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HERBICIDE BIODEGRADATION POTENTIAL OF FUNGI ISOLATED FROM HERBICIDE-CONTAMINATED SOILS IN SELECTED OKITIPUPA LOCAL GOVERNMENT AREA FARMLANDS

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ABSTRACT

This study aims to isolate indigenous fungal species from paraquat-contaminated farmland in Okitipupa, Ondo State, Nigeria, and evaluate their capacity to biodegrade the non-selective herbicide Slasher Dichloride (paraquat, 276 g/L) to mitigate soil contamination and associated ecological and human health risks. Isolation and identification of fungi from paraquat-contaminated sites were carried out using standard cultural and molecular methods, biodegradation assays were conducted in Mineral Salt Medium (MSM) with 100 mg/L paraquat as the sole carbon source, incubated at pH 6.3, 31°C, 120 rpm for 7 days while process optimization of inoculum concentration (1–5 % v/v) was done using response surface methodology, with fungal growth monitored by optical density at 600 nm (OD 600). Optimal fungal growth occurred at a 1 % (v/v) inoculum for *Aspergillus fumigatus* (0.092 AU), *Aspergillus niger* (0.085 AU), *Aspergillus nidulans* (0.078 AU), and *Penicillium allii* (0.050 AU), whereas *Trichoderma harzianum* (0.042AU) peaked at 3 % (v/v). The degradation efficiency ranked as follows: *A. fumigatus* > *A. niger* > *A. nidulans* > *P. allii* > *T. harzianum*. It was then concluded that the isolates have strong potential for the development of myco-based bioremediation strategies to restore herbicide-polluted soils in Okitipupa and similar agrarian regions.

Keywords: Paraquat biodegradation, Indigenous fungi, Herbicide-contaminated soils, *spergillus fumigatus, Trichoderma harzianum,* Myco-bioremediation, Okitipupa farmlands.

INTRODUCTION

Soil is an important natural resource that supports plant growth and helps maintain land-based ecosystems in balance. It is home to many living things, such as bacteria, fungi, actinomycetes, protozoa, and algae. These microbes collectively constitute the soil microflora, which is crucial for soil fertility and nutrient cycling (Bardgett & Van der Putten, 2014). Soil microorganisms play a role in many biological processes that support farming; they break down organic debris, convert nutrients into minerals, fix nitrogen, and alter the chemical makeup of the soil. These activities help plants take up nutrients such as nitrogen, phosphorus, and sulfur by converting them into forms that plants can readily absorb (Prajna et al., 2022; Adeyemo, 2025). Environmental factors and farming practices have a big effect on the presence and activity of soil microorganisms. Soil pH, moisture content, organic matter, temperature, and the presence of agrochemicals can all significantly affect microbial abundance and energy use. The use of agrochemicals, such as herbicides, has received increasing attention in recent years because research has shown that they can have both positive and negative impacts on soil, human health and ecosystems (Cycoń *et al.,* 2017; Wang *et al*., 2024).

Herbicides are chemical agents employed to manage weeds in agricultural fields. Weeds vie for crops' nutrients, water, sunlight, and space, potentially diminishing agricultural yield if not adequately controlled. Farmers frequently utilize herbicides as an efficient and cost-effective strategy for weed management (Kaur *et al.,* 2020). The use of herbicides has proliferated in contemporary agricultural practices due to their ability to reduce labor requirements and increase farm output. Nonetheless, despite their advantages, herbicides may also have unintended effects on soil microorganisms and the physicochemical properties of soil. Herbicide-induced shifts in these properties may indirectly disrupt microbial communities and associated biological processes (Kumar *et al.,* 2021). Herbicides sprayed on farmlands may remain in the soil for extended periods, depending on their chemical structure and environmental conditions (Meena *et al.,* 2020; Das *et al.,* 2022; Ferran *et al.,* 2025). Certain herbicides may suppress the proliferation of beneficial microbes involved in nutrient cycling, whilst others may promote the growth of microorganisms that degrade herbicides (Cycoń *et al*., 2017; Wang *et al*., 2025).

