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Assessment of the amylase activities of aquatic fungi isolated from warm springs of Yankari Game Reserve, Bauchi State, Nigeria

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ABSTRACT

Aquatic fungi are important components of freshwater ecosystems and are recognized for their ability to produce industrially relevant enzymes and degrade environmental pollutants. However, information on the amylolytic potential of aquatic fungi inhabiting the warm springs of Yankari Game Reserve, Bauchi State, Nigeria, remains limited. This study assessed the amylase activity of aquatic fungi isolated from these warm springs and evaluated their potential for hydrocarbon biodegradation. Water samples were collected from four warm springs (Dimil, Gwana, Wikki, and Mawulgo), and fungal isolates were obtained using the baiting technique, followed by purification and identification based on morphological characteristics. Crude amylase was produced through submerged fermentation, and enzyme activity was determined using the dinitrosalicylic acid (DNS) method. The fungal isolates were further evaluated for viable counts, biomass accumulation, and biodegradation of waste engine oil (WEO), diesel, and petrol using standard microbiological and gravimetric techniques. Data were analysed using one-way analysis of variance (ANOVA), with statistical significance set at $P < 0.05$. Five aquatic fungal genera were identified: *Achlya*, *Aphanomyces*, *Saprolegnia*, *Leptolegnia*, and *Brevilegnia*. *Saprolegnia* spp. exhibited the highest occurrence (36.36%), whereas *Achlya* spp. recorded the highest amylase activity (0.93 µmol/min) on day 3, followed by *Aphanomyces* spp. (0.73 µmol/min), with significant differences observed among the isolates ($P < 0.05$). During WEO utilization, all fungal isolates showed progressive increases in viable counts and biomass, indicating active growth on hydrocarbons. *Aphanomyces* spp. demonstrated the highest biodegradation efficiency, achieving 50.09%, 48.23%, and 42.24% degradation of WEO, diesel, and petrol, respectively. These findings demonstrate that the warm springs of Yankari Game Reserve harbour aquatic fungi with significant amylolytic and hydrocarbon-degrading capabilities. The indigenous fungal isolates, particularly *Achlya* spp. and *Aphanomyces* spp., possess considerable potential for industrial enzyme production and the bioremediation of hydrocarbon-contaminated environments.

Keywords: Aquatic fungi, amylase activity, *Achlya*, *Aphanomyces*, hydrocarbon biodegradation, bioremediation.

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INTRODUCTION

Aquatic fungi constitute an ecologically important group of microorganisms that inhabit freshwater, marine, and geothermal aquatic environments, where they play indispensable roles in the decomposition of organic matter, nutrient cycling, and the maintenance of ecosystem productivity (Grossart et al., 2019; Wurzbacher et al., 2017). Through the secretion of extracellular hydrolytic enzymes, these fungi degrade complex organic polymers such as cellulose, lignin, proteins, lipids, and starch into simpler compounds that become available to other organisms within aquatic food webs (Jones et al., 2019). Beyond their ecological functions, aquatic fungi have attracted increasing attention because of their considerable biotechnological potential. They represent valuable sources of industrial enzymes, antimicrobial compounds, pharmaceuticals,

bioactive metabolites, and environmentally friendly biocatalysts suitable for food processing, textile manufacturing, paper production, detergent formulation, biofuel production, and environmental bioremediation (Grossart et al., 2019; Hyde et al., 2019). Consequently, the continuous search for novel fungal isolates capable of producing robust extracellular enzymes has become an important focus of microbial biotechnology, particularly among fungi isolated from extreme environments, where enzymes often exhibit superior physicochemical properties.

Among the hydrolytic enzymes produced by fungi, α-amylase (EC 3.2.1.1) is one of the most commercially important because it catalyses the hydrolysis of α-1,4-glycosidic bonds in starch, yielding dextrins, maltose, and glucose (Pandey et al., 2000). Amylases account for approximately 25–30% of the global enzyme market due to their extensive applications in starch liquefaction, brewing, baking, paper manufacturing, textile desizing, detergent formulation, pharmaceutical production, confectionery processing, and bioethanol industries (Souza and Magalhães, 2010). Although bacteria are important producers of industrial amylases, fungi are generally preferred because they secrete large quantities of extracellular enzymes directly into the fermentation medium, thereby simplifying enzyme recovery and purification while often exhibiting greater substrate specificity (Gupta et al., 2003). Thermophilic fungi are particularly attractive sources of industrial amylases because they produce thermostable enzymes capable of retaining catalytic activity at the elevated temperatures commonly employed during industrial starch processing. Such thermostable enzymes enhance process efficiency, reduce contamination risks, improve substrate solubility, and lower production costs, thereby increasing industrial productivity (Maheshwari et al., 2000; Sharma et al., 2022).

