



Research Article

Morphological and Molecular Characterization of Fungi Isolated from Organophosphate Contaminated Dairy Farm Soils in Kilifi County, Kenya

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Abstract

Organophosphorus (OP) compounds are widely used in livestock farming to control external parasites. However, their extensive application has led to significant environmental contamination, including soil, water, and air pollution, as well as adverse effects on non-target organisms and disruption of ecosystem processes. These challenges necessitate the development of sustainable, cost-effective, and environmentally friendly approaches for OP detoxification. Compared to conventional remediation methods such as chemical treatment, incineration, and landfill disposal, which are often costly and environmentally harmful, microbial-based bioremediation offers a promising alternative. This study aimed to isolate and characterize fungi from OP-contaminated soils collected in Kilifi County and assess their potential for molecular identification and future bioremediation applications. Fungal isolates were obtained using an enrichment culture technique and purified through repeated sub-culturing. Morphological characterization was performed to confirm isolate purity. Genomic DNA was extracted from pure cultures, and its quality and concentration were evaluated using a Nano Drop spectrophotometer, while agarose gel electrophoresis was used to assess DNA integrity. Molecular identification was conducted by amplifying the internal transcribed spacer (ITS) region using ITS1-F and ITS4 primers. The amplified PCR products were purified and sequenced using the Sanger dideoxy sequencing method. Sequence analysis using BLASTn against the NCBI database revealed that most isolates showed 100% similarity with known fungal species. Phylogenetic relationships were inferred using the Maximum Likelihood method in MEGA X software. The findings confirm the successful isolation and molecular identification of OP-tolerant fungi from contaminated soils, highlighting their potential application in the bioremediation of organophosphate-polluted environments. These results provide a foundation for further studies on the development of efficient fungal-based systems for chlorpyrifos and related OP degradation.

Keywords

Organophosphate (OP), Enrichment, BLASTn, Phylogenetic Analysis

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1. Introduction

Because of their great efficacy, organophosphates (OPs) are frequently employed as insecticides in agriculture [2]. One of the most popular acaricides used by dairy farmers in Kenya to manage ticks is chlorpyrifos [O, Odiethyl-O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate] (CP) [10]. It dissolves easily in the majority of organic solvents while having a limited solubility in water. Meat and milk have become contaminated due to the overuse of CP as an acaricide [18]. Because milk is lipophilic, it especially accumulates acaricide residues. In humans, CP residues affect central nervous system (CNS) activity by interfering with the activity of acetyl cholinesterase (AChE-ase) [1, 11]. Symptoms of severe CP poisoning in humans include nausea, headache, twitching of the muscles, disorientation, increased perspiration, weakness, and salivation [6]. There is still a lack of research on the most effective methods for removing organophosphates from contaminated environments, despite studies showing their detrimental effects on human and animal health [25].

Acaricide residues in animal products frequently come from contaminated soil, water, and feed. Specifically, CP residues and its metabolites are stored in the soil. Therefore, it is necessary to use biodegradation to remove the residues from polluted soils [28]. Microorganisms are the main mechanism for breakdown detoxifying in soils, even though abiotic pathways can degrade xenobiotics in water and soil. Although physical-chemical elements including moisture content, temperature, pH, pesticide formulation, and organic carbon content can also affect the process, numerous studies [10, 16, 31] have demonstrated that microbial activity is the most important factor in the breakdown of xenobiotics [12]. According to [23], microbial biodegradation is a very appealing method of eliminating hazardous substances from the environment.

Numerous studies have shown that a variety of terrestrial and aquatic ecosystems may be contaminated with chlorpyrifos [13, 18] which has raised public concern about the need to find an effective, secure, and affordable way to eliminate or detoxify chlorpyrifos residues in contaminated areas. According to [1, 20], microbial degradation may be a helpful strategy for bioremediation of chlorpyrifos contamination. Numerous investigations [24-27] have also demonstrated that *Aspergillus* sp. Y, *Trichoderma* Pres. ex Fr, *Fusarium* LK. ex Fx, and *Verticillium* sp. DSP utilize chlorpyrifos in pure cultures and soil [17]. The 3,5,6-trichloro-2-pyridinol (TCP) and diethyl thiophosphoric acid (DETP) are the primary byproducts of CP breakdown. Few investigations have isolated and described fungi with degrading capacity, despite the widespread use of OP acaricides, including CP, in Kenya [2]. One of the most effective environmental friendly methods for detoxifying organophosphates is soil microflora. Pesticides serve as nutrition for the growth of these microorganisms [31]. Because native microorganisms are more suited to the local environment,

their isolation might be advantageous for *in situ* bioremediation [24]. Therefore, the extraction of native fungi from organophosphate-contaminated soil, morphological and biochemical analysis, and molecular characterisation through DNA amplification, ITS gene sequencing, and phylogenetic tree construction were the main goals of this work.

