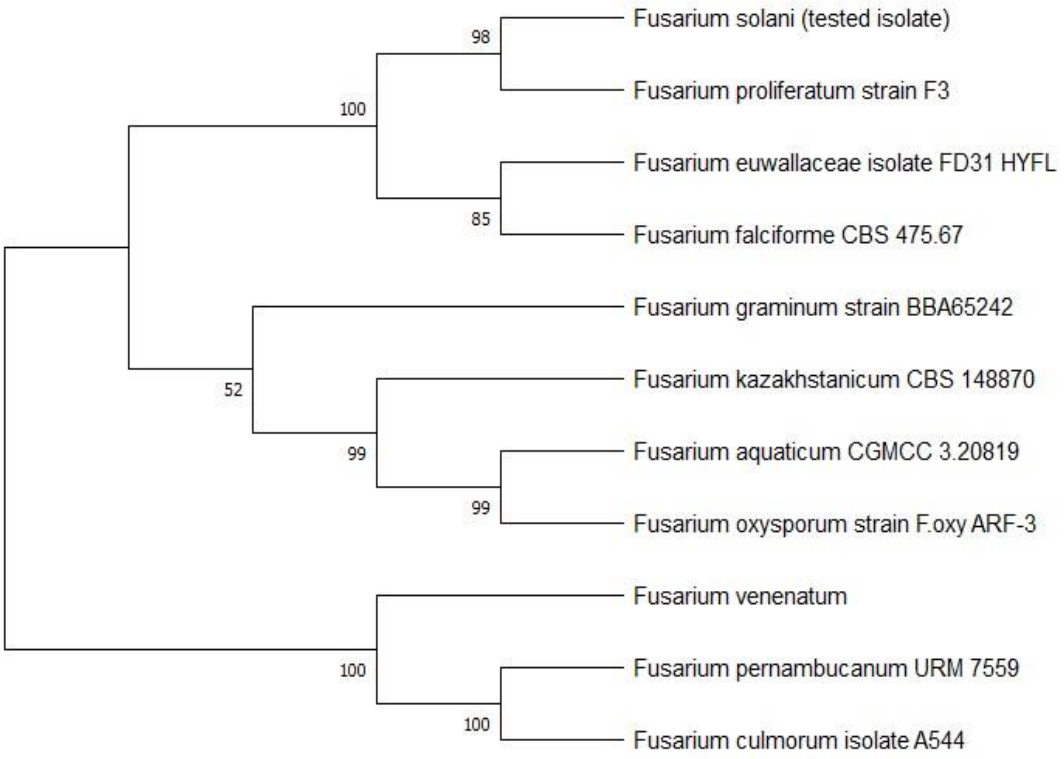

Figure 1: Phylogenetic tree constructed by using neighbour-joining method showing the relationship between *Fusarium solani* (tested isolate) sequence and the sequence of other *Fusarium* spp. selected from Gene Bank at NCBI.