Warm springs are unique geothermal ecosystems characterized by elevated temperatures, dissolved minerals, and distinctive physicochemical conditions that support specialized communities of thermophilic and thermotolerant microorganisms (Maheshwari et al., 2000). Fungi inhabiting these environments have evolved adaptive mechanisms that enable them to withstand thermal stress through the production of heat-stable proteins and enzymes with exceptional catalytic efficiency (Coker, 2016). Consequently, geothermal habitats have become attractive reservoirs for the discovery of novel fungal strains possessing industrially important enzymes, including amylases, cellulases, lipases, proteases, and xylanases (Singh and Kuhad, 2016). Recent advances in microbial biotechnology have emphasized the exploration of extreme environments because microorganisms isolated from such habitats frequently exhibit unique metabolic pathways and enzyme systems that are absent or less pronounced in mesophilic organisms (de Oliveira et al., 2020). Despite the growing global interest in extremophilic fungi, geothermal ecosystems in Africa remain largely underexplored, suggesting that numerous indigenous fungal species with significant industrial potential remain undiscovered.

Yankari Game Reserve, located in Bauchi State, northeastern Nigeria, is internationally recognized for its abundant wildlife, rich biodiversity, and naturally occurring warm springs, including the well-known Wikki, Mawulgo, Gwana, and Dimil springs. These geothermal water bodies maintain relatively stable temperatures throughout the year, thereby providing suitable ecological niches for thermophilic aquatic fungi and other extremophilic microorganisms (Lamido, 2025). Although several studies have investigated the ecological characteristics, tourism potential, and biodiversity of Yankari Game Reserve, information regarding the diversity and extracellular enzyme production of aquatic fungi inhabiting these warm springs remains scarce. In particular, little is known about their capacity to produce industrially relevant amylases despite the recognized importance of thermophilic fungi as producers of thermostable enzymes (Hyde et al., 2019). Characterizing the amylase-producing potential of aquatic fungi from these geothermal ecosystems would therefore contribute significantly to microbial biodiversity research while identifying indigenous fungal resources with potential applications in industrial enzyme production and biotechnology.

Assessing the amylase activities of aquatic fungi isolated from the warm springs of Yankari Game Reserve is therefore of considerable scientific, industrial, and environmental importance. Quantitative evaluation of amylase production among fungal isolates will facilitate the identification of high-yielding strains suitable for further optimization, molecular characterization, enzyme purification, and commercial exploitation. Furthermore, the discovery of indigenous thermophilic fungi capable of producing thermostable amylases could reduce dependence on imported industrial enzymes while promoting sustainable biotechnology development in Nigeria. Such findings may also contribute to advances in microbial enzyme technology, environmental microbiology, industrial fermentation, and the development of environmentally friendly biocatalysts for diverse industrial applications (Sharma et al., 2022; de Oliveira et al., 2020). Therefore, this study was designed to assess the amylase activities of aquatic fungi isolated from the warm springs of Yankari Game Reserve, Bauchi State, Nigeria, with the aim of identifying fungal isolates possessing high amylolytic potential for future industrial and biotechnological applications.

METHODOLOGY

Study area

The study was conducted using water samples collected from four naturally occurring warm springs (Wikki,

Mawulgo, Gwana, and Dimil) located within Yankari Game Reserve, Bauchi State, Nigeria. The reserve lies between latitudes 9°45′–10°30′N and longitudes 10°30′–11°00′E. The warm springs maintain temperatures ranging from 31 to 38°C throughout the year and provide favourable ecological conditions for thermophilic aquatic fungi. Water samples were transported in sterile containers under cooled conditions to the Microbiology Laboratory for immediate analysis.

Collection of water samples

Water samples were collected aseptically from each warm spring using sterile 500-mL screw-capped sampling bottles. Sampling was carried out at a depth of approximately 15–20 cm below the water surface to minimize surface contamination. Three independent samples were collected from different points within each spring to serve as replicates and ensure representative sampling. The bottles were appropriately labelled and transported in an ice-packed cooler to the laboratory, where analyses commenced within 24 hours.

Isolation of aquatic phycomycetes

Aquatic fungi were isolated using the hemp seed baiting technique as described by Johnson et al. (2002), with slight modifications. Sterile hemp seeds were boiled for 30 minutes, rinsed several times with sterile distilled water, and aseptically placed into sterile Petri dishes containing approximately 50 mL of each water sample. The bait cultures were incubated at 28 ± 2°C for 5–7 days. Fungal mycelia emerging from the hemp seeds were examined daily using a stereomicroscope. Hyphal tips were aseptically transferred onto Glucose Peptone Agar (GPA) supplemented with streptomycin (50 mg/L) to suppress bacterial contamination. Pure cultures were obtained through repeated hyphal-tip subculturing until morphologically uniform colonies were produced.