2. Materials and Methods

2.1. Sampling Site

Based on data gathered from the local veterinary officers, a cross-sectional study design was used to interview livestock farmers in three purposefully chosen sub-counties (Kilifi North, Kilifi South, and Kaloleni) in Kilifi County. Kilifi County is situated at 3°37'49.62" S 39°50'59.71" E on Kenya's coast. The names of farmers from each of the selected sub-counties were compiled in order to apply the purposive sampling approach. Primary data on the several acaricides used was gathered by questionnaires, casual in-person interviews, and personal observations. Soil samples were collected from farms with long history of using organophosphate acaricides.

2.2. Sample Collection

After removing the plants and wastes from the soil's surface, soils were sampled at a depth of 0–0.16m using an auger. After mixing, 15g of the soil samples were placed in the plastic zip locks and were appropriately labeled and refrigerated ready for analysis. Each sampling point consisted of three soil samples taken at a rooting depth of 160 mm. A composite sample was created by randomly combining these samples, and was thereafter labeled appropriately [18, 22].

2.3. Isolation of the Fungi from the Soil Samples

2.3.1. Preparation of Enrichment Media

A medium phosphate buffer containing mineral salts (MSM) was prepared using the following components in grams per liter: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; K_2HPO_4 , 4.8; $\text{Fe}_2(\text{SO}_4)_3$, 0.001; NH_4NO_3 , 1.0; KH_2PO_4 , 1.2 and $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 0.4, [2]. Chlorpyrifos (10 mgL^{-1}) was added as the sole carbon source. Fifteen grams agar per liter was added to MSM composition before autoclaving to constitute mineral salt medium agar.

2.3.2. Inoculation of the Media

For each sample, 2g of soil sample were suspended in 10 mL of mineral salt medium (MSM). The samples were incubated in 100 mL culture flasks at 30 °C on an orbital shaker at 110 rpm for 30 days. Cultures were maintained using aerobic techniques which included casing media with sterile cotton wool and constant agitation to allow uniform circulation of air [16].

2.3.3. Colony Isolation

Using sterile swabs, cultures from the flasks were then spread plated into potato dextrose agar plates containing mineral salt medium supplemented with 15mgL⁻¹ of Chlorpyrifos for 15 days. Distinct colonies obtained were sub-cultured by streaking periodically on Chlorpyrifos-supplemented solid medium.

2.3.4. Morphological and Microscopic Characterization of Isolated Fungi

Morphological identification of the isolates was done under the compound light microscope (Olympus, Japan) to determine the shapes, pigmentation and consistency of the colonies. This was followed by the lactophenol cotton blue staining technique and images were captured using Zeiss Primo Star camera microscope [2].

2.4. Molecular Characterization of the Fungal Isolates

2.4.1. Genomic DNA Extraction

The isolates were further subjected to molecular characterization to further identify them based on their ITS gene region. The following steps were followed; genomic DNA extraction, amplification using polymerase chain reaction (PCR), Gel electrophoresis and sequencing of the ITS gene region using the Sanger sequencing method. The gDNA of the fungal isolates was extracted using Zymogen Quick-DNA™ min prep kit (Zymo Research, USA) according to the manufacturers' protocol. In this procedure, young fungal cells were suspended in 200 µl isotonic buffer in a ZR Bashing Bead™ Lysis Tube and 750 µL Bashing Bead™ Buffer was added to the tube. The bead beater was held and fitted with a 2 mL tube holder assembly and was centrifuged at maximum speed for 5 minutes. The ZR Bashing Bead™ Lysis Tube was separated in a microcentrifuge at 10,000 x g for 1 minute, and 400 µL supernatant was pipetted into a Zymo-Spin™ III-F Filter in a collection tube and spun at 8,000 x g for 1 minute. Then 1,200 µL of Genomic Lysis Buffer was transferred to the filtrate in the Collection Tube and 800 µl of the mixture was transferred to a Zymo-Spin™ IICR Column in a Collection Tube and spun at 10,000 x g for 1 minute. The flow in the collection tube was disposed of and this process was repeated. The Zymo-Spin™ IICR Column was then transferred to a clean 1.5 ml microcentrifuge tube, and 50 µl DNA Elution Buffer was pipetted straight to the column matrix and centrifuged at 10,000 x g for 30 seconds to elute the DNA. Next, 200 µl DNA Pre-Wash Buffer was transferred to the Zymo-Spin™ IICR Column in a new Collection Tube and spun at 10,000 x g for one minute. The eluted DNA was kept at -20 °C awaiting sequencing [2].

