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Evaluation of Hydrocarbon Degrading Potentials of Cellulolytic Edaphic Borne Fungi

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Abstract

This study assessed the hydrocarbon degradative potentials of cellulolytic fungi cultured from top soil samples within Benin City, Edo State. Soil samples were collected from 3 sites; a farmland, a fallow plant covered land, and municipal dumpsite. Physicochemical parameters of the soil samples were based on standard methods. Standard cultural and molecular techniques were used to isolate and identify fungi present in samples. Cellulolytic activity of fungal isolates was determined with Czapek-dox agar supplemented with 0.1% sodium carboxymethyl cellulose (CMC). Cellulolytic fungi were subsequently screened for the ability to utilize blended petroleum as sole carbon source. Further, two isolates having the highest optical density were used for growth profile study as axenic culture and in consortium. The growth profile study assessed pH, optical density, total petroleum hydrocarbon content and fungal dry mass. Results showed that the organic carbon value of the top soils ranged from 4.14 g/kg ± 0.55 to 8.56 g/kg ± 0.05. All collected soil samples were sandy, while total hydrocarbon content ranged from 0.38 g/kg ± 0.04 to 1.13 g/kg ± 0.01. The mean fungal counts were highest in dumpsite soils and ranged from 4.0 × 10^3 cfu/g ± 1414 to 7.0 × 10^3 cfu/g ± 1003. Eight fungal isolates with cellulolytic properties were identified from the soil samples. Trichosporon asahii and Curvularia bothriochloae had highest ($p < 0.05$) OD when grown on blended petroleum and were used for growth profile studies. OD was highest ($p < 0.05$) in C. bothriochloae (0.372 ± 0.0005) relative to T. asahii and the consortium at 15 d. THC reduced significantly ($p < 0.05$) in both axenic cultures and the consortium after 15 days, while fungal biomass was highest ($p < 0.05$) in consortium relative to axenic cultures after 15 d. This study showed hydrocarbon degrading capabilities of cellulolytic fungi from different top soils.

INTRODUCTION

Environmental pollution attributed to economic and industrial activities in the upstream and downstream petroleum sector has continued to be an issue despite the increased awareness on the negative environmental effects of crude oil pollution of the surrounding environment (Al-Nasraw, 2012; Abereton *et al*., 2023). Mycelial networks and enzymes production have made several fungi good bioremediation agents (Asemoloye *et al*., 2020a, Harper and Moody, 2023). Mycoremediation has been documented as a viable option in the remediation of petroleum contaminated environments (Al-Dhabaan, 2021; Sadeghian and Mohammadi-Sichani, 2023). Aside from the application of cellulolytic fungi in the bioconversion of agricultural and municipal solid waste streams, this unique group of diverse fungal species can potentially be utilized as

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bioremediation agents (Deshmukh *et al*., 2016; Ramarajan and Manohar, 2017). Cellulolytic ability is comprehensive in many genera in the domain Bacteria and Eucarya (Lynd *et al*., 2002; Ramarajan and Manohar, 2017). Fungal cellulolytic activity is distributed across the entire kingdom, from the primitive, protist-like Chytridomycetes to the advanced basidiomycetes (Lynd *et al*., 2002, Panchapakesan and Shankar 2016). Again, hydrocarbon degrading fungal genera which include; *Graphium Amorphoteca, Talaromyces, Neosartorya*, *Pichia* and *Candida* have been cultured from various crude oil polluted soils (Das and Chandran 2011; Mansi *et al*., 2018). Several terrestrial fungi such as *Pencillium, Cephalosporium* and *Aspergillus* have been known to exhibit appreciable petroleum hydrocarbon degrading attributes (Singh, 2006; Vaksmaa *et al*., 2023). Several fungal genera, primarily ascomycetes which belong to the classes Sordariomycetes (i.e., *Fusarium*) and Eurotiomycetes (i.e., *Aspergillus* and *Penicillium*), and the previously named zygomycetal phylum, are also ubiquitous in contaminated soil environments, and can metabolize a broad range of organic moieties like sugars, cellulose, starch, proteins and lipids, as well as petroleum hydrocarbons, including PAHs, through the activity of intracellular P450 cytochrome monooxygenases (CYPs) (Aranda *et al*., 2017).