Identification of aquatic fungi

Pure fungal isolates were identified based on their cultural and microscopic characteristics using standard taxonomic procedures. Colony morphology, including growth pattern, texture, and pigmentation, was observed on Glucose Peptone Agar (GPA). Microscopic examination was performed by staining actively growing mycelia with lactophenol cotton blue and observing them under a compound light microscope. Identification was based on characteristic features such as hyphal morphology, sporangia, zoospore formation, oogonia, antheridia, and gemmae. The observed characteristics were compared with standard taxonomic keys for aquatic Phycomycetes described by Seymour (1970), Dick (2001), and Johnson et al. (2002). The isolates were identified as *Achlya* spp., *Aphanomyces* spp., *Saprolegnia* spp., *Leptolegnia* spp., and *Brevilegnia* spp. (Dick, 2001; Johnson et al., 2002; Beakes et al., 2014).

Determination of the occurrence of phycomycetes

The occurrence of Phycomycetes isolated from the warm springs was determined by recording the presence or absence of each fungal species at the four sampling locations (Dimil, Gwana, Wikki, and Mawulgo). Following identification, each isolate was scored as present (+) or absent (−) in each spring based on successful isolation and confirmation through morphological characteristics. The frequency of occurrence of each fungal species was subsequently calculated as the ratio of the number of springs in which the species occurred to the total number of fungal occurrences across all sampling locations, multiplied by 100. This approach provided quantitative information on the distribution and prevalence of each fungal species within the study area. The occurrence data were summarized as percentages and presented in tabular form to facilitate comparison of fungal diversity among the four warm springs. Similar methods for determining fungal occurrence and distribution have been widely employed in ecological studies of aquatic fungi (Johnson et al., 2002; Dick, 2001; Wurzbacher et al., 2017).

Preparation of fungal inoculum

Pure cultures of each identified fungal isolate were maintained on Potato Dextrose Agar (PDA) slants and incubated at 28–30°C for five days to obtain actively growing mycelia. Using a sterile cork borer, approximately 5-mm-diameter mycelial discs were aseptically excised from the actively growing margins of each colony and transferred into 100 mL of sterile Potato Dextrose Broth (PDB) contained in 250-mL Erlenmeyer flasks. The inoculated flasks were incubated at 30°C for 72 hours on an orbital shaker operating at 150 rpm to obtain actively growing fungal biomass for enzyme production and biodegradation experiments. The use of young, actively growing mycelia ensured uniform physiological conditions and maximum extracellular enzyme secretion during fermentation (Pandey et al., 2000; Gupta et al., 2003).

Production of crude amylase

Crude amylase was produced by submerged fermentation following the procedure described by Pandey et al. (2000), with slight modifications. Briefly, 100 mL of sterile starch production medium containing soluble starch (10 g/L), peptone (5 g/L), yeast extract (2

g/L), KH_2PO_4 (1 g/L), and $MgSO_4 \cdot 7H_2O$ (0.5 g/L) was dispensed into sterile 250-mL Erlenmeyer flasks and sterilized at 121°C for 15 minutes. Each flask was inoculated with a single 5-mm mycelial disc obtained from an actively growing fungal culture and incubated at 30°C for five days on a rotary shaker operating at 150 rpm. Aliquots were withdrawn every 24 hours throughout the incubation period to monitor enzyme production. The fermentation broth was filtered through sterile Whatman No. 1 filter paper to remove fungal mycelia and subsequently centrifuged at 10,000 rpm for 15 minutes at 4°C. The clear supernatant obtained after centrifugation served as the crude extracellular enzyme preparation for the amylase assay (Gupta et al., 2003; Souza and Magalhães, 2010).

Determination of amylase activity

Amylase activity was determined using the dinitrosalicylic acid (DNS) colorimetric method originally described by Miller (1959). The assay mixture consisted of 0.5 mL of crude enzyme extract and 0.5 mL of 1% soluble starch prepared in 0.1 M phosphate buffer (pH 6.8). The reaction mixture was incubated at 37°C for 10 minutes to allow enzymatic hydrolysis of starch. Thereafter, the reaction was terminated by adding 1.0 mL of DNS reagent, followed by heating in a boiling water bath for 5 minutes to develop the characteristic reddish-brown colour. The tubes were rapidly cooled under running water and diluted with 10 mL of distilled water before the absorbance was measured at 540 nm using a UV–Visible spectrophotometer. A glucose standard curve was prepared under identical conditions and used to determine the concentration of reducing sugars released during hydrolysis. One unit of amylase activity was defined as the amount of enzyme required to liberate one micromole of reducing sugar per minute under the assay conditions. Enzyme activity was determined daily for five consecutive days, and the mean values were expressed as µmol/min and presented graphically (Miller, 1959; Bernfeld, 1955).