2.4.2. Polymerase Chain Reaction (PCR) Amplification of ITS Gene Region

PCR was done in a 20 µL reaction volume, containing 10.0 µL NEB One Taq 2X Master Mix with Standard Buffer, 1µL of 10 µM Forward primer (ITS1-F 5'-TCCGTAGGTGAAC-CTGCGG-3'), 1µL of 10 micrometers (µM) Reverse primer (ITS4-R (5' TCCTCCGCTTATTGATATGC -3')), 1.0 µL of DNA template and 7 µL of Nuclease free water. The PCR reaction was performed using a thermocycler (Nexus Eppendorf™, Malaysia) with the following conditions: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 secs, primer annealing at 50°C for 30 secs, extension at 68 °C for 1 min and final extension at 68°C for 10 minutes. The amplicons were kept at -20°C awaiting sequencing [27].

2.4.3. Gel Electrophoresis of the PCR Products

A 1% agarose gel stained with EZ-vision® Blue light DNA Dye was used to assess the PCR amplicons integrity. Agarose gel in 1X Tris Borate EDTA buffer at 75 volts (V) for 30 minutes was viewed on E-box (Vilber, Australia) and the estimation of molecular sizes of the bands was done using 1Kb ladder (Biolabs) [3].

2.4.4. The Sanger Sequencing of the ITS Gene Region

The PCR products were cleaned prior to sequencing using ExoSAP Protocol where 2.5 µl of the Exo/SAP master mix (prepared by transferring 50 µl of Exonuclease I 20 U/ul and 200 µl of Shrimp Alkaline Phosphatase 1 U/ul to a 0.6ml micro-centrifuge tube) was thoroughly blended with 10 µl of amplified PCR product and incubated 37°C for 15 minutes then heated at 80°C for 15 min. to stop the reaction. Sequencing of fragments was done using the Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions. The cleaning of the labelled products was done using ZR-96 DNA Sequencing Clean-up Kit and injection of the cleaned products was done on an Applied Biosystems ABI 3500XL Genetic Analyser. Sequence chromatogram analysis was carried out using FinchTV analysis software (Geospiza, Inc.).

2.4.5. BLASTn Analysis and Phylogenetic Analysis

The obtained DNA sequences were edited and assembled using Chromas Pro Version 2.3 (Technelysium Pty Ltd., Australia). The edited consensus sequences were then subjected to BLASTn analysis against the National Center for Biotechnology Information (NCBI) nucleotide database to determine their closest genetic relatives based on sequence similarity. The resulting homologous sequences were retrieved and aligned for phylogenetic analysis. Phylogenetic trees were subsequently constructed using the Molecular Evolutionary Genetics Analysis software, MEGA X, to infer the evolutionary relationships among the fungal isolates and reference taxa.

3. Results

3.1. Morphological and Biochemical Characterization of Isolated Fungi

Table 1. Morphological and biochemical characterization of isolated fungi.

Isolate	Colony Colour	Texture	Margin	Growth Rate	Microscopic Features
KA1	Cream-white	Smooth	Entire	Rapid	Oval budding yeast cells
KB2	Yellow-green	Powdery	Entire	Fast	Aspergillus-type conidial heads
KC3	Blue-green	Velvety	Entire	Fast	Columnar conidial heads
KD4	Yellow-brown	Velvety	Entire	Moderate	Septate hyphae, globose conidia
KE5	Green with white margin	Powdery	Entire	Moderate	Brush-like penicilli
KF6	White	Cottony	Irregular	Very fast	Non-septate hyphae, sporangia present
KG7	Brownish	Velvety	Entire	Moderate	Aspergillus-type conidial heads
KH8	Olive-greenish	Velvety	Entire	Fast	Septate hyphae, conidiophores with globose vesicles
KJ10	Black	Granular	Entire	Fast	Black conidia, biserial phialides
KK9	Yellow-green	Powdery	Entire	Fast	Rough conidia in chains