In many developing countries, including Nigeria, farmers rely heavily on herbicides for weed control in crop fields, as they offer a faster, more efficient alternative to manual weeding. These chemicals provide a practical solution for managing unwanted vegetation across large areas, where manual methods prove too slow, labor-intensive, and costly (Bose *et al*., 2021; Nwakoby *et al.,* 2025). Beyond labor and time savings, herbicides enhance farm productivity and profitability by enabling timely land preparation and sustaining weed-free fields through the growing season—especially vital in regions facing labor shortages or high costs. In Okitipupa metropolis, most farmers prefer Paraquat dichloride as their herbicide of choice due to its reported availability and efficacy in controlling weeds that compete with crops. Regrettably, Paraquat dichloride is recognized as one of the most hazardous herbicides due to its detrimental effects on human and soil health. The toxicity is exceedingly high, and even a minimal quantity entering the body, particularly via consumption, can be lethal. (Mohd *et al.,* 2023; Ruuskanen *et al*., 2023). Paraquat exhibits a strong affinity for binding to clay particles and organic materials in the soil. This robust adsorption indicates that it becomes biologically inactive immediately after application and is not readily absorbed by plant roots. Consequently, it typically does not penetrate deeply into the soil or pollute groundwater under standard conditions. Because of this characteristic, paraquat is often considered to have minimal residual activity, allowing farmers to cultivate crops shortly after treatment without experiencing soil toxicity. Nonetheless, frequent or excessive application may adversely impact soil health by diminishing beneficial microbial activity in the short term, particularly at the surface where it is administered, while concurrently elevating the residual concentration of paraquat in the soil, thereby harming the crops (Fargnoli *et al*., 2019; Ahmad *et al*., 2024; Nwakoby *et al*., 2025; USEPA, 2025).

Microbial biodegradation is favored for herbicide removal from farmlands because it uses natural microbial processes to break down these chemicals into harmless byproducts such as carbon dioxide and water, thereby minimizing environmental harm compared to chemical or physical methods (Singh, 2014; Abateh *et al.*, 2017; Ganeshkar, 2022). Microbial degradation avoids introducing additional toxic substances, such as enzymes used by microbes to metabolize herbicides, and does not generate secondary pollutants. It fully mineralizes contaminants rather than merely transferring them to air, water, or soil, thereby reducing long-term ecological risks such as bioaccumulation (Adams *et al*., 2015; Abateh *et al*., 2017; Dar *et al*., 2022). Fungi are among the bioresources that have been widely characterized and applied to the biodegradation and bioremediation of pesticides (Maqbool *et al*., 2016). Fungi have been reported to biodegrade various pesticides in liquid and soil media by breaking down complex compounds into simpler, less toxic forms. They are particularly valuable for bioremediation because their mycelia can penetrate diverse substrates, and they can thrive in contaminated soils where other microorganisms struggle to survive (Magnoli *et al*., 2023). Fungal isolates are an effective bioresource to degrade different pesticides, including lindane, methamidophos, endosulfan, chlorpyrifos, atrazine, cypermethrin, dieldrin, methyl parathion, heptachlor, etc. (Abraham & Silambarasan, 2014; Alvarenga *et al*., 2014; Barathidasan *et al*., 2014; Ortiz-Hernández *et al*., 2021; Díaz-Soto *et al.,* 2024). However, the rate of fungal degradation of pesticides depends on soil moisture content, nutrient availability, pH, temperature, oxygen level, etc. Fungal strains have been reported to harbor various processes, including hydroxylation, demethylation, dechlorination, dioxygenation, esterification, dehydrochlorination and oxidation, during the biodegradation of pesticides with varying functional groups. Moreover, the biodegradation of different pesticides was found to be mediated by the involvement of different enzymes, including laccase, hydrolase, peroxidase, esterase, dehydrogenase, manganese peroxidase, lignin peroxidase, etc (Alvarenga *et al*., 2014; Alsohaili & Bani-Hasan, 2018; Mohy-Ud-Din *et al*., 2023; Díaz-Soto *et al.,* 2024).

Fungal species excel in herbicide bioremediation in soils. Trichoderma spp. (*T. viride*, *T. koningii*, *T. harzianum*) Degrade 2,4-D and alachlor (Nur & Noor, 2020; Jorge & Daniel, 2022). Penicillium spp. (*P. chrysogenum*, *P. spiculisporus*, *P. notatum*) target 2,4-D, diclofop-methyl, and glyphosate; Aspergillus spp. (*A. terreus*, *A. niger*, *A. flavus*) Break down pendimethalin and glyphosate (Rani *et al.,* 2021; Bhatt *et al*., 2023; Magnoli *et al*., 2023; Liu *et al.,* 2023). White-rot *Phanerochaete chrysosporium*, *Fusarium* spp., *Rhizopus stolonifer*, *Alternaria tenuissima, Lentinus crinitus* (atrazine via laccase), and *Trametes versicolor* handle various pesticides (Sánchez, 2024; Krzyśko-Łupicka *et al*., 2025). These fungi employ several degradation strategies, including the production of non-specific extracellular enzymes, such as laccase and manganese peroxidase, by white-rot species, which decompose resistant aromatic rings. Intracellular cytochrome P450 systems, such as those in Umbelopsis isabellina that degrade 2,4-D, enable phase I metabolism. Bioaccumulation occurs through hyphal adsorption, subsequent uptake, and degradation, whereas extensive mycelial networks facilitate soil colonization and pollutant accessibility—both crucial for practical applications in the restoration of agricultural lands.