Although, hydrocarbon degraders could be expected to be readily cultured from a petroleum contaminated environment, similar degree of expectation can be envisaged for microorganisms cultured from pristine or semi pristine environments (Ojo, 2009, Abioye *et al*., 2011). The remediation of petroleum contaminated edaphic environments has increasingly become a focal point of interest among scientists and environmentalists in the course of the preceding decades (Asemoloye *et al*. 2020a). Biological remediation has become a very attractive option amongst other approaches utilized for pollution containment, as it is non-evasive, low-cost incurring, environmentally friendly and sustainable (Asemoloye *et al*., 2020a). With respect to the various bioremediation techniques, microbial degradation has been adopted and applied mostly for the treatment of hydrocarbon contaminated soils (Asemoloye *et al*. 2020b).

Biodegradation rates may slow down as a result of ageing in edaphic environments thereby, end-point levels of HMW crude oil fractions could stabilize at levels that present itself as an unacceptable environmental risk (Ren *et al*., 2018; Medaura *et al*., 2021). The near total reliance on petroleum products as source of energy in both the developed and developing countries has created the enabling situations for the widespread environmental distribution of substantial concentrations of petroleum hydrocarbons. This study therefore evaluated the hydrocarbon degradative potentials of known cellulolytic fungi cultured from several edaphic environments.

METHODOLOGY

Soil Sample Collection

Top soil samples (100 g) were collected in replicate at a depth of 0-15 cm from a farm, a fallow land plot both sited within the Ugbowo campus of the University of Benin, and a municipal dumpsite located at Ikueniro district of Benin City. All collected soil samples were dispensed onto respectively sterile labelled polythene bags.

Physicochemical Assessment of Soil Samples

Soil samples were air-dried for 72 h, sieved using a 2 mm mesh and physiochemical properties were determined. Organic carbon content was based on chromic acid wet oxidation procedure (Black, 1965), mechanical analysis of the soil was carried out by the hydrometer method after the

destruction of organic matter with hydrogen peroxide (Owabor and Ogunbor, 2007) and total hydrocarbon content (THC) was based on the procedure of Onyeonwu (2000).

Enumeration and Isolation of Heterotrophic Fungi

Soil sample (10 g) was suspended in ninety (90) mL of sterilized peptone water in a conical flask, mixed and diluted to 10^{-8} using tenfold serial dilution. Total heterotrophic fungal count was done using Potato Dextrose agar (PDA) and was based on pour plate technique (Harley and Prescott, 2002). Inoculated plates were allowed to solidify before incubation at 28 ± 2°C for 5 days.

Subculturing and Molecular Identification of Soil Mycoflora

Discrete fungal colonies were purified (sub-cultured) via the transfer of a portion of the surface mycelia to an enriched peptone water which contained malt extract (10 g/L), glucose (10 g/L) and 1mL of the antibiotic solution. The pH of the medium was adjusted to 7.2 and broth cultures were incubated at room temperature (28 ± 2°C) for 5 days. One (1) mL of the resultant submerged fungal biomass from each flask was transferred using pour plate method to modified water agar plates and pure isolates were subsequently stored in PDA slants for further studies.

DNA Extraction and PCR Amplification of Fungal Isolates

ZR Fungal/Bacterial DNA extraction protocol was used for the fungal DNA extraction procedure. Touchgene Thermal Cycler (Barloworld Scientific Ltd, United Kingdom) was used for PCR amplification. Universal primer pair ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA-3') and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') respectively were used to amplify the internal transcribed spacer (ITS) regions of rDNA (Gardes and Bruns, 1993).

Sequence Alignment and Phylogenetic Analysis

The resultant ITS sequences were explored for homology with other fungal genera and species in the GenBank database using NCBI BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/). Clustal X program (version 2.0) aided alignments, and phylogenetic analyses were done with the PHYLIP 3.69 package using Dnadist program. Distance trees were assembled with the neighbor-joining (NJ) method (http://evolution.genetics.washington.edu/phylip.html) (data not shown). Bootstrap values were estimated with the Seqboot (1000 replicates), Neighbor and Consense programs (Yuan *et al*., 2011).