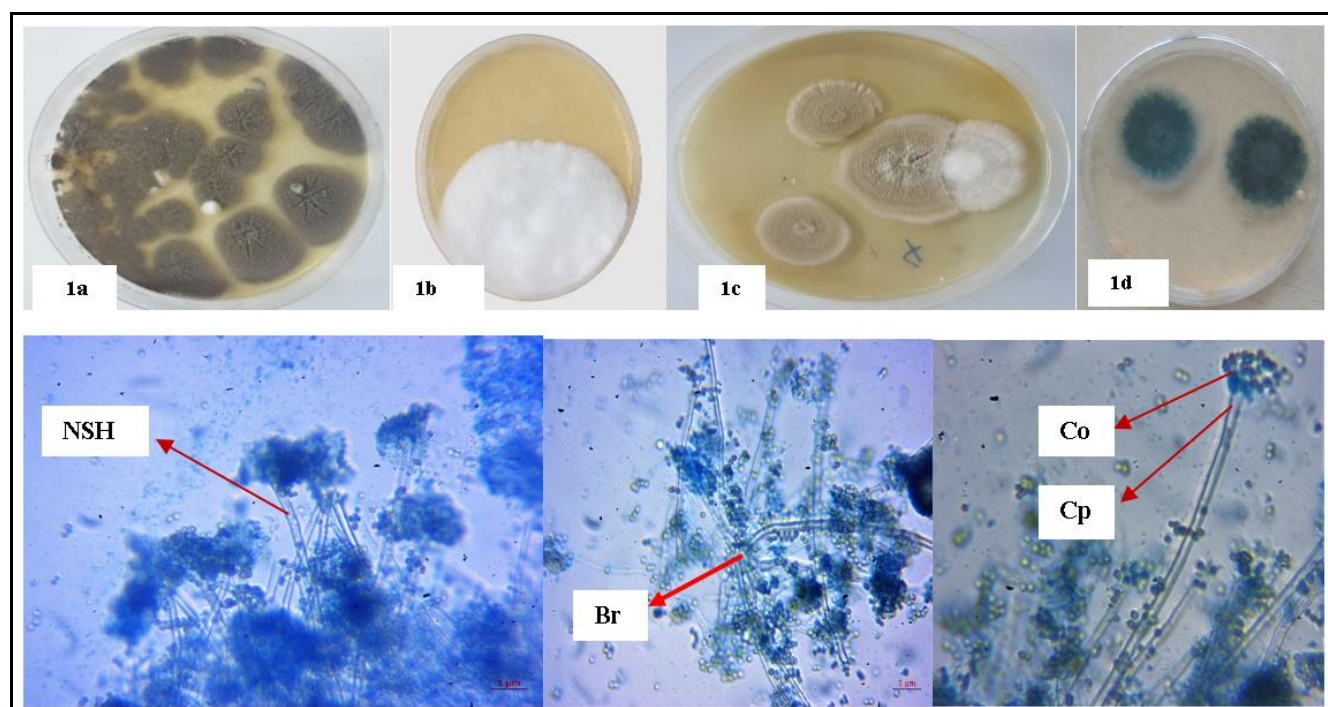


Figure 1. Plate cultures and the microscopic features of some representative fungal isolates, 1a, 1b, 1c and 1d represent 5-day old plate cultures; the microscopic features of some of the fungal isolates, Cp-Conidiophore, Co-Conidia, Br-Branched hyphae, NSH-Septate hyphae.

The fungal isolates exhibited diverse macroscopic and microscopic characteristics typical of filamentous fungi and yeasts. Colony morphology on potato dextrose agar (PDA) ranged from cream-white, yellow-green, olive-green, and black pigmentation, with textures varying from cottony and

velvety to powdery and granular. Most isolates exhibited circular colonies with entire margins and moderate to rapid growth rates (Table 1). Microscopic examination following lactophenol cotton blue staining revealed characteristic fun-

gal structures including septate hyphae, conidiophores, vesicles, phialides, conidia and distinctive brush-like penicillin in one isolate. Some isolates were characterized by broad aseptate hyphae, sporangiospores, oval budding cells with occasional pseudohyphae formation (Figure 1).

3.2. Molecular Characterization of the Fungal Isolates

3.2.1. PCR Amplification of ITS Regions

After PCR amplification, the isolates were shown to have fragments of the amplified DNA of around 700 bp (Figure 2).