Determination of viable counts during waste engine oil utilization

The viable fungal population during waste engine oil utilization was determined using the standard serial dilution and plate count technique. Each fungal isolate was inoculated into Mineral Salt Medium containing 1% (v/v) waste engine oil and incubated at 30°C on an orbital shaker operating at 150 rpm for 16 days. At 4-day intervals (days 0, 4, 8, 12, and 16), aliquots of each culture were aseptically withdrawn and subjected to ten-fold serial dilutions using sterile physiological saline. Appropriate dilutions were spread-plated onto Potato Dextrose Agar supplemented with streptomycin to suppress bacterial contamination and incubated at 28°C for 72 hours. The resulting colonies were counted, and viable counts were calculated as colony-forming units per millilitre (CFU/mL) by multiplying the number of colonies by the reciprocal of the dilution factor and dividing by the volume plated. All determinations were performed in triplicate, and the mean viable counts were reported as CFU/mL (Cappuccino and Welsh, 2017; Benson, 2021).

Determination of percentage hydrocarbon degradation

The biodegradation efficiency of the fungal isolates toward waste engine oil, diesel, and petrol was determined using the gravimetric method described by Atlas (1995). After 16 days of incubation, residual hydrocarbons were extracted from each culture flask using an equal volume of analytical-grade *n*-hexane in a separating funnel. The organic layer containing the residual hydrocarbon was collected and evaporated to constant weight in a pre-weighed evaporating dish. The percentage degradation was calculated using the following equation:

$$\text{Percentage degradation (\%)} = [(Wi - Wf)/Wi] \times 100$$

where *Wi* represents the initial weight of the hydrocarbon before inoculation and *Wf* represents the residual weight after incubation. The degradation percentages for waste engine oil, diesel, and petrol were expressed as mean ± standard error of triplicate determinations and used to evaluate the hydrocarbon-degrading potential of the different fungal isolates (Atlas, 1995; Das and Chandran, 2011).

Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as mean ± standard error of the mean (SEM). Data were analysed using one-way analysis of variance (ANOVA) with GraphPad Prism version 10.0. Where significant differences were detected, Fisher's Least Significant Difference (LSD) post hoc test was used for mean separation. Statistical significance was accepted at $P < 0.05$.

RESULTS

Occurrence of phycomycetes from the experimental Warm Springs in Yankari Game Reserve, Bauchi State

The occurrence of Phycomycetes isolated from the four experimental warm springs in Yankari Game Reserve, Bauchi State, Nigeria, is presented in Table 1. The fungal

Table 1. Occurrence of phycomycetes from the experimental warm springs in Yankari Game Reserve Bauchi State.

Fungal Isolate	Dimil	Gwana	Wikki	Mawulgo	Total	% Occurrence
Achlya spp.	+	+	-	+	03	27.27
Aphanomyces spp.	-	+	-	-	01	9.09
Saprolegnia spp.	+	+	+	+	04	36.36
Leptolegnia spp.	+	-	-	+	02	18.18
Brevilegnia spp.	-	+	-	-	01	9.09
Total	03(27.27)	04(36.36)	03(9.09)	03(27.27)	11	100

isolates identified were *Achlya* spp., *Aphanomyces* spp., *Saprolegnia* spp., *Leptolegnia* spp., and *Brevilegnia* spp. Among the isolates, *Saprolegnia* spp. was the most frequently encountered, occurring in all four springs (Dimil, Gwana, Wikki, and Mawulgo) and accounting for 36.36% of the total occurrence. *Achlya* spp. was isolated from three springs (Dimil, Gwana, and Mawulgo), representing 27.27% of the total isolates. *Leptolegnia* spp. occurred in Dimil and Mawulgo springs and contributed 18.18% of the total fungal isolates. In contrast, *Aphanomyces* spp. and *Brevilegnia* spp. were the least frequently encountered, each occurring only in Gwana Spring and accounting for 9.09% of the total isolates.

Among the sampling sites, Gwana Spring exhibited the highest fungal diversity, with four fungal isolates (36.36%), followed by Dimil and Mawulgo springs, each with three isolates (27.27%). Wikki Spring showed the lowest fungal diversity, with only one isolate (9.09%). Overall, the results indicate that the distribution of Phycomycetes varied among the warm springs, with certain locations supporting greater fungal occurrence and diversity than others, possibly due to differences in local environmental conditions.