Herbicide contamination from intensive farming poses serious risks to soil health, microbial diversity, and food security in agricultural regions of Okitipupa Local Government Area of Ondo State, Nigeria. Fungi offer a promising, eco-friendly solution through mycoremediation, but their biodegradation potential in locally contaminated farmlands remains underexplored.
Farmlands in Okitipupa city rely on herbicides such as Paraquat for weed control, leading to the accumulation of persistent residues that degrade soil fertility and jeopardize groundwater quality. Although global research emphasizes fungi such as Aspergillus and Penicillium species for the degradation of analogous pesticides, there is a lack of focused investigations into indigenous fungi from the herbicide-contaminated soils of Okitipupa. This research finds indigenous fungal strains for sustainable bioremediation, aiding smallholder farmers in Okitipupa by diminishing pesticide persistence and facilitating soil recovery. It is consistent with Nigeria's agricultural legislation on environmental safety and may inform scalable mycoremediation solutions.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from ten (10) farmlands at different location in Okitipupa LGA of Ondo State, Nigeria where Paraquat is regularly used for weed control by the farmers using a sterile soil auger and the samples were taken within two hours in an ice chest to the Microbiology Laboratory, Department of Biological Sciences, Olusegun Agagu University of Science and Technology (OAUSTECH), Okitipupa, Nigeria for microbial analysis.

Isolation and identification of Fungi

10 g of soil samples were mixed to homogeneity and sieved to remove stones and soil debris. Potato Dextrose Agar (PDA) was prepared according to the manufacturer's instructions and autoclaved at 121 °C for 15 min. One (1) ml of dilution from 10^{-3}, 10^{-4}, and 10–5 was pour-plated on PDA and incubated at 30 °C for 72 hours (Sohail & Bayan, 2018). Six colonies were observed on the plates after incubation; each colony was subcultured onto fresh PDA, both on plates and in slants, for identification based on colonial characteristics and subsequent molecular analysis. The identified colonies were later labelled ADE_1 to ADE_6

Degradation of Paraquat by Fungal Isolates

Mineral Salt Medium (MSM) was used to screen herbicide-degrading fungi by preparing it in 21 150-ml conical flasks, with paraquat as the sole carbon source. These flasks were divided into three groups of seven triplicates: six containing fungal isolates (ADE_1 to ADE_6) and one serving as the abiotic control, with paraquat concentrations at 1%, 3%, and 5% (v/v) across groups, using response surface methodology in an orbital shaker at room temperature. Fungal degradation of paraquat was then evaluated in MSM and was amended with 100 mg/L paraquat (pH 6.3, 31 °C, 120 rpm shaking, 7 days). Fungal growth, which corresponds to herbicide uptake, was evaluated using a spectrophotometer at 600 nm on days 0, 1, 3 and 7 (Mark *et al*., 2023). One fungal isolate, morphologically identified as *Rhizopus* sp., failed to yield viable growth across replicate trials; consequently, it was excluded from downstream molecular identification and experimental assays.

Fungi DNA Extraction Protocol

Approximately 100 mg of fungal mycelia was ground with Delaporta extraction buffer (100 mM Tris pH 8, 51 ml EDTA pH 8, 500 mM NaCl, 10 mM mercaptoethanol), and DNA was extracted as described briefly. Each sample was ground in 1000 µl of the buffer in a sterilized sample bag. The mix was collected in a sterile Eppendorf tube, and 40 µl of 20% SDS was then added. This was followed by brief vortexing and incubation at 65 °C for 10 minutes. This was later cooled to room temperature, after which 160 µl of 5 M potassium acetate was added, vortexed and centrifuged at 10000 g for 10 minutes. Supernatant was collected in another Eppendorf tube, 400 µl of cold isopropanol was added, mixed gently, and kept at 20 °C for 60 minutes, after which it was centrifuged at 13000g for 10 minutes to precipitate DNA. The supernatant was gently decanted, and the pellet was ensured not to be disturbed. DNA was then washed with 500 µl of 70% ethanol by centrifugation at 10,000 g for 10 minutes. Ethanol was decanted, and DNA was air-dried at room temperature until no trace of ethanol was visible in the tube. The pellet was then resuspended in 50 µl of Tris-EDTA buffer to preserve and suspend the DNA. (Sohail and Bayan, 2018)