Determination of Cellulolytic Activity of the Identified Fungal Isolates

Czapek-dox agar with sodium carboxymethyl cellulose (0.1%) was used to evaluate the cellulolytic activity of fungal isolates. Matured fungal colonies on the agar surface was mixed with Congo red indicator (0.1 %) and incubated at room temperature (28 ± 2°C) for 15 mins. Subsequently, the dye was discharged and 1M NaCl solution (5 mL) added and the agar plates were incubated at room temperature for 10 mins. Metre rule was used to determine the ratio of the diameter of the clear zone to the diameter of the colony (Doolotkeldieva and Bobusheva, 2011).

Screening cellulolytic fungi ability to use blended petroleum as carbon source

Cellulolytic fungi ability to utilize blended crude oil was according to Obayagbona and Enabulele (2013). Mineral salt medium (MSM; 9 ml) was prepared in test tubes. Sterile Forcados blended crude oil (1 mL) was then placed into each tube and autoclaved at 121°C for 15 mins. Subsequently, cooled tubes were inoculated with 1 mL of each fungal isolate. Suspension was

prepared by placing a portion of the cellulolytic fungal mycelia from the modified water agar plate to obtain similar cell counts of 1.0 $\times 10^3$ sfu/ mL while the control test tube was not inoculated. All samples were incubated for 14 days thereafter, OD (690nm) was measured with a visible UV camp spectrophotometer (Biobase model BK-UV1800PC).

Growth profile of single and mixed consortium of fungi in blended crude oil

Growth profiles of two cellulolytic fungi with maximal differential optical density within the respective incubation period for the screening test was ascertained by the method of Okpokwasili and Okorie (1988). Prepared MSM (2 L; pH 7.2) was dispensed at 250 mL volume onto several conical flasks (500 mL) and 2.5 mL of blended Forcados crude oil was then added to each of the flask. Flasks were autoclaved at 121^{o}C for 15 mins and cooled subsequently, 2 mL (1.0 x 10^3 sfu/mL) of a 96 h MSM broth culture of the two cellulolytic fungi (singly and in consortium) with maximal differential OD was introduced into each respective flask under aseptic conditions while the control had no inoculum introduced into the flasks. All flasks were incubated on an incubator shaker (120 rpm) at 28 ± 2^{o}C for 15 days (Heidolph Unimax 2010). Indicators of blended crude oil utilization [pH, turbidity, residual total petroleum hydrocarbon (TPH) and dry fungal weight (biomass) were measured in each flask.

Statistical Analysis

Analysis of Variance (ANOVA) was used for data analyses at 95 percent probability level. Significant differences in mean values were determined with Duncan Multiple Range (DMR) tests.

RESULTS

Three edaphic environments (a farm, a fallow land plot and a municipal dumpsite) were assessed for cellulolytic fungi, and two cellulolytic fungi with maximal differential optical density for blended crude oil utilization were further used for crude oil degradative studies. The mean physicochemical properties of the three studied soils are shown in Table 1. Total organic carbon was higher in dumpsite soil sample relative to the other soils, while particle size analyses showed higher sand content than silt and clay. Table 2 shows the mean fungal counts from studied top soils, this ranged from 2.7×10^3 cfu/g ± 866 to 3.0×10^3 cfu/g ± 103 to for Farm soils while that of the fallow plant covered land varied from 4.5 × 10^3 cfu/g ± 707 to 6.5 × 10^3 cfu/g ± 901. Again, that of the top soils from the municipal dumpsite ranged from 4.0 × 10^3 cfu/g ± 1414 to 7.0 × 10^3 cfu/g ± 1003 (Table 2).

The molecular identification of fungal isolates is shown in Table 3.

Table 1: Mean physicochemical properties of the three studied soils

Soil sample	Organic carbon content (g/kg)	Particle size (g/kg)			THC (mg/kg)
		Sand	Silt	Clay	
F_1	4.14 ± 0.55^a	843.0 ± 0.0^a	101.0 ± 0.0 ^{a}	56.0 ± 0.0 ^{a}	0.87 ± 0.15^a
F_2	5.02 ± 0.05^a	835.3 ± 4.51^a	123.7 ± 1.5^a	41.0 ± 6.0^a	1.04 ± 0.02^a
F_3	5.10 ± 0.08 ^{a}	818.3 ± 1.53^a	113.3 ± 2.1^a	68.3 ± 1.2^a	0.95 ± 0.05^a
FP_1	6.55 ± 0.05^b	854.0 ± 0.0^a	106.0 ± 0.0^a	46.0 ± 0.0^a	1.13 ± 0.01^a
FP_2	6.69 ± 0.02^b	821.7 ± 2.08^a	105.0 ± 1.0^a	73.3 ± 1.53^a	0.51 ± 0.03 ^{a}
FP_3	6.42 ± 0.02^b	840.7 ± 4.04^a	113.0 ± 2.7^a	46.3 ± 1.53^a	0.38 ± 0.04^a
DS_1	8.56 ± 0.05^c	857.7 ± 1.15^a	118.3 ± 0.6^a	42.0 ± 1.73^a	0.68 ± 0.05^a