Amylase activities (µmol/min) of aquatic fungi isolated from Yankari Hot Springs

The amylase activities of aquatic fungi isolated from Yankari Hot Springs differed significantly among species and across the incubation period, showing a general pattern of increasing enzyme activity that peaked on days 2 or 3 before declining markedly by day 5 (Figure 1). *Achlya* spp. exhibited the highest overall amylase activity, increasing from 0.247 µmol/min on day 1 to a peak of 0.93 µmol/min on day 3 before declining to 0.018 µmol/min on day 5. *Aphanomyces* spp. followed a similar trend, reaching a maximum activity of 0.73 µmol/min on day 3 before decreasing to 0.017 µmol/min by day 5.

Saprolegnia spp. exhibited comparatively lower amylase activity throughout the incubation period, with a maximum value of 0.17 µmol/min recorded on day 3, followed by a sharp decline to 0.004 µmol/min on day 5. *Leptolegnia* spp. and *Brevilegnia* spp. demonstrated moderate enzyme activities, reaching peak values of 0.27 µmol/min and 0.22 µmol/min, respectively, on day 3 before declining to near-zero levels by day 5. The observed differences in amylase activity among the fungal isolates were statistically significant ($P < 0.05$), indicating variation in their starch-degrading capabilities. Overall, *Achlya* spp. and *Aphanomyces* spp. were the most efficient amylase producers among the isolates studied.

Viable counts of selected fungal isolates during waste engine oil (WEO) utilization

The viable counts of selected fungal isolates during waste engine oil (WEO) utilization are presented in Table 2. All fungal isolates exhibited a progressive increase in colony-forming units (CFU/mL) over the 16-day incubation period, indicating active growth and the ability to utilize WEO as a carbon source.

Achlya spp. increased from an initial viable count of 1.0×10^3 CFU/mL to 7.0×10^3 CFU/mL by day 16. *Aphanomyces* spp. consistently recorded the highest viable counts throughout the study, increasing from 1.2×10^3 CFU/mL at the beginning of the experiment to 7.5×10^3 CFU/mL on day 16. *Saprolegnia* spp. and *Leptolegnia* spp. followed similar growth patterns, increasing from initial counts of 0.9×10^3 and 1.1×10^3 CFU/mL, respectively, to final counts of 6.8×10^3 and 7.1×10^3 CFU/mL. *Brevilegnia* spp. recorded the lowest initial viable count (0.8×10^3 CFU/mL) but also demonstrated substantial growth, reaching 6.2×10^3 CFU/mL by the end of the incubation period.

The progressive increase in viable counts across all fungal isolates suggests that they were capable of utilizing WEO as a carbon and energy source. Among the isolates, *Aphanomyces* spp. and *Leptolegnia* spp. exhibited the highest growth during WEO utilization, indicating greater adaptation to hydrocarbon metabolism. These findings highlight the potential of the isolated fungi for application in the bioremediation of hydrocarbon-contaminated environments.

Percentage degradation of waste engine oil (WEO), diesel, and petrol by fungal isolates

The percentage degradation of waste engine oil (WEO), diesel, and petrol by fungal isolates from Yankari Hot