PCR Analysis

To characterize fungi using the Internal Transcribed Spacer (ITS) gene, the universal ITS primer set, which flanks the ITS1, 5.8S, and ITS2 regions, was used. PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each ITS 1: 5' TCC GTA GGT GAA CCT GCG G 3'and ITS 4: 5' TCC GCT TAT TGA TAT GC 3'primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR condition including a cycle of initial denaturation at 94^0C for 5 min, followed by 35 cycles each cycle comprised of 30secs denaturation at 94^0C, 30secs annealing of primer at 55^oC, 1.5 min extension at 72^0C and a final extension for 7min at 72^oC. (Sohail and Bayan, 2018)

Integrity

The integrity of the amplified sequence (about 1.5 Mb gene) fragment was checked on a 1.5% Agarose gel to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare a 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60 °C and stained with 3 µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and emits visible orange light). A comb was inserted into the casting tray, and molten agarose was poured into it. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to submerge the gel barely. Two microliters (2 l) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µl of each PCR product and loaded into the wells after the 100bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes, visualized by ultraviolet transillumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was run alongside the experimental samples on the gel. (Sohail and Bayan, 2018).

Purification of Amplified Product

After the gel integrity test, the amplified fragments were purified with ethanol to remove the PCR reagents. Briefly, 7.6 µl of 3 M Na acetate and 240 µl of 95% ethanol were added to each about 40 µl PCR-amplified product in a new sterile 1.5 µl Eppendorf tube, mixed thoroughly by vortexing and kept at 20 °C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C, followed by removal of supernatant (invert tube on trash once), after which the pellet was washed by adding 150 µl of 70 % ethanol and mixing, then centrifuging for 15 min at 7500 g and 4 °C. The supernatant was decanted, and the tube was inverted on absorbent paper to air-dry in a fume hood at ambient temperature for 10 to 15 minutes. The pellet was subsequently resuspended in 20 µl of sterile distilled water and stored at 20 ^{o}C prior to sequencing. To verify the presence of the purified product, it was analyzed on a 1.5% agarose gel at 110 V for approximately one hour, as previously described, and quantified using a Thermo Scientific NanoDrop 2000 spectrophotometer.

Sequencing

The amplified fragments were sequenced on a Genetic Analyzer 3130 xl (Applied Biosystems) according to the manufacturer's manual, using the BigDye Terminator v3.1 cycle sequencing kit. BioEdit software was used for all sequence editing and cluster alignment, while MEGA 6 was used for all genetic analysis (Sohail & Bayan, 2018).

RESULTS

Only five of the six fungal species were capable of growth on MSM utilizing Paraquat as the exclusive carbon source. The five isolates were provisionally identified based on their morphological characteristics as Aspergillus niger, Aspergillus fumigatus, Penicillium sp., Aspergillus sp., and Trichoderma sp. Only *Aspergillus niger* and *Aspergillus fumigatus* were identifiable at the species level morphologically, while the other three isolates were classified solely at the genus level, necessitating molecular investigation. The five isolates were ultimately classified to the species level using molecular analysis. The findings of morphological identification and the average fungal numbers for each isolate are presented in Table I.

Table I: Mean Fungal Count and Morphological Features of the Fungi Isolated from the Herbicide-Polluted Farmlands

Isolates	Mean Fungal Count (CFU/ml)	Morphological Features	Suspected Organism
Isolate 1	5.93×10^3	Colonies initially appear white to pale yellow and woolly or cottony, becoming dark brown to black as conidia mature. Conidiophores are long, smooth-walled, hyaline, and become darker toward the apex before terminating in a globose vesicle.	*Aspergillus niger*
Isolate 2	5.31×10^3	Colonies are woolly to cottony initially, then turn smoky gray-green. Conidiophores are smooth-walled, colorless, and relatively short, arising from the hyphae and terminating in a dome-shaped or flask-shaped vesicle.	*Aspergillus fumigatus*
Isolate 3	4.25×10^3	Green, woolly colonies with the reverse side of purple-brown. It displays septate hyphae that branch at about 45-degree angles, with some appearing finger-like. The conidiophores are generally short, brownish, and smooth-walled, supporting short, columnar, and biseriate conidial heads. The conidia themselves are globose and rough-walled	*Aspergillus* sp.
Isolate 4	5.15×10^3	Colonies are greyish-green with a white or pale margin; reverse pigmentation is pale yellow to brownish-yellow. Conidia are produced in long, dry chains from the phialides, giving the characteristic brush-like appearance of *Penicillium*.	*Penicillium* sp
Isolate 5	4.75×10^3	Colony is whitish green to yellow-green in coloration with concentric rings in conidia-forming areas. The reverse side appears yellowish-greenish. It has septate hyphae and erect, hyaline, branched conidiophores that are non-whorled, occurring solitary or in groups. Phialides are pear-shaped or ampulliform, often in whorls of 2-6, swollen centrally and tapering at the apex.	*Trichoderma* sp

Overall, the study revealed a diverse fungal community in herbicide-polluted farmland, dominated mainly by Aspergillus, Penicillium and Trichoderma species.