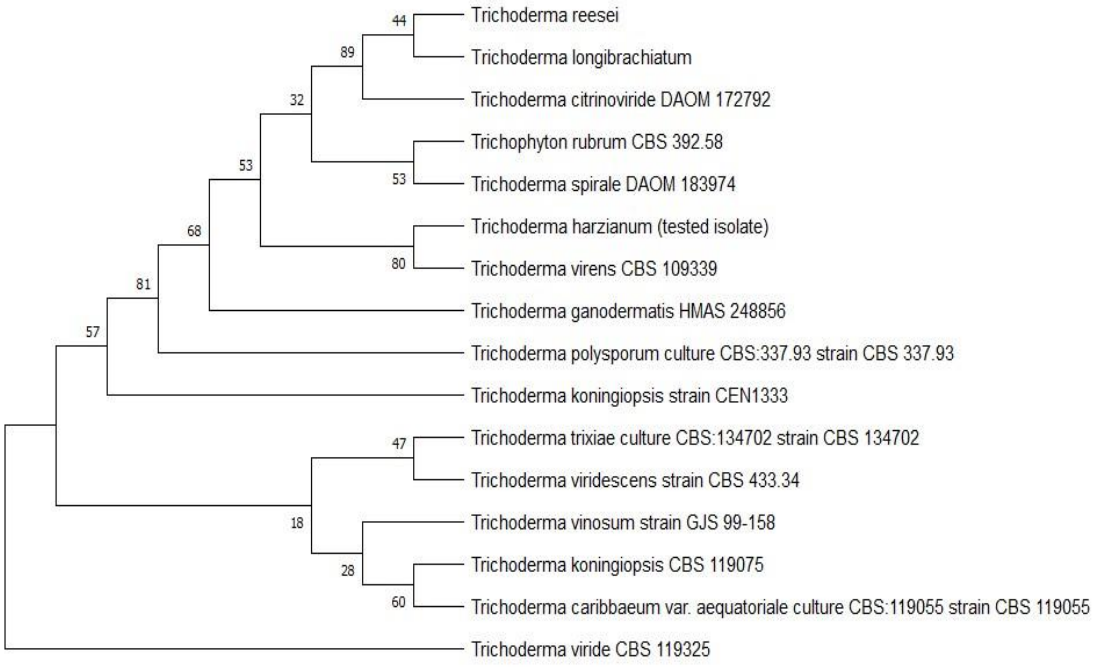

Figure 2: Phylogenetic tree constructed by using neighbour-joining method showing the relationship between *Trichoderma harzianum* (tested isolate) sequence and the sequence of other *Trichoderma* spp. selected from Gene Bank at NCBI.

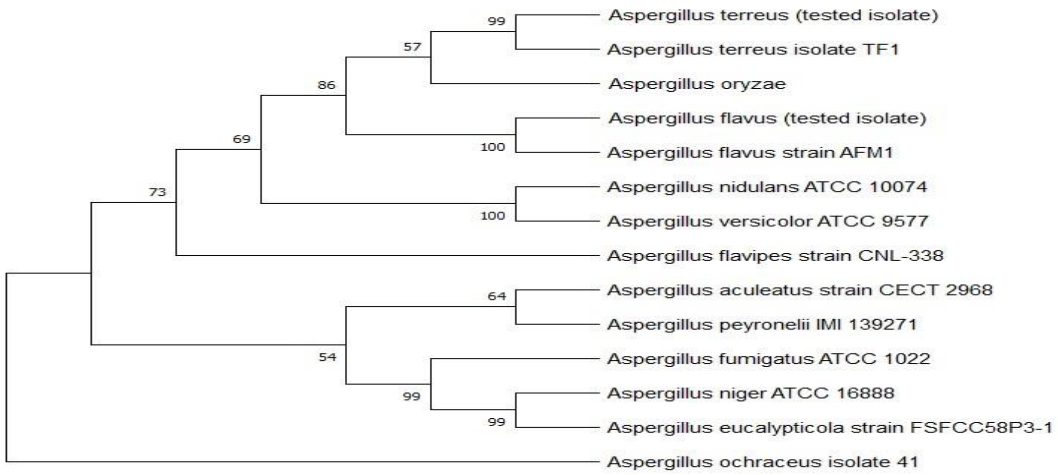

Figure 3: Phylogenetic tree constructed by using neighbour-joining method showing the relationship between *Aspergillus flavus* &*Aspergillus terreus* (tested isolate) sequence and the sequence of other *Aspergillus* spp. selected from Gene bank at NCBI

It was found that Aspergillus flavus was closely related to *Aspergillus parasiticus*, *Aspergillus oryzae*, and *Aspergillus sojae*, (Jackson and Dobson,2011). Moreover, Qi *et al*.(2024) mentioned that closely related species; *Aspergillus fumigatus, Aspergillus niger, Aspergillus nidulans, Aspergillus flavus, and Aspergillus terreus* subjected to analysis whole – genome showing an effective method for species classification.

Hydrocarbon tolerance of the tested isolates of fungal species:

The hydrocarbon tolerance test is an important technique which taken into consideration when tolerant fungal isolates are selected for petroleum oil bioremediation.