DS_2	7.85 ± 0.04^c	852.3 ± 2.52^a	119.3 ± 1.5^a	38.3 ± 1.15^a	1.04 ± 0.02^a
DS_3	7.52 ± 0.06^c	848.0 ± 0.0^a	115.0 ± 0.0^a	37.0 ± 0.0^a	1.12 ± 0.05^a
Significance	P<0.05	P>0.05	P>0.05	P>0.05	P>0.05

KEY: F; Farmland, FP; Fallow plot, DS; Dumpsite, THC; Total hydrocarbon content, P < 0.05-Significant, P > 0.05; Not significant. Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

Table 2: Mean fungal counts obtained from the three studied soils

Soil sample	Mean fungal counts (cfu/g) ± Std. deviation
F_1	$3.0 \times 10^3 \pm 103^a$
F_2	$3.0 \times 10^3 \pm 120^a$
F_3	$2.7 \times 10^3 \pm 866^a$
FP_1	$6.5 \times 10^3 \pm 901^a$
FP_2	$4.5 \times 10^3 \pm 707^a$
FP_3	$5.5 \times 10^3 \pm 1200^a$
DS_1	$5.5 \times 10^3 \pm 807^a$
DS_2	$7.0 \times 10^3 \pm 1003^a$
DS_3	$4.0 \times 10^3 \pm 1414^a$
Significance	P > 0.05

KEY: F; Farmland, FP; Fallow plot, DS; Dumpsite, P > 0.05; Not significant. Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

Table 3: Fungal isolates identified using ITS nucleotide sequencing and blast analysis

Sequence ID	Accession No	Fungal specie	% Similarity
Isolate 1	MN180808.1	*Aspergillus niger*	95.38
Isolate 2	OM956832.1	*Fusarium verticillioides*	87.89
Isolate 3	MH270567.1	*Aspergillus phoenicis*	97.18
Isolate 4	MK751714.1	*Fusarium proliferatum*	92.86
Isolate 5	MN809420.1	*Trichosporon asahii*	86.65
Isolate 6	KJ415543.1	*Curvularia bothriochloae*	80.21
Isolate 7	DQ177877.1	*Geotrichum candidum*	94.76
Isolate 8	KY101879.1	*Candida albicans*	82.74

The cellulolytic activity of the identified fungal isolates is presented in Table 4. *A. phoenicis* displayed the least cellulolytic activity (5.3 mm) amongst the isolates, while *C. albicans* exhibited maximal cellulolytic activity (28.7 mm).

Results of the screened hydrocarbon degrading potential of the cellulolytic fungal isolates is shown in Table 5. The two cellulolytic isolates which displayed the highest hydrocarbon degradation potentials as indicated by differences in their optical density values were *T. asahii* (0.663) and *C. bothriochloae* (0.983), while that of the least was *G. candidum* with difference with OD of 0.143 value.

pH, turbidity, residual total petroleum hydrocarbon (TPH) and dry fungal weight (biomass) were used as indicators of blended crude oil utilization and results generally showed reduction (p <

0.05) in pH with time. pH readings for *T. asahii* ranged from 7.23 ± 0.02 at 0 day to 7.09 ± 0.03 at day 15, while that of the consortium was 7.37 ± 0.02 at 0 day to 7.08 ± 0.03 at day 15. Generally, OD increased with time in both axenic and consortium cultures but not in the control. The mean optical density (OD) value was highest ($p < 0.05$) in *C. bothriochloae* (0.372 ± 0.05) relative to *T. asahii* and the consortium but was least in the control (0.031 ±0.05). Generally, mean TPH values decreased ($p < 0.05$) with time in both the axenic and consortium cultures but not in the control. *T. asahii* and *C. bothriochloae* recorded 0.581 ± 0.001 and 0.580 ± 0.0001 at day 15 relative to 1.117 ± 0.001 and 1.058 ± 0.001 at day 0 respectively, same pattern was observed with the consortium. Overall, there was increase in fungal biomass in both axenic and consortium cultures but maximal ($p < 0.05$) weighed fungal biomass was retrieved from the flasks seeded with the consortium of the cellulolytic hydrocarbon degraders.