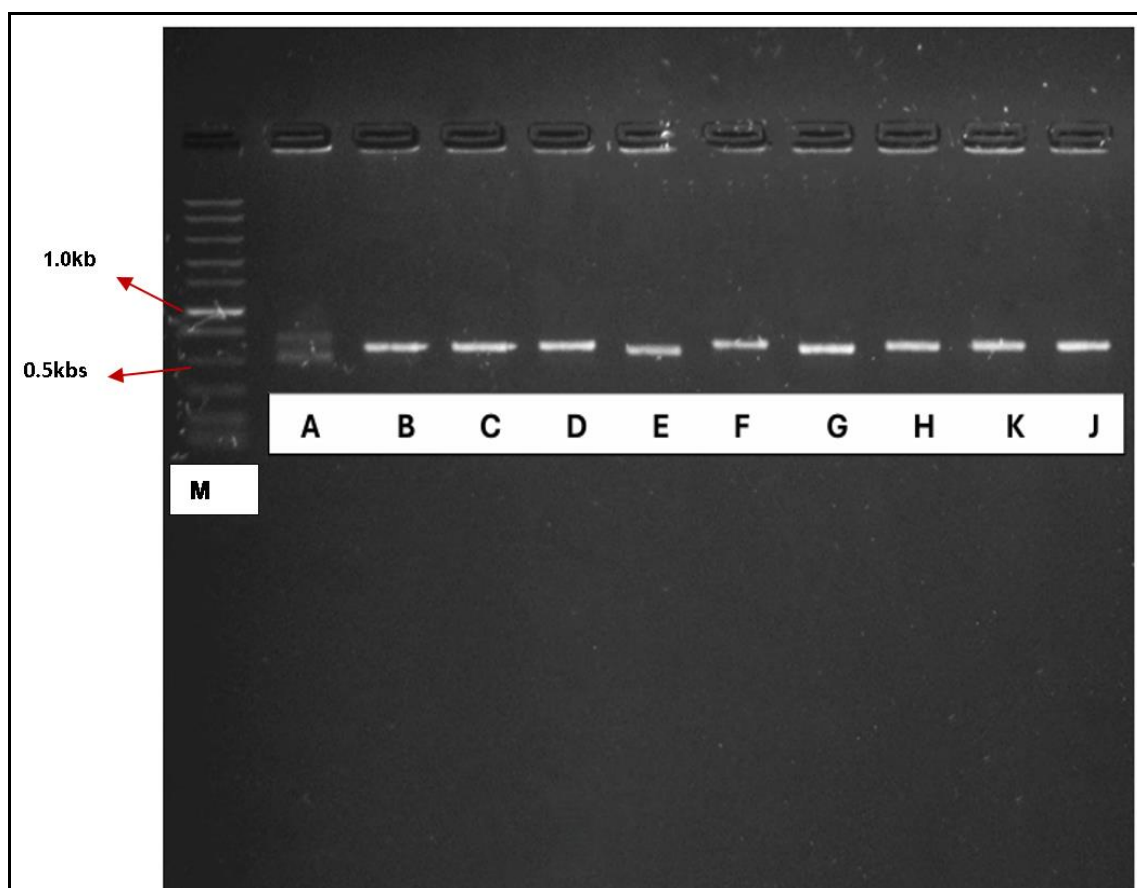


Figure 2. Amplification of ITS gene regions of the fungal isolates. A-J are the fungal PCR amplicons and M represents a 1 kb DNA maker. The expected band size amplified was approximately 700bps.

3.2.2. DNA Sequencing and Fungal Species Identification

Isolates were identified based on the similarity to sequences in the Genbank. The isolates belonged to the genera *Aspergillus*, *Penicillium*, *Pichia* and *Rhizopus* (Table 2). The phylogenetic grouping tree (Figure 3) was constructed using MEGAX Software (MEGA Software Company).

Table 2. Percentage identity of fungal isolates and their accession numbers.

Fungal isolate	Accession	Species (ITS gene analysis)	% Identity
KA1	MT071785.1	<i>Pichia kudriavzevii</i>	100
KB2	OQ438650	<i>Aspergillus fumigatus</i>	100
KC3	OM980663	<i>Aspergillus flavus</i>	100
KD4	KJ173526	<i>Aspergillus ochraceopetaliformis</i>	100

Fungal isolate	Accession	Species (ITS gene analysis)	% Identity
KE5	PP385661	<i>Penicillium citrinum</i>	100
KF6	OM959554	<i>Rhizopus arrhizus</i>	100
KG7	PP385269	<i>Aspergillus brunneoviolaceus</i>	99
KH8	PX970377	<i>Aspergillus flavus</i>	99
KJ10	ON688455	<i>Aspergillus niger</i>	100
KK9	MW250193	<i>Aspergillus oryzae</i>	100