The tolerance capacity was estimated based on the radial growth rate. All tested fungal isolates revealed a decrease in radial growth rate with the increase in diesel oil concentration as shown in Table 2, Figures 4, 5, 6 and 7. All isolates grown on the medium containing 5% Deisel oil differed slightly in radial growth rate compared to the control. *Trichoderma harzianum* recorded the highest values of DIRP (88, 86 at 10% and 15% deisel oil respectively) showing high ability in tolerance capacity followed by *Aspergillus flavus* and *Aspergillus terrus.* (Table 2, figure 4,6and 7) Al-Zaban et al. (2021) mentioned that *Trichoderma harzianum* and *Aspergillus flavus* were promising fungi for petroleum oil biodegradation based on dose inhibition response results. On the other hand, *Fusarium solani* recorded the lowest values of DRIP (63, 50 at 10% and 15% diesel oil respectively) as shown in Table 2 and Figure 5. All isolates showed DIRP values ranging from 50% to 88%, which means that all fungal isolates adapted to the media containing different concentrations of diesel oil and can be used in bioremediation for petroleum hydrocarbons. The same fungal species revealed high tolerance to high concentrations of crude oil by Al-Zaban *et al.* (2021). In addition, fungal strains of *Aspergillus terreus*, *Talaromyces spectabilis*, and *Fusarium* sp. were selected to remove polycyclic aromatic hydrocarbons (PAHs) based on tolerance test to crude oil (Anaisell *et al*,2014). Asemoloye *et al*.(2020b) found that dose inhibition response (DIR) was recorded for *Aspergillus oryzae* and *Mucor irregularis* grown in media containing engine oil. The results revealed that both fungi tolerated and adapted to different concentrations of engine oil.

Table 2: Radial growth rate (cm day-1) of fungi isolates in response to diesel oil concentrations.

Isolates of fungi	Deisel oil concentrations				DRIP at 10%	DRIP at 15%
	Control	5%	10%	15%		
Aspergillus terreus	2.80 ± 0.04	2.70 ±0.16	2.0 ±0.01	1.70 ±0.17	71 %	60 %
Aspergillus flavus	3.27 ±0.02	3.15 ± 0.05	2.5 ±0.5	2.30 ± 0.25	76 %	70 %
Trichoderma harzianum	3.64 ± 0.2	3.61 ± 0.03	3.2 ±0.25	3.15 ± 0.05	88 %	86 %
Fusarium solani	2.20 ± 0.3	2.10 ± 0.1	1.4 ±0.013	1.10 ±0.25	63 %	50 %

DIRP: Dose Inhibition Response Percentage.
The values written after ± represents Standard error values.

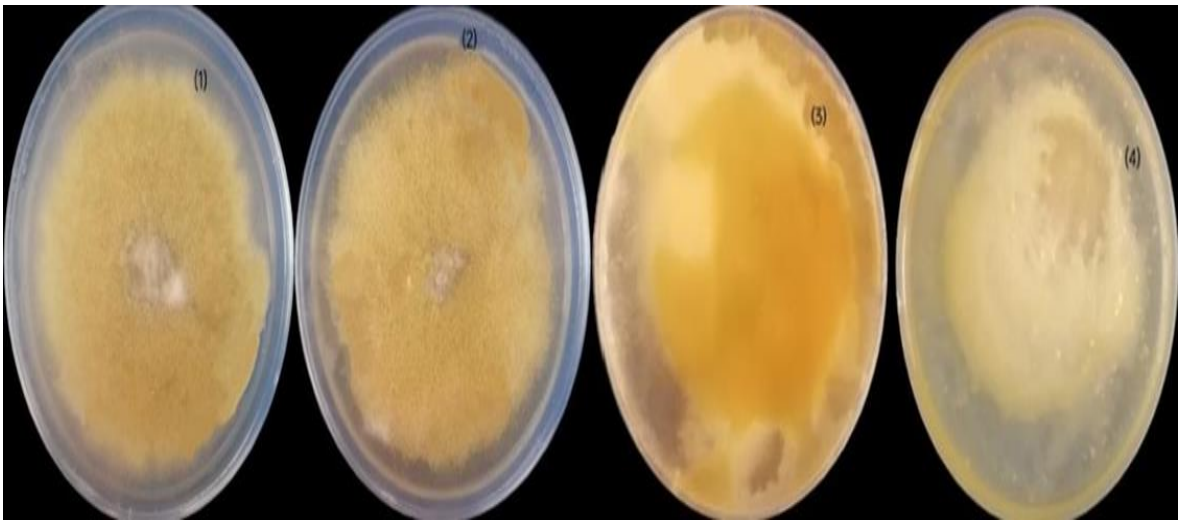

Figure 4: The fungal growth rate of *Aspergillus flavus* was tested on minimal media MM supplemented with different concentration of diesel oil: not treated (1) 5% diesel oil (2) diesel oil (3) 15% diesel oil (4).