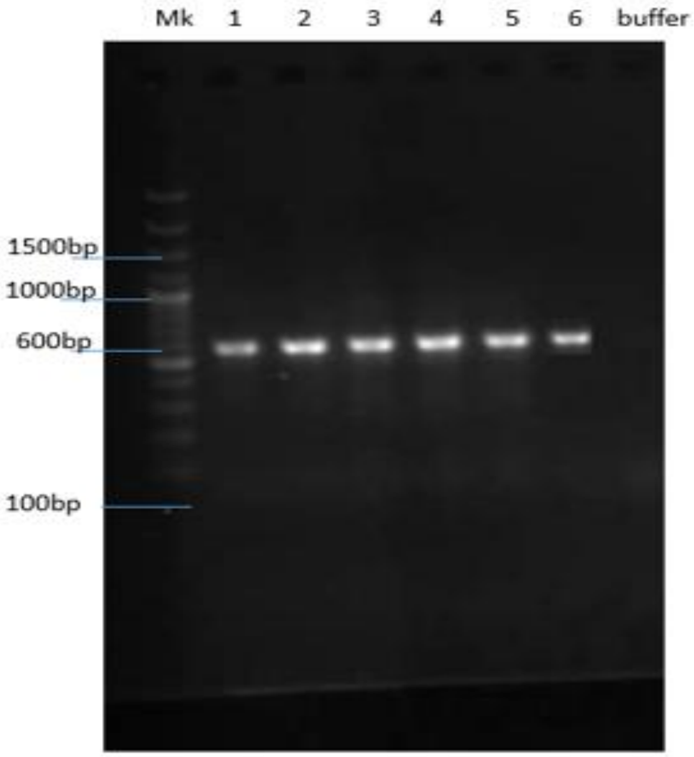

Plate I: Plate X. Agarose gel electrophoresis of PCR-amplified ITS gene fragments from fungal isolates. Lane M: 100 bp DNA ladder; Lanes 1–6: fungal isolates showing amplicons of approximately 600 bp; Buffer: negative control.

Table II: Molecular Identification and DNA Profiling of Fungal Isolates Based on Sequence Similarity Analysis

Sample ID	Scientific Name	E value	% Identity	Accession
ADE 1	*Penicillium allii*	0	98.21%	AJ005484
ADE 2	*Aspergillus niger*	0	100.00%	KX231821
ADE 3	*Aspergillus fumigatus*	0	100.00%	SUMS0106
ADE 4	*Aspergillus nidulans*	0	99.25%	AF138289
ADE 5	*Trichoderma harzianum*	0	97.67%	AY605713

The molecular identification of the isolates revealed that ADE_1 to ADE_5 are Penicillium allii, Aspergillus niger, Aspergillus fumigatus, Aspergillus nidulans and Trichoderma harzianum, respectively, while their phylogenetic trees are shown below in Figures I–III.