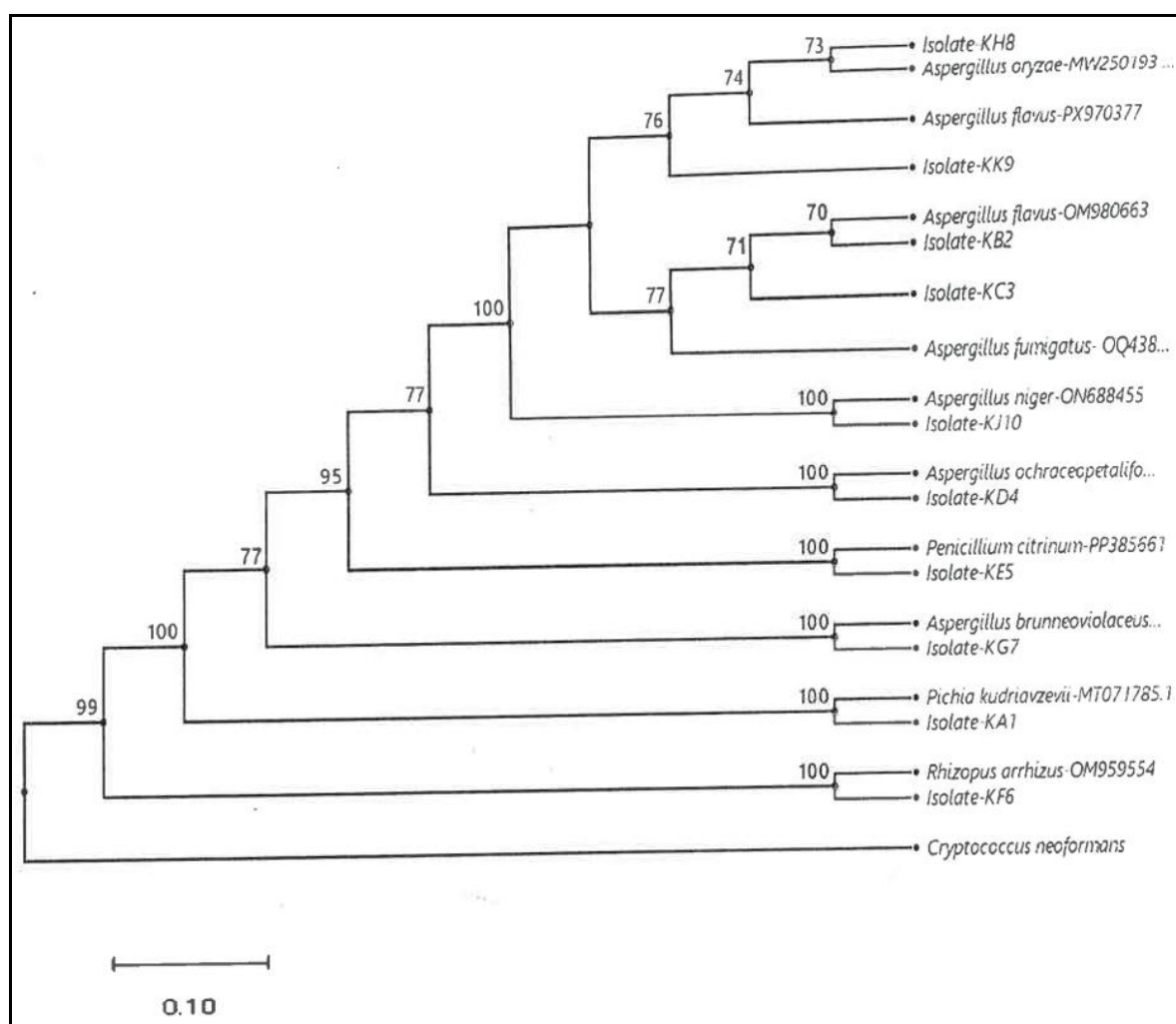


Figure 3. Molecular phylogenetic tree of fungal isolates based on ITS rDNA sequences.

The phylogenetic tree was generated in MEGAX software using the Maximum Likelihood method. Evolutionary distances were estimated using the Kimura 2-parameter model and are shown as the number of nucleotide substitutions per site. The robustness of the tree topology was assessed by bootstrap analysis with 1,000 replicates, and bootstrap values are indicated at the nodes. Bootstrap values of $\geq 70\%$ were considered to represent acceptable support for the corresponding clades. The tree was rooted with *Cryptococcus neoformans*,

which served as the outgroup. Study isolates are shown using isolate codes, while reference taxa are presented with their species names and GenBank accession numbers.