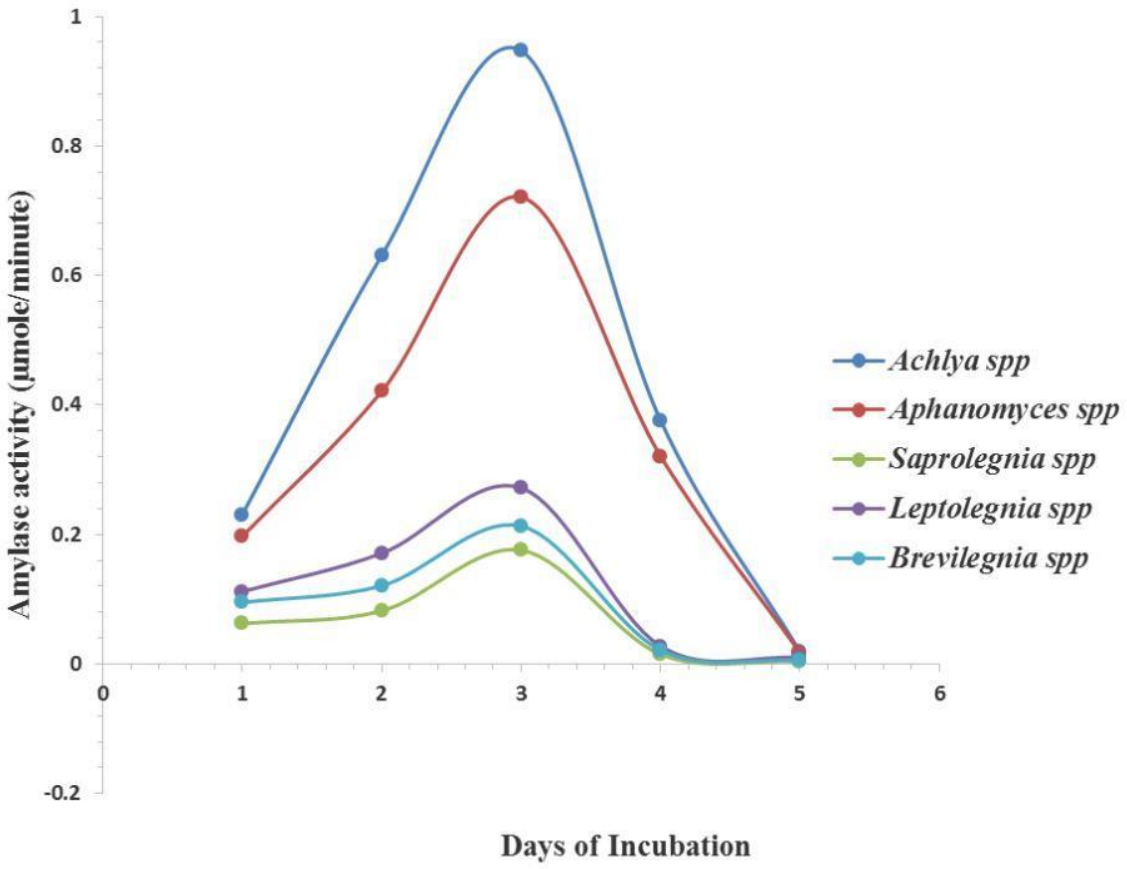

Figure 1. The amylase activities (µmole/minute) of the aquatic fungi isolated from Yankari Hot Springs.

Table 2. Viable counts of fungal isolates during waste engine oil (WEO) utilization from aquatic fungi isolated from Yankari Hot Springs.

Time (Days)	*Achlya* spp. (CFU/mL)	*Aphanomyces* spp. (CFU/mL)	*Saprolegnia* spp. (CFU/mL)	*Leptolegnia* spp. (CFU/mL)	*Brevilegnia* spp. (CFU/mL)
0	1.0 x 10^3	1.2 x 10^3	0.9 x 10^3	1.1 x 10^3	0.8 x 10^3
4	2.5 x 10^3	2.7 x 10^3	2.2 x 10^3	2.4 x 10^3	2.0 x 10^3
8	4.0 x 10^3	4.5 x 10^3	3.8 x 10^3	4.2 x 10^3	3.5 x 10^3
12	5.8 x 10^3	6.0 x 10^3	5.2 x 10^3	5.5 x 10^3	4.9 x 10^3
16	7.0 x 10^3	7.5 x 10^3	6.8 x 10^3	7.1 x 10^3	6.2 x 10^3

Springs is presented in Table 3. Considerable variation in hydrocarbon degradation efficiency was observed among the fungal species.

Aphanomyces spp. exhibited the highest degradation efficiencies for all three hydrocarbons, achieving 50.09% degradation of WEO, 48.23% degradation of diesel, and 42.24% degradation of petrol, indicating its superior hydrocarbon-degrading capability. *Saprolegnia* spp. ranked second, degrading 47.23% of WEO, 44.46% of diesel, and 40.41% of petrol. *Achlya* spp. and *Brevilegnia* spp. demonstrated comparable degradation efficiencies. *Achlya* spp. degraded 45.25% of WEO, 40.16% of diesel, and 35.93% of petrol, whereas *Brevilegnia* spp. achieved degradation rates of 44.38%, 41.51%, and 36.18%, respectively.

Among the isolates, *Leptolegnia* spp. exhibited the lowest degradation efficiencies, with values of 42.10% for WEO, 38.03% for diesel, and 33.13% for petrol. Nevertheless, it still demonstrated appreciable hydrocarbon-degrading capability. The differences in degradation efficiencies among the fungal isolates were statistically significant ($P < 0.05$), indicating species-specific variation in hydrocarbon metabolism. Overall, *Aphanomyces* spp. and *Saprolegnia* spp. were the most effective hydrocarbon degraders, particularly for WEO and diesel, highlighting their potential for the

Table 3. Percentage degradation of waste engine oil (WEO), diesel and petrol by fungal isolates.