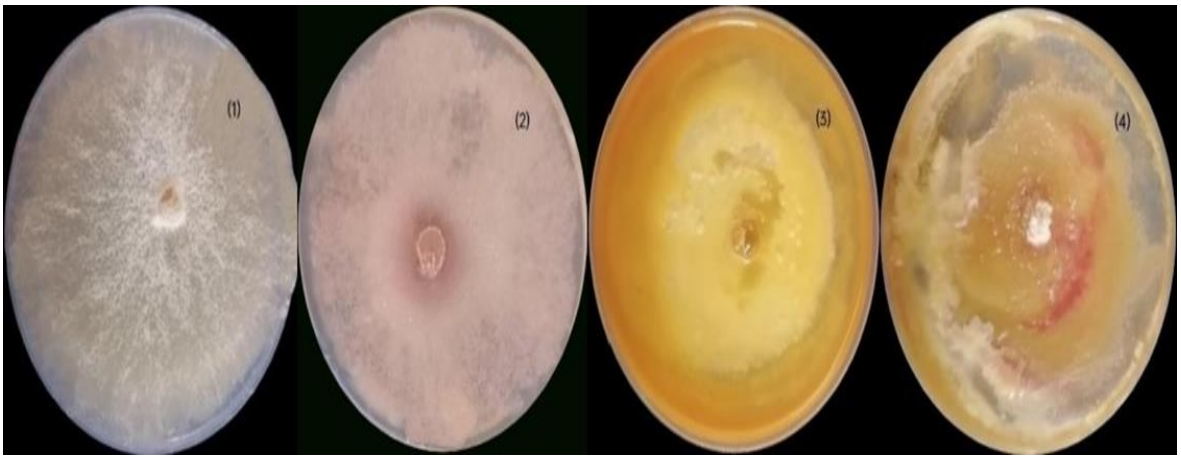

Figure 5: The fungal growth rate of *Fusarium solani* was tested on minimal media MM supplemented with different concentration of diesel oil: not treated (1) 5% diesel oil (2) 10% diesel oil (3) 15% diesel oil (4).

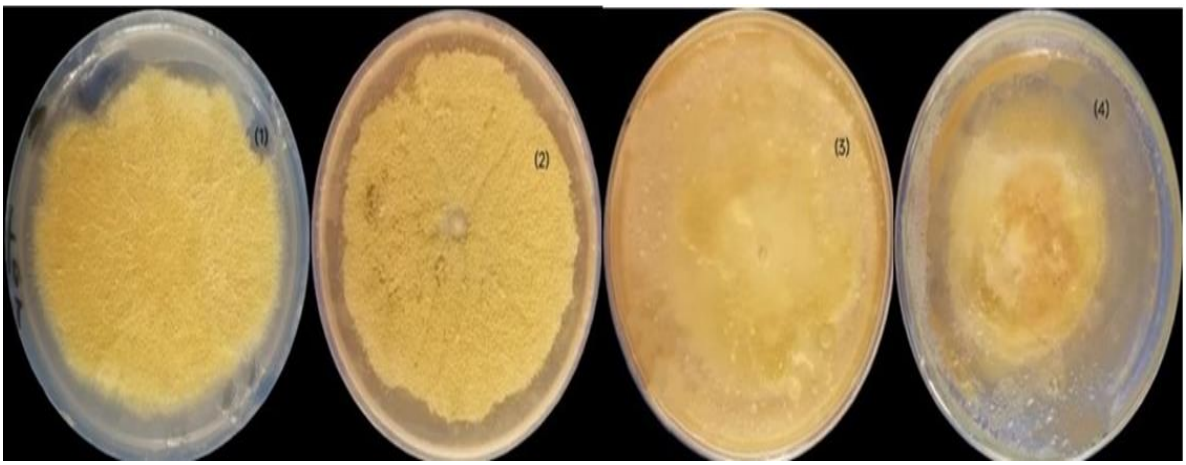

Figure 6: The fungal growth rate of *Aspergillus terreus* on minimal media MM supplemented with different concentration of diesel oil: not treated (1) diesel oil 5% (2) diesel oil 10% (3) diesel oil 15% (4).