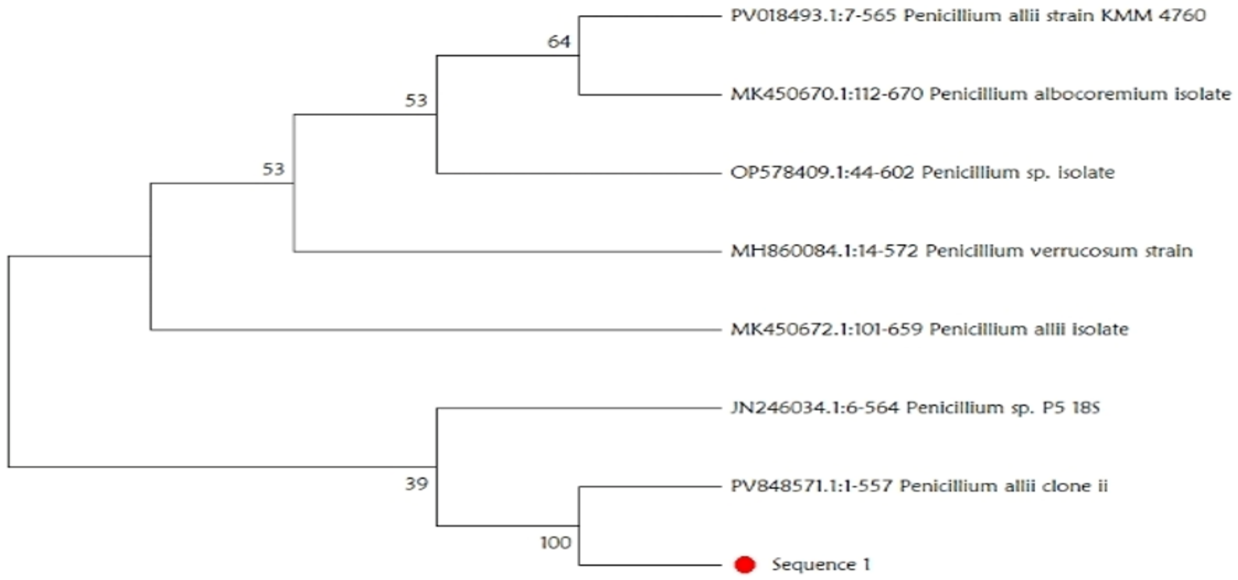

Fig I: Phylogenetic tree of sequence 1(*Penicillium allii*)

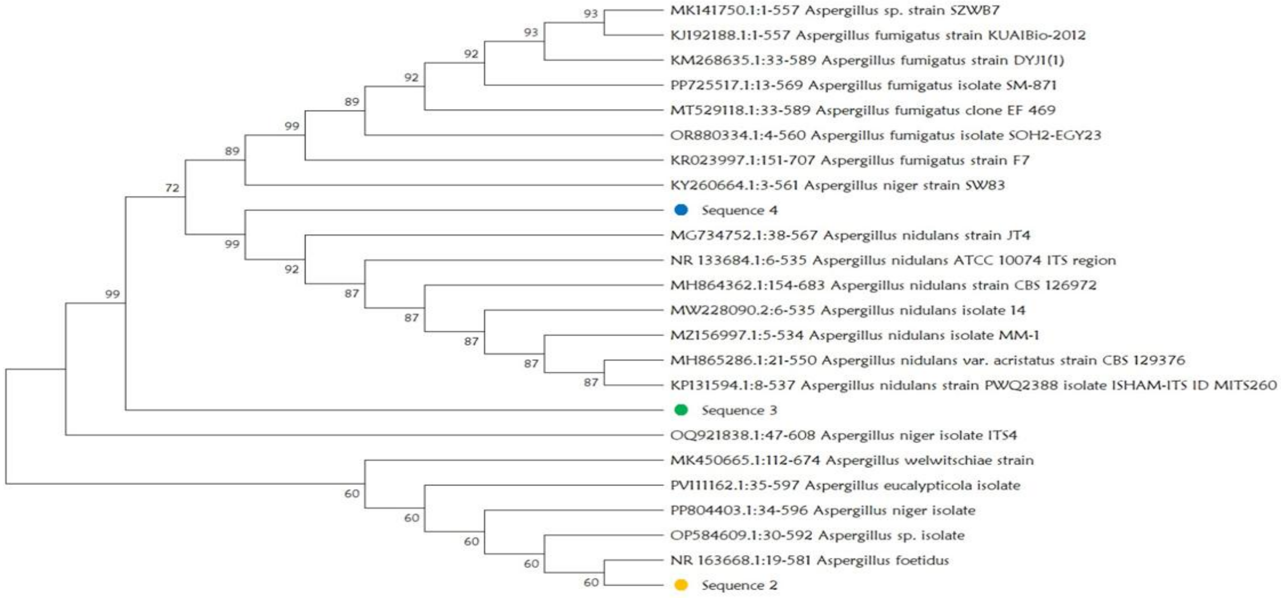

Fig II: Phylogenetic tree of sequences 2, 3 and 4 (Aspergillus genus)