Table 4: Cellulolytic activity of the fungal isolates

Isolate	Mean clearing zone (mm)
Aspergillus niger	7.7 ± 0.01^{c}
Fusarium verticillioides	7.0 ± 0.01^{b}
Aspergillus phoenicis	5.3 ± 0.03^{a}
Fusarium proliferatum	15.3 ± 0.03^{e}
Trichosporon asahii	14.0 ± 0.01^{d}
Curvularia bothriochloae	16.7 ± 0.03^{f}
Geotrichum candidum	20.3 ± 0.001^{g}
Candida albicans	28.7 ± 0.003^{h}

Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

Table 5: Screened hydrocarbon degrading potential of the cellulolytic fungal isolates

Isolate	Day 0 (mean OD reading)	Day 14 (mean OD reading)	Difference between mean OD values	Decision
A. niger	0.114 ± 0.03^{a}	0.287 ± 0.01^{b}	0.173 ± 0.01^{ab}	Not selected
F. verticillioides	0.107 ± 0.01^{a}	0.283 ± 0.03^{b}	0.176 ± 0.02^{ab}	Not selected
A. phoenicis	0.087 ± 0.01^{a}	0.124 ± 0.01^{a}	0.217 ± 0.02^{b}	Not selected
F. proliferatum	0.107 ± 0.03^{a}	0.523 ± 0.02^{c}	0.465 ± 0.01^{d}	Not selected
T. asahii	0.05 ± 0.02^{a}	0.613 ± 0.02^{d}	0.663 ± 0.01^{e}	Selected
C. bothriochloae	$0.097\pm0,04^{a}$	1.08 ± 0.03^{e}	0.983 ± 0.02^{f}	Selected
G. candidum	0.11 ± 0.01^{a}	0.253 ± 0.02^{b}	0.143 ± 0.03^{a}	Not selected
C. albicans	0.118 ± 0.04^{a}	0.520 ± 0.02^{c}	0.402 ± 0.01^{c}	Not selected

Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

Table 6: Mean pH values for the growth profile isolates on blended petroleum-mineral salt medium

Isolates	Day 0	Day 5	Day 10	Day 15
T. asahii	7.23 ± 0.02^{b}	7.07 ± 0.01^{a}	7.65 ± 0.03^{c}	7.09 ± 0.03^{a}
C. bothriochloae	7.20 ± 0.01^{b}	7.08 ± 0.02^{a}	7.62 ± 0.02^{c}	7.24 ± 0.01^{b}
Fungal consortium	7.37 ± 0.02^{b}	7.09 ± 0.02^{a}	7.57 ± 0.03^{c}	7.08 ± 0.03^{a}
Control	6.99 ± 0.03^{b}	6.99 ± 0.01^{b}	7.14 ± 0.015^{c}	6.79 ± 0.01^{a}

Means with similar superscript along a row is indicative of no significant difference, means with different superscript along a row indicate significant difference.

Table 7: Mean optical density values for the growth profile isolates on blended petroleum-mineral salt medium

Isolates	Day 0	Day 5	Day 10	Day 15
T. asahii	0.106 ± 0.02^{b}	0.232 ± 0.01^{ab}	0.332 ± 0.04^{b}	0.249 ± 0.01^{b}
C. bothriochloae	0.233 ± 0.01^{b}	0.301 ± 0.05^{bc}	0.308 ± 0.05^{b}	0.372 ± 0.05^{c}
Fungal consortium	0.135 ± 0.01^{b}	0.332 ± 0.01^{c}	0.272 ± 0.03^{b}	0.254 ± 0.01^{b}
Control	0.009 ± 0.02^{a}	0.030 ± 0.01^{a}	0.049 ± 0.05^{a}	0.031 ± 0.05^{a}

Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

Table 8: Mean total petroleum hydrocarbon (TPH) (mg/l) values for the growth profile isolates on blended petroleum-mineral salt medium