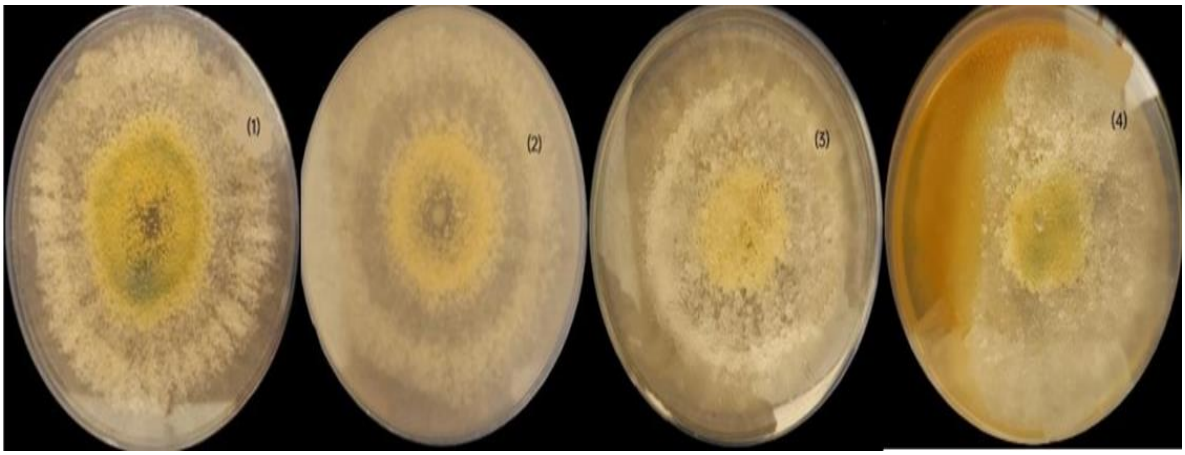

Figure 7: The fungal growth rate of *Trichoderma harzianum* on minimal media MM supplemented with different concentration of diesel oil: not treated (1) diesel oil 5% (2) diesel oil 10% (3) diesel oil 15% (4).

Enzymes production of the tested isolates of fungal species:

The four tested fungal species are filamentous fungi producing extracellular ligninolytic enzymes to biodegrade petroleum hydrocarbon. Most rot fungi produce high redox potential enzymes such as manganese peroxidase (MnP), laccases (Lac), and lignin peroxidases (LiP) for the oxidation of lignin. Range of substances, including pesticides, plastics, and hydrocarbons can be oxidized by these enzymes (Asemoloye *et al*., 2020 a, and Daccò *et al*.,2021). Consequently, the activity of cellulase and Mn peroxidase was recorded in media which are treated with diesel oil and control. Both enzymes cellulase and Mn peroxidase were produced at low levels in control, the highest producer was *Asperagillus flavus* (19.6 U/ml for cellulase and 14.8 U/ml for peroxidase), whereas the lowest producer was *Fusarium solani* (8 U/ml for cellulase and 10 U/ml for Mn peroxidase) as shown in Figures 8 and 9. Different fungal species within the same genus produce different combinations of extracellular enzymes. Consequently, they differ in their ability for biodegradation (Reid, 1995). The fungal isolates used previously in bioremediation, they considered a good producer for LiP, MnP, Cx, and LaC enzymes (Asad *et al.*, 2015).