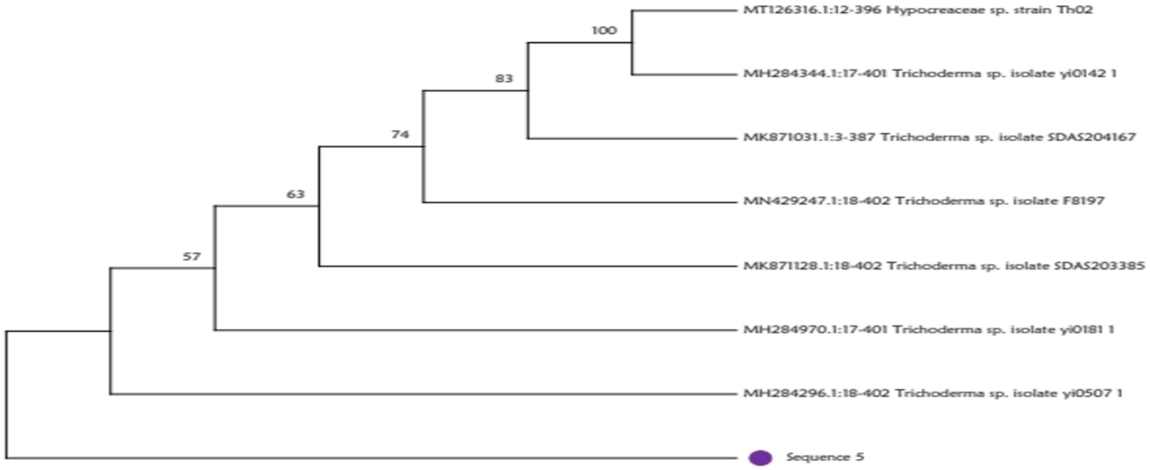

Fig III: Phylogenetic tree of sequence 5 (*Trichoderma harzianum*)

Table III: Optical Density Values of MSM- Paraquat Degradation by Fungal Isolates after 7 Days

S/N	Fungal Isolates	Initial OD (AU)	Final OD (5 %)	Final OD (3 %)	Final OD (1 %)
ADE$_1$	*Penicillium allii*	0.020	0.032	0.037	0.050
ADE$_2$	*Aspergillus niger*	0.020	0.070	0.078	0.085
ADE$_3$	*Aspergillus fumigatus*	0.020	0.082	0.085	0.092
ADE$_4$	*Aspergillus nidulans*	0.020	0.060	0.063	0.078
ADE$_5$	*Trichoderma harzianum*	0.020	0.038	0.042	0.030
Control	No Isolate	0.020	0.020	0.020	0.020

The spectrophotometer readings presented in Table III indicate progressive fungal growth and metabolic activity during paraquat degradation in Mineral Salt Medium (MSM) after 7 days of incubation. Optical density (OD) values increased from an initial reading of 0.020 AU across all treatments, indicating that the fungal isolates were able to utilize the paraquat-containing medium for growth. The increase in OD reflects increased biomass production and active biodegradation processes.

DISCUSSION

Table I shows the mean fungal counts, morphological characteristics, and probable identities of fungi isolated from herbicide-polluted farmlands. The results revealed that the fungal population ranged from 4.15 × 10³ to 5.93 × 10³ CFU/ml, indicating that various fungal species survived and proliferated in soils exposed to herbicide contamination. The presence of these fungi suggests their possible adaptation to polluted environments and their potential involvement in the degradation of herbicide residues in the soil. (Bhosle and Thore, 2016; Magnoli *et al*., 2023). Plate I is the Agarose gel electrophoresis of PCR-amplified ITS gene fragments from fungal isolates. Lane M: 100 bp DNA ladder; Lanes 1–6: fungal isolates showing amplicons of approximately 600 bp; Buffer: negative control. On the agarose gel, most of the fungal isolates produced a single, sharp band corresponding to the expected ~1.5 kb amplicon (as judged against the 1 kb ladder), indicating specific amplification and good template quality. A few samples showed faint bands (low yield), and two samples displayed minor smearing or additional, smaller bands, suggesting partial degradation, nonspecific amplification or primer-dimer formation. Negative control showed no bands, confirming the absence of contamination.

DNA profiling of the fungal isolates revealed the presence of five fungal species from the genera Aspergillus, Penicillium, and Trichoderma, as shown in Table II above. The isolates were identified as *Penicillium allii*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, and *Trichoderma harzianum*. Molecular identifications showed high maximum and total scores ranging from 662 to 1038, with query coverage between 70% and 100%. Percentage identity values were also high (97.67–100%), indicating strong genetic similarity with reference sequences in the GenBank database. *Aspergillus* species predominated among the identified fungi, suggesting their widespread occurrence in the sampled environment. Overall, the molecular characterization demonstrated that the isolates belong to the genera *Aspergillus*, *Penicillium*, and *Trichoderma*. The consistently high percentage identities (97.67–100%) and zero E-values observed across all isolates indicate strong homology with established fungal sequences in GenBank, thereby validating the effectiveness of the DNA profiling approach for accurate fungal identification.