4. Discussion

The main aim of the study was to isolate and characterize the indigenous fungi from the organophosphate contaminated dairy farm soils in Kilifi County (Kilifi North, Kilifi South and

Kaloleni sub-counties) in Kenya. Because the acaricide is poisonous and its widespread use has detrimental effects on human health, it is desirable to remove Chlorpyrifos (CP) residues from the soil [1]. A dependable and economical method for CP abatement is bioremediation, which makes use of microorganisms' capacity for degradation [5, 21]. For *in situ* restoration activities, biodegradation is the optimum choice [10, 25]. Chlorpyrifos, an active ingredient of OP acaricides, is widely used in Kilifi County, Kenya, to manage ticks in dairy farms, which affects the land, water, and animal feed. This study is one of the first to investigate the bioremediation of chlorpyrifos residues using natural bacteria that were recovered from soils in local dairy farms. Additionally, it is the first study to precisely isolate fungus from OP-contaminated soil from farmers employing Duodip acaricide, a commercial CP formulation that Kenyan dairy farmers frequently use. An eco-friendly technique for *in situ* detoxification results from the isolation and characterization of native microbial strains. Because they don't harm the soil microflora, indigenous species are favored [1]. Autochthonous fungal populations have developed to adapt to pollutants in contaminated habitats, including the dairy farm soils studied in this study [24]. As a result, the sites' soils make excellent biological niches for separating microorganisms that may break down the acaricide. The enrichment culture approach, a quick and easy way to separate fungus from polluted soils, was employed in this work [9, 13].

The principle of selective enrichment is based on providing a growth medium containing a specific substrate that can be utilized only by the target microorganism, thereby inhibiting the growth of non-target organisms and serving as a selective medium for the organism of interest [19]. In the present study, Chlorpyrifos (CP)-supplemented minimum salt medium (MSM) was employed as a selective medium to isolate fungal strains capable of utilizing chlorpyrifos and its intermediate degradation products as the sole source of carbon and energy. Consequently, only fungi possessing the metabolic machinery required for chlorpyrifos degradation were able to grow and proliferate on the medium, facilitating their isolation and subsequent characterization [4]. The MSM-isolated fungi had genetic sequences homologous to *Pichia kudriavzevii*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus ochraceopetaliiformis*, *Penicillium citrinum*, *Rhizopus arrhizus*, *Aspergillus brunneoviolaceus*, *Aspergillus flavus*, *Aspergillus niger* and *Aspergillus oryzae*. Continuous application of chlorpyrifos as an acaricide in Kilifi dairy farms may have led to prolonged exposure of soil fungi to pesticide residues. Such conditions impose selective pressure on indigenous fungal communities, potentially favoring strains with adaptive metabolic capabilities. This is evidenced by the ability of isolates to grow in mineral salt medium supplemented with chlorpyrifos as the sole carbon source, suggesting possible utilization or tolerance mechanisms [6, 14, 31].

Some previous studies have reported fungi that have been isolated from OP contaminated soils, which include *Fusarium*,

Aspergillus niger, *Penicillium*, *Lentinula edodes*, *Lecanicillium*, and *Oxyasporum* [6, 27]. In another study, main soil fungi *Trichoderma viride* and *Aspergillus niger* were assessed for the biodegradation of Chlorpyrifos. *T. viride* stood to be more active, with 95.7% of Chlorpyrifos biodegraded by *T. viride* in comparison to 72.3% by *A. niger* at end of incubation period of 21 days [17]. The findings indicate that the fungal isolates were capable of utilizing chlorpyrifos (CP) as the sole source of carbon and energy, without the need for supplementary nutrients to induce the expression of organophosphate (OP)-degrading enzymes. This demonstrates the intrinsic metabolic capacity of the isolates to degrade and assimilate chlorpyrifos [8].

Microbial degradation is considered one of the most effective and environmental friendly approach for the removal of toxic compounds from contaminated environments (Tom *et al.*, 2024). Among microorganisms, bacteria and fungi are the primary agents responsible for the transformation and degradation of pesticides. However, only a limited number of species have been reported to effectively degrade organophosphate compounds due to their complex chemical structures [19].

Bioremediation, which involves the use of microorganisms to detoxify, immobilize, or mineralize environmental pollutants, has emerged as a promising strategy for the management of pesticide-contaminated soils and water bodies. The biodegradation of organophosphate pesticides is particularly important because these compounds can persist in the environment and potentially enter the food chain through livestock exposure, ultimately posing health risks to consumers of animal-derived products [29].