It was noticed that the enzyme activity increased with the increase of diesel oil (figure 8 and 9) concentrations in the media except *Fusarium solani* revealed a decline in cellulase activity at 15% of diesel oil, and *Fusarium solani & Aspergillus terreus* declined in Mn peroxidase activity at 15% of diesel oil. This response to diesel oil may result from the effect of diesel oil on a regulatory system of gene expression of the genes encoding for cellulase and Mn Peroxidase (Hadibarata *et al*., 2009), the transcription level of laccase, manganese peroxidase and lignin peroxidase genes depends on the nutrients and petroleum hydrocarbon composition (Janusz *et al*., 2013 and Yang J *et al*.,2016). *Aspergillus flavus* and *Trichoderma harzianum* grown on media containing crude oil showed high level of transcription of cellulase genes and MnPeroxidase gene, the increase in gene expression leads to the increase of enzymes production(Al-Zaban *et al*.,2021)

Figures 8 and 9 showed that the increase in cellulase activity was the highest in *Aspergillus flavus* (40,70,78.5 U/ml at 5%,10%, and 15% diesel oil concentration respectively) and *Aspergillus terreus* (32,55.4,60 U/ml at 5%,10%, and 15% diesel oil concentration respectively). It was reported that many *Aspergillus* species including *Aspergillus flavus* have petroleum hydrocarbon biodegradation ability, they converted them to nontoxic compounds (Harms *et al*.,2011; Banerjee *et al*.2016 and Barnes *et al*.,2018). *Aspergillus* spp. revealed significant biodegradation of polycyclic aromatic hydrocarbons such as anthracene, naphthalene, and pyrene (Ye *et al*.,2011; Ali *et al*., 2012 and Baniasadi *et al*. 2018)

On the other hand, the highest increase of Mn peroxidase activity was revealed by *Aspergillus flavus* (50,68.5, and 70.5 U/ml at 5%,10%, and 15% diesel oil concentration respectively) followed by *Trichoderma harzianum* (38.6,49,8 and 56.7 U/ml at 5%,10%, and 15% diesel oil concentration respectively) as shown in figure 8 and 9. Peroxidase enzyme has an important role in petroleum hydrocarbon bioremediation. Fungi used in bioremediation are ligninolytic fungi such as *Phanerochaete chrysosporium, Trametes versicolor*, and *Pleurotus ostreatus* attributed to their good ability to produce peroxidases enzyme (Durán &Esposito,2000 and Kersten &Cullen, 2007). The gene expression of laccase and peroxidase genes in *Aspergillus niger* were correlated with

biodegradation of Nigeria Bonny light crude oil (Asemoloye *et al*.,2017 b,c)

The increase in enzyme activity of both enzymes was low at 15% diesel oil compared to the other concentrations of diesel oil as shown in figures 8 and 9. Finally, the tested fungal species revealed different responses in enzyme activity in the presence of different concentrations of diesel oil. The concentration of diesel oil and its chemical composition affected fungi growth and enzyme production. This result is in accordance with previous research which estimated that fungal growth and enzyme production are significantly affected by carbon and nitrogen concentrations and the chemical composition of petroleum hydrocarbon in growing media (Ronne,1995; Janusz *et al*. 2013; Lombard *et al*.,2014 and Asemoloye *et al*., 2020b) Environmental elements such as carbon, nitrogen, heavy metals concentration, the time of exposure to the light and temperature play an important role to induce gene regulation of peroxidase and laccase(Ramirez *et al*., 2010).