Fungal Isolate	WEO Degradation (%)	Diesel Degradation (%)	Petrol Degradation (%)
Achlya spp.	45.25± 0.26^{c}	40.16± 0.29^{c}	35.93± 0.84^{c}
Aphanomyces spp.	50.09± 0.16^{a}	48.23± 0.27^{a}	42.24± 0.55^{a}
Saprolegnia spp.	47.23± 0.29^{b}	44.46± 0.23^{b}	40.41± 0.56^{b}
Leptolegnia spp.	42.10± 0.38^{d}	38.03± 0.26^{d}	33.13± 0.39^{d}
Brevilegnia spp.	44.38± 0.22^{c}	41.51± 0.26^{c}	36.18± 0.27^{c}
L.SD	0.60		
P-value	<0.05 ****		

At P≤0.05 there was a significant difference in the percentage degradation of waste engine oil (WEO), diesel and petrol by fungal Isolates. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same super script are not significant.

bioremediation of petroleum-contaminated environments.

DISCUSSION

The occurrence and distribution of Phycomycetes isolated from the warm springs of Yankari Game Reserve revealed that *Saprolegnia* spp. was the predominant genus, whereas *Achlya* spp. was the second most abundant. The predominance of *Saprolegnia* spp. may be attributed to its broad ecological adaptability, rapid growth, and ability to colonize a wide range of aquatic substrates under diverse environmental conditions. This finding is consistent with the reports of Johnson et al. (2002) and Beakes et al. (2014), who identified *Saprolegnia* as one of the most common and widely distributed genera of aquatic oomycetes in freshwater ecosystems because of its efficient saprophytic and opportunistic lifestyle. Similarly, Czeczuga et al. (2003) reported that species of *Saprolegnia* and *Achlya* frequently dominate aquatic fungal communities due to their high reproductive capacity and adaptability to different physicochemical conditions. The relatively low occurrence of *Aphanomyces* spp. and *Brevilegnia* spp. observed in the present study may be associated with their more specific ecological requirements and narrower habitat preferences, as previously reported by Dick (2001), who noted that the distribution of some members of the Saprolegniaceae is strongly influenced by environmental variables such as water temperature, dissolved oxygen, pH, and nutrient availability. Furthermore, the higher fungal diversity recorded in Gwana Spring compared with the other warm springs suggests that local environmental conditions favoured the establishment and proliferation of a wider range of aquatic fungi. This observation agrees with the findings of Wurzbacher et al. (2017), who reported that environmental heterogeneity significantly influences the diversity and distribution of aquatic fungal communities. Overall, the occurrence pattern observed in this study demonstrates that the warm springs of Yankari Game Reserve provide suitable ecological niches for diverse Phycomycetes, highlighting their importance as reservoirs of aquatic fungal biodiversity with ecological and biotechnological significance.

The amylase activity of the aquatic fungi isolated from the warm springs of Yankari Game Reserve differed significantly among the fungal genera and throughout the incubation period, indicating species-specific variations in enzyme production. The gradual increase in enzyme activity from day 1 to a peak on day 3, followed by a marked decline by day 5, suggests that amylase synthesis was closely associated with the exponential growth phase of the fungi. The subsequent decline in enzyme activity may be attributed to nutrient depletion, the accumulation of metabolic by-products, or enzyme degradation. This pattern agrees with the findings of Pandey et al. (2000), who reported that microbial amylase production generally reaches its maximum during the active growth phase before declining as fermentation progresses. Among the isolates, *Achlya* spp. exhibited the highest amylase activity, followed by *Aphanomyces* spp., indicating their superior capacity for starch hydrolysis. This finding is consistent with Gupta et al. (2003), who observed that fungal species differ considerably in extracellular enzyme production because of genetic and physiological differences that influence enzyme synthesis and secretion. The comparatively lower amylase activities recorded for *Saprolegnia*, *Leptolegnia*, and *Brevilegnia* spp. suggest that these fungi either rely less on starch as a primary carbon source or possess lower intrinsic amylolytic capabilities. Similar observations were reported by Souza and Magalhães (2010), who noted that enzyme productivity varies widely among fungal species and is influenced by substrate utilization efficiency, environmental conditions, and regulatory mechanisms controlling enzyme expression. The statistically significant differences observed among the isolates further demonstrate variation in their starch-degrading potential and indicate that *Achlya* spp. and *Aphanomyces* spp. possess greater promise as sources of industrial amylases. This observation also supports the findings of Maheshwari et al. (2000), who reported that fungi isolated from thermally

influenced environments frequently produce extracellular enzymes with enhanced catalytic efficiency and stability, making them suitable candidates for industrial biotechnology.