Isolates	Day 0	Day 5	Day 10	Day 15
T. asahii	1.107 ± 0.01^{a}	0.618 ± 0.05^{b}	0.598 ± 0.05^{b}	0.581 ± 0.01^{b}
C. bothriochloae	1.058 ± 0.01^{a}	0.616 ± 0.05^{b}	0.598 ± 0.05^{b}	0.580 ± 0.01^{b}
Fungal consortium	1.055 ± 0.05^{a}	0.616 ± 0.05^{b}	0.598 ± 0.04^{b}	0.582 ± 0.01^{b}
Control	1.106 ± 0.06^{a}	1.106 ± 0.01^{a}	1.044 ± 0.01^{a}	1.021 ± 0.06^{a}

Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

Table 9: Mean dry biomass weight (g) for the growth profile isolates on blended petroleum-mineral salt medium

Isolates	Day 0	Day 15
T. asahii	0.20 ± 0.01^{a}	0.78 ± 0.01^{b}
C. bothriochloae	0.23 ± 0.01^{a}	0.69 ± 0.01^{a}
Fungal consortium	0.21 ± 0.02^{a}	0.85 ± 0.01^{c}

Means with similar superscript along a column is indicative of no significant difference, means with different superscript along a column indicate significant difference.

DISCUSSION

Three edaphic environments were assessed for cellulolytic fungi, and two cellulolytic fungi with maximal differential optical density for blended crude oil utilization were further used for crude oil degradative studies. The organic carbon values of the studied top soils showed that municipal dumpsite soil had the highest values ($p < 0.05$). This could be as a result of accumulation of decomposed litre in the dumpsite soils thereby, making the soil more structurally stable (Ikechukwu-Edeh *et al*., 2021). Vijayalakshmi *et al*. (2020) demonstrated low pollution level and low ecological risk of soils near solid waste landfill site. Generally, studied soils were sandy, and THC of soil samples were low. The low THC of studied soils could be due to minimal exposure to hydrocarbons since the soils were from a farm and a fallow plot. However, that of dumpsite soils could be because it is not close to major roads with high automobile traffic.

The observed variations in the top soils borne fungal counts was not statistically significant ($P > 0.05$). Factors such as land conversion patterns and use of fungicides can affect the diversity of fungi in different soils (Hamad *et al.* 2021). Eight fungal isolates (*Aspergillus niger*, *Fusarium*

verticillioides, Aspergillus phoenicis, Fusarium proliferatum, Trichosporon asahii, Curvularia bothriochloae, Geotrichum candidum and *Candida albicans*) were identified molecularly from the soil samples (Table 3). Two of these identified cultures were yeasts; *T. asahii* and *C. albicans.* Generally, Ascomycota and Basidiomycota group of fungi have been reported in fallow land and forest soils (Jaboro *et al*., 2019).

The isolation of cellulolytic fungi from the respective soil samples suggested that these studied edaphic habitats are nutritionally capable of supporting the growth of cellulolytic fungi. The detection of cellulolytic fungi from top soil samples has also been reported by several researchers (Khokhar *et al*., 2012; Ja'afaru *et al*., 2013; Muhammad, *et al*., 2021). However, the definitive identities of the cellulolytic fungi in this study contrasted with fungal isolates previously described by Khokhar *et al.* (2012), while the highest cellulolytic activities in this study were observed in yeast isolates theirs, indicated cellulolytic *Trichoderma* spp. and *Penicillium* spp. Again, Bairagi, (2016) documented the culturing of cellulolytic *Trichoderma* spp. from soil samples collected at various regions within Haryana, India. Isolation of cellulolytic and cellulase producing *A. niger* from different soil samples was reported by Ja'afaru *et al.* (2013) and Muhammad (2021). Similarly, this study also isolated *A. niger* but the other fungal isolates were different from those reported by Ja'afaru *et al.* (2013) and this could be attributed to differences in soil characteristics and conditions.