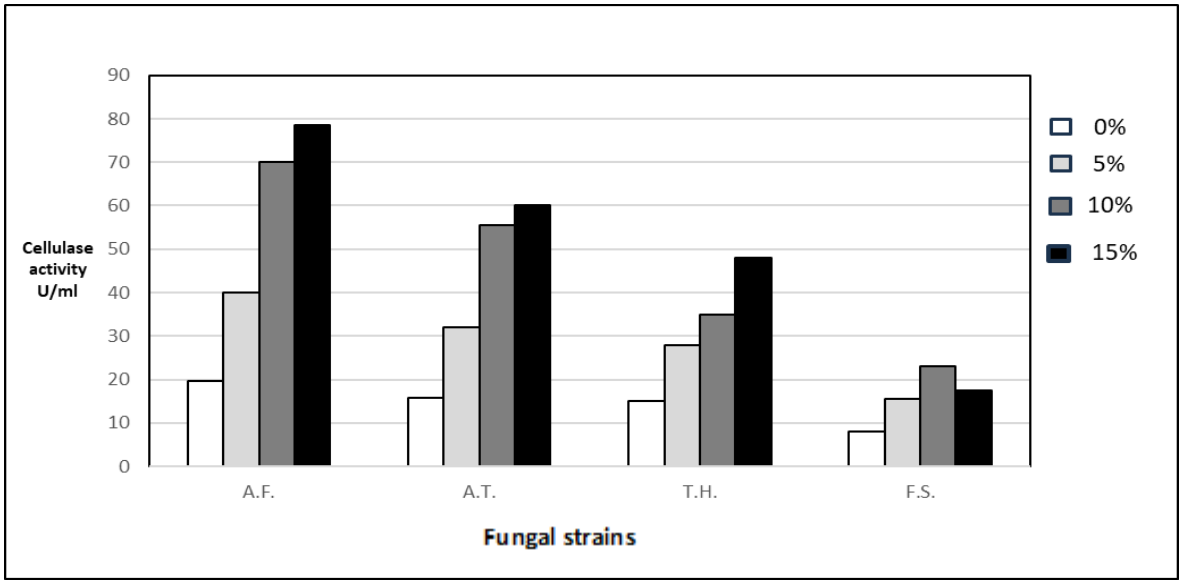

Figure 8: Cellulase activity produced by the tested isolates of fungal species in the presence of different concentrations of diesel oil.

A. F=*Aspergillus flavus* A. T=*Aspergillus terreus*
T.H=*Trichoderma harzianum* F.S.=*Fusarium solani*

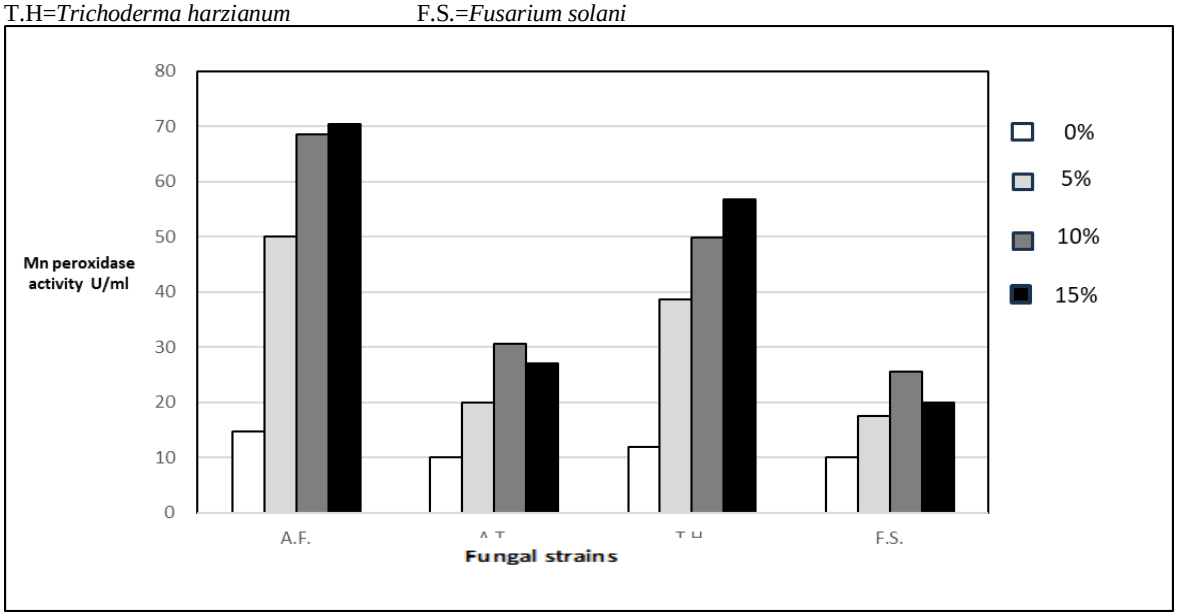

Figure 9: Mn peroxidase activity produced by the tested isolates of fungal species in the presence of different concentrations of diesel oil.