The viable counts of the aquatic fungal isolates increased progressively throughout the 16-day incubation period during waste engine oil (WEO) utilization, indicating that all the isolates were capable of adapting to and proliferating in a hydrocarbon-containing environment. The steady increase in colony-forming units (CFU/mL) suggests that the fungi utilized waste engine oil as a carbon and energy source for growth. Among the isolates, *Aphanomyces* spp. recorded the highest viable counts, followed closely by *Leptolegnia* spp. and *Achlya* spp., indicating their superior adaptability and metabolic capacity for hydrocarbon utilization. These findings are consistent with the reports of Das and Chandran (2011), who stated that microorganisms capable of utilizing petroleum hydrocarbons generally exhibit increasing cell populations as degradation progresses because hydrocarbons serve as growth substrates. Similarly, Atlas and Bartha (1998) reported that an increase in microbial population during hydrocarbon degradation is a strong indication of active biodegradation and reflects the organisms' ability to metabolize complex petroleum compounds. The relatively lower viable counts observed for *Brevilegnia* spp. may be attributed to differences in hydrocarbon tolerance, enzyme production, and substrate utilization efficiency among fungal species. Comparable observations were reported by Chaillan et al. (2004), who found that hydrocarbon-degrading fungi exhibit species-dependent growth responses when exposed to petroleum products because of differences in their enzymatic systems and physiological adaptation mechanisms. The continuous increase in fungal populations throughout the study therefore demonstrates the ability of these aquatic fungi to survive and proliferate in hydrocarbon-contaminated environments. This finding supports the assertion of Harms et al. (2011) that fungi possess extensive extracellular enzymatic systems capable of degrading complex organic pollutants, making them promising agents for the bioremediation of oil-polluted ecosystems. Consequently, the high viable counts recorded for *Aphanomyces* spp. and *Leptolegnia* spp. suggest that these fungi possess considerable potential for application in the biological remediation of environments contaminated with waste engine oil and related petroleum hydrocarbons.

The percentage degradation of waste engine oil (WEO), diesel, and petrol by the aquatic fungal isolates demonstrated marked differences in hydrocarbon degradation efficiency, indicating species-specific biodegradation capabilities. *Aphanomyces* spp. exhibited the highest degradation of all three hydrocarbons, followed by *Saprolegnia* spp., suggesting that these fungi possess more efficient enzymatic systems for the breakdown of petroleum hydrocarbons. The superior degradation ability of *Aphanomyces* spp. may be attributed to its capacity to produce extracellular oxidative and hydrolytic enzymes that facilitate the metabolism of complex hydrocarbon compounds. This finding agrees with the reports of Das and Chandran (2011), who stated that the biodegradation efficiency of fungi depends largely on their ability to synthesize extracellular enzymes capable of transforming petroleum hydrocarbons into simpler metabolites. Similarly, Harms et al. (2011) reported that fungi possess versatile enzymatic systems, including oxygenases and peroxidases, which enable them to degrade a broad range of recalcitrant organic pollutants. The comparatively lower degradation rates observed in *Leptolegnia* spp. indicate relatively lower metabolic efficiency in hydrocarbon utilization, although the species still demonstrated appreciable biodegradation potential. Comparable species-dependent variations in hydrocarbon degradation have been reported by Chaillan et al. (2004), who observed that fungal isolates differ significantly in their capacity to degrade petroleum products because of differences in enzyme production, physiological adaptation, and substrate specificity. Furthermore, the higher degradation of waste engine oil and diesel compared with petrol observed in the present study is consistent with the findings of Atlas and Bartha (1998), who noted that microbial degradation of petroleum products varies according to hydrocarbon composition, with some fractions being more readily metabolized than others. The highly significant differences ($P < 0.0001$) among the fungal isolates therefore confirm that *Aphanomyces* spp. and *Saprolegnia* spp. are the most effective hydrocarbon degraders among the species evaluated and possess considerable potential for the bioremediation of petroleum-contaminated environments.

CONCLUSION

The findings of this study demonstrate that the warm springs of Yankari Game Reserve harbour diverse aquatic Phycomycetes with significant ecological and biotechnological potential. *Saprolegnia* spp. was the most frequently occurring fungal genus, whereas *Achlya* spp. exhibited the highest amylase activity, highlighting its potential as a promising source of industrial amylase. Furthermore, all the fungal isolates were capable of utilizing waste engine oil as a carbon source, as evidenced by their progressive increases in viable counts and appreciable hydrocarbon degradation efficiencies. Among the isolates, *Aphanomyces* spp. demonstrated the greatest hydrocarbon-degrading capacity, indicating its suitability for the bioremediation of petroleum-contaminated environments. Overall, the results indicate that the indigenous aquatic fungi inhabiting the warm springs of Yankari Game Reserve represent valuable biological resources for industrial enzyme production and

environmental biotechnology. Future studies should focus on the molecular identification of these isolates, optimization of enzyme production, purification and characterization of the amylase enzymes, and evaluation of their performance under industrial and environmental conditions.

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