Two cellulolytic fungal isolates (*T. asahii* and *C. bothriochloae*) displayed the highest hydrocarbon degradation potentials as indicated in the difference in optical density values (Table 5). The ability of soil borne fungi to degrade different hydrocarbon moieties present in crude oil has been reported by several authors (Das and Chandran, 2011; Al-Nasraw, 2012; Hamad *et al*., 2021). The specific hydrocarbon degrading potential of *Curvularia bothriochloae* in particular was reported by Hamad *et al.* (2021) which documented the hydrocarbon degrading ability of soil borne fungi from Asal oil field, West Sinai, Egypt. The detection of just two proficient fungal hydrocarbon degraders in this study contrasted with results reported by Hamad *et al.* (2021) which detailed the isolation of about six soil borne hydrocarbon degrading fungi.

pH, turbidity, residual total petroleum hydrocarbon (TPH) and dry fungal weight were used as indicators of blended crude oil utilization using shake flask. Results generally showed reduction ($p < 0.05$) in pH with time. pH values control fungal catabolic activities and composition in soil therefore reduction in pH with time could be as a result of produced organic acids as a result of crude oil degradation (Sari *et al*., 2019). Organic acids and other metabolic products are usually a result of microbial degradation of hydrocarbons (Al-Jawhari, 2014; Sari *et al*., 2019). Generally, OD increased with time in both axenic and consortium cultures but not in the control. The mean optical density (OD) value was highest ($p < 0.05$) in *C. bothriochloae* (0.372 ± 0.05) relative to *T. asahii* and the consortium at day 15. This increase in OD is indicative of growth (aggregation of the mycelia or cellular mass) of these fungal isolates with time as they use up the carbon source present in the crude oil. This is usually accomplished by release of extracellular enzymes and acids which are capable of breaking down the recalcitrant hydrocarbon molecules, and transforming petroleum into simpler forms or products that can be absorbed for fungal growth and nutrition (Sari *et al*., 2019). Generally, mean TPH values decreased ($p < 0.05$) with time in both the axenic and consortium cultures but not in the control. This observation also collaborated results documented from the hydrocarbon screening test which revealed maximal OD of *T. asahii* and *C. bothriochloae* amongst all the other screened cellulolytic fungal cultures. This could be as

a result of the uptake and assimilation of petroleum hydrocarbons present in the culture medium and a higher tolerance of likely toxic end products generated in the process of hydrocarbon assimilation and metabolism by these fungi. Different studies had demonstrated the biodegradation of petroleum hydrocarbon using fungal isolates (Al-Jawhari. 2014; Marchand *et al*., 2017; Al-Hawash *et al*., 2019; Hamad *et al*., 2021; Novianty *et al*., 2021). Al-Jawhari. (2014) reported 90% loss of crude oil by mixed cultures of *A. niger* and *A. fumigatus* from petroleum hydrocarbon polluted soil. Similarly, petroleum hydrocarbon degrading efficiency of two filamentous non-lignolytic fungi (*Trichoderma tomentosum* and *Fusarium oxyspoum*) was demonstrated by Marchand *et al*. (2017). Again, FC1 (*Penicillium chrysogenum* and *Curvularia brachyspora*) and FC2 (*Scopulariopsis brevicaulis* and *Stemphylium botryosum*) consortia exhibited positive synergistic effects with highest significant PHs deterioration abilities than did the individual isolates during petroleum hydrocarbon bioremediation with native fungal isolates (Hamad *et al*., 2021). Olukunle and Oyegoke, (2016) reported an efficient degradation (66,2%) of crude oil from a cow dung fungal isolate (*Trichoderma viridae*). Overall, there was increase in fungal biomass in both axenic and consortium cultures but maximal ($p < 0.05$) weighed fungal biomass was retrieved from the flasks seeded with the consortium of the cellulolytic hydrocarbon degraders after crude oil degradation. This is indicative of the use of the crude oil as a carbon source for increase in cell biomass with time. Daassi and Almaghrabi, (2023) reported improved TPH biodegradation rates for petroleum-degrading fungal isolates with incubation time. The higher dry biomass recorded by *Drechslera spicitera* during biodegradation of used oil relative to mixed oil was indicative of its biodegradation of different hydrocarbons (Al-Zahrani *et al*., 2022).

CONCLUSION

Eight fungal isolates with cellulolytic properties were identified from the soil samples in this study. The ability of identified soil borne fungal isolates to breakdown cellulose was observed, as well as hydrocarbonoclastic activities of two of the cellulolytic cultures; *T. asahii* and *C. bothriochloae*. TPH reduced in both axenic cultures and the consortium after 15 days, while fungal biomass increased with time. Conclusively, hydrocarbon degrading capabilities of *T. asahii* and *C. bothriochloae* is presented and evaluation of the specific type of cellulolytic activity of these soil borne cellulolytic fungi is recommended.

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