A. F=*Aspergillus flavus* A. T=*Aspergillus terreus*
T.H=*Trichoderma harzianum* F.S.=*Fusarium solani*

Our findings indicate that fungi tolerance to diesel oil was not correlated with enzyme production, *Trichoderma harzianum* had the highest tolerance whereas *Aspergillus flavus* was the highest in enzyme production. previous research confirmed that fungal tolerance to complex hydrocarbons is not a function of their degradation ability, and also that the enzyme-secreting ability in fungi enhances hydrocarbon degradation faster than tolerance (Varjani,2016, and Asemoloye *et al*.,2017b and 2020b).

Asemoloye et al.(2020b) emphasized the importance of fungal isolation from petroleum hydrocarbon-polluted soil screening for high enzyme-producing and hydrocarbon-biodegradable fungi. They isolated *Mucor irregularis* and *Aspergillus oryzae* from contaminated crude oil fields in Nigeria showing highly producing laccase enzymes and good biodegradation. Previously, Mahmoud *et al*. (2015) isolated *A. terreus* strain from kerosene-polluted soil that can produce lipase and degrade petroleum hydrocarbons

CONCLUSION

The fungal isolates were identified as *Fusarium solani* PP979702, *Trichoderma harzianum*PP979703, *Aspergillus terreus*PP979704, and *Aspergillus flavus* PP979705 by using rDNA sequencing of ITS rRNA. The results of the hydrocarbon tolerance test showed that *Trichoderma harzianum* had the highest tolerance towards diesel oil followed by *Aspergillus flavus* with DRIP values equaling 86% and 70% at 15% of diesel oil respectively. In addition, diesel oil induced gene-overexpression for cellulase and Mn peroxidase enzymes. *Aspergillus flavus* was the highest producer of cellulase and Mn peroxidase. Finally, all isolates especially *Aspergillus flavus* and *Trichoderma harzianum* can adapt to high concentrations of petroleum hydrocarbon and produce suitable levels of cellulase and Mn peroxidase enzymes for petroleum hydrocarbon biodegradation. It was recommended that gene expression regulation of ligninolytic enzymes needs a lot of work to understand the mechanism that leads to the increase in ligninolytic enzyme levels with the increase of petroleum hydrocarbon concentrations.

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الملخص العربي

الهدم الحيوي للهيدروكربونات البترولية والتحليلات الجزيئية الميكروبيولوجية المتعلقة لبعض أنواع الفطريات

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يعد التلوث بالهيدروكربونات البترولية أحد التحديات البيئية الرئيسية. ووجد ان المعالجة الحيوية هي أفضل طريقة للتغلب على هذه المشكلة باستخدام الفطريات، فهي أكثر فعالية مقارنة بالبكتيريا. تهدف الدراسة الحالية إلى التحقق من تحمل أربع عزلات فطرية للهيدروكربونات البترولية، وتقدير مستوى إنتاج إنزيمات السليوليز وإنزيم بيروكسيديز المنجنيز المشاركه في التحلل الحيوي للزيت البترولي. ولقد تم تعريف العزلات الفطرية كما يلي: *Fusarium solani PP979702*، *Trichoderma harzianumPP979703*، *Aspergillus terreusPP979704*، و*Aspergillus flavus* PP979705 باستخدام تسلسل ITS rRNA. تم إجراء اختبار التحمل الهيدروكربوني باستخدام بيئة MM الصلبة التي تحتوي على تراكيز مختلفة من زيت الديزل، وتم تسجيل معدل نمو الفطريات وقيم DIRP. تم استخدام بيئات BH السائلة المعاملة وغير المعاملة لاختبار قدرة الفطريات على إفراز الإنزيمات المستهدفة، وأظهرت النتائج أن *Trichoderma harzianum* كان الأكثر تحملاً لزيت الديزل يليه Aspergillus flavus. ووجد ان زيت الديزل تسبب في الإفراط في التعبير الجيني لإنزيمات السليلوز والمنجنيز بيروكسيديز. وكان *Aspergillus flavus* هو الأعلى انتاجا للسليلوليز ومنجنيز البيروكسيديز. وأخيرا، فإن جميع العزلات وخاصة *Aspergillus flavus* و *Trichoderma harzianum* لديها القدرة على التكيف مع التركيزات العالية من الهيدروكربونات البترولية. وبالتالي يمكن استخدامها في المعالجة الحيوية.