Table III presents the spectrophotometric absorbance profiles, which serve as a direct proxy for fungal growth and metabolic activity. The progressive increase in optical density (OD) as paraquat concentrations decreased from 5% to 1% indicates that reduced herbicide toxicity significantly improved fungal metabolic output, likely by alleviating metabolic stress and enhancing enzymatic degradation efficiency. Notably, *Aspergillus fumigatus*

(ADE$_3$) exhibited the highest OD across all tested paraquat concentrations (0.082), (0.085), and (0.092 AU at (5 %), (3 %), and (1 %), respectively. This distinct growth pattern highlights its superior tolerance and bioremediation potential, driven by highly efficient detoxification pathways, as reported by Jindakaraked et al. (2023). These findings are also aligned with literature documenting the versatile enzymatic machinery of *A. fumigatus*, which facilitates its survival in pesticide-stressed agricultural soils (Carranza *et al.,* 2017; Efremenko *et al*., 2024). Furthermore, its robust performance here parallels previously observed degradation efficacy against pyrethroid, organochlorine, and organophosphorus compounds (Matúš *et al*., 2023).

This study confirmed that *Aspergillus niger* (ADE$_2$) can develop and utilize the herbicide as a primary carbon source, as evidenced by growth and spectrophotometric absorbance data. The prevalence of *A. niger* in herbicide-contaminated agricultural land is likely due to its metabolic adaptability and significant resilience to environmental stressors, allowing it to decompose complex organic compounds and agrochemical residues, as documented in previous studies (Aimeur *et al*., 2012; Pandey & Choudhury, 2021; Matúš *et al*., 2023). Degradative ability is facilitated by a highly versatile enzyme system that converts complex chemical compounds into accessible sources of carbon, nitrogen, and phosphorus. The fungus secretes extracellular enzymes, including laccases and peroxidases, to decompose refractory chemicals such as glyphosate and clodinafop-propargyl into bioavailable intermediates (Carranza *et al.,* 2017). *A. niger* can function autonomously, achieving up to 95.2% degradation efficiency for specific pesticides such as chlorpyrifos under optimized settings; nevertheless, it frequently collaborates within mixed-species consortia to attain enhanced metabolic diversity and increased biodegradation rates. Moreover, its bioremediation capabilities encompass more than organic compounds; recent studies (Matúš *et al*., 2023; Efremenko *et al.,* 2024) demonstrate that *A. niger* can function in a synergistic consortium with *Aspergillus niveus* and *A. flavus* to eliminate 70–90% of heavy metals (Cr, Zn, Pb, Cd, and Ni).

Aspergillus nidulans (ADE$_4$) exhibited robust increases in optical density (OD), highlighting its strong adaptability and ability to utilize herbicide-contaminated media. Consistent with prior studies, the fungus demonstrates compound-specific degradative capabilities rather than universal bioremediation potential (Florence et al., 2017; Mesgane et al., 2020; Oestreicher et al., 2023; Ward & Golubev, 2026).

The moderate growth of *Penicillium allii* (ADE$_1$) observed in this study may be attributed to its ability to synthesize extracellular enzymes, which facilitate the decomposition of organic matter and the detoxification of soil pollutants. Although there are no previously documented reports of herbicide degradation by Penicillium allii, our findings are consistent with the well-established metabolic versatility of the genus Penicillium in bioremediation. Various species within this genus have demonstrated a robust capacity to degrade, metabolize, or detoxify multiple classes of agricultural herbicides (Alvarenga *et al*., 2014; Rani *et al*., 2021; Bhatt *et al*., 2023; Matúš *et al*., 2023). *Trichoderma harzianum* (ADE$_5$) showed a slightly different pattern, with OD increasing from 0.038 AU at 5% to 0.042 AU at 3%, but decreasing to 0.030 AU at 1%. This irregular trend may indicate strain-specific physiological limitations or possible nutrient competition affecting growth at lower concentrations. This result, however, is consistent with the findings of other researchers, who also reported that Trichoderma harzianum demonstrates moderate to high potency in biodegrading certain herbicides, particularly chloroacetanilides and some organophosphorus compounds, though its effectiveness varies significantly by herbicide class and soil conditions (Nykiel-Szymańska *et al*., 2020; Conte *et al*., 2025). The control setup maintained a constant OD of 0.020 AU throughout the experiment, confirming the absence of microbial activity and demonstrating that the observed increases in OD in the experimental treatments were attributable to fungal growth and biodegradation.

Conclusion

The findings in this study demonstrate that indigenous fungal strains from Okitipupa farmlands possess significant myco-remediation capabilities. Specifically, *Aspergillus fumigatus* and *Aspergillus niger* show the most promise for the biological degradation of paraquat. Consequently, these isolates can serve as highly effective, eco-friendly bioagents for formulating targeted bioremediation strategies to restore herbicide-contaminated agricultural soils in similar tropical and agrarian environments.

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