The ability of the isolates to grow in CP-supplemented medium may be attributed to their adaptation to pesticide-contaminated environments. According to [26], the prolonged and recurrent use of chlorpyrifos as an acaricide in dairy farms in Kilifi County has subjected soil fungal communities to continuous pesticide exposure over many years. Such selective pressure may have favored the enrichment and evolution of fungal populations capable of tolerating and metabolizing chlorpyrifos, thereby enhancing their biodegradation potential. Such chronic exposure exerts strong selective pressure, promoting the survival and enrichment of fungal strains capable of tolerating and metabolizing organophosphate compounds. In this study, the isolated fungal consortia was dominated by *Aspergillus*, *Penicillium*, *Rhizopus* and *Pichia* species, as confirmed through ITS rDNA sequencing and phylogenetic analysis. These genera are widely reported as key degraders of chlorpyrifos and other organophosphate pesticides due to their versatile enzymatic systems, including phosphatases, esterases, and oxidoreductases [7, 12]. Recent studies have demonstrated that *Trichoderma asperellum*, *Aspergillus sydowii*, and *Penicillium spp.* can utilize chlorpyrifos as a sole carbon source in mineral salt media, achieving substantial degradation efficiency under controlled conditions [10, 26]. The observed growth of isolates in chlorpyrifos-amended mineral

salt medium in this study therefore suggests adaptive metabolic capability driven by long-term environmental exposure and substrate-driven selection.

5. Limitation of the Study

It is widely recognized that traditional culture-based techniques capture only a limited proportion of environmental fungal diversity, with estimates suggesting that approximately 10% of fungal species are recoverable using standard laboratory cultivation methods. Thus, culture-dependent methods recover only a fraction of the microbial diversity present in environmental samples [10, 13]. Consequently, many fungal species involved in the degradation of chlorpyrifos and other environmental contaminants may remain uncultured and therefore undetected using conventional laboratory isolation techniques [23]. This limitation suggests that the diversity and biodegradation potential of fungal communities in contaminated soils may be substantially underestimated. To overcome these constraints, metagenomic approaches can be employed to explore and exploit the vast diversity of microorganisms inhabiting soil ecosystems. By enabling the analysis of genetic material directly from environmental samples, metagenomics provides insights into both culturable and non-culturable microbial populations and their functional roles in pesticide degradation [15, 30]. Furthermore, the successful detoxification of pesticides and other organic pollutants often depends on the synergistic activities of microbial consortia rather than individual species. Fungal communities may work cooperatively, with different species contributing complementary metabolic pathways that facilitate the complete degradation and mineralization of complex compounds [19, 27]. Therefore, understanding the composition and functional interactions of fungal consortia is essential for developing effective bioremediation strategies for chlorpyrifos-contaminated environments.

6. Conclusion

The production and application of acaricides have become indispensable in modern agriculture for effective pest control. As demonstrated through the identification and screening techniques employed in this study, the fungal isolates exhibited a notable ability to survive and grow in organophosphate (OP)-contaminated soils, indicating their potential tolerance and adaptability to pesticide-stressed environments.

Accordingly, the findings of the present study are significant for the potential application of these isolates in the bioremediation of OP-contaminated soils, given that microbial-based remediation approaches are generally efficient, cost-effective, and environmentally sustainable, with minimal disruption to indigenous microbial communities. The fungal isolates identified in this study therefore represent promising candidates for further development in chlorpyrifos (CP) bioremediation, particularly in contaminated dairy farm soils.

7. Recommendation

It is recommended that future research focus on the further characterization and optimization of the isolated strains to enhance their degradation efficiency. In addition, the development of a fungal consortium is strongly encouraged, as synergistic interactions among different strains may improve the overall degradation of chlorpyrifos through complementary metabolic pathways. Furthermore, future studies should investigate the molecular and biochemical mechanisms underlying degradation, including the identification of functional genes, key enzymes, and associated metabolic pathways, to support their effective application in large-scale bioremediation strategies and metagenomic approach using eDNA.

Abbreviations

PCR	Polymerase Chain Reaction
eDNA	Environmental Deoxyribonucleic Acid

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Author Contributions

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Gibson Gicharu: Supervision, Conceptualization, Resources, Data curation, Formal Analysis, Writing – original draft, Methodology

Makan Peter: Conceptualization, Resources, Data curation, Formal Analysis, Writing – original draft

Conflicts of Interest

The authors declare no conflict of interest.

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